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Newcastle Disease in Village Chickens

Control with Thermostable Oral Vaccines

Proceedings of an International Workshop held in
Kuala Lumpur, Malaysia, 6–10 October 1991

Editor: P.B. Spradbrow

Australian Centre for International Agricultural Research
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Contents

Preface

'There are no easy victories in the field of vaccine development and application.'

E. Norrby 1989. In: Modern Approaches to Live Virus Vaccines. Advances in Veterinary Science and Comparative Medicine, 33, 267.

THE Australian Centre for International Agricultural Research (ACIAR) has supported two consecutive projects on the vaccination of village chickens against Newcastle disease. The first project involved research at the Universiti Pertanian Malaysia and the University of Queensland. The original research groups developed the concept of a thermostable Newcastle disease vaccine and demonstrated the efficacy of vaccines based on strain V4 when they were administered on food. At the conclusion of the first project, ACIAR sponsored an international workshop in Kuala Lumpur to review these results and to consider the whole problem of village chicken production and Newcastle disease in Asia. The monograph 'Newcastle Disease in Poultry. A New Food Pellet Vaccine' resulted from that workshop. Village chickens were identified as a vital resource in most Asian countries and Newcastle disease was recognised as the key factor limiting full exploitation of this resource. Several Asian countries indicated their interest in participating in any future research project.

ACIAR then funded a second project on a more ambitious scale than the first project. The initial collaborators from the University of Queensland and the Universiti Pertanian Malaysia were joined by colleagues from other institutes in Malaysia and Australia, and the project was enlarged to include Thailand, Philippines, Indonesia and Sri Lanka. During this second project, further basic findings on the interactions of Newcastle disease vaccines, food carriers and chickens were obtained. Malaysia showed that large-scale control programs were feasible, successful efficacy and pilot village studies were repeated in other countries and quantities of field data were generated, collected and evaluated. Some distillations of our findings and of our musings on the project appear in this volume.

This project has been truly international in scope, with the direct involvement of six countries. It is a great strength of the ACIAR organisation that such complex, multinational programs can be accommodated, involving many government institutes and universities in very diverse countries. The project leaders and all those involved in the project acknowledge their appreciation, not only of the financial support from ACIAR, but of the organisational assistance from the ACIAR staff in Canberra and in the Embassies and High Commissions. Countries not directly concerned with the project have indicated their interest and support. In Asia, an important indication of this support has been the continued interest of FAO and its Animal Production and Health Committee for Asia (APHCA) in the project. The presence of APHCA delegates at the workshop was most welcome. Further FAO support was evidenced by the invitation to the project leaders to address an FAO workshop on 'Newcastle disease vaccines for rural Africa' in Ethiopia in April 1991.

Large scientific projects rarely progress strictly to proposed timetables, and rarely bring all the answers that were anticipated. Perhaps vaccine projects are more difficult than most with ' ... no easy victories ...'. We have shown that thermostable Newcastle disease vaccines can be produced and that they can protect chickens against virulent challenge, even when given on the feed. In some areas the project has progressed from the research phase to a development phase, requiring different sources of funding and deserving other forms of expertise. In other areas much research remains to be done, especially on delivery systems. Many people have contributed years of painstaking work to the project, in villages, in laboratories and at the computer. These efforts must eventually lead to an improved science of village chickens and to benefits for the villagers who own them.

As our project concludes, we can attempt an evaluation of our efforts. We know that the concept of a thermostable Newcastle disease vaccine is sound, and that oral vaccines based on these viruses can be highly protective. We acknowledge the need for further work, especially on food delivery systems. We have a greater appreciation and understanding of village chickens. These chickens, with Newcastle disease under effective control, will eventually contribute appropriately to the welfare of rural people. As a result of our project, the control of Newcastle disease in village chickens is changing in the countries that were involved. The governments in Malaysia, Philippines and Indonesia are actively planning or supporting larger trials or actual control programs with oral vaccines. In Sri Lanka and Thailand it may be more appropriate to deliver thermostable vaccines by conventional routes. Other countries, both in Asia and in Africa, are determined to take up our results to improve the performance of their village chickens.

I believe we have done something useful.

P B Spradbrow
University of Queensland

Thermostable Vaccines and Village Chickens

The Origin and Outcomes of the ACIAR Newcastle Disease Project

J. Copland*

Abstract

The origins of the projects reflect the importance of Newcastle disease in Southeast Asia; the lack of appropriate vaccines in village poultry farming systems; building on earlier experience and research interests of Australian and Asian partner countries and their strategies for disease control. The project is a collaborative project between research institutions. The project was based on the high level of mutual interest in sharing the benefits of the research, training, linkages to AIDAB and other agencies, such as FAO (APHCA); and the development of commercial interests in the technology generated by the projects and sustainability of impact.

The projects consisted of two phases: the first, to develop and evaluate a heat-resistant feed-delivered lentogenic vaccine strain of Newcastle disease virus (HRV4), isolated in Australia, to protect village poultry against pathogenic wild strains of the virus in Malaysia; the second, to expand the pilot trial in Malaysia, and determine the appropriateness of the immunology in village poultry farming systems of other countries in Southeast Asia.

The V4 virus was found to be an effective vaccine when coated on specific carriers in large-scale field trials in Malaysia. Success and failures have been reported in the field trials of other countries.

NEWCASTLE disease (ND) is a widespread and important cause of loss in productivity of village poultry. It was first described in Indonesia by Kranveld (1926) and the virus was isolated by Doyle (1927) in the United Kingdom. The name Ranikhet disease is commonly used in Asia. Ranikhet is the site of the first Indian recognition of the disease and the name is still used by communities with Indian backgrounds.

The ACIAR project aimed to increase the take-off of village poultry which in turn had the potential to improve the nutrition and welfare of Asia's communities. Village poultry systems have not benefited greatly by past research on ND, which has resulted in the development of vaccines suitable for intensive poultry industries. The project's objective therefore was to provide a simple and cheap method of protecting village poultry which was safe, appropriate and sustainable in the village poultry systems of Asia.

ND virus is a highly infectious paramyxovirus that is antigenically stable and has four pathotypes. Velogenic strains, particularly the viscerotropic sub-type, cause a severe disease and mortality in Asia; mesogenic strains are moderately virulent and cause mortalities of up to 50% and reduced egg production; lentogenic strains have low virulence, although they can effect egg production and cause mortality in young chickens. The avirulent strains cause no clinical disease when transmitted by natural routes (Spradbrow 1987). One of the avirulent strains, the V4 virus, is the focus of the research reported from this project.

Clinical ND is currently not present in Australia. However, the avirulent strain, V4, is present and was originally isolated in Australia (Simmons 1967).

Previous ND Research in Asia

In the past the Australian Poultry Research Fund and the International Foundation for Science supported a research project at Universiti Pertanian Malaysia (UPM), with the object of evaluating methods of controlling ND with avirulent strains such as V4. These projects, established in the early 1980s, studied the potential effectiveness of V4 to protect poultry kept under intensive conditions from wild strains of ND virus, when vaccinated by the traditional methods of the ocular and parenteral routes.

As Australia does not have wild strains of ND virus, Arthur Webster, a vaccine producing company, in association with UPM, undertook research to test the effectiveness of Australian manufactured ND vaccines. The V4 strain was one of the vaccine strains tested using artificially challenged birds. Again the results were supportive of the hypothesis that V4 could be used as one of the vaccines for the control of endemic ND. These results were later confirmed by Bell et al. (1991a,b) who, in a detailed larger study with meat birds, considered that

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the V4 vaccine was an effective vaccine, and had a role in control and eradication strategies in Australia should virulent ND enter the country.

UPM had also received support from the Malaysian Government, the Australian Vice-Chancellors Committee and others, in establishing the research capacity of the Faculty of Veterinary Medicine and Animal Husbandry.

The earlier Australian supported research on ND is indicative of the importance the Australian poultry industry places on reducing the threat and avoiding the losses which this disease could cause. This was one of the factors and a major influence in ACIAR's decision to support the projects reported in these Proceedings.

The ACIAR Projects

The philosophy of ACIAR is to support collaboration between Australian and overseas institutions that address priority research topics which are mutually important. The results of the ND projects are an excellent example of the mutual benefits to partner countries which can flow from such projects.

The 'spark' that set off the development of the first project was a meeting between Professor Latif, from UPM, and ACIAR at the 4th Conference of Institutes for Tropical Veterinary Medicine in Florida, USA. During the conversation Professor Latif outlined his work in Malaysia and his past collaboration with Arthur Webster and Professor Spradbrow on ND.

This led to Professors Spradbrow and Latif designing a new project for consideration by ACIAR for support. The endorsement of the priority status and the agreement to the proposed project was sought by ACIAR from the Government of Malaysia, as ACIAR operates through a government-to-government relationship, although non-government agencies, such as universities, as is the case in this project, can be supported.

The first ACIAR supported project titled 'Vaccination of Malaysian Village Poultry with a avirulent Newcastle Disease Virus — Phase I' commenced in early 1984 and a second phase based on the results of this project titled 'Control of Newcastle Disease in Village Chickens with oral V4 Vaccine' followed on, and ended in 1992.

Results

The Phase I project

A joint Australian–Malaysian review team which reviewed the first project towards its completion found that:

- the project had achieved the major objective of developing a cheap feed-delivered vaccine;
- the pilot village communities adopted the vaccination procedure and valued the resulting benefits;
- despite the need for further refinements, it was considered that sufficient reliable information was available to extend the vaccine to other Asian countries for wider use, with particular reference to economical and social factors; and
- there were potential commercial interests and opportunities in the vaccine.

The review recommended that:

- the current Malaysian village heat resistant V4 (HRV4) vaccination trial be extended;
- the range of traditional feeds in each country be examined for the most suitable feed vehicle for delivery of the vaccine; and
- the replication site of V4 virus in live chickens be identified and the effect of age on vaccination efficiency should be clarified.

One Malaysian project scientist was able to combine both project work and postgraduate research to complete a PhD at UPM.

The Phase II project

In a joint review of the project the main findings and achievements were considered to be as follows.

- The Webster HRV4 can be stored for at least 24 hours at 32°C without loss of titre.
- HRV4 survived better in cooked rice and resulted in quicker serological response than in uncooked rice.
- There was mixed success with rice bran as a vehicle after 24 hours. Rice bran quality varies between countries.
- Newly isolated Australian lentogenic strains were found to give better serological response than the original V4 when given with cooked rice.
- Viricidal components in paddy rice and wheat are present which could be reduced by washing, resulting in good serological response of feed-vaccinated birds.
- Virulent ND virus challenge of birds vaccinated with UPM virus (V4) coated on wheat and stored for 6 weeks showed between 40% and 90% protection in experimental flocks and approximately 60% in the village trials.
- The Malaysian Government has approved the use of HRV4 feed vaccination as the national control strategy.
- In small-scale semi-commercial systems the vaccine was effective in Malaysia.
- The efficacy trials were successful in most countries after several attempts.

- The pilot field control trials in Thailand, the Philippines and Sri Lanka had mixed success, with some trials not successful.

General recommendations made by the review team included:

- a need for further research on the feed delivery system such as identification and removal of HRV4 toxic factors;
- further research to optimise HRV4 survival on paddy rice;
- alternatives to rice-based carriers should be developed;
- village trials in Thailand and the Philippines should be extended to complete and analyse the data;
- the Australian Animal Health Laboratory be incorporated into the project to aid detection of natural outbreaks of ND and vaccine strains; and
- that the potential benefits of the project be capitalised by preparing 'development packages' with the Australian International Development Assistance Bureau (AIDAB).

An extension of one year was accepted by ACIAR and the partner countries. The overall funding from Australian sources is approximately $A500 000 for Phase I and $A1.3 million in Phase II. AIDAB contributed over 30% of the funding for the Phase II project.

Conclusion

The project origins were based on the importance of the disease, for different reasons, to Malaysia and Australia.

The project has demonstrated the usefulness of the HRV4 which has been adopted by Malaysia as the vaccine of choice. The need for an effective and cheap carrier besides wheat and paddy rice is still an important objective.

The heat resistant V4 vaccine has been shown to be effective against several wild strains of ND and has a role in countries with limited cold chain distribution systems. While the oral vaccine is one method of ND control, there remains a need for research to develop a wider range of delivery systems applicable to village poultry systems.

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Heat Stable Vaccines as One Approach to the Control of Newcastle Disease in Village Chickens

P. Spradbrow*

Abstract

The Newcastle disease vaccines that have been produced for the village chicken project needed to solve two problems — those of heat inactivation of viral vaccines exposed to ambient temperatures and of delivering vaccines to scavenging chickens that could not be individually caught. The solution to the first problem was to select the heat-resistant V4 strain of ND virus for greatly increased heat resistance. The partial solution to the second problem was to use an oral vaccine–heat resistant virus applied to various foods. Oral vaccination is not as efficient as conventional vaccination and there remain problems with virus inactivation on some foods. Improving husbandry may eventually allow conventional vaccination of most village chicken populations with heat resistant vaccines. At present, heat resistant vaccine supplied on food is, in many areas, the only feasible method for control of Newcastle disease in village chickens.

Two problems hamper the delivery of Newcastle disease (ND) vaccines to village chickens. The first is a problem common to the delivery of any vaccine, human or animal, in tropical or subtropical countries. It is the problem of heat inactivation. All vaccines are, to some extent, heat labile. Even inactivated bacterial vaccines, for example those against cholera and typhoid fever, have limited shelf lives at even 4°C. Attenuated viral vaccines are more fragile and even brief exposure to moderate heat will convert an effective living vaccine to an inactivated vaccine with insufficient antigenic mass to induce an immune response. Heat damage is believed to account for many vaccine failures in developing countries. ND vaccines share this susceptibility to heat damage.

The second problem concerns delivery of ND vaccines to individual chickens, after the vaccine has reached villages without inactivation. Most methods of application require catching and handling of individual chickens (eye-drop, intranasal application) or access to groups of confined chickens (aerosol or spray application and administration through drinking water). The conditions of village chickens vary. In some countries they share the house of the owners and might be regarded as companion animals. In a few places they receive good housing and supplementary food and they are kept as commercial farm animals. In most areas they have very primitive housing, or no housing at all, and their condition is nearly that of feral animals.

The delivery of fully potent vaccine to villages is the first problem to solve. One answer is the provision of efficient cold chains, but this is an expensive and usually an impractical solution. Recent developments in the provision of insulated transport containers of high efficiency may offer a partial solution to this problem. A second solution is to improve the thermostability of the vaccine, so that the reliance on expensive cold chains is reduced. There are two methods of achieving this result. The first aims to stabilize the vaccine by physical or chemical means. Attention to the process of freeze-drying vaccine is important, so that virus contained in the dry product has increased heat stability. Great progress has been made with the modification of freeze drying procedures for this purpose, but only the dried product is heat resistant. The reconstituted vaccine has the heat lability of the original virus suspension. The inclusion of various proteins (serum albumen, skim milk, or gelatin) chelating agents, sucrose, lactose or polymers has been used to increase the shelf life of vaccines in liquid form. The second method is to produce virus particles that have innate thermostability. This was the solution sought in the present project.

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Variation in the Properties of RNA Viruses

Viral genomes, as are the genomes of other organisms, are subject to alteration by mutation. RNA genomes, such as those of ND virus, are probably more susceptible to copying errors as the RNA is replicated, than are the DNA genomes of other viruses or the DNA genomes of microorganisms. In even a single egg passage of ND virus, it is unreasonable to suppose that all the progeny virus, possible 10^{11} virions, will have genomes of exactly the same sequence as the input virions, which may have numbered only 10^3. Some of these alterations in genomic sequence will be expressed as minor alterations in amino acid composition of virally coded proteins. Such altered proteins will remain unnoticed, swamped in the mass of virions that have replicated truly. Differences will not be noted unless the altered proteins confer phenotypic changes on the virions and until these altered phenotypes are favoured by selective pressure.

Clones of RNA viruses will all be the progeny of a single viral genome. It may not be assumed that all virions of a clone contain exact copies of the original genome.

Variations in the Properties of Newcastle Disease Virus

Isolates of ND virus contain subpopulations with different biological characters. The properties that are subjected to variation include plaque size when grown in cell culture (Durand and Eisenstark 1962), organ affinities (Spalatin and Hanson 1976), pathogenicity for chickens, pathogenicity for chicken embryos, ability to spread and susceptibility to natural inhibitors (Gustafson and Hanson 1982), receptors for various cell types (Piraino and Hanson 1959), heat stability of the haemagglutinin (Spalatin and Hanson 1976) and heat stability of infectivity (Goldman and Hanson 1955). Doubtless other characters subject to genetic variation could be discovered if suitable tests were designed. It is the last-mentioned character, heat stability of infectivity, that is particularly important for the village chicken project.

Heat Stable Viruses

Many studies with viral vaccines have used differential sensitivity to heat. Most of these studies have been on temperature sensitive mutants, viruses whose replication cycles are impaired at normal body temperatures. Such viruses are of reduced pathogenicity, and often favoured as vaccines.

Heat resistance has received less attention. The interest here is not the optimal temperature for viral replication in cells, but the temperatures to which the virions can be exposed without destroying infectivity. Such studies were first reported with bacteriophages, heat resistance being used as a marker in experiments on genetic recombination. The first heat resistant variants of ND virus were produced more than 35 years ago, for the same purposes (Goldman and Hanson 1955). A few years later Hofstad and Yoder (1963) noted that various strains of Newcastle disease virus had different heat stabilities when prepared as freeze-dried vaccines. They postulated that it might be possible to select stable vaccines that retained immunogenicity.

Heat Resistant Vaccines

The first studies on heat resistant vaccines were made with herpesviruses, the first experiments coming out of Hungary some 25 years ago and dealing initially with Aujeszky's disease virus. The heat-resistant Bartha strain of Aujeszky's disease virus is still used as a vaccine. Heat resistance was found to correlate with attenuation of virulence and this correlation has since been established for other herpesviruses. We developed a heat-resistant avian herpesvirus (infectious laryngotracheitis virus) in 1979. Attenuation has been the rationale behind the selection of heat-resistant herpesviruses for vaccines.

With ND we have another reason for selecting viral subpopulations for heat resistance. This is for the production of a robust vaccine that can be taken into the field with minimal dependence on cold chains. The starting point for our studies has been the V4 strain of ND virus. This virus is avirulent for chickens, producing no clinical signs of disease when it spreads by natural means. Oro-nasal infection has also produced no clinical illness and minimal levels of gross or microscopical pathological changes (Hamid et al. 1990). The V4 strain has a high degree of heat resistance and has been the basis of Websters' commercial vaccine for many years. It had other traits that were useful in a vaccine designed for use in village chickens. It gives good protection, and it will spread from vaccinated chickens to nonvaccinated chickens in contact with them. Similar requirements for robust vaccines have prompted the selection of rinderpest vaccines for heat resistance (Provost and Borredon 1972). Table 1 illustrates some of the uses of heat-resistant virions.

Another feature of V4 populations is the ability to select from them subpopulations of extreme thermostability. This has now been done in several countries including Australia, Malaysia and Indonesia. Websters have selected a heat resistant vaccine strain from their original V4 vaccine seed. With the commercial V4, thermostability is a property of both the freeze dried vaccine and the reconstituted product.

Various strategies have been used to select heat resistant vaccines. Some use stepwise exposure to increasing

Table 1. Heat resistant virions

Virus	Date	Purpose
Bacteriophage	1953	Mutants for
Newcastle disease	1955	recombination experiments.
Aujeszky's disease	1968	
Other mammalian herpesviruses	1969	Attenuation
Infectious laryngotracheitis	1979	
Rinderpest	1972	Robust
Newcastle disease	1984	vaccines

temperatures, with final selection at 56°C. Others have advocated short exposures to 56°C to enhance the proportion of resistant particles in the population. It is also effective to initiate extreme selection at 56°C, using only those few longest-surviving virions as the parental stock for the next generation. Selection at 56°C has been used because it gives rapid results, and it is assumed that particles that have high survival at 56°C will also survive well at the ambient temperatures likely to be encountered in tropical countries — 30°C to 40°C. This needs to be established, and there could be merit in selecting at these ambient temperatures, although selection times would be much greater. It is known, for example, that poliomyelitis virions selected for stability at 50°C have similar stability to that of parental virus at 37°C (discussed in Woese 1960).

It seems that strains of ND virus other than V4 will respond to selection for heat resistance. Some of these may be described at this workshop, and a heat resistant vaccine strain of ND virus has been described from Hungary (Meszaros 1991).

The chemical changes that confer thermostability on ND virus are not known. The changes might involve nucleic acid or protein. It is known that very minor changes will enhance the thermostability of enzymes and such induced changes are used in enzymes that are engineered for industrial use. With glucose isomerase it has been reported that a single amino acid substitution yielded a form of the enzyme with an increase in half-life of nearly threefold (Quax et al. 1971).

The Village Chicken Program

A heat resistant, attenuated virus solves many of the problems of delivering vaccines in countries where cold chains are not readily available. Various heat resistant progeny of V4 virus have formed the basis of the village vaccination program. These vaccines perform well when given by conventional routes, but conventional vaccination was seldom possible in the populations of village chickens with which we were dealing. Catching even a small sample of village chickens for blood sampling proved to be a major task. Catching them all for vaccination would not be feasible.

The second phase of the project involved the development of methods for supplying heat resistant vaccine to chickens on food. Some of the problems encountered with various feed stuffs will be discussed in this workshop, as will the large-scale adoption of the process in Malaysia. Feed vaccine does offer us the beginnings of a process for control of ND in village chickens. As the disease becomes controlled and the value of village chickens is realised, husbandry procedures will certainly improve. This will eventually allow the replacement of oral vaccination with more efficient routes of vaccination. Heat resistant vaccines will remain basic to the program.

Future Vaccines

The late Professor Hanson (Hanson 1988) demonstrated that ND isolates were composed of various subpopulations, and he suggested that such mixed populations were required for perpetuation of the virus in populations of chickens. Subpopulations with various characters can be obtained by applying selection pressures to these isolates. It should be possible to produce improved vaccines by selecting for all the properties required by the vaccine. In the present studies we have selected only for heat resistance. It should be possible to select also for ability to spread, antigenicity and oral infectivity.

The practice of cloning vaccine stocks also needs to be reconsidered. Preparing vaccines from seedlots is, I agree, essential but do these seedlots need to be cloned? Would a vaccine composed of subpopulations be more versatile and mimic closely the mixed populations of ND viruses that circulate in populations of chickens?

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Observations on Some Difficulties Encountered in Trials with Oral Newcastle Disease Vaccination

A.R.B. Jackson*

Abstract

A number of significant problems have been encountered in trials with feed-based oral vaccination of village poultry in Asia. An important early difficulty was the now demonstrated viricidal properties of grains and the resulting variable, usually poor longevity of the virus on inoculated feed. Other problems were associated with inadequate knowledge of the epidemiology of Newcastle disease in village chickens and concurrent diseases and their diagnosis, the different strains of virus used, the birds and the interpretation of trial results. The recognition of these problems is an essential component in the ultimate development of a reliable virus and robust delivery system essential for the adoption of a feed-based oral vaccination program.

BETWEEN July 1988 and September 1990, I visited Malaysia, Thailand and the Philippines at irregular intervals to assist with the efficacy and village trials with feed added Newcastle disease vaccination. My task was to advise project workers in the countries concerned and, in particular, to identify and assist with problems as they arose.

As with all large projects, a number of difficulties did arise and were of major concern to the program. The purpose of this paper is to briefly discuss some of these problems so that they might be clearly identified and allowed for in current and future trials. Experimental work associated with some of these problems is reported by others.

Disease Factors

i. Epidemiology

While velogenic viscerotropic Newcastle disease (ND) in the form of an explosive epidemic in unprotected commercial birds is well recognised, its behaviour as an endemic disease in village poultry is likely to be quite different.

During 1988, ND was prevalent in the villages surrounding the town of Rosario in the Philippines. The trial monitored mortalities in individual households, and birds submitted to the laboratory confirmed ND by virus isolation. Mortalities were quite variable and continued over some months, usually affecting household in groups, lingering or doubling back as new susceptible birds were raised.

Field investigation showed that the presence of busy roads or intervening paddy fields played a part in slowing or inhibiting spread. Many factors, however, probably contributed to the natural disease's irregular spread and variable mortality, all of them worthy of study.

Results of our field trials in village chickens were dependent on ND's natural spread as an imperfectly understood endemic disease.

ii. Mortalities

Paucity of information on causes of mortality is an added difficulty in assessing the value of vaccination.

Our field trials were backed by competent laboratory diagnosis, but the number of birds submitted, even from suspected cases of ND, was low and causes of deaths were largely presumed. Even mortalities themselves may have been masked by the practical expediency of consuming sick and in-contact birds. Although results of disease surveys are available, the actual role of concurrent disease (or inadequate husbandry) in our mortality patterns remains unknown.

Considering the difficulty of extending diagnostic service to the village householder level, significant monitoring, however valuable, would require a substantial input of expertise and funds. In Australia, 'backyard'

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poultry are not normally provided for in any diagnostic program.

Virus Factors

A number of problems can be identified with the virus vaccines used in the trials. Indonesia and Malaysia have used their own locally developed heat-adapted V4 vaccine of varying origin, while Thailand and the Philippines have used the Australian Websters HRV4, resulting in difficulties in direct comparison between trials. In addition, it is now known that one batch of Websters HRV4 did differ significantly from others used.

The development of heat-adapted strains by progressively subjecting the virus to increasing temperatures might also be expected to alter their infectivity, immunogenicity and capacity for lateral spread as compared with the original V4. Any expectations based on trials with V4 may not be valid.

As the oral route of infection is known to be less efficient than the respiratory, maintaining an infective dose is critical. Loss of virus coupled with viral inadequacies has most probably led to some disappointing results.

Feed Factors

A practical period of longevity of vaccine virus on the food vehicle was early identified as an essential property of a feed vaccine.

To this end a period of heat stability to high ambient temperatures was developed in the virus on the assumption that this would be the significant limiting factor.

In trials in Armidale and Brisbane, however, it could never be shown that the heat adapted V4 virus (HRV4) was consistently and reproducible stable at any ambient temperature on feed. With this in mind field trials in the Philippines and Thailand allowed for a minimum time between incorporating the virus with the feed and feeding. This minimum time was minutes in Thailand, and up to a couple of hours in the Philippines.

Probably all grains and seeds protect themselves with antimicrobial chemicals which diffuse to their surface on the stimulus of moisture. The demonstration at Armidale of antiviral substances on grain, and the value of heat, washing and cracking grain has suggested ways for the development of successful vaccine vehicles. The considerable success of the Malaysia trials using wheat and virus coated by a Glatt machine (Uni-Glatt Fluidised Bed Granulator, Laboratory Model; Benzen–Halbiegen, Germany), is probably explained by the rapid drying afforded by that technique.

The development of a practical and reliable feed vehicle to allow for a period of vaccine longevity remains the most important problem in the adoption of a feed vaccine program. A reliable period of 24 hours would enable mixing of vaccine at a veterinary or village centre for distribution that day and feeding at dawn the following morning, and is seen as a minimum practical requirement.

Bird Factors

At an early inspection of a maize-growing area near Batangas in the Philippines, the ability of a rattled tin of maize to attract household birds was amazing. In the trials in rice areas in both Thailand the Philippines the situation was often different.

Particularly during the rice-growing seasons, birds needed to be confined overnight prior to feeding in the morning, or fed immediately on leaving the roost at dawn. Birds irregularly and reluctantly returned from their scavenging to receive the feed vaccine.

In feeding, problems of peck order and chick size was seen to influence the effective dosing of flocks.

Many householder's birds appeared semi-feral in their behaviour and the stress associated with catching, transport and confinement in challenge trials remains an unknown factor.

The Trials

i. Controls

While it has been accepted that adequate control groups are an essential part of the village trials, in no country has it been possible to adequately arrange for them.

To be a negative control holds no attraction for the village householder. Involved in the daily task of making ends meet, she sees no dividends in a project she little understands or which offers her no immediate advantage. At the same time, all those involved in organising the running of the trial are paid in comparison handsome salaries and living expenses. The provision of suitable incentives for controls must be an integral component of a budget.

Positive controls making use of an alternative proven treatment, as was used in Thailand, can be a practical alternative. In the ultimate analysis, however, assessment is difficult without comparison of one of the forms of treatment with the untreated state.

ii. Results

It is always disappointing in experimental work to obtain results contrary to expectations. It is often these unexpected results, however, that ultimately prove the most interesting.

Most of the efficacy trial results were disappointing and were repeated in the hope of demonstrating better protection. The results, however, when understood, provided strong clues — parboiled rice better than white rice, cooked parboiled better again.

Experimental results in general produced inconsistent results suggesting an incomplete understanding of the system. Endless repeating to obtain a wanted result ignores the problem.

Experiments which are technically faulted may be safely discarded, but the selection of good results for reporting while omitting others is poor science. The level of protection measured in a field trial may be disappointing, but it is probably not the measurement that is at fault. Selection of results will certainly not report the true field situation.

There are problems in comparing the results obtained in the different countries. Different strains of virus, birds, feeds and methods of feeding have already been referred to.

Generally there was an effort to standardise laboratory techniques. The lack of a universally adopted standard control serum made direct comparison of serological results an early casualty.

Some differences may be less obvious. In the Philippines birds purchased for challenge trials were drawn from a large number of households and results obtained were probably a reflection of the immune status of the vaccinated village birds as a whole where ND in endemic. In Thailand in an attempt to measure protection achieved in vaccinated flocks, purchases were made from a small number of selected households on the basis of satisfactory vaccination technique and no recent evidence of ND.

Conclusions

While not identifying all problems encountered, I have attempted to describe a number I found important during the period I was involved with the project.

The problem of ND both as a cause of economic loss in Asia and potential threat to poultry producers in Australia needs no stressing. The oral vaccination program has the potential to make a significant contribution in controlling the disease in village poultry.

As with all new ideas, difficulties must be expected. Identifying a problem is an important step in its solution. The general adoption of a feed-based oral vaccination program in village poultry will require a reliable virus and robust delivery system. Current work should aim to develop these objectives. Further advocacy of the system should await such developments.

A Review of the Use of Food Carriers for the Delivery of Oral Newcastle Disease Vaccine

P. Spradbrow*

Abstract

Further work will be required on the treatment of food grains to ensure the attachment of vaccine virus and its survival for a suitable length of time. Best results to date have been obtained with wheat-based vaccines in Malaysia, even after prolonged storage, and with unhusked (paddy) rice-based vaccines in other countries, especially when the vaccine is fed soon after preparation. Trials with other grains will be required. Some well waters and rain water have proved suitable for use as diluents. Polyvinylpyrollidone (PVP) has been the most successful additive to protect vaccine virus after application to grain.

ORAL vaccination with thermostable strains of Newcastle disease virus (NDV) seems not to be the most efficient way of delivering Newcastle disease (ND) vaccine. Nevertheless, in many areas, it remains the only feasible way of reaching large, scattered populations of free-ranging, scavenging, nearly feral chickens. Certain problems associated with survival of vaccine virus on food still need to be overcome. The following short review considers some of the findings that have come from the present project and from other trials that have been published. The review deals with the food carriers and the diluents that are used to carry the virus on to the food carriers. As further countries begin trials with oral vaccine, local foods that have not been used previously as carriers must be tested. The review will indicate that some problems remain.

Food Carriers

a. Commercial pellets and crumbles. Commercial foods have given variable responses. They were the mainstay of the earlier Malaysian trials and have been used successfully in Australian trials. Tests in other countries have not always been successful. Commercial foods vary so much in composition that variable results are not unexpected. Tallow coats may interfere with viral adherence and contents such as coccidiostats, growth stimulants and antioxidants may reduce titres of vaccine virus. Many countries would find commercial pellets too expensive for delivery of vaccine while in some countries commercial pellets are not available at all.

b. An 'ideal pellet'. Would it be possible to produce a pellet that is cheap and virus friendly? The pellet need not be nutritious as long as chickens find it palatable. This would function something like the inert carriers that are used to take rhizobial cultures into the soil with legume seeds, to avoid the toxicity that results when untreated seeds and Rhizobium are in direct contact. The problem deserves attention.

c. Rice in its various forms. Unhusked (paddy) rice is a commonly used carrier in Asia. It seems to be effective when vaccine is fed soon after preparation, but some workers have encountered problems when the vaccine is stored, even if only for a few hours. Mass vaccination campaigns in much of Asia will probably depend on paddy rice as carrier, so the problem of survival of vaccine on this grain needs to be solved. Disadvantages of paddy are its alternative use as human food, its seasonal availability and its absence from many areas of the tropics. The large grain size makes it unattractive to small chickens.

Uncooked white rice has nearly always given disappointing results. The problem is probably one of failure of the virus to adhere, and suitable additives might solve this problem. This food is potentially very useful because of its ready availability. Cooked white rice usually gives good results. The only problem with this vehicle is its susceptibility to rapid spoilage. Parboiled (Indian) rice has given excellent results in a trial in Sri Lanka

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(Jayawardane et al., 1991), but even this food gives better results in its cooked form. Unpolished (brown) rice and broken rice need to be investigated further.

d. Rice bran. Highly processed rice bran has proved to be an excellent vehicle for vaccine in trials in Australia, although the bran is less effective after long storage. This is probably a result of developing rancidity. Less highly processed rice bran and brans from other grains were not effective. It may be possible to devise simple methods for treating bran to make it compatible with virus. Bran deserves further study because it is much cheaper than paddy rice and it is available throughout the year.

e. Wheat. Wheat has proved an excellent carrier in Malaysia. Vaccine is produced in bulk in large food mixers, and survives storage for some weeks, even at room temperature. The large control program in Malaysia will use a wheat-based vaccine. Many countries would find imported wheat too expensive to use as a base for a vaccine and some workers have had poor recovery of virus from certain wheats.

f. Maize. Trials with maize have generally been unsuccessful. Again this may be a problem with a hard, smooth surface not favourable for attachment of virus. There is also evidence for toxic substances on maize. More work is required because maize would be the vehicle most readily available in some parts of Asia and in much of Africa.

g. Popcorn. Indian workers have reported successful vaccination with F vaccine fed on popcorn, after cooking and grinding (Rajiswar and Masillamany, 1991).

h. Cassava. Dried cassava has been used successfully in limited trials reported from Indonesia.

i. Coconut pulp. Coconut pulp is widely used as food for village chickens in Sri Lanka. Unfortunately it seems not to favour survival of vaccine virus.

j. Vermiculite. Vermiculite has been used as a speculative vaccine carrier in Malaysia. It is eaten by chickens and the vaccine was effective.

k. Silicon. Silicon has been used by Russian workers to carry ND vaccine as an aerosol (Mavlikayev et al., 1988). It might also be suitable as a carrier for oral vaccine.

l. Millet and sorghum. There have been no detailed trials yet with millet, sorghum or other small grains. Such trials are required as these foods would be available in some countries.

Diluents

ND vaccine should ideally be reconstituted from the freeze-dried state in the diluent supplied by the manufacturer. This would add greatly to the cost of the vaccine, and this has not been the approach in the ACIAR project. In some countries vaccine is produced in liquid form and must be further diluted in a suitable diluent before use.

Most laboratory trials use distilled water as the diluent. In remote areas, less refined sources of water must be used. Success has been reported with well water, rain water from metal tanks, deionised water intended for use in batteries and even with tap water. It must be appreciated that these products will vary from area to area.

Water from green coconuts has been used as a successful diluent in Sri Lanka.

Additives

Various additives have been used to improve the survival of ND virus after reconstitution. In any trial it is necessary to include a control with distilled water to demonstrate conclusively any advantage that is claimed for the additive.

Polyvinylpyrollidone (PVP) has been very successful in Malaysia. It has been used for vaccines based on both commercial pellets and wheat, and for preparation in either the Glatt machine or large commercial food mixers. PVP is widely used to counter the toxicity of legume seeds for Rhizobium. Sucrose seems to be a useful additive and counters bacterial growth although it will permit fungal growth. Skim milk powder is useful for short-term protection, but spoils very rapidly at room temperature. Starches have given variable results. Methyl cellulose has been used in Malaysia. It gives similar protection to PVP but is more difficult to use. It could warrant further investigation for grains where viral adhesion seems to be a problem. Gelatin has been used with vaccines other than ND vaccine, but it would not be an acceptable additive in Moslem countries.

Powder Vaccine

Experiments on powder vaccine are warranted, to avoid the problems encountered when vaccine is reconstituted to a liquid form before mixing with grain. Freeze-dried vaccine in a suitable neutral extender (for example, starch) could be mixed with food. Such a vaccine could be prepared in small packages (for example, sufficient for 20 or 50 chickens) to avoid wastage.

Discussion

Why are some foods unsuitable for use as carriers for ND vaccine? Three reasons can be suggested. Firstly, the vaccine virus may fail to adhere. We suspect that this happens with uncooked white rice. Secondly, the virus may be bound chemically. The surfaces of grains contain lectins and some of these could bind NDV, as do the surfaces of chicken red blood cells. Attempts to recover

NDV from such preparations would fail and they might be assumed to be viricidal. The bound virus could still be available for chickens, explaining the vaccines that yield little virus in the laboratory, but that still invoke a protective immunity. Thirdly, the grains may produce substances that clump or even inactivate the virus. The suggestion has been made that these are specific antimicrobial substances, produced when the grains that we attempt to use become wet and germination is initiated. This idea deserves further consideration.

Legume seeds have been known for many years to produce antimicrobial chemicals (polyphenols) and methods have been devised to counter these when it is necessary to add Rhizobium cultures to the seeds. Such substances are not known from other seeds, and agricultural microbiologists take no special precautions when adding bacterial cultures to seed wheat or seed rice. Wet wheat or paddy does not, in our hands, inhibit the growth of bacteria on plates. Seeds used for poultry feeds do contain anti-nutritive substances that can be removed by soaking (for example, non-starch polysaccharides – Annison and Choct, 1991) and these could bind virus. Seeds have no biological requirement for an antiviral mechanism and any antiviral activity is likely to be adventitious. It is also possible that chemicals with antiviral activity might be applied to grains during the growing phase or during storage.

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Village Chicken Production: Problems and Potential

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Abstract

The village chicken system is described and the problems discussed. Newcastle disease is a major problem that periodically decimates flocks and the oral V4 vaccine may well be the answer to this problem. Practically all eggs produced are incubated and very heavy mortalities are recorded in these young chickens, resulting in an extremely wasteful system. A system that concentrates on egg production, but also increases meat production, is proposed for villagers.

In this paper, I will describe village chicken production and then outline possible areas for future development of this most numerous domesticated bird. This future development hinges on the successful control of Newcastle disease (ND) at the village level.

Definition

By village (rural or scavenging) chicken production is meant the small scale extensive method of poultry farming carried out in the developing countries of the world, usually in tropical environments.

The importance of the village chicken in the economy of developing countries has been coming to the fore far more noticeably over the past few years. Not only are they the most numerous poultry type in the world, but when they do not die from ND, they can play a very important role in the cash flow of the poor rural population. Therefore, FAO organised a workshop on village chickens in Dhaka, Bangladesh in March 1987. The results from this meeting were summarised by Bessei (1987) and are a very useful compilation of the present state of the art.

The system has been described on many occasions by a great number of authors from Southeast Asia, Africa and South America (e.g. Huchzermeyer 1967; Aini 1990). All describe essentially the same scenario — small flocks, nil or minimal inputs, with a low output and periodic decimation of the flocks by ND.

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The birds are owned by the individual households and are maintained under a scavenging system, usually with little or no inputs for housing, feeding or veterinary care. Typically the flocks are small — 10 to 20 birds per household consisting of 2 to 5 adult hens, a male bird and a number of growers of various ages. In most situations, the small amount of care bestowed upon the birds, such as table scraps or limited amounts of grain each morning, is given by the women of the household.

The birds are usually maintained as a source of meat, with few eggs being consumed or sold, and practically every egg is incubated by the hens. When the young birds reach a live weight of about 1 kg, which takes up to 20 weeks, the birds are either sold or consumed by the household. In most countries the village chicken is highly prized as a gourmet meat and thus the price paid is frequently well above that paid for a similar sized commercial broiler chicken. The production from these flocks is low, seldom exceeding much more than 2.5 birds per month.

In general, the villagers perceive these scavenging chickens as a natural low grade crop that offers a very desirable meat on occasions. However, production is too unreliable to warrant committing any investment of their limited resources.

Research in General

Comparatively little sound scientific research has been published on village chickens, despite the fact that quite often they are more numerous than commercial chickens in many developing countries.

Research Topics

1. Genetics

Despite the fact that the first limiting factors in tropical animal production are disease and nutrition, genetical studies on village chickens have been undertaken by a host of research workers. These studies have generally attempted to establish the genetic potential of the indigenous birds under conditions of good disease control (usually only ND vaccination), good nutrition and good husbandry. The village hens are often housed in laying cages and, under these conditions (where they seldom go broody), they can produce a fair number of eggs — 75 to 200 per hen per year. Egg size is usually low — about 45 g per egg. Usually a commercial egg laying strain is included in such trials and these birds usually lay +250 eggs per year, averaging over 60 g. If the trials are conducted in deep litter pens with nests, the village hens lay far fewer eggs as they usually go broody after laying about a dozen eggs.

This scenario was repeated at CIAWI (see Kingston and Cresswell 1982).

The next step in this type of research has been to 'upgrade' the village birds, usually by the introduction of males of an imported high egg producing strain. A great number of husbandry problems has been encountered in implementing these upgrading schemes — mainly the problem of ensuring that all village male birds are removed and, more importantly, the fact that the introduced high grade cockerels cannot cope with the harsh environment of the village. Crossbred pullets always show a significant increase in egg size and egg production if evaluated in laying cages. However, only a few more larger eggs are recorded if the crossbred pullets are housed on deep litter, as broodiness again markedly reduces the number of eggs laid.

No upgrading scheme of village or scavenging chickens has succeeded anywhere in the world.

2. Diseases

A. Newcastle disease

The major disease affecting village chickens around the world is ND and this generally appears in the severest form, often killing 100% of the birds. Conventional ND vaccines have been used in a number of countries and successful vaccination has been shown to be of considerable value in village chicken flocks (Bell and Mouloudi 1988). However, the problems of the cold chain (to maintain the viability of the vaccine virus during distribution) and the cost involved in catching and vaccinating widely spaced small flocks have proved major obstacles and many of these vaccination schemes have lapsed.

The Australian V4 feed supplied vaccine may well be the answer to this major disease problem. In addition, as the Australian V4 virus is far more tolerant to poor storage conditions it may well prove an excellent tool when used as a conventional vaccine.

B. Other diseases

A number of other diseases have been reported by various authors and, most recently, by the German/Thai team working in north east Thailand (Orawan et al. 1989; Wimolporn et al. 1989). These include fowl pox and fowl cholera, as well as a number of internal parasites.

As well, serological evidence of the presence of ND, infectious bronchitis, Marek's disease, *Salmonella pullorum, Mycoplasma gallisepticum* and *synoviae* and avian encephalomyelitis has been produced (Kingston and Cresswell 1982). In other words, these village flocks are affected by the whole range of known poultry disease organisms. This is hardly surprising when one considers the methods of husbandry and the custom of trading in grown birds.

C. Young chicken mortality

A major source of loss in all village chicken production is the heavy mortality (about 70% of chickens hatched) that occurs between hatching and the end of brooding — about 6 weeks of age. This is reported in all countries, whether ND is present or not. The only detailed report is that by Kingston and Cresswell (1982) from Indonesia. The actual causes of all deaths have not been identified, but are a combination of poor nutrition, predators and various disease factors — in fact, the very hostile environment the village chicken has to confront on hatching.

The answer to this problem is to improve the nutrition and husbandry of the baby chickens, whose first few weeks of life are spent scavenging the over-grazed and bare ground around the owner's house. In this situation they are in constant competition for any feed with the older, more aggressive birds in the flock.

3. Other Areas of Research

Nutrition and husbandry practices have received little serious attention from researchers and most approaches in this area remain speculative.

However, a German veterinarian, working in what was Southern Rhodesia, published a remarkable report nearly 20 years ago on the production of high class hybrid pullets in an African village (Huchzermeyer 1973). The birds were vaccinated against ND and received a daily allocation of maize, as well as ad lib water and calcium chips. The birds were confined from sunset to midday,

when they were allowed to scavenge for their protein, vitamin and mineral requirements, i.e. they were free choice fed, the maize supplying most of their energy and they scavenged for their protein.

The birds produced 170 eggs each at a cost of 8 cents per dozen. This report demonstrates two factors very clearly: (1) modern breeds can successfully select their own diets; and (2) modern breeds can cope with rather rough environments.

Basically free choice feeding is the system where the fowl is offered three feedstuffs:

1. protein source plus vitamins;
2. energy source (whole grain); and
3. in the case of laying hens, calcium in granular form.

The bird then decides exactly what proportion of energy and protein it requires, as well as how much calcium for egg shell formation. In other words, we rely on the bird's energy, protein and calcium appetites, which we have shown are extremely accurate.

This differs from the modern complete diet which is a mixture of both protein and energy sources, ground up and mixed together. Under this system, the fowl can only exercise its energy appetite. When the environmental temperature varies, the bird either over or under consumes protein and calcium.

We at the University of New England have over a period of ten years, rewritten the text books about free choice feeding and shown the advantages of its application to modern intensive poultry production. Subsequent work by the Department of Agriculture in Sydney has confirmed our findings and shown that free choice feeding can double the profitability of modern layers in cages. Free choice feeding has even more application in a scavenging system of husbandry.

Biological Limitations on Village Chicken Production

Viewed overall, the limitations on production in village flocks are as follows.

1. ND periodically decimates flocks, emphasizing to the owner all his birds may die at any time. The result is that the farmer does not invest any of his scarce resources in his chicken flock. In addition, the owner allows each hen to incubate her eggs to replace the birds that have died.
2. The feed supply is variable, depending on the season and rainfall. In most situations, energy is probably the first limiting nutrient, but protein may be more critical in others. Over population can play a crucial role as well (Huchzermeyer 1973).
3. The environment for the newly hatched chicken is very hostile as evidenced by the huge mortalities over the first six weeks of life. Usually, the hen hatches her chickens close to, or in the owners house. Here the ground is bare and checked out for anything edible several times a day by older birds, plus dogs and cats. Very young chickens cannot walk to more fertile areas, e.g. vegetable gardens, paddy fields, where suitable food is more readily available. Thus, young chickens — up to 6 weeks of age — literally starve to death, as well as being affected by predators and common disease organisms.
4. This leads to practically all eggs being incubated, which in turn limits the production of eggs. Two recent reports, one from Indonesia (Prasetyo et al. 1985), the other from Bangladesh (Sazzad et al. 1990), both emphasize how the incubation of eggs by hens markedly reduces the potential egg supply from village hens.

Thus, I conclude as long as ND is prevalent broodiness is essential. Once ND is controlled broodiness is a major problem to be overcome.

The Wasteful Present System

A nutritional balance study of the incubating village hen highlights the waste that occurs when eggs are incubated and the chickens die.

a. To lay a clutch of 10 eggs takes ± 18 days
   Protein intake/day 14 g × 18 = 252 g
   Energy intake/day 240 kcal × 18 = 4320 kcal

b. Incubation for 21 days
   Protein 5 g/day = 105
   Energy 100 kcal/day = 2100 kcal

c. Rearing of chickens to 42 days of age
   Hen – 42 days – protein 10 g/day = 420 g
   energy 200 kcal/day = 4200 kcal

   Chickens – 9 hatched – all bar two dead by 6 weeks of age
   Protein eaten by clutch ± 150 g
   Energy eaten by clutch ± 4000 kcal

d. Total feed consumed for 81 day period
   Protein 927 g
   Energy 14 620 kcal

e. Production by day 81 is two chickens, each weighing approx. 200 g each. This represents a total of 400 g of chicken, equivalent to about 80 g of protein for human consumption.

Thus, nearly a kilogram of protein was harvested from the environment, to return less than one tenth of that as human food.

As suggested by Huchzermeyer (1973), Kingston (1980) and Kingston and Cresswell (1982) how much more protein would be available for human consumption

if the eggs were harvested instead of being incubated? This protein being harvested by these birds from the environment is not being used by humans at all.

A Proposed 'Ideal' Village Poultry Flock

The present system of village chicken production, where practically every egg is incubated, to be followed by huge losses of the young chickens, is extremely wasteful. Yet the fascinating fact is that nearly all the food, and particularly the protein the fowls scavenge to stay alive, grow and reproduce with, is harvested from the environment, but not in competition with humans.

The benefits that will flow to villagers from the successful control of ND by the use of the V4 oral vaccine are substantial. Thus, Johnston and Cumming (1991) have suggested a rise for a typical flock from 30 to 45 saleable birds per year. There are far greater benefits to be gained if the villagers perceive that their chickens are worth investing in and husbandry alterations worth considering.

If we can modify the husbandry to concentrate on egg production and still produce some growers for meat efficiently, the advantages to the villagers will be considerable.

In my opinion, the 'ideal' village flock, should consist of 6 hens and 1 rooster per household. Two of the hens would be the traditional village breed and these would be encouraged to incubate eggs and raise chickens all year. The newly hatched chickens would be supplied with some additional feed over the first 6 weeks of life (creep feeding) and the hen and chickens protected from predators. Each hen might be expected to rear 4 batches of 8 chickens per year — a total of 64 birds for meat or replacements.

The other 4 hens of the 'ideal' village flock would be of a high egg-producing strain, that never go broody. These could be introduced as fertile eggs from a government or commercial farm, and hatched by a village hen. There are other ways of introducing these commercial layers, which would be replaced at regular intervals, say every 18 to 25 months. These hens should be capable of producing at least 150 eggs per hen per year, which could be consumed by the family or sold. Money from these sales could help pay for the creep feeding of the chickens mentioned above.

In summary, to exploit the advantages of the oral V4 vaccine to the full, we need to:

1. Demonstrate to villagers that birds can be satisfactorily vaccinated against ND.
2. Demonstrate that husbandry alterations are economically advantageous. The emphasis will be on egg production but meat production will be significantly increased as well.
3. Demonstrate that it is worth while to invest in the village chicken.

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The Development of Commercial Antigen and Antibody Detection Assays for Newcastle Disease Virus

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Abstract

The development and marketing of commercial products require a progression through the distinct stages of research, development, manufacturing and marketing. Each stage requires the allocation of specific resources and staff with appropriate skills.

A series of research projects conducted at the Graduate School of Tropical Veterinary Science and Agriculture developed diagnostic assays for Newcastle disease using enzyme-linked immunoassays.

They resulted in the development of an indirect ELISA which proved to be more sensitive and more specific than haemagglutination inhibition. Filter paper blood collection was evaluated and the antibody titres were found to correlate closely with serum antibody titres.

The indirect ELISA has now been developed into a simple kit in which the reagents are stable for up to one year at 4°C. The procedure can be carried out in a laboratory with minimal facilities. The sensitivity and specificity are superior to the original test and all of its competitors evaluated to date. Computer software which interfaces with the plate readers evaluates the results and expresses them as flock profiles to assist with easy flock monitoring.

Panels of monoclonal antibodies were incorporated into antigen detection assays. An ELISA was used to detect velogenic virus in tissue suspensions and could be used to type the isolates in allantoic fluids using appropriate monoclonal antibodies.

Antigen detection ELISA kits which satisfy the requirements of the end users are being developed. The capture antibody which is precoated to the plates or strips is stable for more than a month at 37°C. The methods must be simple and the results need to easy to interpret.

A biotechnology company, TropBio, has been established by James Cook University of North Queensland to develop, manufacture and market the products.

THE diagnosis of viral diseases can be based on the demonstration of the organism or measurement of immune responses. The assays used may be developed in the laboratory or carried out using kits which are supplied commercially.

Assays developed in the diagnostic laboratory require specific skills to be used by those staff responsible for the development as well as those responsible for the routine performance of the tests. There is a need to develop and maintain quality control standards. The assays carried out in one laboratory may not produce results similar to those which are obtained in other laboratories using similar tests. This situation can be quite unsatisfactory and uneconomical.

Commercial diagnostic assays should be developed based on appropriate, sound research findings. The kits are designed to operate efficiently in laboratories where there is a minimal level of staff training and the quality control is based on rigid standards applied by the manufacturers and the controls which are integral components of the kits.

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The Commercial Development Process

There are four distinct stages in the development and delivery of a commercial assay, starting with research, followed by development, manufacturing and marketing. Each stage is of vital importance and requires a separate set of skills and resources. The financial input increases as the product progresses through each of the stages.

Research

The research stage commences with the identification of a problem which needs a solution. The problem is clearly defined and the present state of knowledge is ascertained using techniques such as literature reviews, questionnaires and epidemiological surveys. Methods are designed and tested. There is further refinement of the methods and, in many cases, further definition of the problem using the newly defined methods. The methods are verified using surveys and clinical trials. It is common to publish the findings at this stage, to acknowledge the achievement of a distinct milestone. The most important achievement of this phase is the development of a new approach to the problem and a confirmation that the new method has the potential to be applied to solving the problem. Scientists can readily identify with the requirements of this stage of the process and, in many cases, are aware of the likely costs.

Development

Unfortunately, the research phase is just the beginning. The development phase starts with an investigation of how the product of the research can be applied in the laboratories charged with carrying out the diagnostic tasks. A new product can be successful only if it meets the requirements of the laboratory and is consistent with the resources available The present and potential role of the laboratory need to be assessed. The skills of the staff need to be ascertained.

The assays developed in the research phase of the project are redesigned firstly to suit the technical requirements of the end-user. However, this is not enough. The test must also be designed to satisfy a range of additional criteria. A commercial assay must be sensitive, specific, simple to perform, inexpensive, stable, consistent with the technical capacity of the operator and, finally, attractive to the end-user.

For a serological assay, it is important to determine the number of samples to be tested in a batch, the requirements for duplicates, the acceptable time for the completion of the test, the equipment available for performing the test and, finally, the financial resources. For an antigen detection assay it is important to determine the nature of the test sample, the number of samples in a typical batch, the facilities available and the speed required for a result. In some cases it is sufficient to identify the presence or absence of a specific virus. It may be necessary to distinguish between strains of virus, such as the differentiation between vaccine and wild type viruses.

Having identified the requirements of the end user it is then necessary to include the features necessary to conform with the production and marketing criteria. The stability of the reagents is paramount. There is a need to produce the assays and store them prior to dispatch. The agent needs to store the kits prior to supply and, lastly, the end-user requires a working life of more than six months. Every component of the kits must therefore be stable at 4°C for at least 15 months. There must be no significant loss of titre or precipitation.

Packaging can present enormous problems. The outer packaging needs to be attractive to the end user. It also needs to be sufficiently robust to protect the product during transportation. It may need to insulate the kit while it is in transit. All of the individual containers need to be appropriate for the task. They must be just the right size, and bottles must not leak or absorb proteins. The polymer and the seals must be completely resistant to the solvents used in the test. The pack insert must be written in such a way that it is almost impossible to make a mistake. The instructions must be brief and simple, yet adequately covering all aspects of the performance of the assay and interpretation of the results. Pack inserts frequently go through many drafts before they meet all of these criteria. It may be necessary to rewrite the instructions, or even redesign the test, to cater for a specific market.

The development phase requires the application of skills different from those appropriate for the research phase. The staff must not only be aware of the requirements of the end-user but also be fully aware of the limitations imposed by the manufacturing process. Once commercial prototypes are completed there is a requirement for a further round of exhaustive testing. The kit is no longer the assay which was evaluated during the research phase. It has new characteristics and the performance of the test under field conditions must be evaluated. If possible, the testing should be carried out using batches of reagents which would be used in the first few production runs. This may require considerable investment in production prior to the first commercial kit being made available to the end users.

Manufacturing

Having developed the kit, it is then necessary to gear up for mass production. A rigid quality control process needs to be designed and this must be implemented in such a way that all of the product meets the defined quality standards. New problems emerge and must be met by

minor changes in design of the assay or production technique. There may be a requirement for inspection and approval of plant and equipment in order to gain registration of the product.

Marketing

The marketing phase is no less important than any of the other phases. The end-user must be informed of the existence and potential performance of the assay. There must be no unacceptable delays in delivery of the product. It is important that the products receive sufficient technical support from staff with a clear understanding of the potential of the product. The costs of promoting and supporting the diagnostic products may be relatively high and can contribute quite significantly to the selling price.

Research at James Cook University of North Queensland

A series of research projects conducted at the Graduate School of Tropical Veterinary Science and Agriculture, James Cook University of North Queensland developed diagnostic assays for Newcastle disease (ND) using enzyme-linked immunoassays (ELISA). They resulted in the development of an indirect ELISA which proved to be more sensitive and specific than haemagglutination inhibition (HI). The assay used a purified envelope glycoprotein antigen passively adsorbed to the plastic plates. Diluted serum was added to the plates and the bound antibody was detected using an affinity purified anti-chicken globulin conjugated to horseradish peroxidase. The substrate of choice was 2-2'-azino-di-(3-ethyl benthiazoline sulfonic acid) (ABTS) which changes from clear to green. The research program determined the effect of different polymers as the adsorbent surface. The effect of a range of coating buffers, coating parameters, serum and conjugate diluents and incubation times and temperatures was investigated. A series of serum controls was added in duplicate as a standard curve on each plate. Test samples were diluted 1/100 and added in duplicate. The optical densities of the test serum samples were compared with the optical densities of the standard curve and titres were calculated.

The standardised technique was used in a series of studies to determine the ability of the ELISA to detect immune responses to Newcastle disease virus (NDV) infection. Birds were experimentally infected with the V4 and an MC110 like strain, 3245. Field samples were collected from flocks of known NDV status. Serum samples were evaluated with ELISA and HI. The ELISA proved to be slightly more sensitive than HI. There was not a close linear relationship with HI titres. ELISA proved to be more specific than HI when the quality of samples was poor.

Filter paper blood collection was evaluated. Whole blood was collected using adsorbent paper. The antibody was eluted and tested in ELISA. The titres were found to correlate closely with serum antibody titres. This is a simple, cost-effective method of antibody collection. Dried blood is stable at 37°C for more than one week, allowing the samples to be sent by mail.

Further research was directed at the development of assays which could differentiate immune responses to specific strains of NDV. It was clearly demonstrated that this could be achieved using competitive ELISA based on the use of monoclonal antibodies which are specific for the viral strains to be measured.

Panels of monoclonal antibodies were imported and further panels of monoclonal antibodies were produced to two local isolates of NDV, V4 and the MC110 like duck isolate 3245. These monoclonal antibodies were used to develop antigen detection assays. One study examined the various options in the design of antigen detection assays based on ELISA. Hyperimmune rabbit globulin was adsorbed to a plate and monoclonal antibodies were used to recognise the virus. The monoclonal antibodies in turn were recognised by a goat anti-chicken globulin conjugated to horseradish peroxidase with ABTS being used as the substrate.

It was shown that an antigen-capture ELISA could be used to detect ND antigens in allantoic fluid with a sensitivity of approximately 50 to 100 times that of haemagglutination. When specific monoclonal antibodies were used, it was possible to allocate the viral isolates into epidemiologically important groups. The assay has the ability to at least partially replace pathotyping. When tissue suspensions from birds infected with velogenic NDV were tested, the viral antigens could be readily detected with the highest titres being demonstrated in spleen samples.

The monoclonal antibodies were also used to demonstrate NDV antigens using indirect immunoperoxidase staining in tissue sections from infected birds, infected embryos and desquamated allantoic cells. The distribution of the viral antigens in the embryos and the chorioallantoic membrane correlated closely with pathotype.

Commercial Development of the Newcastle Disease Assays

Funding was received from the Australian poultry industry to develop the assays into a form which suited the industry. The first function was to ascertain the requirements of the industry. It was concluded that the antibody detection assay would be developed as a 400 test kit with 80 samples

being tested on each precoated plate. The kit should be complete with seven standards on each plate, a one pack substrate and all diluents and wash buffers in concentrated form.

Stability of the antigen-coated plates was achieved by postcoating and packaging procedures. The serum and conjugate diluent contained a coloured inert dye. The bottle of concentrated wash buffer also assisted with refrigeration in transit.

The development of an antigen detection kit is also well under way. It is planned to have 12 eight-well strips in a kit. One kit will be used to detect all strains of virus and a further series of kits will be developed for strain differentiation.

Unlabelled monoclonal antibodies have been prepared in freeze-dried form and selected antibodies are being conjugated to FITC, horseradish peroxidase, colloidal gold and biotin.

Production and Marketing of the Assays

James Cook University of North Queensland established a holding company JCU Technologies and, in April 1991, a subsidiary company JCU Tropical Biotechnology Pty Ltd was incorporated. This company has specialised in the production of high quality assays and other products to be marketed under the name TropBio. A licensing agreement gives TropBio the exclusive rights to develop, produce and market the assays and other products resulting from the NDV projects carried out in the Graduate School of Tropical Veterinary Science and Agriculture.

Indirect ELISA for the measurement of immune responses, a filter paper collection and elution kit, and unlabelled monoclonal antibodies are the first products to be marketed. The antigen detection assays in strip format are approaching the stage where the commercial prototypes will be ready for field testing. Further rapid test formats are being evaluated.

ELISA Plate Reader Interface

The most efficient method of expressing serological results on a flock basis is the use of flock serological profiles. A suite of computer programs has been written to interface between a computer and a plate reader.

The details of the flocks to be tested are entered into the computer by following a series of logical prompts. The program indicates where the samples should be allocated to the test plates. Once the tests have been performed the optical densities are read in a plate reader which receives its instructions from a computer to which it is interfaced. The data are transferred to the computer and analysed. The results are presented firstly in summary form and a report is then produced for each unit.

The first set of programs has been written for use on an IBM compatible computer interfaced to a Multiskan MCC plate reader. Additional programs will be written to cater for a wider range of readers and hopefully other computers.

Conclusion

A series of research projects conducted at the Graduate School of Tropical Veterinary Science and Agriculture has developed a practical range of diagnostic assays for ND.

Further development has resulted in the definition of kits that can be used in a wide range of laboratories for the recognition of ND viral antigens and for the measurement of immune responses.

A biotechnology company, TropBio, owned by James Cook University of North Queensland has begun manufacturing and marketing the products.

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The Scavenging Feed Resource Base in Assessments of the Productivity of Scavenging Village Chickens

John A. Roberts*

Abstract

A simple model for the production systems of scavenging village chickens is described in which the biomass of the village flock is maximised at the capacity of the scavenging feed resource base (SFRB). The resulting intraspecific competition causes a high mortality rate in the chicks and growers, and diverts the production away from consumption and towards wastage. Two methods for determining the capacity of the SFRB are described and applied to three production systems. Several uses for the data of the capacity of the SFRB are suggested, and the efficiency with which an egg producing flock of village chickens converts the protein in the SFRB into protein for human consumption is calculated as 15%. Some implications of the model concerning the productivity of existing systems, and modifications to existing systems, are discussed.

THERE are around 3 billion scavenging chickens in the villages of the developing world. They provide nutrition for the family, a small cash flow, a reserve for times of celebration or need, a sanitation service and, in some areas, contribute to healing ceremonies, religion and recreation. The high quality protein they provide through meat and eggs is particularly important for subsistence families whose diet is often deficient in protein, and that protein which is available is of low quality for human nutrition. The major input to the production system is the scavenging feed resource base (SFRB) comprising household waste, crop by-products and the gleanings of gardens, fields and wasteland. The beneficiaries, the families of the villages, are among those whose need is greatest. Thus, any increase in the productivity of the system would be multiplied enormously and would be automatically directed to those who would benefit most. In a survey of the objectives of village families keeping scavenging chickens, 100% cited family nutrition, 92% easy care operation and 72% cash flow (Gunawardena et al. 1991). Thus, the village people are motivated to increase the production of the consumable and saleable meat and eggs, particularly if little effort is required.

This paper describes a simple model for the village chicken production system in which the chicken population and the yield from it are determined by the capacity of the scavenging feed resource base (SFRB). Estimates of the size of the SFRB in different systems are made using data from other studies, and the efficiency with which the resource is utilised in the production of high quality protein for human consumption is calculated.

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A Model of the Scavenging Village Chicken Production System

The model is depicted in Figure 1. The village community consists of n families, all of which discard household refuse which is then available to the village flock. The village flock is the sum of the flocks of those families which keep chickens ($\sum^{n-x}$, where there are x families which do not keep chickens). The remainder of the SFRB comes from the environment, plus any supplements which may be provided by the householders. The household refuse portion of the SFRB will be relatively constant, but the portion from the environment will vary with seasonal conditions and with activities such as cultivation and harvest. The families incubate an excess of eggs to replace the birds which are harvested from their flocks, or which die. In the absence of an event which diminishes the flock, such as an outbreak of disease or a festival, the biomass of the village flock will always be the maximum which can be supported by the SFRB. If the biomass exceeds the capacity of the SFRB, then there will be strong selection against the weakest members of the flock, the chicks and growers (Figure 2). The production is divided between that which is utilised by the families in the form

of eggs and/or meat, and that which is used to replenish the flock. Any additions to the village flock which exceed the capacity of the SFRB, increase the competition pressure, and production will then be diverted away from consumption and towards wastage. The main wastage is in incubated eggs, the brooding time of hens and the deaths of chicks and growers.

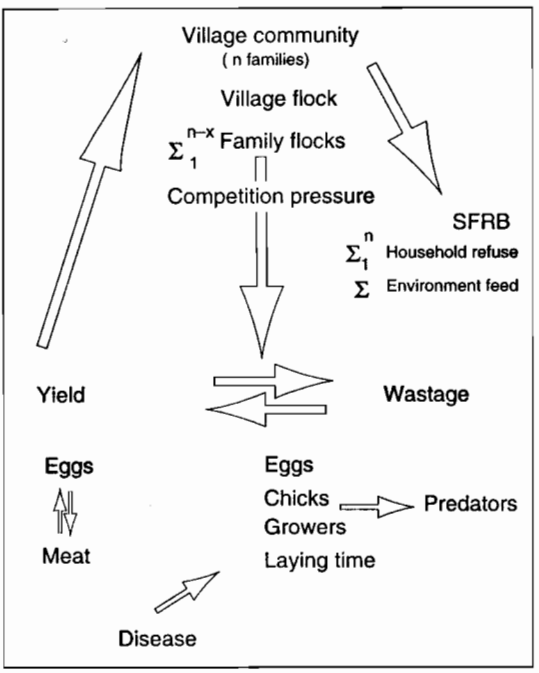

Fig. 1. Diagrammatic representation of a model of the scavenging village chicken production system

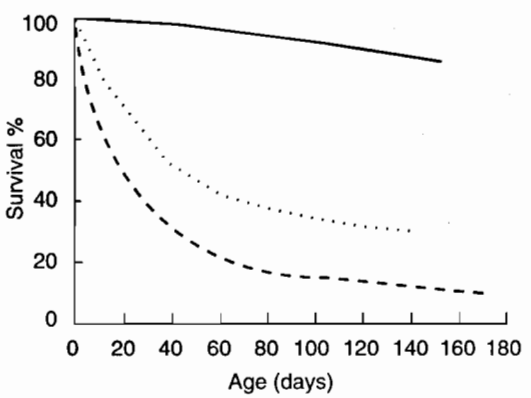

Fig. 2. Survival of chicks in scavenging flocks: — Sri Lanka, supplemented in village (Roberts and Senaratne 1991); ..... Sri Lanka (Gunaratne et al. 1991); – – – Indonesia (Kingston and Creswell 1982)

The Production Systems

The systems examined are a predominantly meat production system in West Java, Indonesia (Kingston 1980; Kingston and Creswell 1982), complemented by data from a similar system in northeastern Thailand (Janviriyasopak et al. 1989), and a predominantly egg production system in Sri Lanka (Gunaratne et al. 1991).

Determination of the SFRB

(a) Measurement

The SFRB for each family flock per year can be measured using the formula:

$$\text{SFRB} = [H/p] \times [n/(n-x)]$$

where: H = amount of household waste/family/day (kg dry weight).

n = number of families in the community.

x = number of families in the community which do not keep chickens.

p = proportion of the crop content which is household waste, as determined by visual inspection.

In the study of Gunaratne et al. (1991) the figures are:

$$0.200/0.72 \times 1/0.5 \times 365 = 203 \text{ kg dry weight per family per year}$$

The amount of protein in the SFRB can be determined from an analysis of the crop content, and the ME can be determined by analysis of the crop content or by reasonable estimation. On the basis of 11.2% protein, ash free (Gunaratne et al. 1991), and 3000 kcal/kg the SFRB has 23 kg protein and 609 Mcal ME.

(b) Calculation

To the extent that there is feed available, the amount of feed consumed by the birds is determined by their energy requirement, and the protein consumption and availability for maintenance, growth and egg laying is determined by the percentage of protein in the diet. The daily consumption of the flock is the SFRB, so it can also be calculated using the formula:

$$\text{SFRB} = \Sigma E_j / E_s$$

where: j = the average number of birds in the family flock

Table 1. Average numbers of birds in different age categories, in family flocks of village chickens

Stage				Total	References
Chicks	Growers	Mature			
		F	M		
6.7[a]	6.3[c]	11.4	3.6	28.0	Kingston and Creswell (1982)
14.0[b]	8.0[d]	4.5	1.4	27.9	Janviriyasopak et al. (1985)
2.4[b]	3.7[d]	4.0	1.4	11.5	Gunawardena et al. (1991)

[a] < 6 weeks; [b] < 8 weeks; [c] 6 weeks–mature; [d] 8 weeks–mature

E_j = the ME requirement for the daily maintenance and production of each bird per day (kcal/kg dry weight)

E_s = the ME in the scavenging feed (kcal/kg dry weight)

j can be determined from a census, or censuses, of the family flocks (Table 1).

E_s can be measured or reasonably estimated from the crop content

E_j can be calculated for each bird from the production data of growth rate (Fig. 3) and egg production (Table 2), using a formula such as that of the National Research Council (1984):

$$\text{ME/bird daily} = W^{0.75}(173 - 1.95T) + 5.5\Delta W + 2.07EE$$

where: W = body weight (kg).
T = ambient temperature (°C).
ΔW = change in body weight in g /day.
EE = daily egg mass (g).

An ambient temperature of 26°C is assumed throughout.

The calculated values for the SFRB are:

- 475 kg/year for the study of Kingston and Creswell (1982)
- 390 kg/year for the study of Janviriyasopak et al. (1989)
- 195 kg/year for the study of Gunaratne et al. (1991), which compares well with the measured value of 203 kg, above.

Using the Information about the SFRB

The data on the SFRB can be used in many ways to assess options for improving the productivity of scavenging village chickens in particular situations. The efficiencies of different production systems can be compared, options

Table 2. Fate of eggs produced[a]

Laid	Consumed	Incubated	Hatched	Matured
Per hen per year				
72	9	63	52	3
49	0	49	36	3
110	102	8	5	0.5
Per family flock per year				
821	103	718	593	27
221	0	221	162	13
440	408	32	20	2.5

[a] Sources as in Table 1

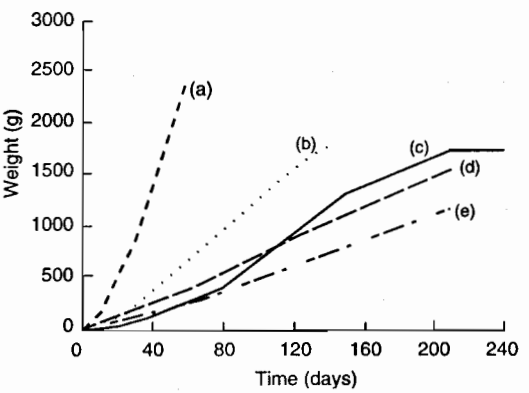

Fig. 3. Growth rates of chickens: (a) commercial broilers in intensive production; (b) Indonesian village chickens in intensive production (Cresswell and Gunawan 1982); (c) village chickens in Indonesian villages (Kingston and Cresswell 1982); (d) village chickens in Thai villages; (e) village chickens in Sri Lankan villages (Gunaratne et al. 1991)

for minimising wastage can be assessed, appropriate nutritional inputs to the system can be planned and preliminary assessments made of the benefits which might be derived, and the potential benefits of simple husbandry changes such as creep feeding can be determined. As an example, in the village flocks studied by Gunaratne et al. (1991), the 22.4 kg of protein in the SFRB produced 0.6 kg of meat protein and 2.6 kg of egg protein, so the efficiency with which the family flock converts the protein in the SFRB into protein for human consumption is 14%.

Epidemic Disease

One of the causes of wastage in the model is disease (Fig. 1), of which Newcastle disease (ND) is probably the most important (Higgins and Shortridge 1988). All three of the production studies cited lasted one year or more, two did not vaccinate against ND and the other had a control unvaccinated population. No disease event was reported as seriously affecting the sizes, or the age profiles, of the populations during the studies.

Some Implications of the Model

The intraspecific competition pressure in the model is intrinsic in the village flock, so the the only adjustments which the individual farmer can make to optimise the productivity of the family flock, are to vary the proportions of eggs incubated and consumed, and the harvest of meat birds. Any reduction made in the size of one family flock will be taken up by additional survivors in the flocks of other families in the community, so no family can reap the benefits of any change made in the biomass of their own flock. Two different production systems have been considered. There is the predominantly meat production system described by Kingston and Creswell (1982), perhaps determined by the preference of the Moslem community for meat, and the predominantly egg production system favoured by a Buddhist community (Gunaratne et al. 1991). In the meat production system, there is a large number of hens (Table 1), a high proportion of eggs incubated (Table 2) and a very high mortality rate in chicks and growers (Fig. 2). It is suggested that the family is seeking to produce more meat by rearing more chickens; but the attempt to increase production only increases the pressure of intraspecific competition, and raises the mortality rate of the chicks and growers in all flocks. In the egg production system there are fewer hens (Table 1), higher hen day production and a high proportion of eggs is consumed (Table 2). In that case it is suggested that the family, in seeking to optimise production, consumes as many eggs as possible, thus relieving some of the competition pressure, and so a higher proportion of chicks and growers survives (Fig. 2).

The survival of chicks and growers is dramatically improved when they receive a feed supplement (Fig. 2) and, presumably, preferential feeding of chicks using a creep feeder would confer the same benefit, while still utilising the same SFRB. However, the additional survivors will increase the competition pressure, and the model predicts that the birds will still die as they grow older, unless there is some countermanding reduction in the constitution of the village flock so that the total biomass is not increased as the chicks grow. Two of the many possible solutions are to either increase the consumption of eggs, and thus to reduce the number of chicks incubated and hatched, or to reduce the number of hens and eggs and to grow the additional survivors for meat. Either scenario should reduce wastage and increase the productivity of the flock.

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Patterns of Newcastle Disease Virus Activity in Village Fowls and the Measurement of Effective Field Protection Following Oral Vaccination

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Abstract

Householders in a rural municipality about 250 km north of Manila cooperated in a trial in which the performance of two groups of flocks of village fowls was compared. One group continued under normal conditions (no vaccination) while the other group was vaccinated monthly with a heat-resistant variant of V4 Newcastle disease (ND) live vaccine applied to paddy rice. The flocks in each treatment were not clustered, but formed part of a larger observational trial involving 170 households selected from throughout the municipality. Blood samples were taken monthly, mainly from tagged birds in the vaccinated flocks, for the purposes of HI antibody assay. Large rises in HI titres provided some of the evidence that corresponding flocks were undergoing natural ND viral challenge. The relatively few blood samples taken from the non-vaccinated flocks were HI antibody negative. Detection of natural viral challenge in this group relied on observation of mortality patterns and gross pathological examination of sick birds, with limited detailed laboratory examination of tissue samples.

The performance of the flocks with respect to reproduction, mortalities, bird and egg production, and population change was monitored monthly, so that any changes in mortality patterns could be related to our suspicions of contemporaneous natural challenge. In the non-vaccinated flocks, in months of suspected viral activity, the mortalities of flock birds rose from normal background levels by 39% (chickens), 37% (growers) and 17% (adults), respectively, indicating the rate at which local virulent virus, when active, attacked flocks. Among the vaccinated birds, monthly mortalities rose by only 17%, 8% and 8%, suggesting that the reduction of deaths due to vaccination was 22%, 29% and 9% of flock birds in the three groups. The monthly effective field protection conferred on the proportion of birds attacked by natural virus was therefore 56%, 78% and 50%. These measures correspond generally with protection estimated for adult and grower village birds when they were taken from villages and periodically subjected to challenge in formal challenge trials.

SOME of the trials over 1988–1991 in the ACIAR-sponsored Newcastle disease (ND) control project were designed to generate information on the productivity response of householders' fowls to a vaccine administered orally, baited onto local feed material. It was thought that any single trial should have sufficient cooperating householders to involve about 3000 birds in all. All householders were asked to continue marketing their birds as they saw fit, as long as records of transactions were kept.

The project area in the Philippines had not been previously subject to any form of ND control, so the treatments became:

(a) control (non-vaccinated), and;

(b) trial treatment (oral vaccination).

For the purposes of evaluating the 'effective field protection' resulting from vaccination, this was the favoured situation. Field research staff would observe flock performance under a range of field conditions, and compare non-vaccinated and vaccinated flocks as naturally occurring diseases were experienced.

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Unlike laboratory challenge trials, it is not possible to introduce ND virus into village flocks to simulate naturally occurring outbreaks of disease. In order to give useful results, the trials required flocks to become exposed at least once to a naturally occurring build-up of wild strains of ND virus, so that any differences in flock performance between vaccinated and non-vaccinated flocks could be recorded and observed. For this reason, project staff in the Philippines decided to run the trials for two years if possible.

The Philippines trial has involved some 170 households, set in five contiguous barangays (villages) in the rural municipality of Rosario, some 250 km to the north of Manila. Some 145 households were still involved after 18 months. It was possible to gain the special cooperation of 30 householders to use the HRV4 vaccine baited onto paddy rice, and also provide both monthly flock productivity information and blood samples for serological study.

Another 20 householders agreed to provide monthly productivity information, and that their flocks would remain as non-vaccinated controls. These households were located randomly through three of the five barangays, sometimes close to, or even contiguous with, vaccinated flocks. The 30 vaccinated flocks were also randomly located, but throughout all five barangays.

Flocks in the other cooperating households had birds which were either vaccinated, vaccinated and sampled for blood, or vaccinated and monitored with respect to flock mortality and productivity. Information from these cooperators will be used for research into issues concerning householders' attitudes to the technique of oral vaccination, as well as providing more serological and productivity information for better understanding the ecology of ND virus in tropical village environments.

Consequently, this analysis is confined to data from the 50 households for which there is sufficient information for judgments to be made on whether and when natural challenge by ND virus was taking place on a monthly basis.

Field vaccination commenced in March 1989. The productivity monitoring data referred to in this paper continued through to July 1990, and the serology monitoring till August 1990.

As a monthly routine, vaccine was made up in 10% skim milk (w/v) and thoroughly mixed with 'palay' (paddy rice) before visiting household compounds to start vaccinations at 0545 hours, finishing at about 0800 hours. The early start was needed because many of the birds were not confined for the night and they ranged away from the houses very quickly after daybreak. Birds for bleeding were caught by the owners the night before, and held in cages provided by the project.

As a backup to the field-based method of estimating the effectiveness of the vaccine, selected trial birds purchased from village householders were subjected to contact challenge by virulent virus under controlled conditions. The results from such contrived trials cannot be directly compared with the performance of birds running under field conditions. There are differences between field and controlled environments which can affect the levels of immunity that the birds display.

For example, transporting village birds from their home territory and the site of the challenge, confining them in housing, and subjecting them to close contact with unknown birds, can affect the performance of their immune system. The contact rate between birds brought in from the villages and the infected birds deliberately introduced to provide the challenge would give rise to rates of virus transmission somewhat higher than would apply in village conditions. Artificially induced challenge is feasible only for birds of adult or grower age groups, which must give rise to results not entirely representative of the whole age-range of the flock.

The Need for Detection of Periods of Active Infection

Crucial to measurement of performance differences between vaccinated and non-vaccinated flocks was the development of a method for detecting with satisfactory precision the times when flocks in the trial were being exposed to quantities of virus sufficient to cause disease outbreaks.

Arrangements were made to take monthly blood samples from a proportion of the vaccinated birds (about one-fifth) at each cooperating household, so that changes in birds' antibody levels could be measured and monitored.

The non-vaccinated birds were blood sampled in a baseline survey before the trials started. Because non-vaccinated birds appeared to die quickly (or be consumed) following natural exposure to virulent virus, before they could be expected to provide an indicative blood sample at monthly bleeding sessions, very few blood samples were taken in non-vaccinated flocks. Towards the end of the trials, this policy was modified somewhat, and attempts were made to collect blood samples from non-vaccinated flocks contiguous with vaccinated flocks, in order to detect any cases of lateral spread of vaccine virus between flocks. A final survey will be done of birds in the non-vaccinated flocks to see if there has been any build-up of antibody there over the trial period.

The blood samples have provided immunological indicators of:

- satisfactory vaccination; and/or
- natural challenge, especially in vaccinated birds.

The distinctively large antibody response of birds surviving natural challenge from wild strains of virulent ND virus (in almost all cases this would apply to birds protected by vaccine, although some rare cases of natural survival could occur) showed up in the serology data.

Flock Mortality and Productivity Data

A basic set of data items describing flock characteristics, size, mortality, reproduction and offtake was drawn up and questionnaires designed. Computerised data entry, storage and analysis were initiated to handle the productivity data and the associated serological information.

Cooperators were interviewed at an introductory visit, and basic household and flock data collected, such as name, address, and numbers of birds in age groupings (chicks recently hatched, chickens to 8 weeks, growers to 8 months, and adults.) During subsequent visits, new information on flock inventories was collected, together with the intervening information on flock, hatchings, and egg and bird production.

Information on sales and purchases of birds and eggs, and estimates of the value of any flock inputs having an alternative use were also collected. Material that would otherwise go to waste, or leisure time spent on the birds, was not valued as input.

Serology, Mortality and Other Indicators of Change

The laboratory blood test which was used to detect antibody changes was the Haemagglutination Inhibition test (HI test). The test, although cheap to perform, was found to have a number of deficiencies, and new technologies being developed now provide hope that more precise measurements will be possible in the future at a cost equivalent to the HI test. Once it has 'infected' a bird, the live HRV4 vaccine can be expected to generate a modest amount of antibody and give rise to an HI value from zero to up to four dilutions, referred to as an HI titre of four (to log base 2). Of course, some operators have reported higher titres, and for some it tends to be lower.

In individual cases, a bird having an HI titre of 4 may not be protected against field challenge by ND virus: and a vaccinated bird with a zero titre (apparent zero antibody) will not necessarily be unprotected. This is because there are other forms of protective reaction which can take place across the immunological spectrum and which are not measured by the HI test. Generally, we found in the Philippines that a group of birds with a geometric mean of around 1.4 or slightly higher seemed to have good field protection.

A bird which has been protected by vaccine and then exposed to a field strain of virulent virus, produces a great deal of protective antibody. The boost of antibody would start within a few days, and the amount would still be rising for up to two or three weeks afterwards. In the next monthly HI test, this antibody increase should show up as a sharp rise in titre, much higher than levels induced by vaccination, to readings of over 5 and commonly to 8 or more. This type of reaction was of the greatest interest to us in the trials because it pointed to cases where the vaccine had indeed been put to a real test. Such rises in antibody (and consequent protection) can be quite long-lasting, enduring commonly for about one year, much longer than immunity induced only by vaccination.

Death patterns in vaccinated flocks should not be expected to provide reliable evidence of field challenge by virulent ND virus, although such evidence should always be examined to support other sources of information. Among vaccinated birds, abnormally high mortalities would not show up, except perhaps among the young birds not yet old enough to have become fully protected by several vaccinations. The better the level of vaccination, the more will the indication from deaths be masked.

Causes of the deaths needed to be defined. Other disease problems occur, and the death rate among young birds, even in the absence of ND, is quite high. Gross pathological observation on site cannot disclose with certainty whether a bird has died from ND. Laboratory tests are needed on organs taken from dead or dying birds. Specimens need to be passed in good condition to the laboratory. Such specimens were not easy to acquire in the village trials, due to problems of trial supervision, lack of refrigeration and transport difficulties.

Nevertheless, assessment of the onset of disease outbreaks in both treatments had to rely to some extent on abnormal death patterns as a source of information. This was especially the case in the non-vaccinated flocks, where serological evidence was extremely rare. The judgments we made for non-vaccinated flocks relied on deaths, the serological patterns of nearby vaccinated flocks, the general observations of flockowners and the local project field staff on conditions of bird health, and some limited specimen material which was sent for laboratory examination.

The serological results for the orally vaccinated group are in Table 1. The geometric mean of the HI titres can be seen to rise as the number of vaccinations increases. However, this rise, as has been explained, can also be attributed in part to birds being challenged in the trial period by localised build up of natural virus. The HI titre distribution (Table 2) provides further information, with HI titres of over five possibly, with eight and nine certainly, being associated with birds having survived field challenge.

A similar picture of the antibody patterns which result from vaccination or challenge at village level is evident in Figure 1. It is based on the same data making up the earlier tables but is presented in the form of geometric mean titres for each of the months of the trial. The build up of antibody from vaccination is reflected in the rising mean titres over the first few months, with many factors accounting for the fluctuations showing up thereafter. The sampling of blood from newly-caught partially-vaccinated young birds as the trial proceeded, the effects of natural challenge, and the gradual loss of natural and vaccine-induced immunity would all play a part.

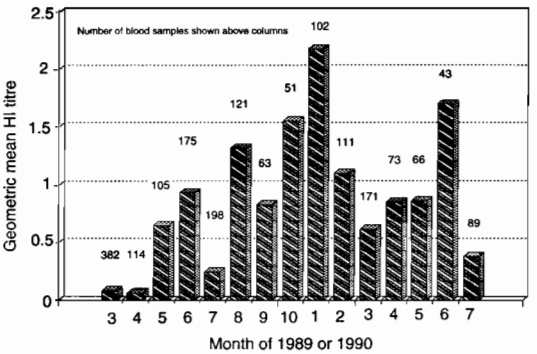

Fig. 1. GM HI titres of village birds vaccinated orally HRV4 monthly.

Table 1. Serological history for the village trials at La Union, Philippines. Serological history for birds orally vaccinated with HRV4. Vaccination began March 1989, ultimate bleeding August 1990

Samples taken from birds in flocks after:	No. of blood samples	HI antibody (GM Titre)
Zero vaccination	114	0.00
One vaccination	477	0.15
Two vaccinations	109	0.61
More than two vaccinations	1168	0.98

Mortality Patterns as Indicators of Viral Attack and Protection

The mortalities rose each month for both treatments for the first five months. Local observation tells of some abnormally wet and cold conditions in July and August 1989, which stressed the birds seriously. Thereafter, the mortalities fell away to a low in January–February 1990, but they then rose again to June 1990. This was probably part of a regular seasonal pattern, and there appears to be no consistent difference between the mean mortalities for vaccinated and non-vaccinated groups: for some months one had a higher death rate, sometimes it was the other.

In many of the months of the trial it was possible to observe for one, or a cluster of two or three flocks, cases of abnormally high deaths across all age groups, ultimately reducing bird numbers by a third, a half, or even more. This type of death pattern gave rise to suspicion that the flock or flocks were under challenge by ND, especially when the deaths were observed to begin in the young, moving later to the older birds. Many cases showing this characteristic pattern of deaths also had sentinel birds contributing blood samples, the serology of which showed sharp rises in antibody levels, thus strengthening the suspicion that challenge had occurred.

Figure 2 shows the overall percentage mortality estimates for the two groups of birds in the Philippines trial. The data relate to 716 months of flock observations, and a special classification has been made to allow those flocks which experienced a month terminating in zero birds (flock liquidation) to be shown separately. No great differences showed up between groups, except that the non-vaccinated flocks had a noticeably greater percentage of months ending up with zero birds.

By using the technique of relating monthly data on flock mortalities to serological information of tagged sentinel birds within the flocks, together with other available pieces of evidence, the flock mortality data were broken up into flock/months of 'no challenge detected' (Fig. 3) and flock/months of 'challenge suspected' (Fig. 4). Challenge in both groups was experienced for about 13% of the total number of flock/months.

Table 2. HI titre distribution

Samples taken from birds in flocks after:	HI antibody distribution (log 2) 0	1	2	3	4	5	6	7	8	9	10+
Zero vaccination	114	0	0	0	0	0	0	0	0	0	0
One vaccination	456	2	6	1	6	5	0	1	0	0	0
Two vaccinations	76	13	10	7	2	1	0	0	0	0	0
More than two vaccinations	837	102	45	28	50	39	29	19	9	7	3

The 'no challenge' situation indicated only minor mortality differences between groups (apart from the flock liquidation factor); while the one showing the mortalities under conditions of suspected challenge showed some remarkable differences.

Under challenge, the two groups seem to have had a common experience, with about 15% of the months having 40–50% mortalities.

At lower mortality groupings, that is below 30%, the vaccinated group was represented more frequently, indicating more challenge time taken up by light mortalities than the non-vaccinated flocks. Flocks experiencing over 50% mortalities, that is 50–60% per month, 60–70%, 70–80% and so on, right through to full liquidation of the flock, were much more heavily represented by non-vaccinated flocks.

It thus appears that use of the vaccine, viewed over a period of many months, did not have a spectacular effect on flock mortalities. As would be expected, when no viral attack or challenge was present, the mortality patterns in each group were similar. However, in those periods when flocks were affected by active infection from natural strains of ND virus, the vaccine appears to have significantly improved the patterns of bird survival.

Estimation of Field Protection Levels

The trials performed in village conditions in some ways were expressing a real-life version of laboratory-style challenge trials. In challenge trials performed in controlled conditions, vaccinated and non-vaccinated

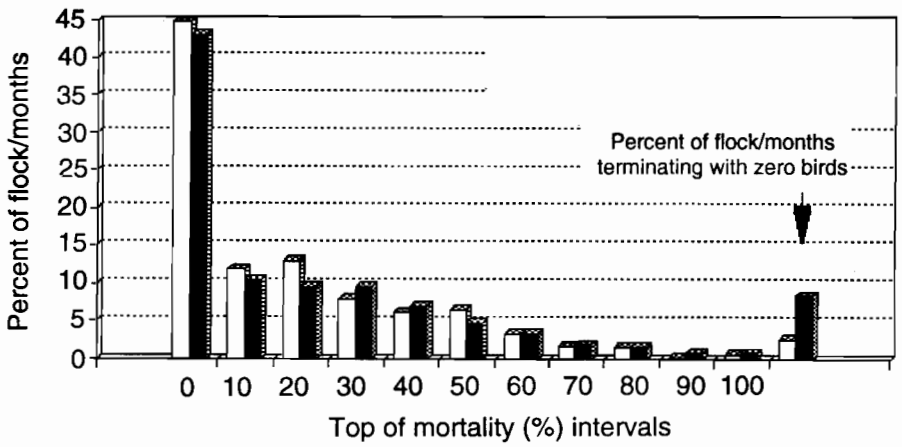

Fig. 2. Distribution of monthly mortalities — 716 flock/months observed: open bars, HRV4 vaccinated; solid bars, non-vaccinated

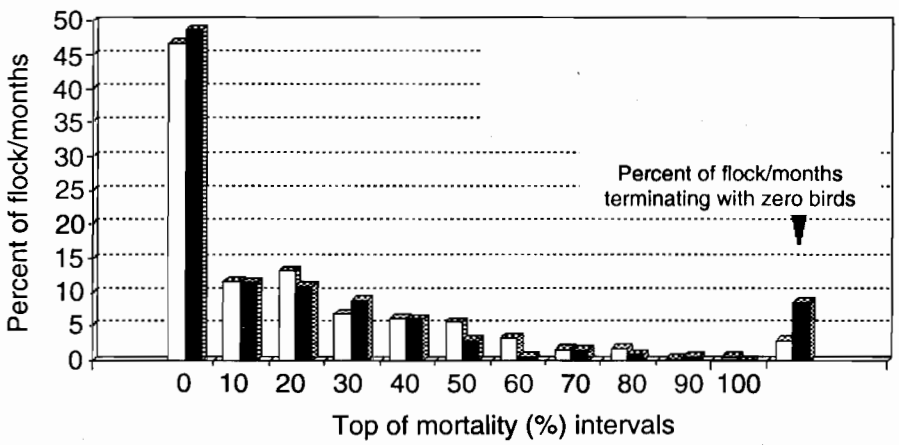

Fig. 3. Distribution of mortalities — no natural challenge detected: open bars, HRV4 vaccinated; solid bars, non-vaccinated

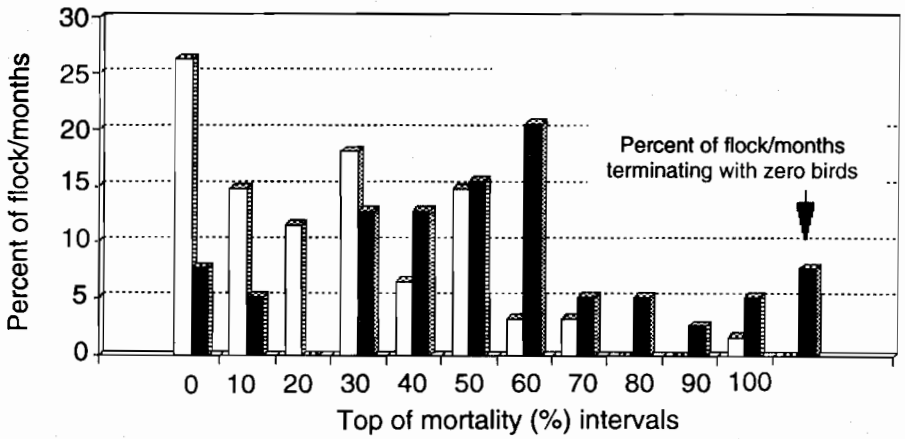

Fig. 4. Distribution of monthly mortalities — natural challenge suspected: open bars, HRV4 vaccinated; solid bars, non-vaccinated

birds are subjected to challenge by contact with birds recently infected by virulent ND virus. As a check that the challenge virus is indeed lethal, some negative control birds are usually included, often commercial birds reared in NDV-free conditions. Usually, the challenge to non-protected birds is so severe that all die. Calculations of protection conferred by the vaccine are quite simple: the surviving percentage of birds in the vaccinated group, perhaps 60% of them, compare with the zero survivals of the controls, which suggests (for this example) a 60% protection rate.

But when observing protection at village level, a number of other factors intrude to complicate the calculation of field protection percentages. The main factor is that, over the prolonged period of the trial, the village environment gives rise to many hazards and many causes of death other than ND. Further, it would be unusual to select control and vaccinated villages with identical conditions, giving rise to identical 'crude' or background mortality rates.

Another factor is that the rate of exposure to challenge virus in village conditions will not be as high as in a laboratory-style trials because contact rates will be lower. Contact may be such that, say, only 40% of birds in any one month become exposed to natural infection. As a consequence, in calculating 'effective field protection' for a vaccine used in a village bird population, some variations are required to the calculations normally performed.

They need to focus not so much on the absolute mortality in the period of challenge, but on the rise which occurs in mortality in periods when challenge is observed to take place. To illustrate this point, the mean monthly mortality rates, for challenged and unchallenged birds respectively, together with the relative rises in deaths when birds were considered to be under challenge are shown in Table 3 for the two treatment groups:

Such information presents an opportunity for calculation of the 'effective level of ND challenge' and the corresponding level of 'field protection' obtained from oral vaccination.

Table 3. Monthly mortality rates of treatment groups under inferred ND viral conditions

	Challenge suspected mortality rate (%)	None detected mortality rate (%)	Rise due ND (%)
Non-vaccinated group			
chickens	56.4	17.8	38.6
growers	39.7	2.8	36.9
adults	17.8	0.75	17.1
Vaccinated group			
chickens	38.5	21.6	16.9
growers	16.8	8.75	8.1
adults	10.6	2.2	8.5

First, there appears to be a basic difference between the groups in terms of basal mortality rates when unchallenged. This is to be expected when relatively small groups (20 and 30 flocks, respectively) are selected for a pilot trial.

Table 4 outlines a simple analysis which focuses on the changes in mortalities associated with challenge periods in each of the two treatments to deduce the challenge intensities and the field rates of protection afforded by the oral vaccine.

At the Philippines site it appears that infection by the local strains of ND is lethal, because serological survey in the region shows no birds which have recovered from ND infection. The rise in death rate associated with ND in the challenged non-vaccinated group can be taken to give an indication of the percentage of birds being attacked in a typical month when active ND infection is under way.

The resulting rate (for the period of a month) is surprisingly low, ranging from 17% (adults) to approaching 40% (chickens and growers), indicating that in the conditions of this trial over 60% of flock birds in any one month escaped challenge. Spread of the virus in the field therefore can be quite slow.

From the experience in non-vaccinated flocks, 38.6 chickens in 100 were challenged in any one month, as indicated by the ND deaths. The deaths from ND in this age group were reduced by vaccination by 21.7 per 100 birds, which leads to the conclusion that the protection level for chickens actually under challenge was 21.7 divided by 38.6, or *56.2%*. Comparable percentage protection levels for growers and adults were *78.1% and 50.3%*, respectively.

These measures generally correspond with the protection levels estimated for adult and grower birds when samples of them were taken from the village site periodically and subjected to challenge in formal trials under controlled conditions.

Table 4. Effective rates of challenge and field protection

	ND rise in death rate (%)	Reduction due use of HRV4 (%)	Effective field protection (%)
Non-vaccinated group			
chickens	**38.6	–	–
growers	**36.9	–	–
adults	**17.1	–	–
Vaccinated group			
chickens	16.9	21.7	56.2
growers	8.15	28.8	78.1
adults	8.5	8.6	50.3

** Represents the mean rate of challenge or viral attack in the non-vaccinated fowl population over one month

The Epidemiology of Newcastle Disease in Village Chickens

P.A.J. Martin*

Abstract

Much work has been done to throw light on all aspects of the epidemiology of Newcastle disease (ND) in intensively managed poultry. Although free-ranging village poultry are a significant potential source of infection for commercial birds, remarkably little work has been published on the epidemiology of ND in village chickens. This paper describes aspects of the known epidemiology that are relevant to village poultry, and existing knowledge of the disease in the village environment.

ND is enzootic in many countries with significant village poultry populations, and it is likely that it is enzootic within individual villages; however, clinical disease often appears to follow epizootic patterns. Velogenic and lentogenic pathotypes of ND virus (NDV) are present in village birds. A reservoir of NDV in the village is probably maintained among chickens and other domestic birds, the physical environment, and wild birds.

Indigenous breeds of village poultry are probably as susceptible to ND as commercial breeds. Factors affecting the occurrence of ND in village poultry include the weather, exposure to virus, virulence of virus, age, immune status, and concurrent disease. Mortality rates in village chickens during outbreaks of velogenic ND are often comparable to those in non-immune commercial poultry.

Newcastle disease (ND) was first described in 1926, and affects poultry populations throughout the world, in all but the most isolated communities. It has been described as a disease of commercial poultry, first appearing at a time when intensification was developing, and its epidemiology has been studied and described primarily in this context. Certainly, ND and its prevention have great economic consequences for the commercial poultry industry, but to concentrate study on the intensive industry ignores the fact that ND is prevalent in village poultry populations throughout the world, where it undoubtedly causes great economic loss (Supramaniam 1988). It is seen by many as a principal factor limiting productivity of village chickens. Although free-ranging village poultry are recognised as a significant potential source of infection for intensively managed poultry, remarkably little has been published on the epidemiology of ND in village chickens.

Recent developments in ND virus (NDV) strain differentiation, such as the use of panels of monoclonal antibodies (Della-Porta et al. 1988), open the door to detailed work tracing the path of NDV infections through individual village flocks and entire village populations. The information from such studies will allow more informed decision-making on control strategies.

Many characteristics of the virus are now well understood, including properties related to invasion of the bird, replication, virulence, shedding from the bird, and survival in the environment. Much is also known about the immune response of the chicken. In addition, how the disease spreads among intensively managed poultry is well understood, as are many of the possible means of spread between poultry houses.

This paper describes aspects of the known epidemiology that are relevant to village poultry, and discusses existing knowledge of the disease in the village environment.

In the context of this paper, village chickens are free-ranging poultry, mostly of unimproved, indigenous breeds. A high proportion of households in the village keeps such birds, and flock size generally ranges from a few up to 100 birds. They are scavengers, and are often not fed, or are only given household scraps. Some are housed at night, while others roost in trees. Nesting boxes may or may not be available. Few, if any, disease control measures are practiced. The physical and climatic

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environments of these birds obviously vary from country to country and region to region.

In non-immune, intensively managed, commercial poultry, the introduction of a pathogenic strain of NDV in sufficient quantity to infect a bird is in itself a sufficient cause of ND. In village poultry, the occurrence of ND is dependent on a combination of factors. The presence of a pathogenic strain of NDV is one factor which is necessary for disease to develop, but is not in itself a sufficient cause. This is because village poultry populations are mixed in terms of susceptibility to infection with NDV (because of immunity due to age and exposure to NDV) and, in extensive conditions, the spread of virus from bird to bird does not occur as readily as in intensively housed poultry. Consequently, other factors affecting the ease of spread of virus and the susceptibility of birds are significant in the epidemiology of the disease in village poultry.

ND is enzootic in many countries with significant village poultry populations, and it is likely that it is enzootic within individual regions, and even villages (Bell and Mouloudi 1988). However, clinical disease often appears to follow typical epizootic patterns; factors contributing to this pattern are discussed below. Velogenic, mesogenic and lentogenic strains of NDV have been isolated in many tropical developing countries (Higgins and Shortridge 1988), and it is likely that a combination of pathotypes is present in most village poultry populations. For a viral disease to remain enzootic, the virus must be maintained in a reservoir.

NDV Reservoir in the Village

The reservoir of NDV in the typical village is not clearly understood; however, the following factors are probably involved to varying extents in different village environments.

1. Cycling of infection within the village poultry population

Spread both within and among village flocks is not as rapid as in commercial poultry houses, and the disease can take weeks to pass through a flock, and months to pass through a village (Martin, unpublished data). The constant introduction of susceptible birds through hatching, and the probability that some flocks or individual birds will have evaded infection during the passage of disease through the village, mean it is possible that within a village poultry population there will always be susceptible birds to whom diseased birds can transmit NDV.

Velogenic NDV (VNDV) can be maintained in a flock of poultry through a cycle of waning immunity followed by immunity-boosting symptomless infection, which is then passed on to other birds with sufficiently lowered immunity (Hanson 1976).

2. Other domestic birds

Other domestic birds, including ducks, turkeys, doves, geese, guinea fowl, etc., can harbour virus. Such birds can become infected with NDV, and can shed the virus, acting as a source of infection for chickens. They may or may not develop clinical ND, depending on the strain of virus and the bird species (see Lancaster 1966 for a review).

3. Carrier chickens

It is unclear whether chickens can become long-term carriers of NDV. Partially immune birds can develop infection with VNDV and display mild or no clinical signs. Such infected birds can then shed virus for up to 5 weeks (Lancaster 1966).

4. Wild birds

Velogenic, mesogenic and lentogenic strains of NDV have been isolated from numerous species of wild bird, all over the world (Hanson 1976; Lancaster 1966; Alexander 1988). Undoubtedly they form a reservoir for the virus, but their significance in spreading NDV to village chickens is unknown. Psittacines are known to be capable of harbouring and spreading VNDV, and lentogenic strains are frequently isolated from migratory water fowl (Hanson 1976; Alexander 1988).

5. The physical environment

Infected birds shed virus in the faeces, where it can survive 3 months at temperatures of 20°–30°C and longer at cooler temperatures. From dried faeces, dusty material containing NDV can become widely distributed, and the virus can survive on many materials for periods of weeks to months at tropical temperatures, and for longer periods in cooler climates (Lancaster 1966).

A small experimental flock of free-range birds kept in a sub-tropical climate in Australia maintained active infection for 2 years (Samuel and Spradbrow 1989) following dosing of half the adults with the avirulent heat-resistant V4 strain of NDV that forms the subject of this volume. Cycles of infection sparked off by each hatching of chicks spread through all birds in the flock. The precise nature of the reservoir of the virus was not established, but the chicks were involved as periodic multipliers, and their source of infection could have been the environment or older carrier birds. The pattern would probably be different for a virulent strain of NDV.

Spread of NDV among Village Poultry

The introduction of NDV to a village is most likely to occur when infected live chickens are introduced. Live bird markets are probably a major means of spread (Cherdchai 1988; Alexander 1988), particularly since many village flock owners take their birds to market as soon as they

become sick, in an attempt to salvage some value from them. Other means of introducing new strains of NDV to a village poultry population include wild birds, live infected chickens being transported through the village, infected carcases, and movement of contaminated objects from an infected site. Cases of contaminated vaccines have also occurred (Alexander 1988).

The incubation period of ND varies with strain of virus, and route and dose of infection; typically for VND it is 4–5 days, but it may be from 2–15 days (Beard and Hanson 1984; Lancaster 1966).

In intensively managed poultry, the most important means of spread within a poultry house is by the respiratory route (Beard and Hanson 1984). NDV readily establishes infection in the respiratory tract of a susceptible bird and, when infection is established, virus is shed from the respiratory tract. The atmosphere of the poultry house then contains a fine aerosol of virus. Spread in larger droplets occurs readily when birds cough and sneeze. This means of spread may occur in extensively managed village poultry, particularly among birds housed at night, but is not likely to be as important as in intensively housed poultry.

Spread by the faecal–oral route is slower than by the respiratory route. Chickens infected with NDV shed virus in the faeces, and infection can become established following its ingestion. This means of spread is important particularly for low virulence viscerotropic strains (Alexander 1988). Virus in the faeces survives well outside the chicken, so allowing the development of many different circumstances leading to oral, or even respiratory, infection of another bird. NDV can survive in faeces, on egg shell, on feathers, on walls and other inanimate objects, and in water. In each of these situations, the virus is more resistant to inactivation when surrounded by protein and, while virus will not remain active in fresh clean water for more than a day or two, it can survive many days in ponds and lakes full of organic material (Lancaster 1966). Chickens may readily become infected by drinking contaminated water. Communal watering points are therefore potentially an important means of spread among flocks.

Sick and dead birds are often eaten by flock owners, and viscera from birds for the table are often fed to poultry. The combination of these two practices provides an excellent opportunity for ND to spread.

Airborne spread from flock to flock is probably unimportant among village chickens, since a dense population of infected birds is necessary to generate a sufficiently dense aerosol for such transmission to occur.

It is unclear whether true vertical transmission (eggs becoming infected in the reproductive tract of the hen) occurs (Bear and Hanson 1984). Hens with clinical ND stop laying, but hens with lentogenic infection and partially immune, infected hens may continue to lay. If the embryo is infected before hatching it will generally die, although with avirulent strains an infected chick may hatch (French et al. 1967). If eggs have surface NDV contamination, e.g. with faeces, the chick can become infected at hatching, since NDV in faeces on the shell can survive egg incubation. An infected egg, following death of the embryo, is a good environment for survival of infective virus: at 37°C, NDV can survive in eggs for over 3 months (Lancaster 1966).

Ectoparasites (lice, mites and ticks) may be capable of transmitting NDV, but are probably not important vectors (Lancaster 1966). The same is true of flies (Beard and Hanson 1984).

Factors Contributing to ND Outbreaks

1. Virus

NDV strains vary in pathogenicity for village chickens, from velogenic to apathogenic. It is likely that NDV strains found in village poultry vary in their ability to establish infections by different routes, the less virulent strains relying more on the enteric route (Alexander 1988). In addition, a strain of NDV comprises several genetically and phenotypically distinct virus 'clones'. The clones that make up a field strain of NDV have varying infectivity, thermostability, shed-ability, replicability and pathogenicity (Hanson 1988). Their combined presence allows the strain to survive under a variety of conditions, and to adapt rapidly to hostile environmental circumstances. There is considerable variation in thermostability of clones and strains of NDV.

These properties of NDV make it hard to generalise concerning the conditions favouring outbreaks of ND among village poultry, especially when the situation in village environments may be complicated by the presence of several strains of NDV. In some villages this is further complicated by the presence of one or more vaccine strains. Assuming the presence of VNDV, it is probably bird and environment factors that are critical for the development of disease.

2. Bird/host factors

Age

Chickens become increasingly resistant to ND with age (Beard and Hanson 1984). Velogenic strains will cause disease in healthy non-immune adults, but some birds will survive.

Acquired immunity

Immunity induced by previous exposure to NDV can be protective. Circulating antibody, secretory antibody,

cell-mediated immunity, and non-specific surface immunity all play a role. Protective immunity is generally accepted as corresponding to a log2 HI titre of 3 or more (Allan and Gough 1974; Sagild and Haresnape 1987), although some birds with low or undetectable HI titres following vaccination or infection are nevertheless protected against ND challenge (Beard and Hanson 1984). The duration of active protective immunity varies with the immune status of the bird at the time of exposure to the immune stimulus, and the nature of the immune stimulus, which depends on the dose of virus, the route of infection, and the strain of virus (Westbury 1984; Ibrahim et al. 1981). The avirulent V4 strain acquired by contact with orally vaccinated birds gives only transient protective HI levels (Samuel and Spradbrow 1989), and at the other end of the spectrum, immune birds surviving artificial challenge with VNDV have maintained a mean log2 HI titre of 7 for 15 months (Urasri Tantaswasdi, pers. comm.). In general, HI antibodies from natural infection decline after 3–4 months, and are gone by 8–12 months, while vaccine-induced protection lasts 2–12 months, depending on vaccine strain and route of administration (Beard and Hanson 1984). Antibodies induced by one strain of NDV are protective against all strains of the virus.

Village chickens are a mixed population in terms of immunity. Maternally derived antibody in newly hatched chickens may be present for up to 5 weeks. Chicks become fully capable of mounting an immune response at about the same age (Spradbrow 1987). An impaired immune response may occur in village poultry, and possible causes include infectious bursal disease, chronic aflatoxicosis and vitamin A deficiency (Spradbrow 1987). Immune birds may still develop infection with VNDV.

The presence of lentogenic NDV in some village chicken populations may result in constant cycles of infection which periodically boost the immunity of all exposed birds (Samuel and Spradbrow 1989). This immunity may or may not be sufficient to protect against VND challenge, the degree of protection depending on the stage of the infection cycle. It is possible that mesogenic strains could behave similarly, and also possible that both lentogenic and mesogenic infection cycles could be superimposed in a village population. Evidence for the presence of such non-velogenic infections in village chickens comes from isolations of virus from healthy village birds (Bell et al. 1990), and from HI titres in high proportions of non-vaccinated village birds, which would normally not be expected to survive VND (Grundler et al. 1988; Sagild and Haresnape 1987; Bell and Mouloudi 1988).

Some village chicken populations, on the other hand, have uniformly low HI titres, implying that the birds sampled have not encountered ND. This could be due to the village being free of ND, or ND occurring in classical epizootic pattern, with close to 100% case fatality rate. In enzootic regions, presumably the latter is the case, and only highly virulent strains are present in the region.

Periodic vaccination complicates the scene further, especially when live vaccine is used, since vaccine strains, particularly V4, spread among the population. Vaccine administered to immune or partially immune birds may not induce protection. There are considerable practical difficulties in ensuring that vaccine is given only to birds that will respond, in a village flock of mixed age and immunity. With periodic vaccinations of all birds or all young birds, it is inevitable that a proportion will not respond, and will be susceptible to VND infection some time thereafter.

In summary, where NDV strains of low virulence are present, or where vaccination is practiced, the village poultry population will be very variable in immune status, and the severity of VND outbreaks will vary with other factors.

Concurrent disease

Village chickens are affected by a wide range of bacterial, viral and parasitic diseases, many of which, particularly parasites, are constantly present. Nutrition is often poor in birds dependent on scavenging for a limited quantity of available food. Post mortem examinations may conclude that the cause of death was ND, but there will usually be other concurrent infections, and debilitating burdens of both ecto- and endo-parasites. These concurrent diseases render village birds more susceptible to ND. In Burkina Faso, where an extensive ND vaccination program was coupled with parasite control, the parasite control was thought to have been critical in ND control (Verger 1986).

Breed susceptibility

Opinions differ on the relative susceptibility of indigenous breeds and commercial breeds. Cherdchai (1988) states that, in Thailand, indigenous village birds are more resistant to ND than commercial breeds, while Higgins and Shortridge (1988) say there is no evidence for differences in susceptibility between local breeds in Hong Kong smallholdings, and imported breeds. It is likely that there are differences in susceptibility among indigenous breeds around the world.

3. Environment

ND occurs year-round in most village poultry populations, but is most common and most severe at times of climatic stress. Outbreaks are often associated with change of season, particularly the start of the wet season (Thitisak et al. 1988; Ronohardjo et al. 1988; Jintana 1987). Cold weather has been cited as a contributory factor in ND outbreaks (Dao Trong Dat and Pham Chuc 1985), as has hot weather (Bell et al. 1990).

A high village poultry density results in greater opportunities for a contaminated environment and for spread of ND, relative to a more dispersed population.

Summary

ND is enzootic in most countries with substantial village poultry populations. The disease occurs year-round, often with major epizootics once or twice a year, which inflict heavy losses on village flocks. Velogenic, mesogenic and lentogenic pathotypes of NDV occur in village poultry. Introduction of ND to a village is usually with a live infected bird. Spread within the village is by the faecal–oral route, and by the respiratory route where close bird-to-bird association occurs.

NDV survives for weeks to months in the environment in tropical and sub-tropical climates, although this varies with viral strain. NDV is also maintained in the village by cycling of virus through the village in chickens, other domestic species, and wild birds.

Epizootics occur at times of climatic stress, notably seasonal changes. Spread of disease through a village poultry population takes weeks to months. The susceptibility of an individual bird depends on the strain and dose of virus it receives, its age, its immune status, and concurrent disease and parasitism.

While the interactions of different strains of virus within the village chicken population are not understood, they do affect the immune status of the individual and of the flock.

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Computer Modelling to Expand Our Understanding of Disease Interactions in Village Chickens

Joe Johnston*

Abstract

The impact of Newcastle disease on the productivity of village fowls is not well understood. Scavenger birds, in the harsh environment in which they exist, are good survivors, although they have little capacity to produce as well. Should it become feasible to incorporate oral vaccination against Newcastle disease into the routine activities of rural communities, the associated productivity benefits are likely to remain largely unrealised until village communities learn to make appropriate changes to their bird handling traditions. New management strategies are likely to call for controls to be imposed on incubation and harvesting, and the possible administration of supplementary feeds.

Based around a flock population routine, a state/transitional computer model was developed to reflect the changing 'state' of a flock as time passes. The flock is compartmentalised into young birds, growers and adults, and the processes of reproduction and wastage are built in to allow the population to rise and fall within environmental and man-made constraints. The flock is also broken crosswise into disease status compartments, and the processes of infection, latency, immunity and reversion to susceptibility can be modelled for different bird densities and contact rates to simulate disease/mortality interactions.

To demonstrate the use of the model, vaccinations of hypothetical flocks of village fowls at intervals of 4, 8 and 12 weeks are compared in a simulation exercise, to show the potential effect on household income of the different vaccination regimes and the impact of different vaccination efficiency levels.

THE impact of Newcastle disease and of vaccination on village fowls needs to be defined. The concept we have followed in the laboratory work and the village trials performed so far has produced a panorama of results, which when scrutinized, give the appearance someone would get when looking at a landscape through some small gaps in the wall of an observatory. The picture one gets can be distorted, incomplete, even misleading. Present data is unlikely to provide clear cut information to guide international policy-makers and health administrators in their desire to make decisions on whether or not to proceed with oral vaccination, nor will it tell them at what frequency vaccination should be done.

The trials simply have not had a sufficiently broad spectrum of treatments. To have investigated all feasible vaccine feeding techniques, dosing frequencies, and other methods of implementing vaccination, would have been immensely expensive.

Another factor which limits our ability to prescribe directly from the results of laboratory work and the field trials, is our lack of knowledge as to how potential new users of the technique might regard it and the rate at which they might adopt it.

Householders have received vaccine free. Technical people have generally done the mixing of vaccine and feed, and in some cases, the feed has given to the birds by the technicians, too. People in the villages have watched vaccination going on, but we have no way of knowing how successful villager-administered mass vaccination would be. At this stage, the only way to find out if village people would be prepared to continue to use the vaccine, perhaps even to pay for it, is to ask them.

A series of questions, carefully phrased, associated with the issue of technological adoption, will be asked in the closing stages of some of the ACIAR sponsored trials to obtain better information on the potential for adoption. To be realistic, when we deal with a traditional and scientifically neglected resource such as village fowls, it seems likely that the best answers will be obtained after some years of vaccination, when householders have worked out for themselves the full implications of the

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technique. Our observations to date of the reactions of household flockowners and the veterinarians and technicians involved in the oral vaccination work in the field are encouraging. The research into disease control has awakened and heightened interest in village fowls as a productive resource with more potential than is indicated by this project.

The Role of Modelling

Modelling can help to overcome the shortcomings of the pilot trials and enhance the use of the available results, whose relevance can be expanded to give coverage to new situations. Modelling can also provide some foresight of the possible impact of vaccination in the longer term, and of how people might react to the changes resulting from the new technique.

So, even though the view we have of vaccination is incomplete, modelling can be used to open up the chinks between the boards in our observatory giving us impressions of what the whole landscape might look like.

Modelling Flock and Disease Interactions

Bird raising, embracing the natural phenomena of population reproduction, growth, ageing, and death all follow the rules of nature, and the essence of these interactions can be laid down as simple mathematical equations. The process of Newcastle disease viral infection being passed from an infective bird to susceptible birds largely depends on the rate at which birds come into contact with each other, as well as the ability of the virus to spread. These processes, in turn, are eminently capable of being encapsulated in mathematical terms, as are the transitions which birds make when they survive infection, to become immune for some months, or gain immunity from vaccination.

Village poultry represent an epidemiologically more complicated system than is found in commercial units. The nutrition, reproduction and productivity of village birds, even their resistance to diseases, is influenced strongly by the variability of day to day conditions. All age groups are represented: ranging from adult males of varying levels of sexual and physical dominance, with adult (say, above 8 months) females either ranging, brooding or mothering small chicks. Young chickens (up to 8 weeks of age) often remain as clutches, and male and female grower birds form daytime groups. With the flock replenishment process going on more or less all the time, influenced by the seasons, there is a steady supply of young, disease-susceptible birds entering each flock. Antibodies passed on to the very young through the egg (maternal antibodies) possibly play some part in protecting very young chicks from the range of local diseases.

Bird densities are rarely as high as in enclosed commercial flocks. The level of contact between infected and clean birds can be quite low, partly because birds of different age-groupings tend to avoid each other, and partly because there is usually open foraging and ranging space available. The initial results for non-vaccinated flocks in the Philippines trials (Johnston and Cumming 1991) attest to the relatively low monthly effective field challenge rates. We have observed that even in months when infection is actively spreading approximately 60% of birds escape challenge.

The general form of the model described here is sometimes referred to as a Markov chain model or 'state/transitional' model, because it is made to represent the various compartments or 'states' of the flock as time progresses. Probabilities, gained from field observation, deduction, or plain guesswork, are assigned to time-dependent and disease-related movements between states. Such models were recently reviewed by Gani (1989), and are techniques similar to those used by Anderson et al. (1985) in fox/rabies work in Europe and by Andrews and Johnston for tuberculosis in feral cattle in northern Australia (Stoneham and Johnston, 1987). A diagrammatic representation of the model is shown in Figure 1. The three-tiered boxes depict the age structure of each subgroup, and for simplicity, the ageing process of birds passing from, say, chickens to growers, etc. has not been shown.

Under computerisation, the calculations take the first record entry from a database, the fields (representing states) of which have been 'seeded' with numbers representing cocks, hens, male and female growers, chickens, and very young chicks. The data seeded into the model should preferably be from real-life flock situations. Reference is then made to another data base containing rates of reproduction, offtake, death and the probabilities of birds changing from one disease status to another. These rates are applied to the first entries and generate coefficients representing the flock structure which could be expected to develop 14 days later. The characteristics of this emergent flock are stored as the next flock record, which in turn is used as the basis for generating yet another possible outcome after 14 further days have elapsed. The number of fortnightly periods that the model iterates to generate a simulated chain of outcomes is set at 200, but this can be varied.

The user has the facility to vary the flock composition and size and to insert different rates to conform with field observations. As well as the 5 population categories (cocks, hens, male and female growers, chickens and chicks) the following disease states are used:

- susceptible (that is never infected, or acquired immunity has been lost)
- latent (infected, incubating virus, but not a source of infection)

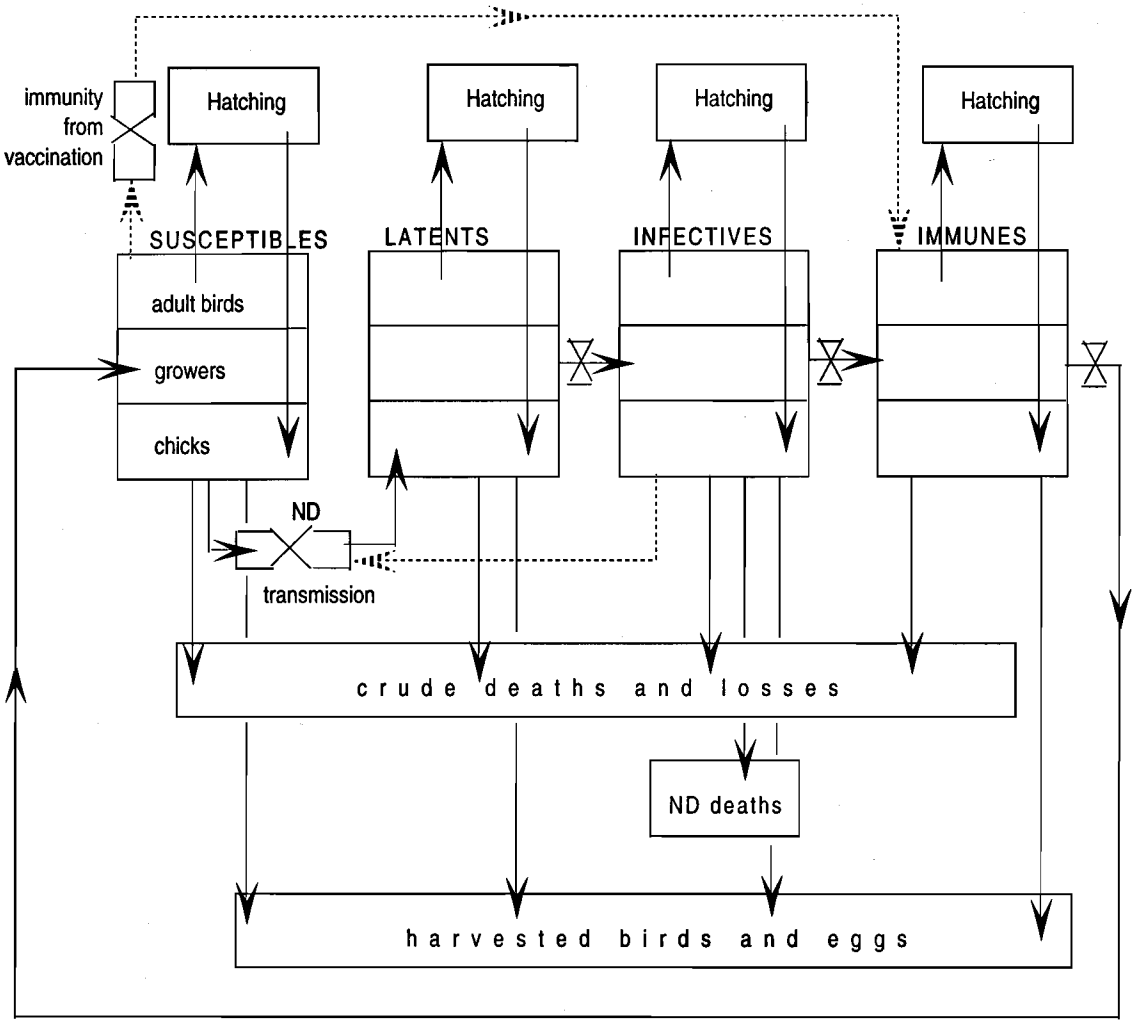

Fig. 1. Flow diagram: state/transitional model of Newcastle disease in village chickens

- infected and infective (source of infection to other birds), and,
- immune (recovered and no longer susceptible).

In order to keep the population replenished the 'adult females' were given mathematical licence to produce a certain number of fertile 'eggs' (the rate of production, as for many other rates, can be varied by the person using the model). A proportion of the eggs are incubated to give rise to chicks each fortnight. The remaining eggs would be regarded as surplus for family consumption. All chicks were taken to flow into the chicken group at the end of any one fortnight, after mortalities. If the chicks were to become infected, according to the probabilities set, then the mortality rate applied would also be that set as appropriate for Newcastle disease in young birds; otherwise the death rate applying would be the normal crude death rate. The same principle is applied with respect to deaths in all other age-groups.

In each fortnight the chicken group gains replenishment from the chick group, as the chicks age, but they are eroded by mortalities (either crude or due to Newcastle disease), and an appropriate proportion are aged out of the group to become either female or male growers, thus replenishing those groups. Growers, after deaths, become subject to the householders taking a certain proportion for sale or consumption (termed offtake), and an appropriate proportion flow out of the group to become adults. The adults, after deaths and after receiving aged ex-growers, are also subjected to harvesting.

A routine was applied to reflect the cost of any inputs

of value used by householders for their birds. When bird numbers are rising, then the value of inputs/bird were set at the level observed to apply when householders' flocks were recovering from a setback, (in the Philippines this was 0.60 pesos/bird). When numbers were stable or falling, the rate observed was 0.40 pesos, and this was applied appropriately in the modelling process.

The value of bird output, either consumed or sold, was calculated in the model by multiplying the offtake numbers by the prices obtained from survey data collections in the trial locality. With the output and the inputs valued, a surplus measure was calculated representing a net fortnightly value of the birds to the household. With all inputs and outputs having been valued the calculation of flock productivity measures and changes is possible.

Model Testing

In testing the model, the following topics were addressed:

- the performance of a village flock in the absence of Newcastle disease, but subject to the other disease and accident rates of the village environment
- the reaction of the flock to the introduction of Newcastle disease
- the possible survival of enzootic Newcastle disease within the flock
- the impact of mortalities induced by Newcastle disease on flock productivity and flock replenishment
- the likely influence of the vaccination programs now being developed.

In these examples the foraging environment was deemed to be capable of supporting 100 birds including recently hatched chicks. One hundred birds is more than the typical flock, in fact it represents about four household flocks in the Philippines trials area, and about two in Thailand. Nevertheless, in view of the sharing of the forage base which takes place, with all the consequent risks of interflock disease transmission, it was taken as a convenient number. In the interests of maintaining relatively stable flock numbers, and to simulate a carrying capacity restriction, the model simulates automatically some variation in the proportion of eggs incubated according to whether flock numbers in any one fortnight are above or below the target number. Proportional bird offtake is also varied around the target, for the same reasons. These measures represent the maximum lengths that householders have been observed to adopt to manipulate flock numbers.

Using the Philippines trial data as a basis, the flock performance parameters were set as fortnightly rates as follows:

Crude fortnightly death rates —

adult males:	0.0035
adult females:	0.0045
female growers:	0.0300
male growers:	0.0300
chickens:	0.0650
chicks:	0.0900

Other data supplied to the model, again based on interpretation of the monthly estimates obtained from the Rosario trials were:

eggs laid per hen	1.60
proportion incubated	0.55 (varies according to flocksize)
proportion hatching	0.57

The householders' desires with respect to removing birds for sale or home consumption appeared to be:

offtake of cocks	0.15
offtake of hens	0.06
offtake female growers	0.12
offtake male growers	0.12

These were target rates of offtake, subject to influence of flock size relative to the forage base capacity.

These data, run on a 'seed' flock of 100 birds generated the following stable flock structure:

cocks	8.23
hens	22.52
male growers	15.60
female growers	18.15
chickens	25.41
chicks	10.52

This conforms well with survey information on flock composition gained from the field.

As a productive unit, the flock would be able to provide the following potential offtake for consumption or sale:

adult males	1.24 per fortnight
adult females	1.35 per fortnight
male growers	2.43 per fortnight
female growers	0.12 per fortnight
eggs per fortnight:	35.83 minus 19.63 incubated, i.e.16.22 per fortnight for consumption

Crude mortalities per fortnight were:

adults	0.14 birds
growers	1.01 birds
young birds (chicks and chickens)	2.60 birds

making 3.72 birds in all, that is slightly less than the 5.14 birds capable of being removed for consumption.

Infection by Newcastle Disease

The process of infection by the Newcastle disease virus was modelled in two ways.

Our observations led us to the conclusion that Newcastle disease can remain endemic in village communities, but the introduction of disease from outside, by human movement and bird trafficking, and by contact with other bird species, etc, is also a significant risk.

Two options are available to the model user: one, which models the interaction of the disease as an endemic influence is done by 'moving' a couple of clean bird from the susceptible group to the infecteds. This simulates the introduction of infection into a completely susceptible flock.

Where disease is active, the process of infection goes as follows: as each 14-day period evolves, virus is transmitted according to a probability set by the user. The probability seeded for virulent Newcastle disease virus was 0.030, that is to say that for an infective bird in the same flock and in contact with a susceptible bird for one time period (a fortnight), then the chance of virus being transmitted to infect the susceptible bird would be 3.0%. This probability appeared to give conditions of spread in the flock at an overall rate similar to that observed in the previously unvaccinated village conditions of the Philippines trial area.

Thus for one infective bird among 99 susceptibles, at 3.0% probability, the number of 'latents' generated by the end of the fortnight would be 1 by 0.030 by 99 or just under 3 birds. These latents would ultimately give rise to infected/infective birds, and as these build up in numbers, so does the rate of buildup of latents, giving rise in turn to a spiralling effect in subsequent fortnights of yet more infectives. This, as well as fuelling further infection of the now dwindling pool of susceptibles, would be accompanied by the infecteds beginning to die at typically high rates, and it is the elimination of infecteds by disease-induced mortality which would tend to extinguish an outbreak. Infection would be reduced overall and the supply of susceptibles to become infected would be severely curtailed.

In trials performed in Indonesia (Parede and Young, 1990) the period of latency was found to be less than 14 days, averaging only five days. Accordingly, the latent group was made to retain at the end of each fortnightly period only that proportion of the birds which would have become infected in the last five of the 14 days period; the remainder being made to flow on in that same fortnight to the infecteds.

The rates of change for each fortnight between the different population compartments were initially set as follows:

• transmission probability	0.030
• latents becoming infective	0.95
• surviving infecteds gaining immunity	0.50
• immunes reverting to susceptible	0.12
• deaths among adults if infected	0.85
• deaths of young birds, infected	0.95
• deaths of growers, infected	0.90

These rates were thought to be appropriate to reflect the impact of a velogenic viscerotropic (fast-acting gut-attacking) form of Newcastle disease virus such as occurs in many parts of Asia.

The pattern of population fluctuations, deaths and the possible offtake of birds over the first three-and-a-half years of a 200 fortnight period is shown in the next graph. Because the amount of information generated by running the model for 200 14-day periods is large, the essence only of the results is presented in the form of Figures 2–8.

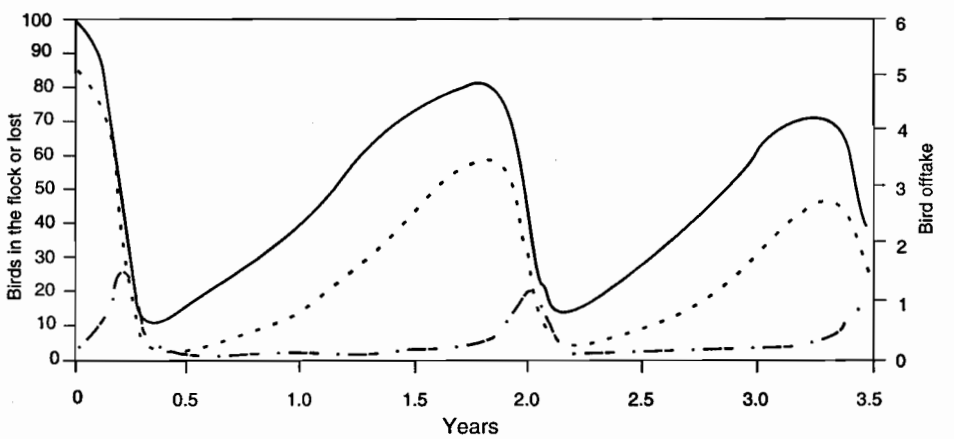

Fig. 2. Endemic Newcastle disease in a closed population of 100 fowls. Inter-epidemic traces of infection are assumed to persist in the flock. The upper curve gives the fowl population, the middle curve bird offtake, and the lower curve total losses.

The model shows how the disease appears to have the capacity to erode population numbers severely, as successive waves of infection pass through the flock (Fig. 1). Moreover, it holds numbers to levels substantially below the carrying capacity of the habitat.

The death rate is modest most of the time, at 2 to 4 birds per fortnight. As infections build up, the deaths rise sharply, to 20 or so birds, to bring the population down.

In the period after an outbreak, the amount of infection remaining would not be readily detected, certainly not at the surveillance levels which could be implemented in many developing countries. Indeed at the levels of surveillance of the trials in the Philippines, for example, any form of infection, active or residual, has not been easy to detect. This probably has helped to nurture the view that Newcastle disease is an epizootic at flock level. Studies from Morocco have confirmed the persistence of Newcastle disease virus over wide areas in village chickens (Bell and Mouloudi, 1988).

The flock generated by simulation modelling had characteristics similar to those observed in villages in the Philippines and in other parts of SE Asia. The death rates, offtake and composition is similar to those that we observe in cooperating countries. Other workers, such as Orawan et al. (1989) surveyed the flocks of 115 village farmers in Thailand over 15 months, and it is of interest to note that the ratios of mature to young birds in those flocks and the proportion of male to female among the mature birds was very close to the estimate generated by the test model. Other studies attest to the typically high losses in young chicks (Wimolporn et al., 1987). A clutch of 8 newly hatched chicks would, in their study, lead to the production of 2 growers and but a single adult bird.

Others have noted the importance of the reproductive cycle influencing the start of outbreaks. Certainly, the model demonstrates that the fuel for an outbreak is the build up of a good supply of susceptible birds, so that even a single residual infective bird can trigger a burst of infection and mortality. With seasonal peaks in egg laying and hatching, there would be a tendency for outbreaks to follow seasons favouring buildup of bird numbers.

An alternative method of running the model was created to simulate the possibility that birds carrying residual infection between epidemics would die out or be deliberately slaughtered, to extinguish the last trace of flock infection. The period that the flock remains obviously infected (for example, infection in an individual householder's flock rarely has been observed to last more than eight weeks) is capable of being restricted before running the model. But to simulate the risks of infection being reintroduced from outside, a random 'disease generator' was adopted which could seed into the population in any disease-free fortnight some further infection, the probability of introduction being set according to the observations or the requirements of the model user. Figure 3 shows that when considering 100 birds in unvaccinated conditions the impact of the disease is not much different from the first option. The random generator in the model in the run depicted below has merely chosen to allow the flock to escape infection for the first 14 or so fortnights of the simulation run, and a further significant outbreak is suffered half way through the recovery phase.

The results of using this facility are again described when dealing with the issue of vaccination.

Vaccination of Fowls

To simulate vaccination, the model was given the scope to convert susceptibles directly into immunes. Different

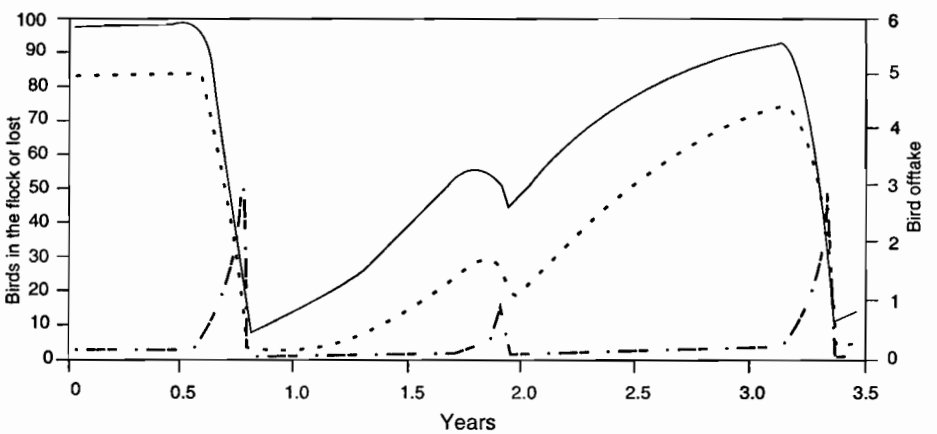

Fig. 3. Influence of Newcastle disease in a susceptible population of 100 fowls. The probability of infection being introduced is 0.06. The upper curve gives the fowl population, the middle curve bird offtake, and the lower curve total losses.

rates of protection were capable of being simulated, according to age group.

The vaccination rates most favoured were as follows:

- protection conferred to adults: 0.46
- protection conferred to growers: 0.67
- protection of the young: 0.37

These rates are those observed (Johnston and Cumming 1991) in the village trials in the Philippines resulting from feeding out vaccine coated grain, using good birdcraft and husbandry.

Protection in field conditions has rarely been successfully measured in field trials yet, so in view of the likelihood that protection levels could fluctuate quite widely in different situations, a description is given later of simulation runs which test the sensitivity of the system to vaccination turning out to be both more and less effective than the favoured rates.

In Figure 4, a model sequence shows the effect of beginning a regime of monthly vaccinations which achieve the favoured rates of protection.

What has been simulated here is the onset of wild infection and the start of vaccination at the start of the run. In such circumstances, a race ensues between two infection processes: the vaccine infecting the birds to give immunity; and the more damaging process whereby Newcastle disease virus infects birds, causing deaths. The deaths can not be expected to cease immediately the vaccine is administered, because infection by the wild strain is winning the race early on. Several months are necessary before the immune group builds up.

After a period of half a year, the impact of vaccination significantly overcomes the effect of the initial disease outbreak to bring the deaths in the flock down from as many as 12 birds per fortnight to about 4 so that the flock can grow back towards its original size. Bouts of infection introduced stochastically into the flock from time to time cause only small blips subsequently in the number of deaths.

This point is of importance in making inferences from the project trial results. It is suspected that vaccination was started in several of the ACIAR project trials just as, or even after, the infection process became more active. As a consequence, it was probably unreasonable to have expected that the patterns of deaths and bird productivity in the first 18 months of the pilot trials would have been representative of the patterns which would apply in a fully stabilised vaccinated population.

Once the regime is firmly established, with vaccination firmly dominating the ravages of new outbreaks, the performance in the 100-bird community is as shown in Figure 5.

Flock numbers hold between 100 and 95 birds, offtake correspondingly ranges between narrow bounds averaging 4.7 birds per fortnight, and deaths averaging 3.9 birds rise slightly above 4 only when disease is actively spreading.

Variations in Vaccination and Protection

Various techniques, such as creep feeding the young birds, are being explored to use the current strain of vaccine more effectively. In addition, it may be that a more invasive vaccine can be developed which still retains the favourable characteristics of the current strain.

Given that the objective is to avoid bird wastage, so that the productive capacity of the flock need no longer be diverted periodically into patching up the decimation

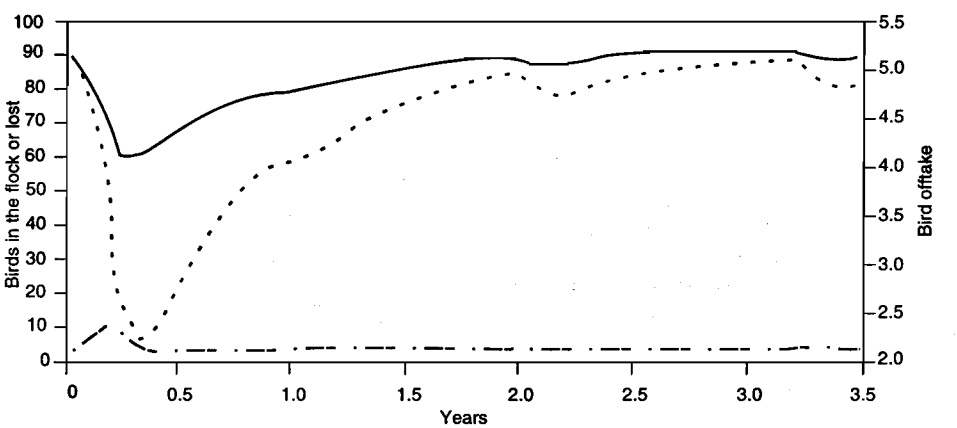

Fig. 4. Initial vaccination experience in a population of 100 village fowls. Monthly vaccinations started at the beginning of year one, at the same time as first infection was introduced. The upper curve gives the fowl population, the middle curve bird offtake, and the lower curve total losses.

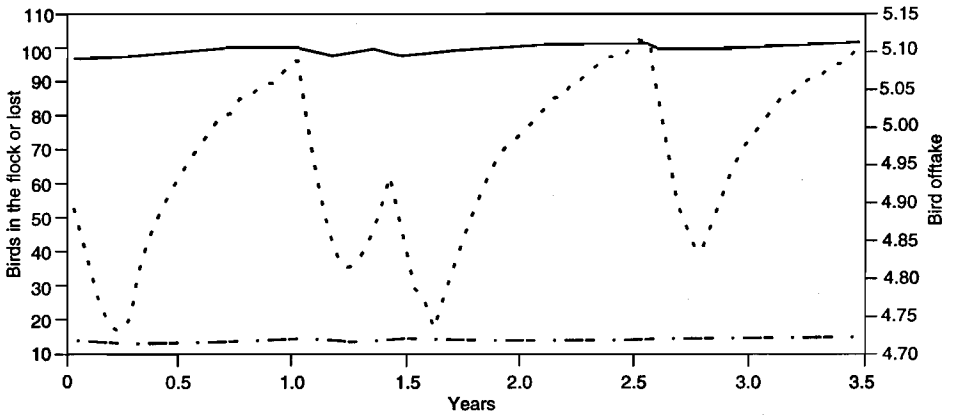

Fig. 5. Flock stability under monthly oral HRV4 vaccination. The upper curve gives the fowl population, the middle curve bird offtake, and the bottem curve total losses.

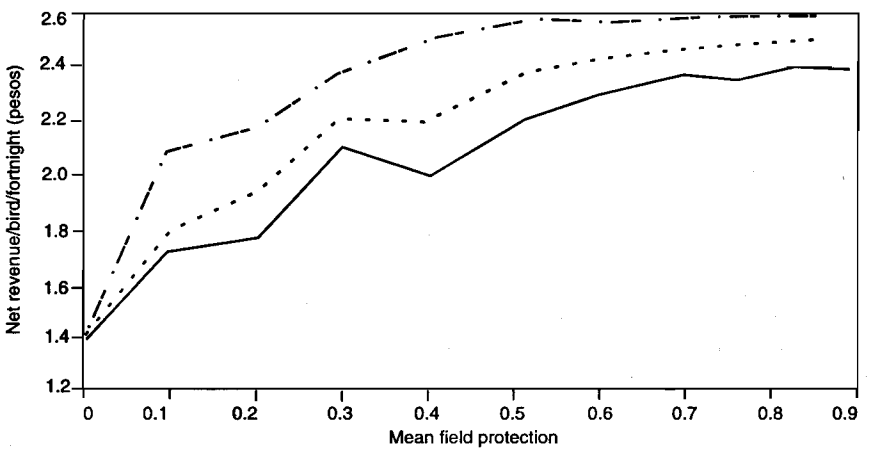

Fig. 6. Protection and net revenue from oral vaccination of village fowls, estimated from a stochastic flock/disease model using data from field trials. Upper curve is monthly vaccination, middle curve at 8-week intervals, and bottom curve vaccination at 12-week intervals.

caused by sporadic Newcastle disease outbreaks, it may be that substantial savings could be achieved by reducing the number of vaccinations after an initial introductory period. In the graphed results which follow (Fig. 6), the model has been run to compare the results from monthly vaccination with alternative intervals set at 8 and 12 weeks respectively. The efficiency of vaccination has also been varied, in steps, in a sensitivity analysis to give rise to mean field protection levels ranging from just over zero to almost 100%. The results are given in terms of long term mean net revenue/flock bird/fortnight: that is profit per bird in the standing flock.

To reiterate, the favoured rates giving rise to the central results were as follows:

- protection conferred to adults: 0.46
- protection conferred to growers: 0.67
- protection of the young: 0.37

The results indicate that at the higher levels of protection the net revenue (profit) per flock bird per fortnight at different vaccination frequencies varies only slightly. At the higher efficiencies, some 0.23 pesos/bird or about 8% of total net revenue of the monthly vaccinated performance are lost by spacing out vaccinations to once every three months.

At medium and lower efficiencies, the losses associated with less frequent vaccinations appear to be more serious, increasing both absolutely (0.5 pesos/bird) and as a

proportion (20%) of the revenue associated with monthly vaccination.

So, from the example, it appears that possibilities exist for spacing vaccinations out beyond the monthly ones which have been mainly adopted in the field trials. However, it is suspected that the advantages of vaccination are perceived by householders to be equally in terms of increasing their long term cash earnings, as in avoiding periodic heavy bird losses.

In Figures 7 and 8, the patterns of flock and production stability for the most favoured levels of protection and for extended vaccination intervals are presented to give a picture of the capacity of 8 and 12-weekly vaccinations to avoid serious bird losses. These can be compared with the results presented earlier of the performance stability of monthly vaccination.

As one would expect, 8-weekly vaccinations give greater variability in flock performance from fortnight to fortnight, but not greatly so. The occasional losses associated with 12-weekly vaccination appear to be even higher than the other vaccination regimes, possibly to level which would be quite noticeable and unacceptable to householders.

Summary

This model, using real-life data from the Philippines trials, has been used (Johnston and Cumming 1991) as the technical basis for interpreting field trial information so that an economic evaluation of the ACIAR Newcastle disease project could be performed.

In the example described here, to show the ways in

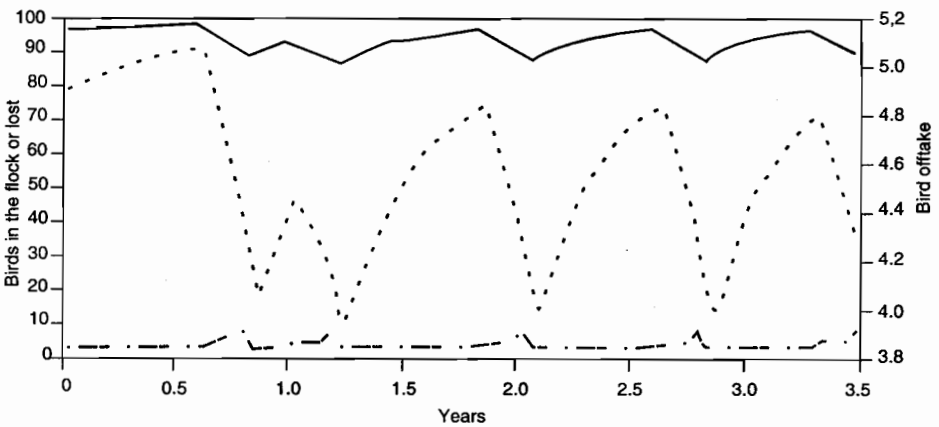

Fig. 7. Flock stability under oral HRV4 vaccination at 8-week intervals. The upper curve gives the fowl population, the middle curve bird offtake, and the bottom curve total losses.

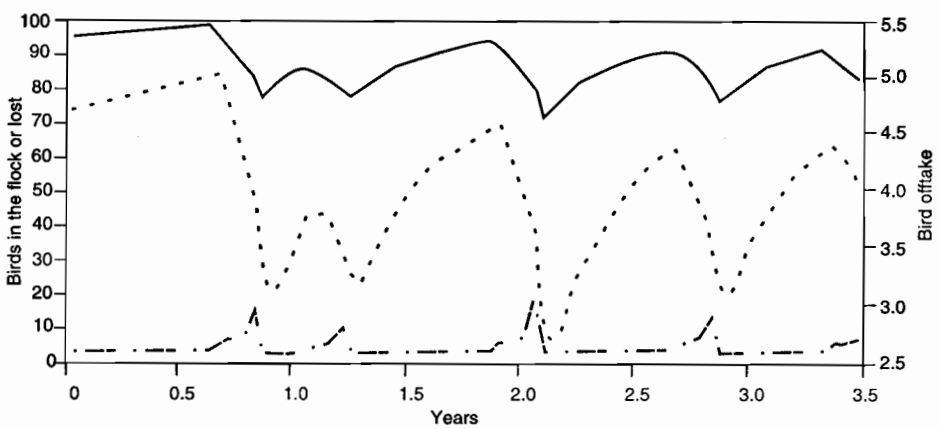

Fig. 8. Flock stability under oral HRV4 vaccination at 12-week intervals. The upper curve gives the fowl population, the middel curve bird offtake, and the bottom curve total losses.

which population/disease models can be used, the issue of vaccination efficiency and intervaccination interval has been examined to see if any economies might be gained from spacing out vaccinations beyond the four-week interval which has been commonly adopted for the pilot trials.

There appears to be a trade-off between vaccination spacing and vaccination efficiency. If protection rates equivalent or better than those found to apply in 1989–90 in the Philippines field trials can be achieved, the small reduction in protection associated with vaccinating village fowls every two months instead of monthly would probably be acceptable. The savings in vaccine and feed would probably exceed the value of the production forgone. Should it turn out to be the case that only much lower levels of protection can be achieved, then monthly vaccination intervals would probably be best continued.

Spacing out vaccinations to once every three months, would probably not reduce susceptibility of bird populations sufficiently to avoid the occurrence of sometimes severe outbreaks. This would give the impression that 'the vaccine isn't working!', and could throw the concept of oral vaccination into disrepute.

Modelling of the style described is merely a means of making better use of available information. At the time of writing this note, there is some new information coming to hand on the two forms of immunity which can be created in village birds by the use of oral vaccine. This information might be important and be used to improve the structure of the model.

Successful vaccination by adequate dosage of oral vaccine can give a bird protection which lasts approximately for three months: but successful vaccination, boosted by field exposure to wild virus, can give birds an immunity lasting about one year.

The difference between the protection resulting from simple vaccination and that resulting from vaccination plus exposure opens up scope for improving the resolution of the model by structuring both forms within the model. It is too early at this stage to do this, but the measurements which are being made in Thailand, the Philippines and other countries should soon provide the necessary information.

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Oral Vaccines and Mucosal Immunity

P. Spradbrow*

Abstract

Newcastle disease virus vaccines delivered on the food could be expected to infect through the mucosal surfaces of the digestive tract. In doing so they would invoke mucosal immunity rather than systemic immunity. There is evidence that the avian mucosal immune system has a similar function to that of the mammalian mucosal immune system. In the latter, there is local production of secretory IgA, and migration of IgA-producing cells from the intestinal mucosa to other mucosal surfaces. The mucosal immune system has a further function of suppressing, rather than initiating, systemic immune responses. The production of circulating antibody may be irrelevant to the protection induced by a Newcastle disease vaccine that remains confined to the intestine. Systemic immunity may develop if the vaccine virus also reaches other portals of entry or if a virulent virus is later encountered and resisted.

ORAL vaccines are not novel, although we believe that the delivery of Newcastle disease vaccine on food is a novel use for that vaccine. Newcastle disease vaccine has been given in drinking water, as have many other attenuated avian vaccines, but this approach probably targets the mucosal surfaces and the lymphoid tissues of the upper respiratory tract, rather than the intestinal mucosa. Other common methods of delivering attenuated Newcastle disease vaccines are by nose-drop or eye-drop, and by aerosol or coarse spray. These have largely replaced the earlier inactivated, injected vaccines whose use was reviewed by Brandly et al. (1946). The production of circulating antibody, especially as detected by the haemagglutination-inhibition test, is held to indicate the development of immunity in the vaccinated chicken. This view appears to be valid in many circumstances, and discourages a consideration of other forms of immunity that may be invoked by vaccination. Cell-mediated immunity is overlooked, as is the influence of the mucosal immune system. It is particularly important that the mucosal immune system be part of any consideration of oral vaccination, for we frequently encounter chickens that resist challenge with virulent Newcastle disease vaccine after oral vaccination, but that possess insufficient circulating antibody to explain their resistance.

Oral poliomyelitis vaccine is the best known oral vaccine. Continued oral vaccination of children has practically eliminated human poliomyelitis from some countries. Attenuated rabies vaccines are delivered on baits to feral animals, and to uncontrolled populations of urban dogs in some countries. The development of baits that will specifically target certain species is becoming a science, and oral vaccines seem the only immunological approach to prevention of infectious disease in feral or near-feral populations. Oral vaccines find use in some other diseases, for example transmissible gastroenteritis of pigs, where mucosal immunity must be developed in the dam to ensure a supply of protective colostrum and milk to the young. Trials are now being initiated of oral vaccines against agents that infect through mucosal surfaces. One that may be cited is the protection of mice against influenza virus infection after oral vaccination that induces, at least in aged recipients, very little circulating antibody (Waldman et al. 1987).

Mucosal Immunity

The mucosal immune system has been studied more extensively in mammals than in birds. Where avian studies

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are lacking, we must assume similar mechanisms exist in birds and that they are subjected to similar controls.

Secretory immunoglobulin (IgA), the common feature of immunity on mucosal surfaces, was described by Tomasi et al. (1965), although Brantzaeg (1989) traced knowledge of the phenomenon of mucosal immunity back to 1919. In the latter review, it is noted that in some species 80% of all immunoglobulin producing cells are in the intestinal mucosa, and that many of the intraepithelial intestinal lymphocytes are suppressor T cells. Local immunity depends on cooperative responses between epithelial cells and lymphoid elements, probably with neuroendocrinal control. Two elements of the mucosal immune system could be of importance to oral vaccination with Newcastle disease. The first is the local production of IgA and its selective transport to the lumen. This could result in a bird immune to superinfection with virulent Newcastle disease virus, but lacking circulating antibodies. The second important concept is that of oral tolerance. There is evidence that suppressor T cells in the intestinal mucosa are involved in reducing the systemic response to ingested antigens. This regulation appears to target both IgG and IgE production, and the induction of cell mediated immunity. It may not be realistic to expect major responses of circulating antibody to oral vaccination. Subsequent systemic reactions may represent antibody responses to challenges with virulent virus that have been contained, or to vaccine virus reaching reactive sites other than the intestine.

Mestecky and McGhee (1989) emphasise the concept of a common mucosal immune system not restricted to the intestine. Cells dedicated to the production of excretory antibody migrate and relocate selectively at other mucosal surfaces. In mammals, the udder tissue is part of this system and a source of protective IgA for the the neonatal intestine. In chickens, the role of the gall bladder and of the antibody content of the bile has been emphasised (Leslie et al., 1976).

Bienenstock et al. (1989) review the interrelationship between the nervous system and the mucosal immune system, and the response of this system to neuropeptides and similar chemicals produced by non-neural cells. Citing other workers (Furness and Costa, 1980), they point out that the intestinal tract contains as many nerve cells as are found in the spinal cord. Control mechanisms in the mucosal immune system are obviously complex, and depend on stimuli other than exposure to antigen.

An Avian Mucosal Immune System

The avian mucosal immune system has not been the subject of intensive study. There is an avian immunoglobulin structurally and functionally similar to mammalian IgA, and it is commonly referred to as avian IgA (Powell, 1987). Cells that produce immunoglobulins are sparse in the intestine of the young chick, but their numbers increase with age and microbial experience. Lymphoid cells of the bursa and the caecal tonsils probably have a similar function to those of the mammalian Peyer's patch in the sampling of antigens.

Chickens also have antibodies that are functionally equivalent to mammalian IgG and IgM. There is still doubt (Benedict and Berestecky, 1987) about the detailed structural relatedness of the mammalian and avian antibodies, especially for the avian molecules that resemble IgG and IgA. The IgA-like molecule of chickens is present in relatively large amounts in mucosal secretions and in bile and in small amounts in serum. In the chicken, as in mammals, secretory IgA seems to be the product of components produced in two different types of cells (Parry and Porter, 1978).

Avian rotaviruses are agents that have been used to define the mucosal immune system. Myers and Schat (1990) found that when the virus was delivered into the oesophagus, there was a short-lived serum response involving IgM and IgA. By contrast, serum IgG and intestinal IgA were present for at least 70 days. It was believed that the intestinal IgA was concerned with both recovery from infection and resistance to reinfection, but it was not the only protective response. Similarly, in intestinal coccidiosis in chickens it appears that protective immunity correlates with the presence of specific secretory IgA in the intestine (Davis et al., 1978).

Newcastle Disease and Mucosal Immunity

Many studies have indicated local immune reactions or mucosal immunity in response to encounters with Newcastle disease virus.

Involvement of the intestine in the pathogenesis of Newcastle disease is obvious when the lesions induced by the viscerotropic, velogenic strains are examined and the lesions, especially those in the intestinal lymphoid system, are noted. However, even infection with lentogenic vaccine strains has an intestinal component. Kohn and Ebert (1960) noted a sequence that involved a primary site in the intestinal wall, migration to lymphoid tissue and return to the intestine. They demonstrated this by introducing B1 virus into exteriorised intestinal loops. Virus disappeared within two hours but was recovered later on the first day and throughout the second and third days. Faecal excretion was maximal on days five and six.

Some instructive experiments were described by Kono et al. (1969), who were trying to develop a model for poliomyelitis oral vaccination rather than protect chickens against Newcastle disease. They used an avirulent, enterotropic Japanese strain of Newcastle disease virus,

which was delivered by tube to the oesophagus. Chickens excreted virus after the first administration, but they excreted little or no virus after a second administration 4 or 6 weeks later, when faecal extracts possessed neutralizing activity, probably associated with IgA antibody. Local immunity depended on the bursal lymphoid system, with bursectomised chickens failing to develop intestinal immunity.

Various factors in the intestinal tract may be deleterious to Newcastle disease virus. Acidity is one factor, although Newcastle disease virus is reported to be stable over a wide pH range (pH2 to pH10) for many hours. Proteases may be another barrier, although cellular proteases are required for the cleavage of surface proteins on the envelope of virions of some paramyxoviruses, converting non-infectious virus to an infectious form. Bile salts reduced infectivity nonspecifically (Lee and Hanson, 1975), probably by solution of the lipid component of the viral envelope. The bile of vaccinated birds or of birds that had survived challenge with Newcastle disease virus contained specific IgG and IgA and had specific neutralizing activity.

Heuschele and Easterday (1970) studied elements of local immunity in the respiratory tract by using tracheal organ cultures. They found that gammaglobulin was present in tracheal secretions in chickens and that specific inhibitory substances for Newcastle disease virus persisted in cultures for more than a month.

In a series of experiments Malkinson and Small (1979) demonstrated that chickens could be rendered immune at one site (the eye or the air-sac) by local vaccination while remaining susceptible to infection at the non-vaccinated site. They attributed these results to local immunity, but did not explore whether the underlying mechanism was mediated by cells or by excretory antibody.

Newcastle Disease Vaccines and Mucosal Immunity

Consideration should be given to the induction of mucosal immunity when oral Newcastle disease vaccine is given to chickens. Much of the evidence for such a response is indirect – the resistance to challenge of chickens having low levels of circulating antibody or that lack detectable circulating antibody. Gamini Jayawardane (personal communication) has produced some direct evidence. He gave V4 virus by various routes, including introduction into the crop, and observed increased levels of IgA and of haemagglutination-inhibition activity in the lachrymal fluid and increased numbers of plasma cells in the Harderian gland.

Further work needs to be done to show that oral Newcastle disease vaccine does invoke a mucosal immune response. Levels of circulating antibody should not be the sole criterion for judging the development of immunity. In future research projects it may be necessary to measure excretory antibody, and resistance to challenge must remain the important indicator of immunity.

There is also a need to consider the possible uses of adjuvants to enhance the protection induced by oral vaccines. Pierce and Sacci (1984) for instance, indicated that it may be possible to develop adjuvants to enhance the response to oral vaccines. They used Avridine and demonstrated enhancement in the priming activity of the vaccine, with a marked increase in immunological memory.

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Alternatives to Oral Administration of Newcastle Disease Vaccines to Village Chickens

P. Young*

Abstract

The emphasis on oral vaccination for Newcastle disease in the ACIAR project has been a response to conventional husbandry practices in villages, where chickens scavenge for food and are provided with poor housing or are unhoused. One result of the protection afforded by oral vaccination will be improved husbandry and housing, as the perceived value of village chickens increases. Adoption of conventional vaccine techniques, that are more efficient than oral vaccination will then be possible. Vaccination will still require a thermostable, avirulent strain of vaccine virus.

THE ACIAR funded project on the control of Newcastle disease (ND) in village chickens has been directed towards the oral delivery of vaccine. This has been in direct response to the problems of delivering vaccine to scavenging birds. During the course of the field trials there has been the opportunity to make some observations on how village people perceive the use of the vaccine and how the use of the vaccine affects husbandry practices. In summary, it seems likely that oral vaccination of village chickens will lead to improved husbandry systems which in turn will lead to the use of conventional vaccine delivery practices and still further improvement in immunity to ND. The HRV4 vaccine will continue to be used for both oral and conventional routes because of its thermostability and low virulence.

If a comparison is made of vaccine delivery to commercial and village poultry, it can readily be seen that husbandry is the major factor limiting the efficient delivery of vaccine to village chickens. In intensive commercial systems, birds are housed in single age groups and are in close contact with each other. It is relatively easy to use mass vaccination methods or birds may be easily caught for individual vaccination. In both layer and broiler flocks vaccination is given regularly. In the village environment birds may not be housed at all and the village flock is typically multi-aged with new birds hatching throughout the year. The birds can be quite difficult to catch which means that when individual bird vaccination methods are used, many birds will miss out. Because the birds are not in close contact, transmission of vaccine virus from vaccinated to non-vaccinated birds may be inefficient. Mass vaccination methods such as drinking water or spray vaccination are not appropriate. Vaccine is usually provided by Government agencies and is administered by them so that village people have no control over the frequency of vaccination.

For vaccination of village poultry the HRV4 vaccine used in the ACIAR field trials has substantial advantages over other vaccines. Because the vaccine is relatively thermostable, especially in the freeze dried form, it is not necessary for the cold chain to extend to the village. Secondly, because the vaccine is avirulent it is possible to vaccinate all ages of birds with the one vaccine without causing any respiratory distress in young birds.

Oral vaccine can provide adequate flock protection. Other papers presented at this workshop will suggest that oral vaccination reduces the number of clinical cases due to virulent ND virus (even though infection may still occur) probably by increasing the numbers of immune and semi-immune birds in the village flock. However, it should be appreciated that protection by oral delivery will usually not equal that provided by eye-drop on an individual bird basis.

During the course of the oral vaccination project, it has been observed that once village people have some confidence that their birds are able to be protected against ND, then they are willing to invest some of their resources in housing and nutrition. Once birds are housed, even if this is only overnight to protect them from predators or theft, then administration of vaccine by conventional routes e.g. eye-drop becomes feasible. Numerous experiments have shown that vaccination with V4 using the eye-drop method results in high levels of protection. Other advantages are: ten times less vaccine per dose is required; the dose per bird is known; the identity of the

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vaccinated birds is also known so that each owner can make sure all his birds are vaccinated. Because of close contact between birds when they are housed, lateral transmission of vaccine virus is also more likely to occur. However, it must be remembered that none of the current Newcastle vaccines provide long lasting immunity and it is therefore important to vaccinate regularly, especially where new susceptible birds are entering the flock on a regular basis.

Provision of simple housing also has major benefits which result from improved husbandry of birds. Simple brooding facilities and the provision of a little extra nutrition can dramatically reverse the huge mortalities observed in young birds up to the age of 6 weeks.

In due course one can envisage the situation where village people will want to have control over the purchase and delivery of vaccine. As this occurs, the responsibility for vaccination will shift away from Government to the user, with appropriate advice being provided by Government agencies. The responsibility of the Government will then be directed towards manufacture (quality and quantity), distribution and storage.

The Economic Impact of Vaccinating Village Fowls: a Case Study from the Philippines

Joe Johnston*, Benjamin Fontanilla† and Florence Silvano†

Abstract

Large scale oral vaccination of village fowls could possibly become established to augment available techniques of Newcastle disease control. Oral vaccination would be particularly suitable for those village fowls which lack overnight housing. The village bird resource, only rarely considered by householders to be worthy of investment and husbandry action, is not very productive. Nevertheless, reduction of even one source of deaths in village birds could make the resource produce a more sustainable output and thus enhance householders' welfare.

Against a background description of the importance of village fowls relative to the national poultry scenes of Malaysia, Sri Lanka, Thailand, the Philippines and Indonesia, an analysis is presented describing the potential economic impact of staged implementation of large scale oral vaccination in one country. The example is based on data collected in village trials conducted in the Philippines, together with official poultry population and marketing statistics. In a market and investment framework, the costs of infrastructure and implementation of vaccination to the year 2010 are estimated and compared in a benefit/cost model with the stream of benefits expected to emerge over the same period. Given that the technique might be developed for convenient implementation as a normal part of rural community life, the benefits in the example were 13.8 times greater than the costs.

The original research topic and the work done in this project were aimed specifically towards people who have birds living under the most primitive conditions of housing and husbandry.

Vaccination by the oral route to control Newcastle disease is appropriate only for such birds. Those that can be handled at will or those having as a minimum, night-time housing protection, could be considered for treatment by other means which give more reliable results, such as vaccinating by eye or nosedrop, putting vaccine in drinking water, or administering vaccine by injection.

In this part of the technical monograph, some information showing the importance of village birds in the overall poultry scene of selected countries is presented. Then baseline data collected from householders in cooperating countries is described. This data gives some measures of the relevance of village fowls to household welfare.

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Then, in order to provide an example of an economic appraisal, the study focuses on the results obtained in a trial set up in a group of Philippine villages. These results show the gains in productivity which could be expected under different vaccination regimes. This leads into a market analysis showing the possible impact of increased productivity over the whole of the nation for that part of the poultry economy for which the technique is appropriate.

Statistical Estimates of Village Poultry

Some statistics relating to village fowls as a separate grouping are available for countries in Southeast Asia and the Pacific region. They were reviewed by Aini (1990) and some additional information from national published statistics is added here to give rise to the data in Table 1, covering the countries involved in the ACIAR project.

In another project paper, (Johnston and Cumming, 1991), the supply characteristics of scavenger village birds are discussed. The conclusion is reached that under conditions of low exploitation, the village bird resource

Table 1. Estimates of numbers of village chickens in countries involved in ACIAR-sponsored research into Newcastle disease control

Statistic	Country				
	West Malaysia	Thailand	Sri Lanka	Philippines	Indonesia
Village chicken population (millions)	6.6	120	2.5	43	174
Annual bird offtake (millions)	17	115	1	42	175
Annual egg offtake (millions)	152	30	160	1162	43.5
Estimated % of total population	15	65	–	72	38

can respond positively to increased demands being placed upon it. However, when harvesting pressures increase past the optimum, and in the absence of any increase in purchased inputs to sustain the flock, it is postulated that the supply response changes from positive to negative. This is suspected to be the situation which has been reached in many of the highly populated countries of Southeast Asia.

Current evidence backs our assertion that in most countries the point of maximum sustainable yield has been passed, and the bird resource is being progressively and increasingly exploited so that future yields can only shrink further. This is borne out, for example, by the Philippines statistics (Bureau of Agricultural Statistics, 1989) on poultry which describe how backyard poultry numbers have diminished slightly over the last decade, while the numbers of birds harvested has gone down by several percent. In contrast, over the same period, the national commercial poultry flock expanded slightly, but the industry gave rise to a substantial increase in output.

It is interesting to note that in much of the developing world, the statistics of numbers of village chickens and rurally-based people indicate a ratio of approximately one person to one bird. Swan (1987) has noticed a similar ratio in the tropics. The project is involved at present with countries having about 330 million people and 350 million village birds.

Reference to the FAO 1988 Production Yearbook, (FAO, 1987) discloses global chicken numbers of 10,000 million. Eliminating those from the developed countries, which are bound to be run commercially, and eliminating those from areas where Newcastle disease has not been reported to be a problem, it would appear that the African continent has at least 300 million village fowls, Central America 100 million, South America 200 million, and Asia (including the countries involved in this project) about 2000 million, all of relevance to this project. People from Africa with interests in poultry would consider the estimate of 300 million for the continent to be grossly understated.

Baseline Estimates at Household Level

The range of production systems applying in different countries is described in Copland (ed) 1987. In earlier studies Huchzermeyer (1973) refers to the possibilities for introduction of hybrid chickens and better husbandry into African tribal life. More recent survey work sometimes involves assessment of flock productivity and health, for instance that reported from Java by Kingston (1980), and for Thailand by Wimolporn et al. (1989) and Orawan et al. (1989).

In this project, field data collection has been aimed at assessing the productivity of small village flocks under different Newcastle disease control systems and disease situations. Some results from 1989 and 1990 household survey work in four countries are given in Table 2.

The data should be regarded as baseline estimates, reflecting the systems traditional to the areas where project surveys have been initiated. In Malaysia, Sri Lanka and Thailand, the birds had in the past gained some protection against Newcastle disease from previous vaccination programs: in the Philippines the data refer to flocks with a history of some decades of no vaccination.

To define the age groupings, chickens were birds judged to below eight weeks of age, growers were below 8 months, and adults were above this age.

Thus, the association of a flock of scavenger birds with a village household improves the cash situation for families by a few dollars/month at comparatively low input cost.

The systems of poultry keeping in the countries involved in the ACIAR research vary considerably, from the harvesting of a completely feral resource subject to no bird husbandry at all, to systems which involve some housing, supplementary feeding, and controls on breeding. For example, the estimates shown contrast the management of the birds in the project area of Sri Lanka with that of the other countries. In the Sri Lankan project area flocks were managed more for egg production, as it is egg consumption which mainly takes place there. Such a large egg output and harvest as is indicated for Sri Lanka

Table 2. Flock monitoring survey results baseline estimates of monthly performance

Statistic	Country			
	Malaysia	Thailand	Philippines	Sri Lanka
Number of households	21	12	20	36
Flock-days observed	7131	3665	9177	13417
Mean monthly flock size				
chickens	22.9	15.5	9.2	1.7
female growers	5.5	7.3	3.2	2.5
male growers	2.6	5.2	1.8	1.5
adult males	2.6	1.4	1.1	0.9
adult females	13.2	4.3	3.0	4.7
total birds	46.8	33.7	18.3	11.3
eggs laid/month	2.2	3.3	2.8	9.4
% brooded	39	99	55	3
% hatchability	66	63	67	72
birds purchased (number)	0.0	0.1	0.0	0.2
Monthly mortalities				
% of chickens	9.8	26.7	23.1	17.0
% of growers	2.5	7.1	9.1	4.9
% of adults	3.3	3.5	3.6	1.8
Sale or consumption of produce/month				
birds sold (number)	1.0	2.6	0.1	0.3
birds consumed (number)	0.8	0.6	0.9	0.2
eggs sold (number)	4.2	0.0	0.0	18.3
eggs consumed (number)	13.2	1.5	0.0	24.8
Value to household				
(local currencies)	20.2	185.7	41.6	129.3
Costs of inputs (local currencies)				
cost of birds purchased	0.0	3.4	0.7	7.4
feed purchases	14.8	56.4	6.2	30.1
medicines	0.0	0.3	1.4	0.6
other purchases	0.0	0.0	0.2	0.0
Total costs	14.8	60.1	8.6	30.7
Net value to household	5.4	125.6	33.1	98.6
(approximate equiv. A$)	2.7	6.3	1.9	3.3
Revenue/bird	0.43	5.5	2.3	11.4
Cost/bird	0.31	1.8	0.5	2.7
Net revenue/bird	0.12	3.7	1.8	8.7

would not be possible from the type of birds which one commonly encounters in countries such as Indonesia, Thailand and many areas of Malaysia, where the emphasis is on meat offtake. The birds appear to have some infusion from egg-laying strains, tend not to go broody as readily as hens from the other countries, and are more strictly managed (mainly by the womenfolk) with respect to when and where they nest. Consequently, compared with flocks from the other countries, the flocks in the Sri Lanka project area have higher egg production, with most eggs consumed rather than incubated, and fewer young chickens in the flocks.

Because of the high wastage (deaths and losses), especially among the young birds, the limited egg production capacity of the meat producing birds is almost entirely taken up by hatching for flock replenishment.

There are a number of other features associated with village birds which are to be found in the almost symbiotic

relationship they have with human communities, but which would defy valuation. There are no garbage collection services in the developing rural world, so the free service provided by poultry has undoubted benefits in terms of locality cleanliness and hygiene. They also benefit the human communities in terms of insect control and weed reduction, and they provide their 'owners' with a form of savings account which can help out in times of need or be exploited when seasonal peaks in poultry demand occur such as at religious festivals or celebrations.

On the negative side, the free-ranging birds do some damage to crops and gardens, not necessarily those belonging to their own households, which obviously can cause social discord.

Boosting the number of poultry harvested in villages would contribute specifically to the future welfare and nutrition of rural people. A proportion of birds reaching markets would go for specialist purposes (traditional and feast day consumption), often at premium prices. By improving village dietary standards, the inequalities which are becoming more apparent in many countries between urban and rural incomes would be to some extent redressed.

In most countries in SE Asia, the proportion of the population located rurally predominates, and despite the drift towards urban centres, this situation is likely to continue for many decades. Attempts through research and technology to improve the living standards of rural people are therefore unlikely to be rapidly outdated by social change.

In this particular animal health issue, the tendency of many governments to implement policies which selectively help rural society would lead logically to official sponsorship of vaccination, perhaps by the subsidisation of vaccines, as already occurs in parts of Malaysia and Thailand for example, where official vaccinators, too, are made available.

Village birds appear to involve the menfolk less than the women and children. This is perhaps because the men often work away from the house, perhaps growing crops or employed by someone else. Any improved conditions of production for the poultry resource, leading to better disease control, will undoubtedly reflect mainly on the women and children of rural communities.

Impact of Vaccination on Flock Protection

After initiating regular vaccinations in villages, some months need to pass before the protection afforded the birds becomes apparent. The technical Working Paper (Johnston and Cumming, 1991) and the paper 'Patterns of ND virus activity in village fowls and the measurement of effective field protection following vaccination' in this Monograph (Cumming, Fontanilla, Silvano and Johnston) describes the work carried out in the Philippines which led to measurements being taken of the percentage of birds which derived protection from a course of orally-administered vaccinations.

The work focused on 30 household flocks which received vaccine monthly, and 20 flocks which acted as controls. The activity of the naturally-occurring virulent virus was monitored by taking regular blood samples from the birds and testing for antibody to see if, and how many, birds had suffered exposure. Monthly records of flock numbers, bird and egg production, losses, and reproduction were carefully kept, so that any unusual patterns in monthly flock performance could be compared with serological events.

Overall, with Newcastle disease virus quietly maintaining a presence among the flocks in the municipality, occasionally causing in one or two flocks short episodes of serious deaths, it was difficult to make any immediate observations in the data we collected that the vaccinations were having any effect, favourable or otherwise, on bird mortalities. But eventually, after about 18 months, it became possible to detect in the serological data some patterns of heightened virus activity among the households of the five villages involved in the project. The periods of active infection showed that a flock on average was subject to challenge by the local virus strains for 13% of the time. In times of challenge, it was not necessarily the case that every bird in any particular flock became exposed: about 60% in any one month escaped challenge, perhaps to become challenged in the subsequent month. Of the birds found to be challenged, the vaccine gave protection to:

- adult birds 46%
- grower birds 67%
- birds younger than 8 weeks 37%

Obviously, in the 87% of the time when flocks were not obviously subject to challenge, there were no significant differences in flock performance, with the exception that flocks which had recently suffered heavy mortalities were obviously less able to produce birds for consumption or sale until they had regained a productive flock status.

Impact of Protection on Flock Productivity

By avoiding the wastage of birds in the period of challenge, and the subsequent need to rebuild numbers, vaccinated flocks could be expected to be more stable and more biologically efficient in terms of capability of producing a sustainable offtake.

It has not yet been possible to assess from field survey work the increase in production which one could expect over the long term due to vaccinating, but an estimate has been made by running the flock/disease model described in another part of this monograph. The mathematical model was made to depict a community of 100 village birds, and simulation runs were done to subject the flock to the same amount of challenge as had been detected in the field. Production was calculated, under the same harvesting rules as householders appeared to apply in the villages. Maximum use was made of the data collected from householders in monthly survey collections. The costs of any inputs which had some tangible value were calculated, as was the value of output. With productivity being defined as the value of outputs divided by the value of inputs, unprotected flocks had productivity ratios of 3.5, that is householders derived from their birds three-and-a-half times as much as they put in. Gains in productivity were modelled, by simulating vaccination and the resulting immunity conferred, giving to the different age classes of birds in the flock the same protection as we had measured in the trials. But to gain a better understanding of the impact of vaccination, the model was run several more times, with:

- protection levels set at levels ranging in steps somewhat higher and lower than those observed; and
- vaccinations spaced out from monthly to two and three-monthly intervals.

The results therefore give some idea of the productivity trade-offs which could eventuate between vaccination spacing and vaccination efficiency, and are summarised in the Figure 1.

The graphed output is not smooth because in the interests of realism, viral challenges were allowed to intrude into the population on a random basis. Some variation would have occurred in the frequency of the challenges in each simulation run. In one case a three-monthly vaccination run at very high levels of protection happened to give such favourable conditions of challenge that the outcome was the same as the equivalent situation for 8-weekly vaccination.

The horizontal axis is calibrated in terms of mean protection afforded the three groups of birds, with the simulation runs stepped around a basic value of adults 45%, growers 67% and young birds 37%, averaging 50%.

It should be noted that the productivity gains would be derived almost entirely from reduced bird wastage and the consequent extra output. The inputs would be about the same for all situations. The only extra cost (not included at this stage in the analysis) would be for vaccine and the feed vehicle.

The results show that, if vaccination is done monthly, a minimum of 50% increase in productivity is possible for bird protection levels of 35%. For protection levels higher than this the gains in productivity are modest, never rising to a gain exceeding 60%.

For eight-weekly vaccination, protection efficiency needs to be 50% (the levels we actually measured to apply in the field) to derive a productivity gain of 50%. Again, higher protection levels result in only very modest further gains.

Twelve-weekly vaccination appears to give slightly less productivity gain than the eight-weekly routine, but as is described in the paper on modelling, detailed examination of the output shows that sometimes severe, possibly unacceptably severe, losses of birds could be suffered under this regime. Under the other treatments, (monthly and 8-weekly vaccinations) the occasional episodes of deaths are comparatively mild.

The result leads to conjecture as to how effective would be a routine based on monthly vaccination to the young birds, with the others receiving vaccine less frequently.

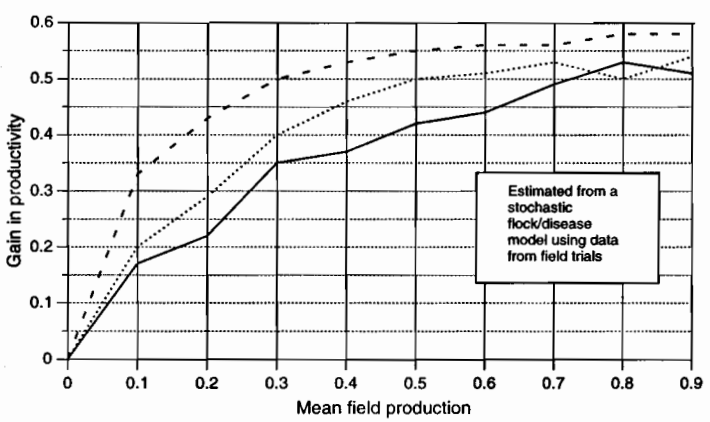

Fig. 1. Newcastle disease protection and productivity gains in village fowls following oral vaccination with HRV4: – – – = vaccinated monthly; ····· = vaccinated 8 weeks; ——— = vaccinated 12 weeks.

Or perhaps all birds could be vaccinated on a two-monthly basis after an introductory period of monthly vaccination?

Analysis at Market Level — the Shift in Supply

The impact of a shift in supply, such as would result from a 50% increase in productivity from a standing flock of several million village birds could significantly affect prices in any national poultry market. An appropriate way of assessing possible increases in supply is through the 'producer/consumers' surplus approach'. In a typical graphical exposition of the method, with prices depicted on the vertical axis and quantities on the horizontal one, a demand function is used to show in a downward sloping line how consumers are willing to buy greater quantities of a commodity as that commodity becomes cheaper. The supply function is usually taken to slope upwards from left to right to signify how producers attempt to produce more as they see unit prices increasing. Where the two functions intersect is the point identifying market equilibrium, representing (on the X axis) the quantity traded, and (on the Y axis) the price of the commodity.

Gross market turnover is represented by the rectangle of intersection price multiplied by the quantity of commodity traded. A triangle sitting on top of this rectangle, but remaining below the demand function, is called 'consumers' surplus' and represents the amount that consumers would be prepared to pay, but because of the way the market has settled out, they don't have to.

The concept of 'producers' surplus' measures the amount by which the gross market revenue exceeds the variable costs of production. It is usually represented by the triangle above the supply function, within the gross revenue rectangle. The corresponding area below the supply curve, of course, would represent the costs of producing the commodity.

The uptake of new information or new techniques flowing from a research project successfully completed can cause supply or demand functions to shift or go through a change in slope. The resulting change in consumers' and/or producers' surplus can be calculated to give measures of who benefits from the research; and by how much.

We can use our knowledge of the Philippines market for 'backyard' chickens to demonstrate the possible impact in the Republic of the technique of oral vaccine being adopted.

The statistics for the Philippines indicate that the 'backyard' population of birds of 43 million now has the sustainable supply capacity of some 42 million birds which can be harvested. This productivity relationship of one bird in the standing flock to one bird harvested annually conforms well with our observations in villages in the project area and the modelled estimate for a non-vaccinated situation.

Our surveys in the village indicates that a typical village bird has a value at the nearest local market of about 60 pesos.

Based on the factors described earlier in this report, it is postulated that the market for village poultry in the Philippines can be depicted as in Figure 2.

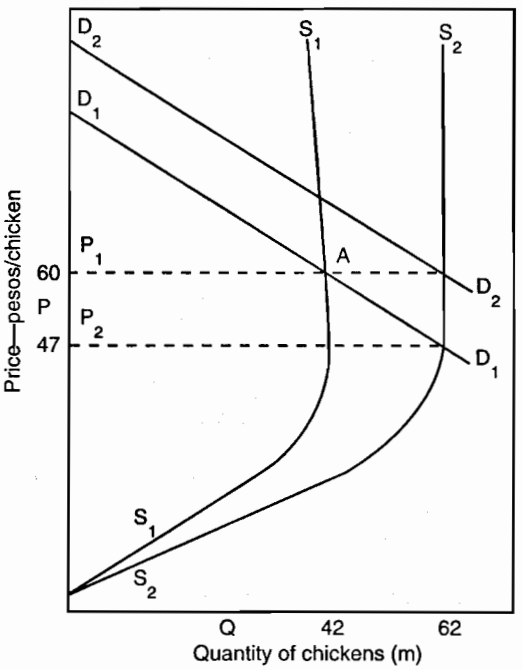

Fig. 2. Supply and demand characteristics in the market for village poultry in the Philippines. See text for explanation.

The current demand, shown as D_1D_1, is set at a slope which postulates that if bird prices change by one percent, then bird purchases will change, in the other direction, by 1.5 percent. The elasticity of minus 1.5 is considered to be a suitable figure for the Philippines market situation and corresponds with the demand elasticities which have been calculated for comparable proteinaceous food products in similar economies.

Supply is depicted as the sustainable supply function S_1S_1, initially rising with a slope of about 1.5, indicating that producers in earlier times were not exploiting their flocks heavily and had slack foraging resources available around their houses so that they could respond to any one percent change in price by producing 1.5 percent more of birds for the market. Later the function rises more

steeply to reach zero elasticity, indicating the point where the maximum yield is taken off from the aggregate resource. Under higher exploitation levels (higher demand), the curve bends back modestly, the slope chosen being minus 0.05. This function intersects the demand function at a price of 60 pesos and a market quantity of 42 million birds to depict the current market equilibrium.

The overall size of the market annually is thus 2520 million pesos. It should be borne in mind that in many cases the produce is not traded on conventional markets, but is consumed by the household at source. Producer and consumer are one and the same, but it is likely that decisions made in the household with respect to home consumption would be made with some reference to local market prices.

Consumers' surplus, that is what consumers would be prepared to pay for village chickens, but don't need to because of the way the market has found equilibrium, is represented by the triangle under the demand function but above the price line: this is 588 million pesos. Producer surplus, the value of the market net of costs, or the area above the supply function but below the price line, calculates out as pesos 1734 million.

The line S_2S_2 represents the supply curve which could be expected following adoption of the technique of oral vaccination. The magnitude of the supply shift above the initial supply function S_1S_1 has been taken from an earlier modelling exercise, and is 47%. Because the avoidance of some wastage of birds from disease would reduce the pressures of exploitation on the flock, and the standing flock of 43 million could be expected to remain the same size, it is postulated that the sustainable rate of harvesting would move back towards the maximum, and the supply function would therefore become less perverse, perhaps attaining zero elasticity (a vertical line) as depicted.

Given no change in demand, price would decline to 47 pesos/bird (about the price of today's commercially produced broiler), with the quantity rising to 61.74 million birds. Consumers' surplus would take over some of the area previously taken up by producers' surplus, rising to 1271 million pesos annually.

Producers would be less well off due to the lower price, but some compensation would accrue in the form of increased market volume, shown in the diagram as the band running between the old and the new supply curves and below the price line. Producers' surplus would rise marginally to 1790 million pesos.

The Gross Annual Research Benefits, referred to by the acronym GARB in some of the economics literature (e.g. Lindner 1989), thus work out to an expected pesos 739 millions, the difference between aggregated producers' and consumers' welfare, before and after adopting the research.

Because it could be expected that vaccine would be introduced to rural people on a subsidised basis, (financed by governments or from international sources, on the grounds of bettering selectively the conditions of the rural poor), the costs of administering the vaccine have not been included in the analysis. The supply/demand diagram depicts the market response which could be expected with householders' seeing price signals which allow them to ignore the cost of vaccine. The costs of vaccine production, packaging and distribution will be taken into consideration at a later stage.

Analysis at Market Level — the Shift in Demand

The demand for protein in many countries in Southeast Asia has risen in recent years, partly due to increasing populations, but also due to changes in food consumption patterns associated with some sectors of the population attaining better income levels. In many countries, the initiation and expansion of commercial poultry units is in response to these demand forces. As a result, prices for proteinaceous foods have risen faster than food prices generally, and the dietary outcome for those who are able to afford it is a move away from starchy and staple foods towards more eggs, meats, milk, even in some countries, to more pre-packed and fast foods. Perhaps not originally conceived as having a role to play in meeting future demand pressures, the research towards better Newcastle disease control could nevertheless be a significant force in stemming price rises in future markets.

In the supply/demand diagram, a second demand function D_2D_2 has been drawn which is 20% vertically above D_1D_1. This higher function intersects the technology-driven supply function S_2S_2, coincidentally to cause price to rise again to 60 pesos/bird. Consumers' surplus remains at 1271 million pesos, but producers' surplus increases to 2593 million pesos. This signifies that the new technology can become much more valuable than we might currently expect because of technology-induced supply combatting future demand pressures. In this example, GARB increases by a further 803 million pesos to 1542 million.

Adoption of a new technique can take a considerable time, so that it can be expected that as adoption proceeds, demand pressures will be growing too. The research outcome is therefore enhanced under such conditions.

Implementation Costs

The gross annual benefits of research have been calculated under an assumed regime of subsidy to householders, and although the supply response is conceptually correct, the benefits would be overstated by the costs of vaccination.

In the benefit cost scenes described later, the costs of vaccination have been taken into consideration.

The two significant ongoing costs of adopting the technique are for vaccine and for the feed vehicle (or bait). In the Philippines, paddy rice has been used, costing six pesos for one kilogram. Research is under way to improve the compatibility of rice and the virus vaccine, but it is unlikely that the treatment of the rice vehicle, when perfected, will attract large costs. Other research currently under way may lead to cheaper baits being used. One kilogram of rice has been found to be enough to carry the vaccine for 100 birds. The cost for vaccinating a standing flock of one million birds twelve times annually therefore works out at pesos 720 000.

Possibilities are being explored in some countries for the local production of vaccine, to serve the needs of indigenous populations of village fowls. It is too early to firmly predict the likely cost of producing vaccine to standards adequate for local use among village birds.

It should be recognised that the technique of monthly vaccination of a flock of birds of mixed ages in village conditions suffers the disadvantage that the number of birds which inevitably receive vaccine is large relative to the number ultimately saved from viral attack. Cost of vaccine is therefore a critical issue, as a costly vaccine could significantly erode the benefits to be gained from vaccinating.

The Australian firm of Arthur Webster Pty. Ltd., holder of the reference HRV4 seed virus, has supplied the vaccine to the project so far in pack sizes suitable for experimental purposes. This firm is active in many countries, not only to market its normal range of products, but also to advise on the issue of Newcastle disease vaccine production.

Techniques are being developed to help potential producers of vaccines overseas to produce a vaccine which is much cheaper than the experimental packs, yet of an adequate standard for the task of protecting village birds. If economies can be effected, it could be expected that vaccine could be produced locally in SE Asian countries for a cost of less than ten pesos for 1000 doses. However, the packaging, labelling, distribution and storage of the vaccine could bring the cost, excluding profit, up to ten times the cost of the actual vaccine. For this demonstration, a future price for vaccine has been assumed to be 19 doses per peso (1 peso taken to be A5.7 cents); and to allow some contingency for distribution inefficiencies in rural areas, this has been set in the analysis at 15 doses per peso.

This price, when raised to cover the annual needs of one million birds, works out to 800 000 pesos, so that the cost of the vaccine and vehicle combined is pesos 1.52 million.

For the Philippines future scene, therefore, where vaccine and vehicle for a standing flock of 43 million birds could ultimately be required, the future expected GARB of 549 million pesos under a supply shift only should be reduced by 65.4 million pesos to 483.6 million pesos. The long term GARB, including an expected growth in demand, of pesos 1542 million, would be reduced to pesos 1477 millions.

Potential Benefits and Cost

Johnston and Cumming (1991) present in an ACIAR Economic Monograph and a Working Paper an appraisal of the potential benefits which could flow from this research project, and compare the value of these benefits with the estimated project research costs. The analysis assumes that some uptake of the technique will take place in the five participating countries of Malaysia, Sri Lanka, Indonesia, the Philippines and Thailand. With conservative assumptions as to the number of birds which might appropriately become vaccinated, and a relatively slow rate of adoption, it was estimated that the benefits could eventually rise, in present day values, to almost fifty times the costs of research.

If the authorities of a particular country are interested in evaluating the new vaccine for domestic use, it is more useful to cast the benefit cost analysis in a different way. The benefits would be those which one would estimate from some form of market analysis rather like the one described in the previous section. The costs would be the costs of technological implementation, including factors such as the provision, quality control, and distribution of vaccine supplies, and setting up and maintaining the necessary training and extension facilities.

Again, using the Philippines data to create an example, Table 3 gives an evaluation of the costs of implementing vaccination for 43 million village fowls.

Critical to worthwhile benefits flowing from the technology is the rate at which it is taken up by people who have a dependence, however small, on scavenger poultry. Obviously productivity increases would not eventuate immediately; the vaccine manufacturing facilities would need to be built or rehabilitated, potential users would need to get to know about the vaccine, and how to feed it to birds. Vaccine production and distribution infrastructure would need to be developed, as well as a householder advisory service. The rate of uptake is assumed to be no uptake until 1996, rising to 50% adoption by the year 2010.

The costs involve an assumed investment of just over 8 million pesos to create vaccine production and distribution facilities, spread over 1991 and 1992. This estimate has been made at this stage for the purposes of supplying an illustrative guesstimate and, like many

others in this analysis, would need to be checked carefully.

This example depicts the type of analysis which might help individual countries to appraise vaccination as a national investment.

Nations will wish to set their own standards for vaccine according to the use to which it is put. Australian advice will continue to be available to help with problems of vaccine standards, production and utilisation.

If Newcastle disease can be effectively controlled in

Table 3. Benefit–cost ratio and internal rate of return. Implementation of oral vaccination to control Newcastle disease in village chickens — example based on circumstances in the Philippines

Basic parameters	
Discount rate	10%
Base year	1991
Project time scale including base year (years)	20
Bird population eligible for oral vaccination (millions)	43
Expected response to implementation of vaccination per million birds:	
Gross Annual Research Benefit pesos (millions)	17.186
Benefit enhancement due to expected growth in demand pesos (millions)	18.674
Vaccination costs pesos (millions)	1.520
Training and extension costs pesos (millions)	0.500
Initial investment in vaccine producing infrastructure pesos (millions)	8.600

Adoption profile, benefits and costs:

Year	Assumed adoption profile % of fowls	Assumed increase in demand %	Benefits due adoption of vaccination (supply) pesos (M)	Enhancement of benefits due increased demand pesos (M)	Benefits due supply and demand shifts pesos (M)	Costs of vaccine & feed base pesos (M)	Costs of training & extension pesos (M)	Total costs of implementation pesos (M)	Net benefits ofimplementation pesos (M)
1991	0%	1%	0.0	0.0	0.0	4.3	0.0	4.3	(4.3)
1992	0%	2%	0.0	0.0	0.0	4.3	0.0	4.3	(4.3)
1993	0%	3%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1994	0%	4%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1995	0%	5%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1996	1%	6%	7.4	2.4	9.8	0.7	0.2	0.9	8.9
1997	2%	7%	14.8	5.6	20.4	1.3	0.4	1.7	18.7
1998	3%	8%	22.2	9.6	31.8	2.0	0.6	2.6	29.2
1999	4%	9%	29.6	14.5	44.0	2.6	0.9	3.5	40.5
2000	6%	10%	44.3	24.1	68.4	3.9	1.3	5.2	63.2
2001	9%	11%	66.5	39.7	106.3	5.9	1.9	7.8	98.4
2002	12%	12%	88.7	57.8	146.5	7.8	2.6	10.4	136.1
2003	15%	13%	110.8	78.3	189.1	9.8	3.2	13.0	176.1
2004	20%	14%	147.8	112.4	260.2	13.1	4.3	17.4	242.8
2005	25%	15%	184.7	150.6	335.3	16.3	5.4	21.7	313.6
2006	30%	16%	221.7	192.7	414.4	19.6	6.5	26.1	388.4
2007	35%	17%	258.6	238.9	497.5	22.9	7.5	30.4	467.1
2008	40%	18%	295.6	289.1	584.7	26.1	8.6	34.7	549.9
2009	45%	19%	332.5	343.3	675.8	29.4	9.7	39.1	636.7
2010	50%	20%	369.5	401.5	771.0	32.7	10.8	43.4	727.6
Undiscounted total (million pesos)	–	–	2194.8	1960.5	4155.3	202.7	63.9	266.5	3888.8
Discounted pesos (millions)	–	–	528.5	442.0	970.5	54.9	15.4	70.3	900.2

Ratio of Benefits to Costs = 13.81 Internal Rate of Return = 65.0%

the world village environment, then it opens up substantial research and technical opportunities for the future. Fundamental to introducing husbandry innovations is to assist the bird population to produce at a level closer to the maximum sustainable yield. If the risk of mortalities can be reduced, householders' perceptions of the value of the chicken as a provider of protein and cash will change. This, in turn, would open up possibilities for introducing a range of practical husbandry improvements which could further act towards the improvement of world rural living conditions.

Indeed, the more that the practice of vaccinating birds orally is adopted, the more will householders discover better ways of husbanding fowls, which in turn will make the practice obsolete and lead to other methods of controlling Newcastle disease.

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Results from the ACIAR Project

An Overview of the Use of Food-Based Newcastle Disease Vaccine in Malaysia

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Abstract

Since 1985, Universiti Pertanian Malaysia (UPM) has been involved in two research projects funded by ACIAR. The main objective of these projects was to develop a new, simple and effective Newcastle disease (ND) vaccine for village poultry. Many trials or experiments were conducted and these included:

- selecting and cloning a heat tolerant variant of the V4-ND virus (NDV) which was used as vaccine;
- preparation and testing of food-based ND vaccine using various food grains as carriers of the vaccine;
- determining the efficacy of the food-based vaccine under laboratory, simulated village and field conditions;
- transfer of technology;
- a large scale control program incorporating epidemiological study and analysis of economic benefits; and
- application of vaccine for commercial broilers and layers.

It has now been established that village chickens vaccinated with the vaccine are protected against challenge with virulent NDV. The benefits to the rural farmers resulting from the vaccine include increased survival of chickens and increased income.

POULTRY are the only livestock species that are widely accepted by people from a wide variety of cultures and backgrounds in the Asia–Pacific region. It is estimated that 35% of the world poultry population comes from this region. Three systems of poultry production are practiced: the intensified commercial system; the smallholder 'rural' system, which normally requires small capital inputs; and the village or backyard system. The village chicken is the dominant form of poultry in many developing countries. The rural households keep a few chickens of local breeds that survive by scavenging for food and water. They are sometimes supplied with simple housing and provided with supplemental food. The poultry are maintained virtually without cost, and they return some meat and eggs and, because of the premium prices paid for village chickens and eggs in the market place, some income.

The village chickens, however, are not very productive, mainly because diseases cause enormous losses. One of the most important diseases is Newcastle disease (ND). Vaccination is the only safeguard against endemic ND. The disease in commercial poultry is usually adequately controlled by vaccination, but the delivery of conventional ND vaccine in village poultry has not been practicable. The chickens, which are in multi-age flocks, are scattered all over the villages. The birds are difficult to catch for conventional vaccination. Moreover, the vaccine is not heat-stable and complex cold chains are required to link the vaccine from the producers to the users. A new approach is therefore required to control ND in village poultry.

Cloning of Heat Tolerant Variant of V4 Newcastle Disease Vaccine

A research project was undertaken at Universiti Pertanian Malaysia (UPM) to develop a new, simple, cheap and effective ND vaccine for the village chicken. The main objective of the project was to produce an oral vaccine, which seemed the only feasible way of delivering the vaccine virus to the village chicken. Our objective was to isolate an immunogenic and heat-tolerant ND virus

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(NDV) which would then be incorporated into chicken feed and be given to the village chickens. It was assumed that chickens fed with the vaccine would be protected against ND.

The virus selected for the project was the avirulent Australian V4 strain of NDV. Early work with the V4-NDV had demonstrated that V4-NDV induces an adequate antibody response in chickens, spreads readily by contact and that naturally infected chickens and V4 vaccinated chickens resisted challenge with virulent NDV.

Development of Food-based Newcastle Disease Vaccine

A heat-tolerant variant of the V4-NDV, designated V4-UPM, was isolated and used as a vaccine. The vaccine was prepared by coating the V4 vaccine virus onto food pellets in a laboratory model Uni-Glatt Fluidised Bed Granulator. A nominal dose of vaccine was 10 g of pellets containing 10^6 fifty percent egg infectious dose (EID_{50}) of vaccine virus. Various food grains, such as wheat, barley, rice and maize were also tested as the vaccine carrier.

The Efficacy of the Food-based Vaccine

Experiments were then undertaken to test the efficacy of the food pellet vaccine. Studies on broiler chickens raised under laboratory conditions showed that two doses of the vaccine were sufficient to protect against the virulent challenge virus, which killed all control chickens even by contact spread. The experiments were extended to village chickens kept under simulated village conditions at UPM. These chickens had housing at night, access to an enclosed grass area during the day and were fed scraps with grain supplements. After two exposures to the oral vaccine, they were found to be substantially resistant to challenge. Field trials were then undertaken in 15 villages which were supplied with the vaccine once each month. Some 60% of the village birds resisted artificial challenge and outbreaks of ND were not recorded in the participating villages.

Transfer of Technology

A notable feature of the project in Malaysia was the effort devoted to transfer of technology before and during the field trials. We realised that the most important phase of the application of any new technology is the successful introduction of results into practice. Results of research conducted at universities or research institutes generally find their way to the user group through some type of extension education. In this particular case, the transfer of technology was undertaken with UPM's Centre for Extension and Continuing Education, which developed an excellent working relationship with the farmers. The programs were explained carefully in the villages, and field days were arranged on campus where farmers could vaccinate the chickens. There was an early appreciation of the simplicity of the technology. Villagers began asking to be included in the scheme and some individual owners indicated willingness to pay for the vaccine.

Control Program

A large-scale control program involving studies on the economic impact of the disease and the benefit of the new technology was also undertaken. The control program was being undertaken in the district of Hulu Langat which comprises seven subdistricts. It was carried out by the Department of Veterinary Services with technical assistance from the Faculty of Veterinary Medicine and Animal Science, UPM. In the past, vaccination programs against ND have been carried out using the F and Mukteswar strains of NDV. The routes of administration were intranasal/intraocular and intramuscular, respectively. The aim of the control program was to cover five out of the seven subdistricts with the feed vaccine. The two smallest, most urban subdistricts were to continue with their previous vaccination methods and served as control. The main activities of the control program include:

- census of the village chicken population;
- selection of villages and farmers for the monitoring program;
- dialogue, discussion and meeting with village leaders and farmers;
- initial data collection;
- initial and monthly bleeding program;
- distribution of vaccine;
- monthly collection of data;
- challenge trial;
- monitoring of incidence of ND; and
- analysis of data.

A screening survey was carried out to identify farmers who would participate in the monitoring program. Four villages from the control subdistricts and nine villages from three other subdistricts were identified. A series of meetings and discussions was carried out with the village headmen and farmers. The main objective of the meetings was to explain to the farmers the application of the new vaccine against ND and to seek full participation and cooperation of the farmers. Initial data, which included flock description, ND incidence and

vaccination, and flock inventory, were collected from each farmer involved in the monitoring program. Initial bleeding was also carried out before vaccinating the chickens. HI results showed that most of the village chickens have low HI antibody titres. The vaccine was prepared at UPM using wheat as the carrier. To date, about 50 000 doses of the vaccine have been distributed during the project. The vaccines are distributed to the farmers in the following manner:

- by field officer to farmers who participate in the monthly monitoring group;
- through village leaders; and
- through the subdistrict veterinary office/centre.

The chickens were vaccinated at monthly intervals, and 2 weeks after the third vaccination some of the chickens were artificially challenged at UPM. The chickens were challenged in the following manner:

- Group A — 60 chickens vaccinated with food pellet vaccine.
- Group B — 60 chickens vaccinated by the conventional method.
- Group C — 60 chickens with no history of vaccination against ND.

The results showed that between 50–60% of chickens in Group A were protected while 50–80% of chickens in Group B survived the challenge. All chickens from Group C died.

Preliminary analysis on the economic impact of the vaccination showed that there is an increase in (i) the number of chickens, (ii) the number of eggs and poultry meat consumed, and (iii) income from sale of chickens.

Application of Vaccine for Commercial Broiler and Layer Chickens

The vaccine is also being evaluated for commercial broiler and layer chickens. There is already evidence in Malaysia that the food-based vaccine is an effective booster vaccine for commercial chickens that have received primary vaccination with lentogenic virus in commercial hatcheries. The ease of application of ND vaccine through the feed supply has obvious commercial benefits to the industry.

Conclusion

In conclusion, a new food-based ND vaccine for the village chickens has been developed. It is effective, safe, cheap and has a moderate shelf life. It has now been established that village chickens vaccinated with the food-based vaccine are protected against virulent NDV. The food-based ND vaccine will undoubtedly revolutionise the vaccination of village chickens against ND in Malaysia. The benefits to the rural farmers, in terms of increased survival of chickens, improved nutrition of rural poor villagers and an increased income to the farmers, could be enormous.

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Results of the Newcastle Disease Research Project at the University of Queensland

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Abstract

Our work has addressed the following problems: survival of the vaccine virus during transport and between application onto feed and consumption by chickens; production of protective levels of antibody; ensuring that all birds in a flock are exposed to vaccine.

The virus was found to survive in a variety of diluents, with little or no drop in titre, for several weeks at 4°C, for one week at 22°C, and for 24 hours at 32°C. Skim milk was the least effective diluent because it supported bacterial growth. After application to uncooked grains survival was very poor, with significant loss of titre after a few hours. Use of 50% sucrose as diluent somewhat improved survival on paddy rice over 24 hours.

Antibody responses of chickens to feed-vaccination were extremely variable, even when treatments were apparently identical. Uncooked white rice was uniformly ineffective, producing virtually no response. Cooked white rice was more effective, particularly when the amount of rice allowed per bird was increased from 10 to 25 g. Paddy rice was effective in most trials, provided that a short-grain variety was used and that the birds had been trained to eat it. Wheat and maize were used successfully on some occasions. Use of grains that had been stored for 24 hours after application of virus usually resulted in very poor responses. However, in one experiment, when sucrose-diluted virus was used on paddy, a better response was obtained with stored than with freshly prepared grain. Rice bran was a satisfactory vehicle, producing a good antibody response even after storage for 24 hours.

The amount of vaccine consumed varied greatly between birds. When they were kept in small rooms, contact birds achieved levels of antibody equivalent to those of vaccinated birds, but spread of infection was less efficient in open-range birds. However, creep feeding vaccine to young chicks was shown not only to produce antibodies in the chicks but also to boost the antibody levels of adults in an open-range flock.

Another phase of the research topics in the laboratory was undertaken by Gamini Jayawardane. He demonstrated in chickens receiving oral vaccination an increase in plasma cell numbers in the Harderian glands and increased IgA and haemagglutination-inhibition activity in lachrymal fluid. His results support the hypothesis that an avian equivalent of the mammalian mucosal immune system is responding to oral vaccination.

THE aim of this project is to protect village chickens against Newcastle disease (ND) by vaccinating them with the Australian avirulent (V4) strain of ND virus in feed. For this to be successful, several conditions must be met:

1. the vaccine virus must be able to be transported from the supply source to the chicken-owning household with minimal loss of titre;
2. there must be little loss of titre between the time the vaccine arrives at the household and the moment it is eaten by the chickens;
3. the vaccine must reliably produce a protective immune response when it is eaten by a chicken; and
4. all susceptible birds must be exposed to the vaccine. This means *either* that every bird must eat the vaccine *or* that the birds that eat the vaccine must excrete the virus and infect any birds that missed out.

Work at the University of Queensland during the period 1985 to 1990 has been directed towards finding the best ways of ensuring that these conditions will be met. Most of the work up to March 1987 has been reported in Copland (1987) and in the scientific literature. This paper will deal chiefly with work that has yet to be published, while briefly covering the work published since 1987.

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Survival of Vaccine During Transport

The initial idea was for a 'food pellet vaccine' which would be manufactured at the supply source and distributed to households. The virus was found to survive on feed pellets at ambient temperatures for several weeks (Ideris et al. 1990). However, there are several problems with the use of pellets: they are expensive to buy or produce; they are bulky and therefore costly to transport; and variations in their composition may result in toxicity to the virus. For these reasons, we have adopted the aim of using cheaper, less processed feeds which are readily available in the country where the vaccine is to be used. We looked at three different grains: wheat, maize and rice; and the rice we tested in several different forms: 'paddy' or rice in the husk, brown (unpolished) rice, and white (polished) rice, both cooked and uncooked.

We also tested the use of several different fluids for diluting the vaccine. These were plain water and water with one of the following additives: skim milk (1%), sucrose (50%) and polyvinylpyrrolidone (PVP) (1%). The water used was either sterile distilled water or rainwater. Webster's diluent was also tested. This is a commercial diluent produced by the manufacturer of the commercial form of the vaccine.

We found that uncooked grains were not entirely satisfactory vehicles for the vaccine. When the vaccine was added to the grain and then immediately washed off and titrated, there was often a drop in titre of at least one log: i.e. only one-tenth of the virus could be covered. The drop in titre was greatest for white rice and least for paddy; this difference was possibly related to differences in the absorptive capacities of the grain surfaces. With cooked white rice, on the other hand, it was usually possible to recover the full dose of vaccine. Of even greater concern was the fact that the titre dropped further with storage; usually there was a fall of one log or more within 4 hours, and by 24 hours the virus was sometimes undetectable. The reason for this drop is uncertain. We suspected that it might be due to viricidal substances on the grains and we were able to demonstrate this for maize: when maize was added to a suspension of virus and then kept overnight, the titre of the suspension dropped several logs, but other grains did not have this effect. We attempted to remove possible viricidal factors on paddy by treating it in various ways before the virus was added: boiling for two minutes, washing for 10 minutes, or dry heating for 30 minutes; but none of these treatments had any effect. We did, however, find that survival on paddy was better when the diluent used was 50% sucrose. The temperature of storage, whether 4°C or 22°C, made little difference to survival on grain, and indeed the standard V4 strain did no worse (and no better) than the heat-resistant strain.

The poor survival on grain means that the idea of coating the vaccine onto grain before distribution is unlikely to be successful. (We should add that we did few experiments with wheat, which is used in Malaysia, because this grain is not widely available in Southeast Asia.) Obviously, the most efficient and safest way of transporting the vaccine is in its freeze-dried form, but this is not suitable for distribution to households at least as it is packaged at present: first, because of the large number of doses per vial, and second because of the difficulty of ensuring that it is correctly diluted. A good compromise would therefore be to reconstitute and dilute the vaccine at a central point, such as a provincial town, and distribute it in this form. We examined the survival of the vaccine in the five diluents previously described and found that at 4°C there was no drop in titre for at least four weeks. At 22°C, there was no drop after 24 hours and after one week there was a maximum drop of one log. At 32°C, the virus either survived unchanged or dropped by one log after 24 hours, but after a week there was a severe drop. The best diluents were water and water with PVP. Milk was the least effective because it supported the growth of bacteria. The sucrose suppressed bacteria but sometimes grew fungi. As might be expected, the standard strain of V4 did not survive as well as the heat-resistant one. It seems then that the vaccine could be diluted and held at any place where refrigeration was available; it could be distributed from there in cold boxes; then within a village it could be distributed to households, and kept overnight for administration in the morning: all this without significant loss of titre.

Survival of the Vaccine from Its Application onto Feed to Its Consumption by the Chicken

This period is a critical one and it is in this area that more work needs to be done. Theoretically, it should be possible to issue each householder with a measured amount of diluent and the grain to correspond, with instructions to mix them early in the morning and feed immediately to the chickens, before they have had a chance to eat. The mixing can be readily accomplished by stirring in a bowl, or by shaking in a sealed container or plastic bag. However, there are obviously several things that can go wrong with this system, and it would be better to prepare the feed, at least at the village level, and distribute it for feeding each day. This however, would be contingent on finding a means to ensure survival of vaccine on feed for 24 hours.

We found that, in order to ensure that the food was eaten quickly, it was necessary for the birds to be starved beforehand and for the food to be familiar to them. This would mean that the birds had to be fed early in the morning, and that the feed used in any area was one in common use there. It was for this reason that our early experiments used white rice. Cooked white rice, which

is fed to chickens throughout most of Southeast Asia, proved a good carrier in many ways, with good survival of the virus for two hours after application, and ready acceptance by the birds, but its use would be limited by its tendency to spoil.

Production of Immune Responses in Chickens

Measurement of immune response at the University of Queensland has been limited to measuring haem-agglutination-inhibition antibodies, except for some work on neutralising antibodies carried out by Gamini Jayawardane. We have not attempted to measure cellular immunity, and we are not able to challenge with velogenic viruses. It should be borne in mind that, while an HI titre of 3 or more is a fairly reliable indicator of immunity to ND, lack of antibody does not necessarily signify lack of protection. Most of our vaccination experiments have been carried out in young layer pullets, (3 to 6 weeks at first vaccination), while a small number of experiments have used bantams. The birds were housed in concrete floored rooms; in some experiments they were also let out into adjoining dirt-floored runs. The standard amount of feed-vaccine per bird was 10 g, and the titre of vaccine was calculated to be at least 10^6 per gram.

The most striking finding of our vaccination experiments has been the variability of the responses, even between apparently identically treated groups. This variability appears to be an effect of the oral route of dosing and not of the V4 virus itself. In two experiments in which feed vaccination was compared with vaccination by intramuscular injection, eye-drop, and drinking water, rapid responses were obtained to the intramuscular and eye-drop methods on both occasions, whereas in one there was very little response to feed vaccination, and in the other there was a very good response (Spradbrow and Samuel 1991). It was noteworthy that, although the injection and eye-drop methods produced high titres initially, these fell rapidly, so that by 40 days the titres were lower than in feed-vaccinated birds.

The only consistent result obtained with feed-vaccination has been an almost complete lack of response in birds given uncooked white rice. This is presumably linked to the poor recovery and rapid loss of titre of virus on white rice. Even when rice was force-fed to birds immediately after application of virus, there was little response. This is unfortunate, because white rice is so widely available in many countries. Brown (unpolished) rice, either whole or broken, performed slightly better, with around 50% of birds gaining HI titres of 3; the maximum titre reached was 4. Although brown rice is little used as human food in Asia, broken brown rice, a by-product of milling, is fed to chickens in some areas.

Of the other grains tried, paddy rice, cooked white rice and cracked maize each produced protective titres (i.e. 3 or more) in a majority of birds in at least one experiment and each failed to produce satisfactory responses on at least one occasion. Most of our work concentrated on cooked white rice and paddy, as maize is less widely used in Asia. We noted that, in several trials using cooked rice, some birds in a group obtained titres of 5 to 7, while others failed to rise above 2. We thought this might be because 10 g of cooked rice contained fewer grains than 10 g of uncooked rice and this, with the tendency of the grains to stick together, might mean that some birds received no vaccine. Accordingly, we designed an experiment to compare the effect of the same dose of virus given on either 10 g of cooked rice or 10 g of raw rice, cooked (about 25 g). We did this test in duplicate — two groups of birds given each treatment — and again the most striking finding was the variation between groups given identical treatments. However, taken together the birds given more rice mounted a better response.

We found that even birds that had never seen cooked rice before ate it very quickly. In contrast, we had considerable difficulty in getting birds to eat paddy rice. Initially we were using a long-grain rice and this seemed to be difficult for the birds to eat. Even when hungry, although they picked up the grains they often dropped them again. A shorter grained variety appeared to give them less trouble but it was still necessary to train them to eat it. The short-grain paddy was coated with vaccine using all five of the diluents mentioned earlier (water, skim milk, sucrose, PVP and Webster's diluent) and fed to 5 groups of chickens. Titres rose above 3 in at least 70% of birds from every group except the one given vaccine in skim milk; in this group the rise was very slow and only 55% of birds gained protective titres. The experiment was repeated for three of the diluents — water, milk and sucrose — and this time some groups of birds received the vaccine after it had been stored on the grain for 24 hours. The results were perplexing: even with the vaccine that was given immediately after mixing, there was very poor response in the milk and water groups, and in the sucrose group only 30% of birds responded. Yet, paradoxically, the group that had received sucrose-diluted vaccine that had been stored for 24 hours gave a response in 100% of birds, with some reaching titres of 9. When this experiment was repeated, sucrose vaccine stored for 24 hours gave only a 30% response, and stored water vaccine 20%. Thus, there is no consistency in the results obtained with paddy; in the majority of cases when it is fed immediately after mixing there is a protective antibody response in most of the birds but this cannot be relied upon; and when the paddy is stored for 24 hours before feeding, the results are even less predictable. Of course it is not possible to say whether the birds that failed to show a rise in titre were in fact protected. It is possible that they developed a local

immune reaction in the gut which prevented a systemic response.

Some experiments were also done using bran as a carrier for the vaccine. In two experiments using processed rice bran, responses were obtained in 100% of birds, whether the vaccine was fed immediately or after 24 hours. However, when unprocessed bran was used, whether rice, maize or wheat bran, there was no immune response. The processing of bran involves steam treatment, which possibly inactivates antiviral substances. Unfortunately, such processed bran may not be available in rural areas and simple methods for transforming unprocessed bran into a suitable carrier should be investigated.

We have tried to elucidate the reason for this variability in response by killing birds at set times after vaccination and trying to recover the virus. Some of this work which used direct administration of high doses to the crop has been reported (Samuel 1987). This work showed that, up to 4 days after vaccination, virus was present chiefly in the walls of the crop and proventriculus. We later repeated this experiment, using vaccination with standard doses of virus on various feeds (pellets, maize, cooked and uncooked white rice, and paddy), killing some birds at 1, 3, 5 and 24 hours after vaccination and keeping others in order to measure antibody responses. In this experiment, the most successful feed-vaccine was paddy (100% protective titres), followed by cooked rice (60%). There seemed to be a correspondence between the length of time that virus persisted in the crop and the success of vaccination, although the numbers were too small for this to be tested statistically. Disappearance of virus from the crop was in some cases due to the fact that the feed had all passed through; in other cases it seemed that the virus had failed to survive on the grain after ingestion. Perhaps the ideal carrier should not only be one that allows the virus to survive both outside and inside the bird but also be a feed that has a slow passage time. It was notable that only in the paddy-vaccinated chickens was virus isolated from the walls of the proventriculus or intestine.

Exposing All Birds to Vaccine

Our findings in birds that were killed after vaccination led us to question whether all the birds ate the vaccine. We therefore did a 'sham' vaccination of 40 birds with paddy and, as soon as all the rice had been eaten, we killed the birds and weighed the contents of their crops. The weights varied from 0.4 g to 16 g with a mean of 8.6 g. Thus there is considerable variation in intake even between birds of the same age and size, and this means that many birds will not get an adequate dose of vaccine. One bird's crop contained only dehusked grains; presumably it dehusked them before swallowing them and thus would have avoided eating the vaccine.

The fact that some birds do not get an adequate dose will not be important if the virus is then spread to them by the vaccinated birds. We have several times demonstrated that this occurs when the birds are held in concrete-floored rooms (Spradbrow et al. 1988; Spradbrow and Samuel 1991). Birds in such close contact with vaccinated birds tend to develop a similar GMT and range of titres to the vaccinated birds, whether these are low or high. Where birds are kept under more open-range conditions, simulating what happens in a village, transfer of infection to in-contact birds does occur but not so effectively; the GMT of the contact birds is lower, and falls more rapidly than in the vaccinated birds. We demonstrated that the V4 virus would persist in such a flock for years, causing antibody production in new clutches of chicks, usually at around 60 days of age (Samuel and Spradbrow 1989). However, when some of these contact-vaccinated birds from our flock were sent to UPM for challenge 2 years after the last introduction of vaccine to the flock, they all succumbed. It seemed that they had partial immunity, because they died later than control birds, but obviously it is not enough to introduce vaccine into a flock once and expect it to continue to provide protection indefinitely.

On the other hand, continual revaccination of an entire flock seems unnecessarily costly. The ideal, it seems to us, could be to vaccinate new chickens as they are introduced to a flock and to allow natural spread of virus from these vaccinated birds to boost the immunity of the rest of the flock. In most cases, new chickens will be young chicks hatched into the flock, and therefore we experimented in our open-range flock with creep-feeding vaccine to young chicks. We creep-vaccinated a total of 8 clutches, using vaccination at fortnightly intervals over 3 months. As we have reported (Samuel and Spradbrow 1991) the experiment was successful. All the clutches showed a rise in titre, generally after the second vaccination although the last clutch responded after only one vaccination, perhaps because there was a great deal of virus in the environment by then. The adults showed a rise in titre at the same time as the first batch of chicks. After the third vaccination, neither the chicks nor the adults showed any further response, and the chicks' titres fell fairly rapidly. Presumably this was because of solid local immunity preventing further invasion by virus. One would expect that, as this local immunity waned, the vaccinated birds would be able to respond to later introductions of virus to the flock. We used both wheat and crumbles as the vaccine carrier and found that the wheat was more effective, probably because the chicks preferred it. It was necessary to train the chicks to use the creep-feeders, and also to be alert for aggressive adults that kept the chicks away from the feeder. Scattering feed for adults in another area was helpful. It might be difficult to introduce the practice of creep-feeding to areas where it is not familiar, but it has other advantages for chick

survival as well as vaccination and could perhaps be made part of an integrated approach to improving chicken husbandry.

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Newcastle Disease Research at the University of New England

R.B. Cumming*

Abstract

When the V4 vaccine is applied to seeds by mixing with water, inconsistent results have been obtained. This is due to the presence of water soluble anti-microbial materials, that apparently are present on the outside of seeds. If seeds are killed by briefly boiling, dried, coarsely ground and then washed overnight, the virus will survive far longer — up to 24 hours. This results in consistent sero-conversion in vaccine coated seed stored for 18 hours at 25°C before feeding out.

RESEARCH workers in all participating countries have experienced extremely variable results when applying the heat selected V4 vaccine to feedstuffs with water. This is in stark contrast to the very repeatable results recorded when the vaccine was applied to either feed pellets or wheat grains by the Glatt machine.

Our efforts over the past 18 months have been concentrated on producing a practical method of maintaining a vaccinating dose of the V4 virus on seeds for at least 18 hours. To ensure that all birds receive a dose of vaccine, it is essential to feed the vaccine out at first light in the mornings. It is quite impractical to distribute the feed vaccine to householders early in the mornings in the tropics and ensure that the scavenging birds are all close at hand and all hungry. Further, any vaccine-treated grain that lies around on sunny days in the tropical sun would rapidly lose its infectivity, while in wet weather the virus can be washed off grain that is not consumed immediately.

Rationale

After many months of frustrating work, in which we obtained a serological conversion rate of about one batch of chickens in three, I approached Dr Graham Blair of our Department of Agronomy and Soil Science, to discuss the technology of seed dressings. Dr Blair advised me to consult Dr James Scott in his Department, an authority on the application of nitrogen-fixing rhizobia to seeds.

Dr Scott was of immense help to us and I wish to place on record our appreciation for his advice and stimulating discussions.

Procedures

Initially, we attempted to evaluate, on the V4 virus, the techniques used in rhizobia seed applications. The procedures used for rhizobia are to coat the seeds with sodium caseinate with the addition of sterilized peat moss, activated carbon and/or polyvinyl pyrrolidone.

It was not possible to evaluate these procedures quickly in eggs, but we commenced work in groups of chickens. As this work proceeded slowly, we came to the realization that the various grains we were attempting to coat with the V4 virus probably contained water soluble anti-microbials. These water soluble anti- microbials were necessary to protect the grains when planted in moist soil, to enable the grains to germinate successfully.

Following this, we evaluated various practical procedures to kill the grains (so that more anti-microbials were not produced) and to leach away the anti-microbials. This work was evaluated in both eggs and in chickens. Finally, we evaluated cracking the grains coarsely before applying the vaccine virus.

Results

We now have what we feel is a sound procedure that will enable large scale application of the V4 vaccine on feedstuffs to produce serological conversions in chickens

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with a high degree of certainty. This has been applied successfully to paddy rice, wheat and maize. Sorghum still gives us problems.

The technique is as follows:

1. Boil for 2 minutes — i.e. bring water to boil, put in grain at 1 kg per 3 litres of water — bring again to boil then leave for 2 minutes.
2. Cool down — let water go off boil and when cool enough to move (in about 5 minutes) — cool down under running tap water.
3. Dry grain — i.e. get as much water off as possible by straining and put grain on plastic on bench in as much sunlight as possible and allow to dry. This takes up to 48 hours.
4. Crack grain using a household blender. Blend for 3 seconds — about 100 g at a time.
5. Soak grains — place grains either cracked or whole into container at 100 g/400 g of water. Soak all day — pour off twice with running water and repeat overnight.
6. Dry — as in step 3 above.

Using this technique we now obtain a high correlation between recovery of virus in eggs and serological conversion in chickens in grains stored at 25°C. If we can recover a vaccinating dose of virus from the 10 g of grain (paddy rice, wheat or maize) 18 hours after application of the vaccine virus, the chickens will sero-convert when fed the grain. If we cannot recover a vaccinating dose of virus only about one batch of chickens in three will sero-convert.

Discussion

The consistent survival of the V4 vaccine on pellets and grains after application by means of the Glatt machine tended to confuse our thinking on this problem. Careful reading of the Glatt machine technique reveals that very little water is used and the feedstuff is maintained in an environment of 40°C during treatment. Thus, the water is rapidly driven off, preventing anti-microbials solubilising and attacking the virus. This probably explains why Glatt machine applied virus vaccinates so well.

The procedure we have developed is a technique that can be applied anywhere, as it does not involve elaborate equipment or techniques. However, it does involve additional labour.

With a shelf life of at least 18 hours, this vaccine-treated grain can be made up and distributed to poultry owners the afternoon before vaccination. The poultry owners can then feed their birds at first light the next morning, when the birds are hungry and rapidly consume the vaccine-treated grain.

While killing and washing the grain significantly extended the shelf life of the virus on the grain, consistent recovery of virus in eggs and sero-conversion was not obtained if vaccine-treated grain was stored for more than 8 hours. Coarse cracking gave a further significant extension. This may well be due to the virus being able to be attached or absorbed to the grain contents, rather than the outside of the grain where the anti-microbials are concentrated.

Sorghum has been a problem in that we can recover virus from treated grain up to 18 hours after mixing, but only succeeded with sero-conversion when the grain was fed within two hours of mixing. This anomaly is presently being investigated.

We may now have an explanation for the rather inconsistent results obtained with the administration of the vaccine virus to commercial chicken starter rations in Sri Lanka, Thailand and even the Philippines. Certain feed ingredients, particularly milling by-products like rice bran or wheaten bran may well be high in water soluble anti-microbials. Rations containing little of such materials could be a more satisfactory vehicle for the vaccine, and high energy broiler diets, which usually do not contain low energy milling by-products, may be more suitable for vaccination.

The water soluble anti microbial materials on the grains have not yet been identified. They may be phenolic compounds of low molecular weight, such as p-hydroxybenzoic, vanillic, p-coumaric and ferulic acids, which are known to have anti-microbial properties. Further research in this area may yield useful results.

An alternative approach to the problem could be to use a non aqueous material (e.g. light oil) to distribute the virus on the grains. Other techniques, such as the use of powders, may be useful. There are areas for some innovative future research.

Laboratory Trials of Heat-Adapted V4 Vaccine Strains of Newcastle Disease Virus in a Simple Feed-Delivery System for Vaccination of Village Chickens

Darminto* and P.W. Daniels†

Abstract

Laboratory trials were conducted to test the efficacy of two heat-adapted strains of vaccine derived from separate stocks of the V4 strain of Newcastle disease virus (NDV). The delivery system trialled was one developed in Indonesia to take account of logistical problems, in which it was proposed that vaccine concentrate be taken to villages for dilution with local water and mixed with local foodstuffs directly before feeding to chickens. Trials were conducted in both commercial chickens and village chickens of differing ages and levels of maternal antibody.

Both strains of V4 NDV gave good antibody responses in commercial chickens and protected them against challenge with an Indonesia strain of viscerotropic velogenic NDV.

Village chickens with either high or low levels of maternal antibodies vaccinated at one and 4 weeks of age with 10^7 EID_{50} failed to respond and all succumbed to challenge. Another group of village chickens vaccinated at 4 and 7 weeks of age with 10^7 EID_{50} also failed to show any antibody responses. Revaccination at 10 weeks elicited responses in 4 of 20 birds, and only these responders subsequently survived challenge.

The food delivery system was compared with eye and mouth drop administration in village chickens vaccinated with calculated doses of 10^7 EID_{50} at 7 and 10 weeks of age. Antibody titres were significantly lower in the food-delivered vaccination group, and only 21% (3 of 14) birds survived challenge, while 100% of eyedrop vaccinated and 82% (14 of 17) mouth drop vaccinated chickens survived the same challenge.

These results indicate a problem in vaccinating village chickens with feed-delivered vaccine in the particular delivery system under trial, and that in this respect village chickens gave a different result from commercial chickens although eye drop and mouth drop delivery systems in village chickens gave satisfactory results.

Since direct application by eye or mouth drop of V4 strain of NDV protected village chickens against challenge with a highly pathogenic strain of NDV, even after only two vaccinations, V4 must be considered a useful and adequate vaccine strain for routine use. Where direct individual dosing is feasible, mouth drop application may be as effective as eyedrop, but further research is needed to confirm this aspect.

Further studies are also needed to determine the duration of immunity and hence the recommended interval between vaccinations for each delivery system. The efficacy of V4 depends on replication of virus in the bird, which requires an adequate dose of live virus. The heat-stable strains should allow delivery of a sufficient titre of virus to ensure responses under tropical field conditions. More experience is needed to confirm the duration of heat stability of any products developed for routine use.

Before a feed-delivery system can be recommended, further research must be conducted to study the multiple interactions of vaccine strain of virus, carrier feeds and village chickens. Such studies must overcome any inactivation of virus on feed or non-release of vaccine from feed in the GI tract, as well as identifying sites of replication of virus in the GI tract, and studying possible natural resistance of village chickens to infection and replication of NDV as well as studying the mucosal and cell mediated immune systems of village chickens.

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Newcastle disease (ND) is identified as one of the animal health problems of major economic impact in Indonesia (Anon. 1991). Since the disease is preventable by vaccination, its continued severity in many countries must be seen as a failure of modern technology and animal health services to deliver achievable benefits to farmers. Commercially reared chickens are protected by vaccination programs with variable success, depending on management considerations. Indonesia also has 187 million village chickens spread among more than 13,000 islands with a land area of 1.8 m sq. km. Protecting this population poses enormous logistical problems, that could be partially addressed by effective heat stable vaccines.

Efficacy trials in Malaysia of ND vaccine based on a heat inactivated variant of strain V4 (V4-UPM) of Newcastle disease virus (NDV) showed 80 to 100% survival of birds challenged (Ideris et al. 1987) where vaccine was coated on feed for administration to birds as a food pellet vaccine. Such a strategy offers a means of vaccinating village chickens raised under free range or semi feral systems.

Since vaccination in the above experiment was with a variant of V4 developed in Malaysia, and challenge was with an isolate of viscerotropic velogenic NDV (VVNDV) isolated and characterised in that country (Lai and Ibrahim 1987), it was considered necessary to test whether other heat adapted NDVs available in Indonesia could protect against challenge with VVNDV from that country.

In the design of the trials, note was taken of the further logistical problems in delivery of a food-pellet vaccine to large numbers of village chickens in widely dispersed rural areas. A strategy of coating vaccine on feed, described by Ronohardjo et al. (1988), was adopted, of diluting concentrated vaccine with water or locally available diluent and mixing with a locally available feed at the point of use.

General Materials and Methods

Village chickens

Poultry of village stock were available from a government breeding unit. Day-old chickens were purchased and reared in isolation until the time of first vaccination. Chickens were free of antibodies to infectious bursal disease as assessed at various times during the experimental period by a gel diffusion test.

Viruses

Two heat-adapted variants of V4 NDV were tested as candidate vaccines. In each case the process of heat adaptation involved cycles of heating at 56°C for several hours. The viruses were designated as RIVS.V4 (Ronohardjo et al. 1988) and HRV4, Batch No. 80236 of HRV4 from Arthur Webster Pty Ltd, Sydney, supplied under the ACIAR project.

The challenge virus used was a plaque purified strain of VVNDV isolated in Indonesia and characterised by Parede and Young (1988, 1990).

Vaccination

Feed-delivered vaccine was prepared and administered according to a protocol described by Ronohardjo et al (1988). Vaccine concentrates containing 100 doses of 10^7 EID_{50} were diluted in 100 mL of distilled water and mixed with 1 kg of unhusked rice by manual stirring in a plastic bowl immediately before vaccination. The coated grain was fed to birds which had access to other food withdrawn overnight prior to vaccination the next morning.

Where oral vaccination without feed-delivery was used, 0.1 mL of diluted virus stock containing 10^7 EID_{50} was squirted into the open mouth using a tuberculin syringe.

Challenge

A contact challenge method was used, in which unvaccinated control birds with undetectable antibodies to NDV were inoculated intraocularly with 10^7 EID_{50} of ITA strain of VVNDV. After 24 hours, inoculated and experimental birds were mixed in an isolation facility and held together for 3 weeks. Birds were examined twice daily for signs of illness or mortality. A representative proportion of dead birds was necropsied and reisolation of challenge virus attempted from brain tissue to confirm the cause of death.

Serology

Antibody responses to vaccination and to challenge were determined from blood samples collected weekly during the course of the experiments. A haemagglutination inhibition (HI) test using the beta procedure, as described by Hanson (1980), was used. The test was based on 4 HA units of antigen prepared from strain V4 of NDV.

Reisolation of Vaccine and Challenge Viruses

At regular intervals, as described in individual experiments, oropharyngeal and cloacal swabs were taken from selected birds for the purpose of detecting excretion of vaccine or challenge viruses. Swabs were stored in 1 mL transport media containing antibiotics until testing by inoculation of media into embryonated eggs.

Experimental Design and Results

Efficacy in commercial chickens

The efficacy of both variants of heat adapted V4 NDV and the patterns of excretion of vaccine and challenge virus were tested in commercial broiler chickens (Darminto, unpublished data). Chickens vaccinated at 5 and 8 weeks of age with a calculated dose of 10^8 EID_{50} of RIVS.V4 responded serologically and were substantially protected against challenge with ITA strain of VVNDV given 3 weeks after the second dose of vaccine.

A group in which vaccine was mixed with distilled water before coating of unhusked rice had a group mean titre (GMT) of 3.4 ± 0.6 (log_2) prior to challenge, and all 19 birds tested survived. A second group, for which 5% skim milk powder was incorporated in the diluent, had a GMT of 3.6 ± 0.6 before challenge, and 17 of 19 birds in this group survived. A control group of 17 birds vaccinated with the same vaccine by eyedrop at a dose of 10^8 EID_{50} had a GMT of 4.7 ± 0.9 prior to challenge: 16 birds survived. In an unvaccinated control group of 19 birds, with a GMT of 0.1 ± 0.3 prior to challenge, only 1 bird survived.

Most vaccinated birds excreted vaccine virus after first vaccination, but not after the second vaccination. Virus was more frequently detected from mouth rather than cloacal swabs. Nearly all challenged birds excreted challenge virus from both the oropharynx and the cloaca. Vaccinated birds excreted virus for up to 18 days after challenge.

In a second experiment, the efficacy of HRV4 diluted in distilled water and given at calculated doses of either 10^7 EID_{50} or 10^8 EID_{50} mixed with unhusked rice was tested. Controls included unvaccinated birds and birds vaccinated with 10^7 EID_{50} of HRV4 by eyedrop. These latter had a GMT of 7.2 ± 0.6 prior to challenge, and all 15 birds in the group survived. Birds vaccinated with the higher dose had a GMT of 6.1 ± 1.2 and 15 of 17 survived, while birds vaccinated with 10^7 EID_{50} had a GMT of 5.3 ± 1.1, and 13 of 17 birds survived.

For the birds vaccinated by eyedrop, all excreted virus from the oropharynx a few days after first vaccination, and 70% via the cloaca 6 to 8 days post vaccination. Detection of virus was poor in birds vaccinated with the lower dose on feed, but was comparable to the previous experiment with those dosed at 10^8 EID_{50}. In this experiment, reexcretion of vaccine virus was detected after the second vaccination at 10^7 EID_{50} in several birds. This was not observed at the higher dose rate in either experiment. Isolations of challenge virus were made from all challenged birds from both oropharyngeal and cloacal swabs. Excretion from the cloacal route lasted up to 21 days post challenge.

The two experiments successfully demonstrated that the heat adapted variants of V4 under trial could successfully protect against challenge with a local strain of VVNDV, even where administered in a feed-delivery system on unhusked rice. On the basis of these results, field trials were initiated as per the project protocol, and further laboratory trials commenced to define more precisely recommendations for use in the field.

From the attempted isolations of vaccine and challenge viruses, it was noted that excretion of vaccine was more easily detected after dosing with 10^8 EID_{50} rather than after 10^7 EID_{50}, but that detection of virus after a second vaccination was possible only in birds dosed at the lower dose rate. The size of the dose of primary vaccination may be deduced to play an important role in any lateral spread of vaccine virus.

It is well established that vaccinated chickens reexcrete challenge virus (Lancaster 1966) and this proved to be the case after feed-delivered vaccination with heat-adapted variants of V4. This has epidemiological significance for village vaccination programs, for birds protected by vaccination may still be able to spread the infection to other, inadequately vaccinated birds. It is not yet known whether the titre of excreted virus is less from orally-vaccinated fowls, but the duration of excretion will be longer in survivors.

Feed-delivered vaccination of week-old village chickens

Maternal antibodies of village chickens 6 days old were determined by HI tests and chickens divided into a control group and 3 experimental groups depending on whether HI titres (log_2) were 0 or 1, 2, or 3 and above. Chickens were vaccinated at 1 week and 4 weeks of age with 10^7 EID_{50} of HRV4 delivered on unhusked rice, coated with virus in aqueous solution. HI titres were measured weekly. Challenge was by the contact method at 7 weeks of age, 3 weeks after the second vaccination.

The GMTs (log_2) of the experimental groups, presented in Table 1, show that there was little antibody response to vaccination. All challenged chickens died. It was observed that chicks did not eat all the vaccine-coated feed on offer in the first vaccination. The residues from the three experimental groups were 98.3 g, 87.6 g and 84.8 g. Most vaccine-coated feed was eaten at the second vaccination. A problem with the feed-delivery system was not identified, since confounding factors were the suboptimal dose of vaccine eaten and the young age of the chickens.

Feed-delivered vaccination of 4-week-old village chickens

A pen trial was designed to test the effect of vaccine dose of feed-delivered HRV4 on protection and lateral spread

Table 1. Vaccination of village chickens with various levels of maternal antibodies, at 1 and 4 weeks of age, with 10^7 EID_{50} of HRV4 feed-delivered Newcastle disease vaccine

Level of maternal antibody	Number tested	Weekly GMT* after vaccination						Mortality
		1	2	3	4	5	6	
1*	15	0.3	0.3	0.1	0.1	0.1	0.5	100%
2	15	1.2	0.7	0.5	0.5	0.4	0.5	100%
3 or 4	15	2.3	1.5	0.8	0.7	0.7	1.0	100%
Unvaccinated controls	15	1.9	1.4	0.9	0.9	0.8	0.6	100%

* HI titres (log_2)

to in-contacts and aerosol contacts (W. Van der Linden, unpublished data). Groups of individually identified village chickens were raised from day old and were free of detectable maternal antibodies by HI tests at 4 weeks. Four groups were placed in adjacent pens, with half the birds in the first three groups being vaccinated by the feed-delivery system at calculated doses of 10^3, 10^5 and 10^7EID_{50}, respectively. The remaining birds in these three groups were reared as in-contacts, while birds in the adjacent fourth pen were considered to be exposed by aerosol contact. A fifth group of unvaccinated controls was kept in isolation.

Not all food was consumed on first vaccination, for chickens were observed to open the husk and eat only the grain in many instances. This problem was addressed by more stringent withdrawal of food prior to subsequent vaccinations. Birds were given vaccine 3 times at 3-week intervals and challenged by the contact method 3 weeks after the third vaccination.

HI antibody responses were not detected after the first or second vaccinations. After the third, 4 of 20 birds in the group dosed with 110^7EID_{50} gave responses, whereas not any of the in-contact birds or birds receiving lower doses did. The only birds to survive challenge were the four that seroconverted.

Feed-delivered and oral vaccination of 7-week-old village chickens

Since the feed-delivery system gave good results in commercial chickens, and since the two previous experiments had not included all possible controls, it could not be concluded that a problem existed with the feed-delivery system under trial. However, this was obviously a possibility. There was also concern that vaccinating immunologically immature birds by the oral route may have been contributing to failures. Hence, an experiment was designed in which village chickens from the same source were held in isolation and, at 7 weeks of age, vaccinated with 10^7EID_{50} of HRV4 delivered by eye drop, by mouth drop and by the feed-delivery system on unhusked rice. A fourth group was reared as unvaccinated controls.

The overall results of the trial are presented in Table 2. Eyedrop and mouth drop delivery gave GMTs prior to challenge of 4.4 and 3.4, respectively, and 100% and

Table 2. Results of challenge with ita strain of velogenic ndv of village chickens vaccinated with HRV4 (82360) vaccine with a dose of 10^7 EID_{50} at 7 and 10 weeks of age

Groups*	No. tested	GMT HI antibody BC[1]	Survivors	GMT HI antibodies PC[2]
I	17	3.4 ± 1.4	14/17 (82%)	7.8 ± 0.6
II	17	4.4 ± 1.3	17/17 (100%)	7.1 ± 0.9
III	14	1.4 ± 1.3	3/14 (21%)	8.0 ± 0.0
IV	18	0.6 ± 0.5	1/18 (5.6%)	8
V	20	0	0/20 (0%)	–

* Groups : I. Mouth drop vaccination II. Eye drop vaccination
III. Food delivered vaccination IV. Unvaccinated control
V. Extra-unvaccinated control (broiler)

[1] Geometric mean of HI antibody titres (log_2) before challenge
[2] Geometric mean of HI antibody titres (log_2) after challenge

82% survival of challenge. The food delivery system was poor by comparison, with a GMT of 1.4 and survival of 21%. Hence, a problem with the feed-delivery system seems to be confirmed in these immunologically mature chickens.

Challenge virus was isolated from all birds sampled by both oropharyngeal and cloacal swabs at one and two weeks post challenge. Several birds were still excreting challenge virus via the cloaca at 3 weeks after challenge.

Discussion

The overall results were examined to partially identify the problems and to make recommendations for successful vaccination. In the limited number of experiments that has been possible, the feed-delivery system gave acceptable results in commercial chickens when used at $10^7 EID_{50}$ (76.5% protection) but not village chickens of an equivalent age (21% protection). Vaccination of younger village chickens gave no protection with an equivalent number of doses.

The apparent difference between commercial and village chickens is noted, but it is not yet felt necessary to confirm this with further trials. The task is to develop a system of vaccination that works for village chickens.

Where administered by eyedrop, the HRV4 gave excellent protection against challenge with VVNDV in every case. Hence the V4 strain, heat adapted, can be used with confidence to protect chickens against ND. The question of the delivery system and other aspects of vaccination protocol remain to be resolved.

A starting point is to reconsider the need for a feed-delivery system in every situation. The feed-delivery system is promoted for farmers who have difficulty catching their birds. Two considerations are relevant. People may not be able to expect their governments to give them something for nothing, without work or effort on their part. An appropriate effort in this context may be to catch the chickens for vaccination. A second major consideration is that protection of poultry against ND should frequently lead to improved husbandry, since such investment of time or money will be protected against the current major source of loss, ND. Improved husbandry will make birds easier to catch for individual application of vaccine and, in turn, will create a demand for the higher levels of protection that individual application of vaccine can supply at the present time.

In a country the size of Indonesia, where 1000 million doses per year may be needed, the farmers may have to organise to give the vaccine themselves. Hence, it is important that the system of vaccination be one that does not need equipment more properly used by trained staff, especially the syringes for intramuscular inoculation. Delivery of heat stable V4 by eyedrop (or, alternatively, intranasal drop) should replace current programs using the intramuscular inoculation with live mesogenic strains such as Komarov. This would have the added advantage that potential problems of disease caused by inappropriate use of the mesogenic vaccine could be avoided, which is also seen as an important problem (Moerad 1987).

Consideration is currently being given to whether mouth drop application will also give a high level of efficacy. The mouth may be an easier target for farmers that the eye, which is smaller and blinks, and requires closer restraint of the bird to apply the vaccine. The preliminary results reported here, of 82% protection, are encouraging. The oropharynx has for a long time been recognised as an effective route for administration of V4 vaccine (French et al. 1969).

If a need for a feed-delivered vaccine is still identified in some areas, then consideration must be given to why the current results are different from those of Ideris et al. (1987). These differences appear to be the use of different variants of the V4 strain; different feeds; the use of a stabiliser, PVP, in the successful protocol and the use of a wet coating system in the current work in contrast with a much drier method of application of vaccine previously. Since the vaccines used in this study gave good protection by conventional application, the problem probably derives from the latter three points. R. Cumming (pers. comm.) has advised that grains contain water soluble antiviral compounds on their surface, and that wet coating systems will lead to killing of vaccine virus. Any further development of the feed-delivered vaccine approach needs urgent confirmation and clarification of this knowledge to allow development of a cheap effective and simple system that can be adopted by farmers in remote villages.

In Indonesia a full investigation of the limits of the heat stability of available vaccines has not yet been made. Neither has the most appropriate interval between vaccinations been determined. Further pen and village trials are needed to clarify these points, as well as extension research to identify successful strategies for teaching farmers to administer vaccine themselves and to identify the most cost-effective efficient supply lines for farmers to obtain the vaccine. Protection of chickens against ND is an achievable goal, but continuing, well- planned efforts are needed before systems are in place that can reliably deliver the benefit to rural families.

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Field Trials of Heat-Adapted V4 Newcastle Disease Vaccines for Village Chickens Using a Village-Based System of Vaccine Coating of Feed. I. Virological Studies

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Abstract

Indonesia comprises over 13,000 islands with a land area of 1.8 m sq. km spanning a length of 5,600 km and a breadth of 1,600 km. In most places, village chickens are raised semi-ferally or free-range. There is an estimated population of 187 million birds. The country lies on the equator and so has a tropical climate, with considerable variation in rainfall patterns depending on topographical features and monsoonal influences, which also affect the local environments in which village chickens are raised.

The potential of heat-adapted, feed-delivered V4 strains of Newcastle disease vaccine to protect village chickens in these environments was investigated in field trials at three locations. The first site was at Bogor in West Java, with an annual rainfall of 3500 mm falling in a prolonged wet season with a short drier period. The second site was near Pekanbaru in central Sumatra, with an annual rainfall of 3000 mm with a similar seasonal distribution to Bogor but with a different social and agricultural environment, while the third was at Kupang on the island of Timor in eastern Indonesia, with an annual rainfall of 1500 mm with a prolonged dry season.

The experimental design was to vaccinate all the chickens in whole villages with either of two strains of virus, and to compare results with the situation in unvaccinated control villages. Birds classified as chickens, growers and adults belonging to 20 respondents in each treatment group were individually identified and tested serologically each month immediately prior to monthly vaccination. It was aimed to test up to 100 birds of each age group in each treatment group. Vaccine was administered in an aqueous solution coated onto unhusked rice grains at a calculated dose of 107 EID50 per 10 grams of grain per bird. The experimental protocol for each site was the same, so that each treatment could be considered to have been replicated in the three distinct geographical areas.

Vaccinations were organised by government agencies interacting with local government officials and farmers. Considerable time and manpower was required to achieve a reasonable vaccination coverage of birds in the village areas, even though vaccine and paddy rice were provided free. Much work needs to be done to devise effective extension strategies once a proven technology is available. Serological monitoring of chickens from each site did not show any marked differences in antibody titres between vaccinated and control villages. Results of challenge tests were variable, but in each case the survival of vaccinated groups was not markedly better than control groups. Many unvaccinated birds, and birds without detectable antibody levels, survived effective challenge, perhaps indicating a level of natural resistance to Newcastle disease in village chickens.

VILLAGE chickens are an important source of animal protein in Indonesia, where their population has been estimated at 187 million (Anon. 1990). Although some village chickens are reared semi-intensively, the majority are kept extensively, even semi-ferally, in rural areas. They are maintained at very low cost, usually scavenging for food. Newcastle disease (ND) is a major constraint to further development of this resource.

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ND is endemic in village chickens (Ronohardjo et al. 1985; Moerad 1987), but the disease is preventable by vaccination. However, vaccination techniques that are effective in protecting commercial chickens against ND are not practical in the rural environment where there may be no electricity or refrigeration, and the chicken populations are of low density and unconfined. None-the-less, vaccination against ND for village chickens is very important to avoid devastating outbreaks of ND and to reduce the level of challenge to the commercial poultry industry by controlling ND in rural areas, which is believed to be a source of infection (Spradbrow 1987).

Consequently, ND vaccines which are suitable for application to village chickens need to be developed. Such vaccines should be easy to handle and simple to apply. Since village chickens are reared semi-ferally and sometimes difficult to catch, it would be efficient if such vaccines were able to be given orally without catching individual birds.

The V4 strain of avirulent ND virus (NDV) which was isolated by Simmons (1967) has been studied in detail. The strain was found to be avirulent (Anon. 1966; French et al. 1967; Westbury et al. 1984; Hamid et al. 1990) and immunogenic (French et al. 1969; Turner et al. 1977; Ibrahim et al. 1980; 1981) and therefore suitable as a vaccine for mass application. Since V4 has been considered to have a route of infection via the digestive tract (French et al. 1967; Samuel 1987), to have lateral transmissibility from vaccinated to unvaccinated birds (French et al. 1967; Hall et al. 1967; Bancroft and Spradbrow 1978; Spradbrow and Samuel 1989) and to have haemagglutinin and infectivity resistant to heat thereby allowing selection of heat-resistant variants (Kim and Spradbrow 1978; Spradbrow and Samuel 1987), the strain has potential to be developed as a heat stable, live, oral vaccine. At least three heat resistant variants of V4 have been selected; HRV4 selected in Australia by Arthur Webster Pty Ltd (Claxton and Leonard 1987), V4-UPM selected by the Universiti Pertanian Malaysia (Aini et al. 1987) and (RIVS)V4 selected at the Research Institute for Veterinary Science (RIVS), Bogor, Indonesia (Ronohardjo et al. 1988).

Indonesia has over 13 000 islands with a land area of 1.8 million km^2 spanning 17° latitude and 46° of longitude. The country lies on the equator and so has a tropical climate, with a considerable variation in rainfall patterns depending on topographical features and monsoonal influences, which also effect the local environments in which village chickens are raised. A food-delivered vaccination scheme was specifically developed for the country to take account of the special logistical problems. This scheme involved taking a concentrated vaccine to the villages, diluting it with local well water and mixing with local feed (unhusked rice grains) before directly feeding to chickens (Ronohardjo et al. 1988).

This paper describes field trials to investigate the potential of heat-adapted, food-delivered V4 strains of ND vaccine to protect village chickens in such environments, using the delivery system described above. The trials were conducted in three locations selected to represent different environmental conditions. Several criteria were used to evaluate the potential of the heat adapted variants of V4 as food-delivered vaccines. Antibody responses and challenge tests are described in this paper, while population studies and mortalities in the face of field challenge, are described in a companion paper in this volume (Allen et al. 1991).

Materials and Methods

Field sites

The first site was at Desa Cinangka, Bogor, in the province of West Java, with an annual rainfall of 3500 mm falling in a prolonged wet season with a short dry period. The second site was at Sei Tapung, near Pekanbaru in the province of Riau in central Sumatra, with an annual rainfall of 3000 mm with a similar seasonal distribution to Bogor but with a different social and agricultural environment, while the third was at Kupang in the province of NTT on the island of Timor in eastern Indonesia, with an annual rainfall of 1500 mm with a prolonged dry season. Each site had two vaccinated villages and an unvaccinated control village, except the Riau site where a third vaccinated village was included. At the Bogor site the control sites were replicated within the field site.

Viruses

Two variants of V4 strain from different sources were used as vaccines in the trials, HRV4 (Arthur Webster Pty Ltd) Batch No. 82360 provided by Australian Centre for International Agricultural Research (ACIAR) and RIVS2.V4 (Ronohardjo et al. 1988). In addition to these, a heat stable variant derived from another vaccine strain and called RIVS3 (Ronohardjo, unpublished data) was used in the trial in Riau. The calculated dose for vaccination was 10^7 EID_{50} (50%-embryo infective dose) per bird.

The Ita strain of viscerotropic velogenic Newcastle disease virus (VVNDV) which had been purified three times by plaque purification (Ita pl.3) (Parede 1987; Parede and Young 1990) was used as a challenge virus.

Feed for vaccine delivery

Paddy (unhusked rice) was used as a vaccine vehicle at all trial sites. It was planned that 10 grams of paddy

should contain 1 dose of vaccine. One vial of vaccine containing 10^9 EID_{50} was reconstituted and diluted into 100 mL of local well water. The diluted vaccine was then mixed with 1 kg of paddy by stirring in a plastic bowl before being used to feed 100 birds.

Vaccination

Vaccinations were carried out monthly over a year and were organised by government agencies interacting with local government officials and farmers. In Bogor and Kupang, HRV4 and RIVS2.V4 were used for vaccination in two separate villages. In Riau, the three vaccines were used in different sections of one large village. At all sites, an unvaccinated village was monitored as a control.

In vaccinated villages, the protocol required that all chickens be vaccinated. The system of vaccination at each site was similar. The vaccinators usually stayed at a central point mixing the vaccine with paddy. Farmers visited the central point to collect the vaccine-coated paddy to feed their chickens.

An exception to this procedure was at Riau where vaccination was conducted by door-to-door visit. The vaccinators came to the house of the group leader, mixed the vaccines with paddy and distributed the vaccine-coated paddy directly to the chickens at every house.

Virological and serological monitoring

Samples for virological analysis, sera and cloacal swabs, were collected each month, immediately prior to vaccination. Although vaccination of all birds in the villages was attempted, the serum samples and swabs were collected from birds belonging to 20 respondents in each treatment group. Chickens belonging to the respondents were loosely classified by age as chicks, growers and adults, and were individually identified by wing tag.

Blood samples were collected in syringes, then transferred to sterile glass tubes for clotting and centrifugation. Sera were removed from the clots aseptically, transported to RIVS in ice, and stored in serum storage bottles at –20°C until haemagglutination inhibition (HI) antibody tests by the method of Allan and Gough (1974) were performed. It was aimed to test up to 100 birds of each age group in each treatment group.

Swabs were collected from both vaccinated and unvaccinated villages. It was aimed to collect swabs from young birds less than 2 months of age. Swabs were taken from the cloaca of birds and put in 1.8 mL ampules. The ampules were transported to the local laboratory on ice and stored at 4°C before being taken back to RIVS, Bogor. At RIVS, the swabs were placed in 1 mL PBS pH 7.4 containing 5000 IU penicillin and 5000 µg streptomycin and allowed to stand at room temperature for 1 hour before being centrifuged at 2000 rpm. The supernatants from the immersed swabs were then used as inocula for virus isolation by the method of Alexander (1988).

Challenge tests

Challenge tests for each site were conducted three times, except for the field site at Kupang, where a challenge test was conducted once, at the end of the trial.

Challenge tests for the trials at the sites of Bogor and Kupang were conducted at RIVS, Bogor. In each test, 20 birds (growers) were selected from each treatment group. Another 20 NDV-antibody-free village chickens raised in isolation at RIVS were also included as laboratory controls.

At the Riau site, the challenge tests were conducted in an isolated area in a forest about 10 km from the villages. As laboratory control birds for this site were not available, another 20 village chickens (growers) were obtained from a separate area about 25 km away from the field site where there was no history of vaccination, and included as external controls.

Challenge tests were conducted by the contact method. Two days before challenge, 10 NDV-antibody-negative birds were inoculated via the eye drop route with 0.1 mL per bird of undiluted allantoic fluid infected with the challenge virus (the stock of challenge virus contained 10^8 EID_{50} per 0.1 mL allantoic fluid). The infected birds were then placed in the room for the challenge test. Two days later, when the infected birds started showing clinical signs of ND, the birds to be challenged were placed together in the same room with the inoculated birds. Before and after challenge the surviving birds were bled for antibody measurement.

Observations in the challenge tests were carried out daily over three weeks. Any dead birds were recorded and 5 dead birds per treatment group were selected for virus isolation from brains as a confirmation of diagnosis.

Results

Vaccination and sampling coverage

It was difficult to achieve regular monthly vaccination coverage, especially at the Kupang site, where the farmers needed to collect the vaccine from central points on a single day each month. Nonetheless, a reasonable vaccination coverage of the village was achieved when local animal health staff visited and informed the village people on the day prior to vaccination. Fewer difficulties

were encountered at Bogor, as the villages were smaller and the population density was higher than at Kupang. There was no problem with vaccination coverage in the village site at Riau since the vaccines were distributed directly door to door by the vaccinators, two of whom lived in the village.

Difficulties were also encountered in virological sampling in all trial sites. Most farmers were not happy with bleeding of very young chickens. Hence, most blood samples were taken from grower and adult birds. The numbers of serum and swab samples were lower than the anticipated target on most occasions.

Serological tests

Antibodies to NDV were detected using the HI test. For the purpose of presenting data in this report, individual serum samples which had HI antibody titres of 3 (log2) or more were considered to be reactors (Allan et al. 1978). At all field sites, reactors were detected before the vaccination programs were started. The percentages of reactors among all the birds sampled each month are presented in Figures 1, 2 and 3 for field sites at Bogor, Riau and Kupang, respectively.

There were no major differences in the proportion of reactors between treatment groups for the Bogor site, except in June 1991 when reactors in the vaccinated areas were significantly more numerous than in the other villages (Fig. 1).

At the Riau site, before vaccination and after two vaccinations, the proportion of reactors in the village vaccinated using HRV4 was lower than the other villages. Subsequently, the proportion of the reactors in each treatment group varied between 30% and 60% (Fig. 2).

At the Kupang site, the proportion of reactors each month was not significantly different between treatment groups, except towards the end of the trial when an epidemic of ND with high mortalities confused the picture. All villages showed the highest proportion of reactors in October to December 1990 (Fig. 3).

The results of HI tests which were conducted in association with challenge tests are presented in Table 1. On all occasions it was observed that the geometric mean HI titres of the serum samples taken from surviving birds after challenge were significantly greater than those taken before challenge.

Virus isolation

Viruses were not isolated from cloacal swab samples from birds prior to vaccination each month at the Bogor and Riau sites. The swab samples from the Kupang site have not yet been processed, but at the end of August 1990 two isolates of NDV were obtained from sick birds suspected of being infected by NDV in the control village.

The results of the viral isolations conducted in association with challenge tests are presented in Table 2. On all occasions, the challenge virus could be isolated from brains of dead birds.

Table 1. Results of HI tests of serum samples before and after challenge of birds at three field trial sites

Sites	Treatments	Geometric mean of HI titres					
		1st challenge		2nd challenge		3rd challenge	
		Pre	Post	Pre	Post	Pre	Post
Bogor	HRV4	0	7.2±1.0	0.8±0.6	7.2±0.9	2.2±1.1	7.6±0.2
	RIVS2.V4	0	6.8±0.7	0.5±0.8	7.3±0.8	2.7±0.8	7.7±0.2
	Control	0	6.9±0.8	1.2±1.0	6.8±0.9	0.6±0.3	7.9±0.2
	Lab.control	0	NA	0	NA	0	8
Riau	HRV4	0	6.3±1.6	1.6±2.1	7.9±0.3	0	11.0±0.0
	RIVS2.V4	0.2±0.8	5.5±2.2	0.5±0.6	8.0±0.0	1.7±0.7	10.3±0.3
	RIVS3	5.6±2.7	7.1±1.0	0.5±0.5	8.0±0.0	0.4±0.3	9.4±0.6
	Control	1.5±2.5	8.0±0.0	0	8.0±0.0	0	9.6±0.5
	Ext.control	0.6±1.5	7.0±1.0	0.5±0.5	8.0±0.0	0	8.0±0.0
Kupang	HRV4					1.3±0.3	8
	RIVS2.V4					0.9±0.4	7
	Control					0.7±0.3	8
	Lab.control					0	NA

Pre: Before challenge
Post: After challenge
NA: Not applicable

Table 2. The isolation of challenge virus from brain samples of dead birds from three field trial sites

Sites	Treatments	Virus isolation after challenge:		
		1st	2nd	3rd
Bogor	HRV4	5/5[a](100%)	5/5 (100%)	5/5 (100%)
	RIVS2.V4	5/5 (100%)	5/5 (100%)	1/1 (100%)
	Control	4/4 (100%)	5/5 (100%)	3/3 (100%)
	Lab.control	5/5 (100%)	5/5 (100%)	5/5 (100%)
Riau	HRV4	5/5 (100%)	5/5 (100%)	5/5 (100%)
	RIVS2.V4	5/5 (100%)	5/5 (100%)	5/5 (100%)
	RIVS3	2/2 (100%)	5/5 (100%)	5/5 (100%)
	Control	5/5 (100%)	5/5 (100%)	5/5 (100%)
	Ext.control	3/3 (100%)	3/3 (100%)	5/5 (100%)
Kupang	HRV4			5/5 (100%)
	RIVS2.V4			5/5 (100%)
	Control			5/5 (100%)
	Lab.control			5/5 (100%)

[a] No. virus isolated/No. tested

Challenge tests

The results of the tests are presented in Figures 4, 5 and 6. The number of serological reactors with HI titres of 3 (log2) or above are included in parentheses.

At the Bogor site, marked differences in survival rates were observed between vaccinated chickens and laboratory control birds, but there were no significant differences between chickens from vaccinated villages and those from the unvaccinated control village.

At the Riau site, marked differences in survival rates between vaccinated and unvaccinated villages were observed in the first challenge test. At that time the survival rate was not significantly different between the three strains of vaccines. In the second and the third challenge tests, survival rates did not reach the previous level, and no significant differences were observed between treatment groups.

The challenge test from the Kupang site gave much lower survival rates than the other sites, and most challenged birds succumbed except one bird from each vaccinated village (5%) and two birds (10%) from the unvaccinated control village. The differences between treatment groups were not significant.

Discussion

The field trials were initiated on the basis of the demonstrated potential of the food-delivered vaccine technology (Copland 1987), combined with the results of efficacy tests in commercial chickens showing that vaccination with heat-adapted variants of strain V4 of NDV protected against challenge with a velogenic strain of NDV isolated in Indonesia (Darminto and Daniels 1991). In retrospect, field trials were premature since further efficacy tests conducted in village chickens with the delivery system chosen for the study showed different results from those obtained with the same delivery system in commercial chickens (Darminto and Daniels 1991). Nevertheless, useful information can be found in the results of the trials.

At all three sites, reactors to NDV were detectable in all trial groups before vaccination programs started. Since most selected villages were reported to be without ND vaccination programs implemented by government institutions for at least two years before the trials started, the antibodies detected in village chickens in those sites were probably induced by field NDV infections. The same food-delivered vaccines had been used previously at Riau, except for the settlement where HRV4 was used, and so in this site antibodies may have been influenced by the vaccines under test. Because of preexisting antibodies the chosen villages were not ideal trial sites, but villages uninfected by NDV are almost impossible to find in Indonesia.

The proportion of reactors in each treatment group at the Bogor site remained similar each month except for June 1991, when the reactors in the vaccinated villages reached more than 80%. The fluctuations of the percentage of reactors in vaccinated villages were almost always accompanied by similar changes in the proportion of reactors in the unvaccinated control village, and hence seemed to be unaffected by the vaccination. The proportion of reactors at the Riau and Kupang sites increased after several vaccinations. However, as at the Bogor site, these increases seemed to be uncorrelated with vaccination, since the unvaccinated control villages

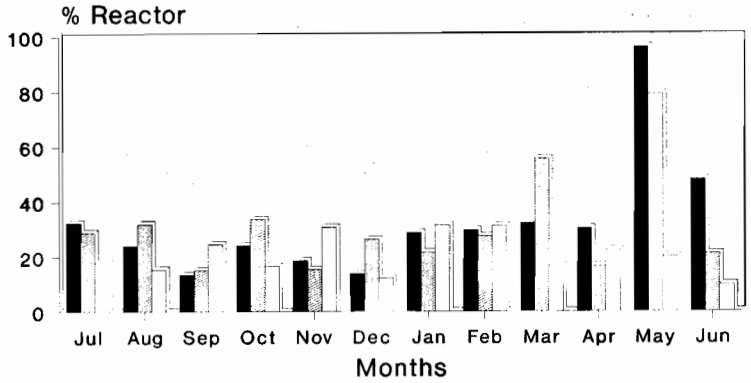

Fig. 1. Monthly distribution of serological reactors with HI titres of 3(log_2) and above from the Bogor site:

■ = HRV4 (82360) ▨ = RIVS2.V4 □ = Control

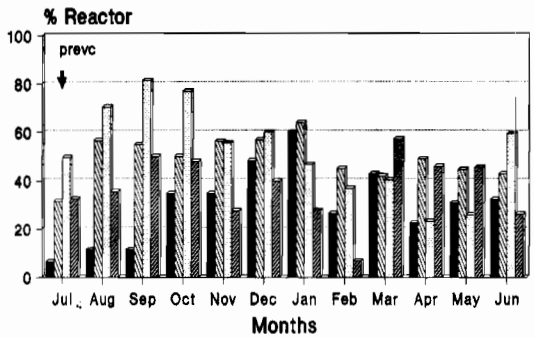

Fig. 2. Monthly distribution of serological reactors with HI titres of 3(log_2) and above from the Riau site:

■ = HRV4 (82360) ▨ = RIVS2
▒ = RIVS3 ▨ = Control

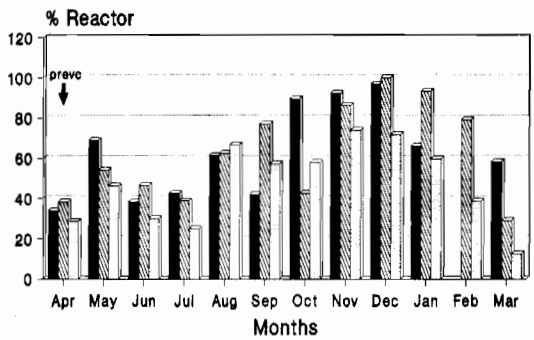

Fig. 3. Monthly distribution of serological reactors with HI titres of 3(log_2) and above from the Kupang site:

■ = HRV4 (82360) ▨ = RIVS2.V4
▒ = Control

also showed a similar pattern. A possible exception was Kupang in the month of March 1991, when the HRV4 vaccinated village had significantly more reactors than the control village. Individual bird analyses are needed to ascertain whether the effect was due to population dynamics associated with the ND epidemic in the preceding months.

The physical distance separating control from vaccinated villages was such that free-ranging chickens from villages would not normally intermingle. Spread of vaccine from vaccinated to control villages, while possible via mechanical or aerosol spread, was thought unlikely with such apparent efficiency. Thus, the possibility that other strains of NDV circulated in the trial villages must be considered.

The potential usefulness of a serological test that would distinguish between antibodies to V4 and other strains has been recognised, and the development of a competitive ELISA based on a monoclonal antibody specific for the V4 strain proposed (Daniels et al 1987). Although approval has been given by ACIAR for such test development, a suitable monoclonal antibody has not yet become available. It is expected that an ELISA in which field sera competed with such a monoclonal antibody for binding to V4 antigen would identify, through lack of competition, sera whose anti-NDV antibodies were unrelated to field strains. Conversely sera competing with the monoclonal antibody in the ELISA would clearly have antibodies to V4, and could be considered to have been exposed to V4. Such a laboratory tool would allow further analysis of the situation described above.

Evaluation by challenge tests failed to demonstrate a benefit from vaccination. At the Bogor site the survival rates in vaccinated birds were considered sufficiently high, 60% to 70%, but were not significantly different from unvaccinated birds. These survival figures were

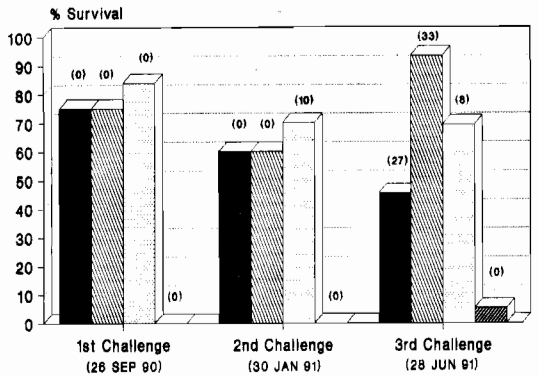

Fig. 4. Survival rates of chickens from the Bogor site in challenge tests:

= HRV4 = RIVS2.V4

= Field control = Lab. control

considered to reflect protection against disease because the high level of mortality in the control birds raised under isolation demonstrated that an effective challenge was given. If the protection demonstrated in the challenge tests at the Bogor site was not induced by vaccination, the possibility of exposure of the birds to field virus is again indicated. Birds without detectable HI antibody responses also survived, and hence were presumably protected. It remains to be determined whether some village chickens have an innate resistance to ND, or whether such surviving birds had been infected and effectively vaccinated without developing HI antibody titres. It was noted that after challenge the HI titres increased markedly.

More sensitive serological tests, or measures of different aspects of the fowls' immune response, may help to answer this question. Current research by the senior authors in collaboration with AAHL and JCU is directed towards assessing whether indirect ELISA tests have a greater sensitivity that will allow detection of responses to infection not detected by the HI test. Another avenue of investigation is whether competitive ELISAs based on monoclonal antibodies to individual viral proteins, including nonstructural proteins and particularly the nucleoprotein (NP), may detect infections not eliciting HI responses. Alternatively, tests of immune responses other than circulating antibodies may be needed to understand the immunity to NDV infection. The mucosal immune system and cell-mediated immune responses are largely unstudied in village chickens.

Results with birds from the Kupang site were quite different, and demonstrated low levels of protection. Almost all challenged birds succumbed to challenge, with the mortalities not being prevented by vaccination or, in some cases, HI titres of 3 or above. Since the birds

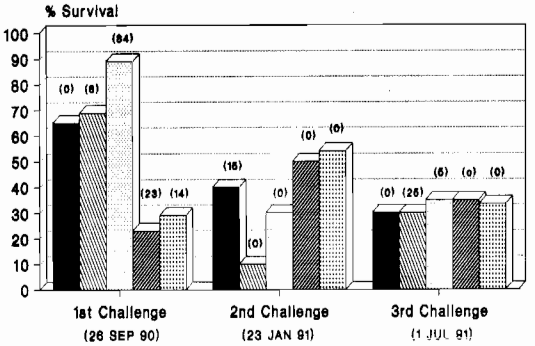

Fig. 5. Survival rates of chickens from the Riau site in challenge tests:

= HRV4 (82360) = RIVS2.V4

= RIVS3 = Internal control

= External control

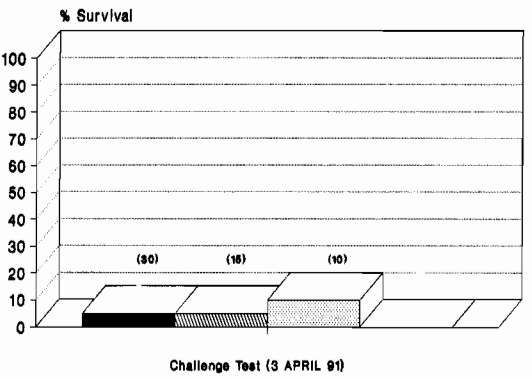

Fig. 6. Survival rates of chickens from the Riau site in challenge tests:

= HRV4 = RIVS2.V4

= Field control

() = percentage of birds with HI titres of 3(log2) and above.

were challenge tested after air freighting to Bogor, stress may have played a role in making birds more susceptible to challenge, even those with antibody titres.

The challenge test at the Riau site demonstrated good protection in the first test. Chickens from villages vaccinated by either HRV4, RIVS2.V4 and RIVS3 showed higher proportions of survivors than those from unvaccinated villages, either internal or external controls. Less satisfactory results were obtained in subsequent tests. It is difficult to explain this situation, but the level of protection demonstrated in the second and the third

challenge tests seemed to be unaffected by vaccination. The results overall illustrated a feature of the trials: that birds with lower HI titres than could be considered protective survived challenge.

The serological monitoring and viral isolations in the challenge tests (Tables 1 and 2) demonstrated that the challenge virus functioned in each test, since seroconversions after challenge were demonstrated in HI tests and the viral isolations from brains of sampled dead birds all yielded NDV.

Although serological results indicate that active infections occurred in most villages during the study, routine viral isolations failed to detect firm evidence for an efficient circulation of the vaccine virus in the field. Heat-adapted variants of the V4 strains, either HRV4 or RIVS2.V4, have been excreted by vaccinated commercial chickens in the laboratory for up to 17 days after primary vaccination (Darminto and Daniels 1991). Although results from commercial chickens cannot be extrapolated to village chickens with confidence, vaccinated village chickens may still be expected to excrete vaccine viruses and possibly infect in-contact birds. Swab samples taken from young chickens were used to evaluate the circulation of the vaccine viruses. However, no virus was isolated from the swab samples at the Bogor site over 12 month collections, or from the Riau site over 6 months of collections.

The sampling protocol was based on the hypothesis that younger birds may not eat as much grain as older birds, and may therefore be the birds most likely to be vaccinated by a second cycle of infection rather than by primary vaccination. If adequately vaccinated birds excrete virus for 1–2 weeks after vaccination, then susceptible birds may become infected and begin to excrete virus 1–2 weeks later. Such excretion after a second cycle of infection in a flock may possibly be detected through swabbing one month after primary vaccination without extra visits to the field sites. Although a more rigorous sampling protocol would be desirable to fully describe patterns of excretion, reinfection and reexcretion, this was not feasible in the present study. Any reisolation of vaccine virus under the sampling conditions used would be evidence of some maintenance of the virus in the ecosystem over the one month period.

The adequacy of the sampling protocol cannot be assessed from the negative results, for it is not clear whether birds even established a pattern of primary infection and excretion. Efficient spread of vaccine to in-contacts may require vaccination with a high dose, but the poor serological responses observed in the trial and the poor responses to vaccination observed in efficacy tests (Darminto and Daniels 1991) suggest that cycles of infection were usually not established by vaccination using the food-delivery system. Further analysis of the serological data for individual birds is needed to see if seroconversions were observed in the months before and after swab sampling. The failure to reisolate excreted virus in this study does not preclude its occurrence after effective vaccination, and further trials should again examine this aspect.

Overall, the field trials yielded much useful information. Additional reliable data have been added to existing information on the population dynamics of village chickens, which will be presented in accompanying papers (Allen et al. 1991). Serological monitoring has revealed that a high proportion of village chickens have antibodies to NDV. Since this was so both in vaccinated and unvaccinated villages, it is difficult to attribute the effect to vaccination. Patterns of seroconversion in individually identified birds and the relationship to mortalities (Allen et al. 1991) may give evidence for infections with velogenic NDV, but the fact that seroconversions occurred in the absence of large-scale mortalities and that birds with low titres were observed may mean that less-pathogenic strains of NDV were also circulating in the villages under study.

Since independently conducted efficacy tests of HRV4 strain in village chickens using the delivery system under trial showed poor protection of birds (Darminto and Daniels 1991), these field trials could not reasonably be expected to have yielded a good result. Hence, the apparent ineffectiveness of vaccination should not discredit the potential of a feed-delivered approach. However, it is clear that much more work needs to be done to develop a practical food-delivery system that takes account of all possible interactions between village chicken gastro-intestinal physiology and immune systems, the strains of virus used, and the food carrier. Considerable practical problems were encountered in organising the mixing of virus with feed in villages and its subsequent distribution. The amount of organisation necessary to achieve a satisfactory system may in fact be more than that required for farmers to catch their birds at night for individual application of vaccine by the farmer. All aspects of the problem, including financial, should be considered before more funds are committed prematurely.

Acknowledgments

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Widescale Implementation of Oral Newcastle Disease Vaccination in Malaysia

A. Latif Ibrahim,* Aini Ideris,* and A. Mustaffa Babjee†

Abstract

Oral Newcastle disease vaccination, using techniques developed during the project supported by the Australian Centre for International Agricultural Research, will be used by the Department of Veterinary Services Malaysia to control Newcastle disease throughout Malaysia. Oral vaccination has already been implemented in four States. Staff have been trained in the production of wheat vaccine, which is prepared in 50 kg batches in large feed mixers.

NEWCASTLE disease has always been a major disease of poultry in Malaysia. Although the disease is under control, outbreaks have been reported from time to time. Parallel with the modern poultry farming, there exists backyard poultry keeping, where 30 to 50 chickens are raised per household. Chickens that are reared under the backyard operation are seldom vaccinated.

A new food-based Newcastle disease vaccine for the village chicken has been developed. It is safe, cheap and has a moderate shelf life (Aini et al. 1990). Extensive laboratory trials, simulated field trials, and field control trials in pilot villages, conducted in Malaysia over a number of years, have established that chickens vaccinated with the vaccine were protected against Newcastle disease vaccine (Ibrahim et al., these proceedings). With the success of the trials, the Department of Veterinary Services (DVS) Malaysia decided to use the vaccine for its vaccination program in the villages of Malaysia. Initially four States, namely Selangor, Negri Sembilan, Malacca and Kelantan, would use the vaccine for their vaccination. Each State would prepare its own feed vaccine and Universiti Pertanian Malaysia (UPM) would be responsible for supplying the vaccine virus. The feed vaccine would be distributed by the veterinary authority to the farms.

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Implementation of Oral Vaccine

As a first step in the transfer of technology to the DVS, a demonstration was held at UPM where veterinary officers and veterinary assistants were shown the technology of preparing the vaccine. The feed vaccine was prepared in batches of 50 kg of wheat in a feed mixer. The wheat was added gradually in the mixer housing chamber and 1.5 litres of aqueous solution of the vaccine was sprayed into the chamber while it was rotating. When spraying was completed, rotating was continued for another 15 minutes for all the feed to dry. The vaccine-coated wheat was then packed and distributed directly to the farmers or stored at 4°C until further distribution to the farmers. Demonstrations were also held at the State veterinary headquarters and district veterinary offices. At present, 50–60 thousand doses of vaccine are distributed monthly.

Conclusion

A new technology has been developed for the preparation of feed vaccine in bulk. It is very practical, as the feed vaccine can be prepared even at the regional veterinary centres, with production geared to the current population of village chickens in that area. This avoids the problem of having to transport the feed vaccine. Due to the ease of application of the vaccine and the interest shown by the farmers in the new technology, the demand for the vaccine is very high. According to the Director General of Veterinary Services, Malaysia, the Department would

be able to prepare 2 million doses of the vaccine by the end of the year for distribution to the farmers.

Acknowledgments

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Epidemiological Studies of Newcastle Disease in Malaysia

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Abstract

Newcastle disease (ND) is the most important viral disease of poultry in the world. Although there is a downward trend in the incidence of ND since vaccination was introduced in Malaysia, more than 40 years ago, the disease continues to be a major threat to the industry. The classical form of ND which causes respiratory and nervous signs with high mortality (approaching 100%) is not commonly seen now except in poorly managed and nonvaccinated village flocks. More frequently the disease is seen in partially immune flocks kept under poor conditions. In spite of the intensity of research on ND locally, there has been little research carried out on its epidemiology. Not much is known about the ecology of ND virus (NDV) in the Malaysian situation. The role of wildlife reservoirs and the persistence of NDV in vaccinated birds and in those that have recovered from the natural infection have not been studied. The present investigations were undertaken in an attempt to address some of these questions. The findings of a retrospective descriptive study and a seroepidemiological survey were presented. The persistence of excretion of virulent NDV from vaccinated chickens and its infectivity for susceptible chickens was demonstrated in trials conducted under experimental conditions. The role of vaccinated birds as an important source of virulent virus in countries where NDV is endemic is subsequently highlighted.

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Webster's Newcastle Disease Vaccine for Village Chickens

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Abstract

Arthur Webster Pty Limited has developed a Newcastle disease vaccine (NDV4HR) for both commercial and village chickens which can be administered by all recognised routes including mixing with food. The vaccine combines the considerable benefits of safety, efficacy, purity and transmissibility of Websters V4 strain with the enhanced thermostable infectivity of the selected heat resistant V4HR form.

Newcastle disease (ND) is an acute, contagious and pathogenic disease of poultry with mortality rates of up to 100% being recorded. It is considered the most important poultry viral disease in the world and has had a devastating effect on large scale commercial and domestic poultry production in most countries. Control of ND is principally based on vaccination measures.

For intensively reared chickens, traditional mass vaccination regimens often employ a live lentogenic strain of virus in young chickens followed by a live mesogenic virus strain. Immunity is often boosted in egg producing flocks at or near the point of lay, using a killed mesogenic or lentogenic virus vaccine which also provides high levels of maternal antibodies to progeny chickens.

The advantages of using avirulent strains of ND virus (NDV) (e.g. Webster's V4) to protect all susceptible birds from day-old to adult against virulent ND viruses are now being recognised. The heat resistant form of Webster's avirulent strain (V4HR), has been developed by the Webster company for particular application in village poultry.

Criteria for an Ideal Newcastle Disease Vaccine

The ideal vaccine for commercial use should satisfy the following important criteria:

1. Safety — The vaccine should not cause clinical signs when given by any route of vaccination in birds of any age.

2. Purity — The vaccine must be free of adventitious contaminants, and the virus seed lot for production should contain only one ND virus strain.

3. Stability — The vaccine must have an acceptable shelf life. The vaccine strain must be genetically stable and not revert to a more virulent type on bird passage.

4. Efficacy/Potency — The inoculated dose of virus (usually between 10^6 and 10^7 (EID_{50}) per dose) must be capable of stimulating a satisfactory immune response.

5. Transmissibility — Spread by direct contact will assist the flock 'vaccination take' when mass vaccination methods are employed.

Webster's Newcastle Disease Vaccine Strains V4 and V4HR

The V4 strain is a naturally occurring Australian ND virus isolated, identified and typed in 1966 by G. Simmons. The strain type is described as slow-eluting with heat-stable haemagglutinin and infectivity (Westbury 1979). It was developed by Webster as a vaccine strain because of its avirulence and high immunogenicity (Webster et al. 1970). Webster's Newcastle disease vaccine now has official approval for use in many countries with annual usage running to hundreds of millions of doses. Although Australia is free of the disease associated with NDV, the V4 vaccine is registered for use in this country should it be required for the control of an outbreak of virulent ND.

Webster's V4HR vaccine has been developed specifically for use in hot climates, so as to reduce dependence on a cold chain for vaccine transport and to

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improve virus stability when applied mixed with feed for village poultry. The efficacy and economic benefits of Webster's oral NDV4HR vaccine for village poultry have been assessed in South East Asia by the Australian Centre for International Agricultural Research (ACIAR) (Johnston 1990; Johnston and Cumming 1991). The results show that it will play a vital role in the improvement of world rural living conditions.

Safety

Webster's V4 and V4HR vaccine strains are safe when administered to all ages of chickens including day-old. There is no evidence of clinical respiratory symptoms, weight loss, mortality in young chickens or egg production drop after vaccination with these vaccines.

The safety performance of V4 (avirulent) is superior to both the B1 (lentogenic) and La Sota (mesogenic) vaccine strains (Webster 1984) (see Table 1).

Purity

Webster's V4HR vaccine is a living, egg adapted, freeze-dried product, produced at the Webster viral vaccine manufacturing plant at Glenorie, Sydney, NSW, Australia. The premises and manufacturing methods employed in the production of viral vaccines, conform to the Australian and United Kingdom Codes of Good Manufacturing Practice (GMP). All Webster's avian viral vaccines, are prepared according to the General Standard for Live Viral Vaccines set out in the Therapeutic Goods Act, 1966, Therapeutic Goods Order No. 21 of the Australian Commonwealth Department of Health, Canberra 1986.

Production methods for V4HR have been described by Claxton and Leonard (1987). Purity of the vaccine is ensured by employing a tested and pure seed lot system, using Specific Pathogen Free (SPF) egg substrate and testing the finished product for freedom from extraneous agents. The Webster SPF flocks are routinely tested and demonstrated to be free from twenty (20) different pathogenic agents including all the known vertically transmitted major viral pathogens of poultry. Each batch of vaccine is tested and demonstrated to be free from thirty two (32) designated pathogenic agents including viruses, bacteria, fungi and mycoplasmas before release for sale.

Stability

The natural characteristics of thermostable haem-agglutinin and infectivity demonstrated by the V4 strain (Westbury 1979), have been utilised in the cloning of the heat resistant (V4HR) form of the original V4 strain. This V4HR virus demonstrates enhanced thermostable infectivity when compared to the parent V4 virus (see Fig. 1), whilst retaining all the established characteristics of avirulence, transmissibility, safety and efficacy.

Because of the demonstrated greater thermostable infectivity of Webster's V4HR virus in freeze-dried form, the vaccine is ideally suited for distribution in tropical countries where maintenance of cold chain storage is a problem and to enhance the stability of reconstituted vaccine.

Efficacy/Potency

Spradbrow and Samuel (1987) carried out dose response studies with Webster's V4 vaccine using three vaccine dose rates, $10^{5.4}$, $10^{6.4}$ and $10^{7.4}$ EID_{50} when administered

Table 1. Comparative safety of Newcastle disease vaccine strains

Symptoms of vaccinated birds	Vaccine strain		
	V4	B1	La Sota
Sneeze test	Nil	Definite symptoms	Pronounced symptoms
Respiratory disease	Nil (or mild)	Clinical respiratory symptoms	Clinical respiratory symptoms
Weight gain	No effect	Significant reduction suppression	Highly significant
Mortality in young chickens	Nil	Yes	Yes
Egg production drop	Nil	5–10%	>10%

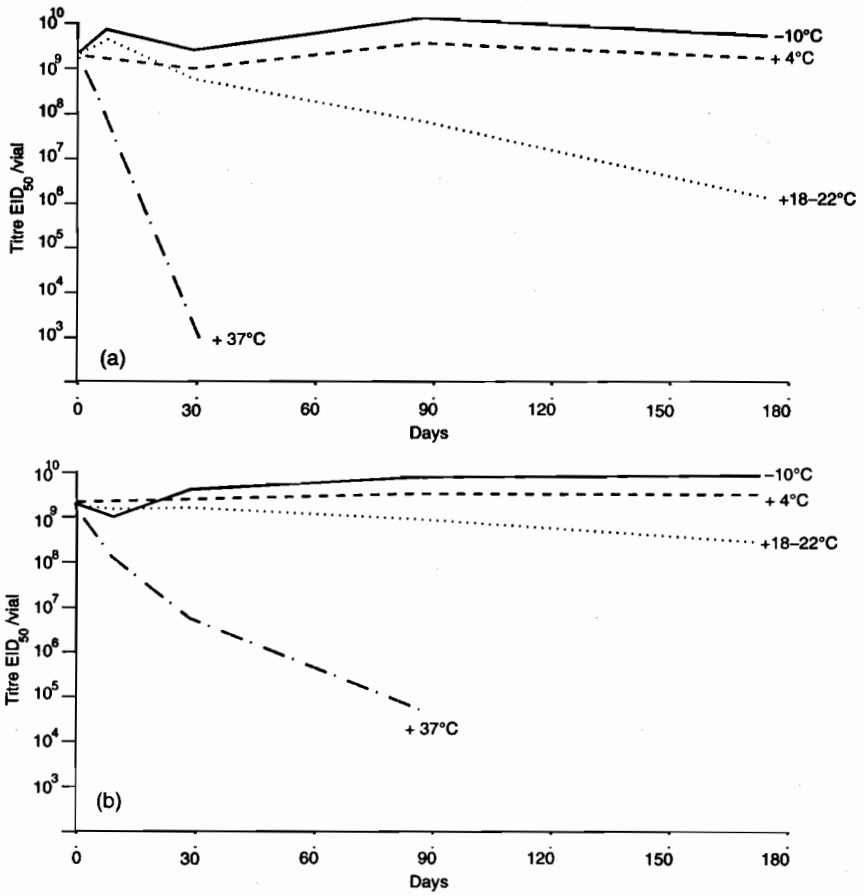

Fig. 1. Stability of (a) Webster's V4 and (a) V4HR freeze dried vaccines

on food. They recorded high antibody response regardless of dose, with peak response 2–4 weeks after vaccination. In-contact birds developed high levels of antibody when placed with the vaccinated birds. Webster recommends a dose rate of $\geq 10^{6.0}$ EID_{50} by all routes of administration.

The V4 vaccine strain is highly immunogenic, capable of protecting vaccinated birds against numerous mesogenic and velogenic viruses used as challenge strains throughout the world (Webster et al. 1970). The level of protection offered by the Webster's vaccines against a variety of velogenic challenge strains is summarised in Table 2.

In comparison with other vaccine strains Webster's V4 vaccine strain offers equal or superior protection. For example, Spalatin et al. (1976) found complete protection against virulent Fontana 1083 following vaccination with La Sota (mesogenic), B1 and F (lentogenic), CT, Ulster and V4 (avirulent) strains. Westbury (1984) found in laboratory trials that when the V4 strain was given in the drinking water a better degree of protection was produced than with B1 strain.

Table 2. Protection conferred by Webster's V4 and V4HR vaccine

Challenge strain	Protection (%)	Reference
Roakin	99	Turner et al. 1976, 1977
Texas G.B.	99	Turner et al. 1976, 1977
Herts 33	100	Turner et al. 1976, 1977
Fontana 1083	100	Spalatin et al. 1976
Ipoh AF 2240-226	91	Spradbrow et al. 1978
Malaysian strain	96	Ibrahim et al. 1979, 1981
Herts 33/56	<100	Westbury 1984
SL88/Ceylon	66* & 100	Jayawardane et al. 1990
Ipoh AF2240-226	66–80*	Ideris 1990 et al.

* V4HR on feed

Table 3. Vaccination response to Webster's ND V4 vaccine applied by various routes

Vaccination route	Vaccination response			
	HI antibodies		Protection against challenge	
Natural Exposure	++	(i)	++	(ii)
	++	(iii)	++	(i)
Intramuscular	+++	(iv)	+++	(xv)
Oro-tracheal	++	(iv)	++	(v)
Intranasal	+	(vi)	+	(vi)
			++	(vii)
			++	(viii)
Intraocular	++	(xiv)	+++	(xiv)
	++	(xviii)	+++	(xviii)
	++	(xv)		
Aerosol	++	(x)	++	(xi)
	++	(iii)	+++	(iii)
	+++	(xii)		
Coarse Spray	++	(xiii)	++	(xiii)
	++	(xi)	++	(xi)
	+	(iii)		
Drinking Water	++	(ix)		
	++	(xi)	–	(xi)
	+++	(vi)	++	(vi)
	++	(iii)		
	+++	(xiv)	++	(xiv)
	+++	(xv)		
On Feed	+	(vii)	+++	(vii)
	++	(xv)	+++	(viii)
			++	(xvi)
			++	(xvii)

Legend: – Negative ++ Satisfactory
+ Inferior +++ Superior

References:
(i) Spradbrow et al. (1980) (ii) Turner et al. (1976) (iii) Bell et al. (1991d) (iv) Kim (1977)
(v) Spradbrow et al. (1978) (vi) Westbury (1984) (vii) Tantaswasdi et al. (1989) (viii) Jayawardane et al. (1990)
(ix) Kim et al. (1978) (x) Schalkoort et al. (1980) (xi) Ibrahim et al. (1981) (xii) Ibrahim et al. (1980)
(xiii) Ibrahim et al. (1979) (xiv) Sagild et al. (1987) (xv) Spradbrow et al. (1991) (xvi) Spradbrow (1989)
(xvii) Johnston (1991) (xviii) Salgild et al. (1982)

In Malawi, Sagild and Haresnape (1987) concluded that chickens vaccinated with Komarov and LaSota mesogenic vaccine strains were often inadequately protected. They recommended V4 vaccine as an alternative to Komarov and La Sota vaccines for small holder flocks, where the large scale use of V4 has reduced the incidence of ND in rural parts of Malawi. They suggested that the success of the V4 strain is due to its thermostability, transmissibility and ease of administration.

In Thailand, Tantaswasdi et al. (1989) showed that V4 was superior to F strain in protecting against challenge with viscerotropic velogenic NDV using feed and intranasal routes of vaccine administration.

Transmissibility

Direct bird to bird transmission has been demonstrated with the V4 strain (Westbury 1979), and Kim (1977) found the V4 strain was highly transmissible compared with other Australian avirulent strains. Rapid in-contact spread of V4 has been referred to by a number of authors including French et al. (1967), Turner et al. (1976), Spalatin et al. (1976), Kim and Spradbrow (1978) and Jayawardane (1990). This characteristic is considered an important attribute of the strain for effective mass immunisation of all birds in a flock.

The lentogenic strain B1 has been shown to be less transmissible than the avirulent V4 strain (Spalatin et al. 1976). Superior transmissibility of the V4 strain, compared with the Komarov and La Sota vaccine strains, has been cited by Sagild and Harsnape (1987).

Egg transmission is not regarded as having epidemiological significance for the more virulent strains of NDV or for the avirulent V4 strain in Australia (French et al. 1967).

Route of Vaccination

A large number of workers have shown that all traditional routes of vaccination with Webster's V4 will provide protection against challenge with a velogenic NDV. Protection afforded by V4 vaccination has been found by Bell et al. (1991a) to be superior in the field compared with laboratory tests.

Vaccination response to Webster's V4 and V4HR vaccines applied by various routes is summarised in Table 3.

Unexpectedly, the low serological response of birds to Webster's V4 vaccine in some reports do not reflect a low protection against challenge. Bell et al. (1991b), showed all vaccinated chickens with a $\log_2$ HI titre of 2 or greater and many of the vaccinated birds with no detectable antibody, survived challenge. This observation confirms earlier work of Ibrahim et al. (1981) who concluded that no correlation existed between HI antibody titres due to NDV4 vaccination and resistance to challenge. Similar observations have also been reported by other workers including Turner et al. (1976).

Timms and Alexander (1977) suggested that local immunity and cell mediated immunity plays a role in the protection of vaccinated chickens against velogenic ND viruses. Spradbrow and Samuel (1991) postulate that oral vaccination may induce the production of secretory immunoglobulins by stimulating the intestinal lymphoid tissue.

Recommended Vaccination Routes for Village Poultry

For optimal protection it is recommended that the birds are vaccinated by the intraocular route.

For birds that cannot be caught and vaccinated individually, the vaccine can be applied by mixing with food of a suitable type (Spradbrow 1989).

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Vaccination of Sri Lankan Chickens against Newcastle Disease with Oral V4 Vaccine Delivered on Cooked Rice

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Abstract

Laboratory trials were conducted to determine the efficacy of an oral V4 strain Newcastle disease (ND) vaccine against local virulent ND virus strains, and to find a suitable vehicle for administration of vaccine. Two field trials investigated the efficacy of the vaccine. Survival of the vaccine virus in different diluents and on different food stuffs was also determined at different temperatures.

It was found that a variety of parboiled rice (PBR) named 'Samba' was a suitable vehicle for oral V4 vaccine, protecting all birds against experimental challenge after two doses. However, protection after repeated vaccination (second dose was given 3 weeks after the initial dose and 3 monthly vaccinations thereafter) was lower under field conditions. In the first field trial, serum antibody levels in the vaccinated group remained higher than that of the control group throughout the trial period. Protection levels in buy-back challenge tests increased from 48% to 75% in the vaccinated group over 12 months. A natural outbreak which occurred only in the vaccinated flocks caused 17% death. In the second trial, 40% of the total population in both vaccinated and control flocks has died of ND after 2 vaccinations.

After reconstitution in different diluents, vaccine remained viable for a considerable period of time at room temperature and under refrigeration. When coated on cooked 'Samba' rice, regardless of the diluent used, it survived up to five hours without any significant drop in titre. Survival on coconut residue was also reasonable for up to four hours. It was concluded that individual vaccination (intraocular or intranasal) with V4 strain is more suitable under Sri Lankan village conditions as chickens can usually be caught.

THE village chicken population in Sri Lanka consists of about one-third of the total poultry population. In many rural areas, families keep backyard poultry. The average flock size is about 15, ranging between 5 and 60. The eggs and meat derived from these birds constitute a supplementary source of protein and also help to earn additional income. This system is very popular among rural farmers, because it needs very small input.

There is a popular belief among villagers that the meat and eggs of village chickens are more nutritious than those of commercial chickens. Therefore, village chickens always fetch a higher market price than commercial chickens. Meat from village chickens is preferred for local dishes.

Village chickens face threats from several infectious diseases, notably Newcastle disease (ND), which occurs almost every year. Control of ND is mainly done by the intramuscular injection of 'Komarov' strain vaccine, which is produced by the Department of Animal Production and Health. Unlike many other Asian countries, village poultry rearing in Sri Lanka is a semi-intensive system and mainly for egg production. In most places, birds are kept in the pen for at least 14–16 hours. Therefore, individual vaccination is possible, but it needs the help of a vaccinator. The State Department of Animal Production and Health produces a vaccine in 200 dose vials which must be used within a short period after reconstitution. Unless the farmer has sufficient birds, it is very difficult to get a vaccinator or vaccine. If the freeze-dried vaccine is reconstituted without a sufficient number of birds for vaccination, the leftover vaccine has to be discarded. For these reasons and also lack of interest in farmers themselves, many flocks are left unvaccinated. Though vaccine is used in some instances, it may not be properly handled. Thus, ND outbreaks occur every year, causing considerable damage to the industry. If a vaccine that can be used by the farmers themselves is developed, we would be able to have a larger proportion of the village poultry population adequately immunised.

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It has been found that the Queensland V4 strain of ND virus is heat stable, spreads laterally, is easy to handle and can be administered orally (Westbury 1981). These characters were considered the ideal basis for a vaccine to be used in village chickens. Therefore, vaccination trials were started in 1988 with financial assistance from the Australian Centre for International Agricultural Research (ACIAR).

Conventional Vaccination Programs

Several types of ND vaccines including Komorov, La Sota and Hitchner B1 strains are used in Sri Lanka. Komorov vaccine is produced by the State Department of Animal Production and Health and is used mainly by small and medium scale farmers. This vaccine is provided free of charge with the recommendation that it be given at 3 and 12 weeks of age. Steps have been taken to replace vaccination at 3 weeks of age with F strain vaccine. Other types of vaccine are imported by private enterprises and are used by the more affluent farmers.

Laboratory Efficacy Trials with V4 Oral Vaccine

After an unsuccessful preliminary trial with a commercial feed as a vehicle for vaccine administration, successful results were obtained with a variety of parboiled rice (PBR) called 'Samba". This variety is small grained and the grains tend to remain separate after cooking, making it possible for the vaccine to be evenly mixed and available to even small chicks. This work was reported by Jayawardane et al (1990). The results of the trial are summarised in Table 1. Other varieties of rice gave less consistent results.

Field Trials With V4 Oral Vaccines

An area with a high village chicken population was identified and three village organisers were appointed. Each organiser handled one control village and two vaccinated villages. A preliminary survey was conducted with 156 households which owned 2515 birds, consisting of 16% chicks (0–8 weeks), 42% growers (2–8 months), 35% layers (> 8 months) and 7% breeding males. Among these 156 households, only 20% of flocks has been vaccinated previously. Suspected ND outbreaks had occurred in nearly 70% of flocks, with losses around 60%. The average flock size in this area was 15 and ranged between 5 and 50.

Nearly 30% of the birds were designated as controls and vaccination commenced in August 1989. A total of 487 birds was tagged initially and bled to determine serum antibody levels before vaccination. Young chicks (3–7 weeks) were vaccinated twice at two week intervals between the ages of 3–7 weeks and thereafter at 3 month intervals. Birds older than 7 weeks were vaccinated at the onset and boosted a month later. Booster doses were then administered every 3 months, as for young birds. Newcomers to the flock at the later stages were also vaccinated in the same manner. The vaccine was reconstituted in 1% skim milk and applied on cooked samba rice immediately before use. The turnover of birds in Sri Lanka is not rapid when compared with many other countries. Furthermore, vaccination every month is considered impracticable under field conditions in Sri Lanka. For this reason, repeated doses were given at 3-month intervals instead of monthly intervals as used in Malaysia (Ideris et al 1987).

Two months after the first vaccination (October 1989), 185 birds were bled and antibody levels were determined. Two more bleedings were done: the first 5 months later (March 1990) and the other one year later (October 1990) involving 402 and 315 birds, respectively.

Four months after first vaccination (February 1990) and later at the time of termination of the trial (January 1991) birds were purchased from the villages and subjected to challenge with virulent virus under laboratory conditions. Birds bought as day-olds from a hatchery and reared in isolation at the Veterinary Research Institute (VRI) were used as unvaccinated controls. During the trial period, each household was given a printed daily record sheet to mark the number of eggs laid, sold and consumed, the number of birds sold, consumed or purchased, daily expenses and income. Village organisers visited each household, collected the record sheets and prepared a weekly report. While collecting the daily record sheet, village organisers were instructed to cross-check the information given. This weekly report, together with daily record sheets, was collected by the VRI team visiting the trial area periodically. Based on these records a monthly report was prepared and analysed.

Village organisers were also trained to identify clinical ND, so that whenever they suspected an outbreak they could inform the VRI. Samples were collected from the outbreaks and confirmed in the laboratories at the VRI.

While new farmers joined, some who enrolled at the beginning left the program during the course of the trial. Altogether 165 farmers were involved.

Results

Serology

Results of the serological tests conducted before vaccination, 2 months after, 5 months after and a year later are shown in Table 2. Geometric mean titre (GMT)

Table 1. Response of chickens vaccinated twice by oral route with V4 vaccine to challenge with a virulent local strain of Newcastle disease virus

Group	No.of chickens	Vaccination procedure	HI antibody response*				Response to challenge		
			Before 1st vaccination	Before 2nd vaccination	Before challenge	After challenge	No. clinically ill	No. dead	No. unaffected
1a	30	Cooked rice	1	1	6.9	9.6	0	0	28+
1b	10	Contact with 1a	1	1	8.1	9.7	0	0	10
2a	30	Uncooked rice	1	1	1	8.2	6	6	18
2b	10	Contact with 2a	1	1	1	6.5	2	3	4++
3	14	Intra Nasal	1	2.3	4.7	10.1	0	0	14
4	40	Control	1	1	1	8.9	9	22	9

Notes:
1st vaccination at 21 days of age; 2nd vaccination at 35 days of age.
Challenge at 56 days of age.
Chickens were monitored for 14 days after challenge.

* Log_2 Geometric mean, V4 antigen
+ 2 deaths before challenge
++ 1 death before challenge

Contacts were kept in the same room with vaccinated birds, but separated at the time of vaccination.
Birds were kept without food or water for 16 hours before vaccination.
Reference: Jayawardane et al. 1990

Table 2. Antibody titres of birds in the vaccinated and control area

	Vaccinated area			Control area		
	Total	GMT	% birds with >3	Total	GMT	% birds with >3
Before vaccination	355	1.9	24	132	1.4	19
2 months after 1st vaccination (2 vaccinations)	128	2.0	35	57	1.4	15
5 months after 1st vaccination (3 vaccinations)	312	2.3	37	90	1.2	14
One year after 1st vaccination (>3 vaccinations)	254	3.0	44	61	2.0	26

GMT = Geometric mean titre, log 2.

of serum antibody levels in the vaccinated group increased gradually with the repeated vaccination and was always higher than that of the control group.

Results of buy-back challenge tests

The results of buy-back challenge tests are shown in Table 3. Birds that resisted artificial challenge had increased from 48% to 75% between the two tests conducted 12 months apart. These protection values are considerably higher than those of the control birds.

Mortality Patterns in the Field

Mortalities in the control group remained higher than those in the vaccinated group for the first four months. Subsequently, mortalities in the vaccinated group rose above those of the control group. An outbreak of ND had occurred in April 1990, continuing till September that year. Unfortunately, only the vaccinated villages were affected and this was the main reason for higher mortality among vaccinated birds after the first few months.

Although a total of 165 flocks has been involved, only 129 flocks consisting of 3265 birds were active participants at the onset of the outbreak. Of these, 35 flocks in the vaccinated area were affected resulting in a total of 509 deaths, which amounted to 15% of the total population. This consisted of 41% chicks, 37% growers, 19% hens and 3% cockerels. In 10 of the 35 flocks almost 100% mortality was observed, while 50–60% mortality was observed in another 12 flocks, with very few deaths being reported in the remainder of the flocks. There were 19 flocks with zero or very low mortality (total 34 deaths). The causes of these deaths were not confirmed in the laboratory, but these flocks showed an elevated haemagglutination inhibition titre of >6 (log 2) after the outbreak. These were identified as flocks in which natural challenge has taken place. Therefore, we determined that natural challenge had taken place in a total of 54 flocks in the vaccinated area with 542 deaths (17% of total population). In the control area, only one outbreak was observed throughout the trial period. A further 7 flocks showed elevated antibody titres and were therefore identified as flocks which faced natural challenge.

Flock Productivity and Household Income

The results of flock productivity studies are shown in Tables 4 and 5. It should be noted that the proportion of total time which was assumed as under natural

Table 3. Results of buy-back challenge tests of vaccinated and control chickens in Sri Lanka

	First test			Second test		
	Total	No.dead	% survived	Total	No.dead	% survived
Controls from hatchery	21	21	0	10	10	0
Controls from trial area	12	10	16	8	6	25
Vaccinates	23	12	48	29	7	75

challenge for the two groups was almost the same: 5% for the vaccinated group and 3% for the control group. However, more birds had died during this period in the vaccinated group. Flock size was larger in the vaccinated group and hatchability and deaths within a month of hatching were almost the same. The total bird and egg offtakes were similar in both groups. Therefore, the net value for each month was almost the same.

Table 4. Flock monitoring survey results (monthly productivity estimates) March 1989–December 1990

	Pre-epidemic phase	Epidemic phase	Recovery phase	All phases
Orally vaccinated flocks				
Flocks	127	54	51	128
Flock days	50156	2984	4060	57200
% flock days	88	5	7	100
Average monthly flock size				
Chickens	3.9	6.5	2.0	3.9
Female growers	3.8	4.2	2.3	3.7
Male growers	2.8	3.4	1.8	2.8
Adult males	1.0	0.9	0.6	1.0
Adult females	7.4	8.7	6.0	7.4
Total birds	18.9	23.7	12.7	18.8
Flock replenishment				
Egg laid/month	6.5	4.3	6.5	6.4
% brooded	3.9	6.5	4.7	4.0
% hatchability	71.7	74.9	83.3	72.6
% died from hatching to the next visit	1.0	23.0	49.0	2.0
Birds purchased	0.05	0.07	0.09	0.05
Monthly deaths and losses				
% chickens	6.6	62.0	13.2	11.6
% growers	1.5	25.0	0.7	2.9
% adults	0.75	13.5	0.7	1.5
Birds and eggs offtake				
Birds sold (No.)	0.5	0.4	0.5	0.5
Birds consumed (No.)	0.3	0.5	0.2	0.3
Total bird offtake (No.)	0.8	0.9	0.7	0.8
Eggs sold (No.)	25.7	21.6	23.8	25.3
Eggs consumed (No.)	25.7	21.6	23.8	25.3
Value to householder (Rs)	156.2	136.7	125.5	152.8
Opportunity cost of inputs				
Birds purchased (Rs)	7.9	5.0	17.3	8.4
Feed purchased (Rs)	36.0	40.44	28.9	35.8
Medicine (Rs)	0.7	6.6	0.8	1.04
Other purchases (Rs)	0.3	0.2	0.0	0.3
Value other inputs (Rs)	0.04	0.0	0.0	0.03
Total costs (Rs)	44.94	52.24	47.02	45.57
Net value (Rs/month)	111.25	84.53	78.52	107.24
Revenue/Bird (Rs)	8.3	5.8	9.9	8.1
Costs/Bird (Rs)	2.4	2.2	3.7	2.4
Net value/Bird (Rs)	5.9	3.6	6.2	5.7

Table 5. Flock monitoring survey results (monthly productivity estimates) March 1989–December 1990

	Pre-epidemic phase	Epidemic phase	Recovery phase	All phases
Non-vaccinated flocks				
Flocks	37	8	8	37
Flock days	16497	443	326	17266
% flock days	95	3	2	100
Average monthly flock size				
Chickens	2.1	8.0	5.6	2.3
Female growers	3.4	4.0	3.9	3.4
Male growers	2.2	2.3	2.7	2.2
Adult males	1.0	1.0	1.3	1.0
Adult females	5.9	7.1	7.9	6.0
Total birds	14.6	22.4	21.4	14.9
Flock replenishment				
Eggs laid/month	7.7	9.3	5.6	7.7
% brooded	2.4	5.3	0.0	2.5
% hatchability	72.9	76.9	0.0	72.9
% died from hatching to the next visit	0.0	55.0	0.0	2.0
Birds purchased	0.2	0.4	1.3	0.2
Monthly deaths and losses				
% chickens	7.8	35.2	0.0	9.9
% growers	2.5	18.1	0.0	2.9
% adults	1.0	3.3	0.0	1.1
Birds and eggs offtake				
Birds sold (No.)	0.5	0.3	1.1	0.4
Birds consumed (No.)	0.2	0.0	0.0	0.3
Total bird offtake (No.)	0.7	0.3	1.1	0.7
Eggs sold (No.)	19.1	26.7	20.5	19.3
Eggs consumed(No.)	25.1	36.3	23.4	25.4
Value to household (Rs)	141.06	165.31	164.46	142.18
Opportunity costs of inputs				
Birds purchased (Rs)	6.76	20.99	12.33	7.22
Feed purchased (Rs)	28.88	32.40	35.43	29.09
Medicine (Rs)	0.43	2.44	3.31	0.54
Other purchases (Rs)	0.03	0.00	0.00	0.03
Value other inputs (Rs)	0.00	0.00	0.00	0.00
Total costs (Rs)	36.30	55.83	51.07	36.88
Net value (Rs/month)	104.76	109.48	113.39	105.30
Revenue/bird (Rs)	9.7	7.4	7.7	9.5
Cost/bird (Rs)	2.5	2.5	2.4	2.5
Net value/bird (Rs)	7.2	4.9	5.3	7.0

Despite the fact that more deaths occurred in the epidemic phase in the vaccinated group, a population growth of 3.9% was observed at the end of the trial. In the control group on the other hand, the population dropped by 4.6%.

Problems Faced in the Field

There were many problems that constrained the conduct of the field trials. They were as follows.

1. Obtaining sufficient households to represent non-vaccinated controls.
2. Many farmers believed that the vaccine should protect birds from diseases other than ND.
3. High mortalities occur due to other diseases, especially in chicks. In the event of such deaths among vaccinated birds, there was a tendency to blame the vaccine.
4. In some households, birds were fed or discharged from the pens before our arrival.
5. Many farmers objected to bleeding of birds.
6. Catching tagged birds for bleeding was a difficult process.
7. Some owners were aware that vaccine administered individually gave better protection than oral vaccine. These people were always pressing for injectable vaccine.
8. Daily record sheets were not completed in many instances. The enumerators had to seek information personally from farmers in order to complete the data sheets.
9. Birds previously vaccinated with Komorov vaccine have survived the natural challenge (almost 100%). The same protection level was not observed with V4 vaccine. This discouraged some farmers from joining the trial and others already enrolled had their birds vaccinated with injectable Komorov vaccine.
10. Private vaccinators in the trial area had lost their usual income after the trial commenced and discouraged farmers from using the oral vaccine on their birds.

All these above factors should be taken into account when developing a program for the use of an oral feed-delivered vaccine for ND in village chickens.

Other Laboratory Tests Done with V4 Vaccine

1. Survival of V4 vaccine reconstituted in different diluents and coated on different foodstuffs was investigated. Recovery was attempted soon after preparation of the vaccine. The results of this test are given in Table 6.

Table 6. Recovery of V4 virus from food

Foodstuffs	Diluents			
	1% skim milk	50% glycerol	40% sucrose	2% starch
Cooked broken parboiled rice (PBR)	>6.6	>6.6	>6.6	>6.6
Cooked unbroken PBR	>6.7	>6.7	>6.7	>6.7
Cooked unbroken SAMBA PBR	6.2	>6.8	>6.6	>6.6
Uncooked broken PBR	<2.8	4.0	4.0	<2.8
Uncooked unbroken PBR	5.2	4.7	5.6	6.2
Uncooked unbroken SAMBA PBR	<4.0	>6.7	<3.9	<3.0

Figures are logs of the EID_{50} of virus recovered.

From the results in Table 6, it can be concluded that the amount of recoverable virus was greater when cooked rice was used.

2. Survival of V4 vaccine in different diluents at room temperature. The diluents used for this test were as follows.

   PHS 15 – Moltodextrin for spray drying
   Gelose 50 – High amylose maize starch (50%)
   3401 C – Regular maize starch (29% amylose)
   3401 X – Waxy maize starch (0% amylose)
   A 282 – Gluten for use in aquaculture
   PBS – Phosphate buffered saline

   Data on the survival of the virus at room temperature in these diluents (5% in distilled water) for a period of up to 5 weeks are given in Table 7.

   Survival was similar to PBS in all the diluents except in A 282. Gluten may have had adverse effect on the vaccine virus.

3. Survival of vaccine virus on SAMBA parboiled rice. Vaccine reconstituted in different diluents, coated on cooked SAMBA rice was also tested to investigate the amount of recoverable virus, at varying intervals after coating. The results are given in Table 8.

   Vaccine survived at least for 5 hours on cooked SAMBA rice at room temperature, irrespective of the diluent used.

4. Survival of vaccine virus on coconut residue. In another test, survival on coconut residue which resulted when coconut milk is extracted from grated coconut kernel, was investigated. This is a common component of kitchen refuse in Sri Lanka. The results are shown in Table 9.

   The recovery was not as good as that from cooked SAMBA rice and also recoverable titre dropped after one hour.

Table 7. Survival of V4 virus in various diluents

Diluent	Titre					
	Day 1	1 Week	2 Weeks	3 Weeks	4 Weeks	5 Weeks
PHS 15	7.1	5.9	3.7	2.7	2.9	<1
Gelose 50	6.3	6.3	3.0	1.7	<1	<1
3401 C	6.9	6.5	3.3	2.5	<1	<1
3401 X	6.7	5.9	2.8	2.0	2.0	<1
A 282	5.9	2.7	<1	<1	<1	<1
PBS	7.1	6.2	3.8	2.0	2.0	<1

Table 8. Recovery of vaccine from samba rice at different time intervals

Duration	Recoverable virus titre (log EID_{50}) at room temp.		
	1% skim milk	Distilled water	PBS
0 hr	4.7	4.1	4.7
1 hr	4.9	4.1	4.9
2 hr	4.7	ND	ND
3 hr	4.7	4.1	4.3
5 hr	4.1	3.7	4.3

ND – not done
Expected initial titre – 5.3

Discussion and Conclusion

The efficacy trial demonstrated that V4 vaccine is capable of protecting birds against local virulent strains of ND virus. The 'SAMBA' variety of parboiled rice appeared to be a good vehicle to administer vaccine orally under laboratory conditions. Conditions in the field, however, are different and variable. Vaccine is presented under less favourable conditions and the village chickens are undernourished, and possibly immunodepressed, as compared with laboratory chickens. The degree of undernourishment is indicated by an analysis of the crop contents of village chickens in Sri Lanka (Gunaratne et al. these proceedings).

A gradual increase of the GMT of serum antibody was observed in the vaccinated group during the progress of the trial. The GMT decreased gradually in the control group during the first few months before rising again. This increase may be due to natural infection, obtaining a higher proportion of blood samples from birds surviving previous outbreaks or from birds previously vaccinated with injectable Komorov vaccine. Survival of older birds with high antibody titres from the outset to the end of the trial have made interpretation of the serological results difficult.

This field trial conducted over a limited period gives only some index of the protection invoked by the vaccine. Significance of the 17% mortality due to ND in the vaccinated flocks cannot be accurately ascertained since the extent of natural challenge is not known. The buy-back challenge tests on the other hand showed a much higher level of protection. Birds purchased for these tests had received at least 2 vaccinations and a special effort was made to purchase growers. This was done to avoid the purchase of older birds that may have received Komorov vaccine prior to this trial or were survivors from previous outbreaks of ND. The protection level in buy-back challenge tests increased from 48% to 75% in the vaccinated group over a period of 12 months. It should be noted that the second buy-back was conducted after the natural challenge. These birds could have been the survivors of natural challenge. Some of the survivors in both groups could have had natural immunity to explain

Table 9. Recovery of vaccine from coconut residue at different time intervals

Duration	Recoverable virus titre (log EID_{50}) at room temperature			
	1% skim milk	PBS	Distilled water	Immature coconut water
0 hr	6.1	5.9	5.9	5.7
1 hr	5.9	5.9	5.7	5.7
2 hr	5.1	5.7	5.1	5.2
4 hr	5.1	4.7	4.7	5.1

Expected initial titre 7.5.

their survival rather than immunity acquired from vaccine. However, the protection level obtained from the second trial is considerably higher than that of the corresponding figure for controls.

The near 100% mortality in 10 vaccinated flocks has had an adverse impact on the progress of the trial. After these outbreaks, some farmers had their birds vaccinated with Komorov vaccine and the cooperation received from others also diminished. This situation led to the termination of this trial in December 1990.

A major error in the design of the trial was the selection of vaccinated and control flocks from different villages. Thus, the outbreaks which occurred in the vaccinated villages did not spread to the control area making the challenge experienced by the two groups unequal. Therefore, one should make it a point, when designing a field vaccination trial to select control flocks randomly, so that control flocks are found around vaccinated flocks. Our own efficacy trial and work done by many other scientists (Bancroft and Spradbrow 1978; French et al 1967; Hall et al 1967; Spalatin et al 1976) revealed that V4 virus spreads from one bird to another. Separate villages for controls were selected to prevent controls obtaining vaccine virus by lateral spread between birds.

It is interesting to note that population growth of 3.9% was detected in the vaccinated group. This increase is shown even after the outbreaks which had a drastic effect on the vaccinated group. In the control group, the population dropped by 4.6%. Average flock size increased in the vaccinated group from 16.8 to 18.8, whereas it remained at the same level in the control group. This observation is of some significance. Had an outbreak of the same magnitude occurred in the control group, it would have had a drastic effect on the productivity and on the total population.

The baseline study conducted before the field trial commenced, revealed a higher death record than is observed in the pilot flocks. Therefore, we believe that by oral vaccination, we have been able to reduce death rate to some degree. This fact was confirmed by the devastating effect of outbreaks of ND in other non-vaccinated areas (not included in our trial area) reported to us during the trial period. No detailed information was collected.

A second field trial is proceeding, with a protocol designed to minimise some of the problems encountered in the first field trial. At present the chickens have been under observation for only four months.

The overall studies in Sri Lanka indicate very clearly that the V4 strain of ND virus can give complete protection against local strains of ND virus in laboratory trials. However, due to various, yet unresolved problems in the delivery system, complete protection of orally vaccinated birds cannot be achieved in the field. The applicability of these findings has to be viewed in the background of the nature of the village chicken industry in Sri Lanka. In contrast to the situation prevailing in many other Asian countries, Sri Lankan village chickens are reared in relatively smaller flocks (15 birds), and are housed for part of the day. Thus, vaccination by other means, which are known to give a higher level of protection, may be acceptable to the farmers. Individual inoculation of the Komorov strain vaccine may not be feasible since the services of a vaccinator are required. However, the administration by the farmer of the thermostable V4 strain by intraocular or intranasal routes, which give high, reliable protection levels, may be most appropriate for Sri Lankan village chickens.

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Control of Newcastle Disease in Village Chickens with Oral V4 Vaccine in Thailand

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Abstract

The results of trials undertaken in Thailand on the vaccination of village chickens against Newcastle disease are summarised. Observations have included efficacy trials conducted in the laboratory and pilot village trials that were evaluated by serological responses, resistance to field challenge and resistance of birds purchased from the villages to experimental challenge. Data were also collected to allow an evaluation of the economics of keeping village chickens and of the impact of Newcastle disease and of vaccination against Newcastle disease.

In the final efficacy trial, in which chickens were given vaccine on paddy rice or cooked white rice at 4 and 6 weeks of age and challenged at 9 weeks of age, there was almost complete protection. Earlier efficacy trials were not successful, possibly because the chickens were younger and because they were kept on wire rather than on the floor.

Field trials have used oral HRV4 vaccine on paddy rice (raw or autoclaved) and cooked white rice, HRV4 vaccine in drinking water, and a comparison of HRV4 and F vaccines given intranasally. Some of the field trials have been running for 2 years. In the absence of natural challenge, the HI antibody response to HRV4 vaccine by any route is modest. Highest titres were obtained by intranasal vaccination, and were similar to those achieved by giving F vaccine by the same route. Buy-back challenges have given variable results, with protection levels of from 23% to 85% for oral vaccine. Two intranasal doses of either HRV4 vaccine or F gave 92% protection on buy-back and challenge while only 40% of unvaccinated control chickens survived contact challenge. Field data was used to calculate protection in the face of outbreaks, using techniques that made allowance for the 'normal, background' death rate. For flocks receiving oral HRV4 vaccine the protection was calculated as 63% and for HRV4 in drinking water as 76%.

Heat resistant vaccines are considered more practical than conventional Newcastle disease vaccines in tropical countries. HRV4 and F vaccines gave similar levels of protection after intranasal vaccination, but thermal stability was an advantage of the HRV4 vaccine. Oral vaccination produced detectable immunity, but this was less certain than that resulting from conventional routes of vaccination. Oral vaccination will be the only practical way of protecting against Newcastle disease the near-feral chickens in some parts of Thailand and other Asian countries, until improved husbandry practices allow better application of heat resistant vaccines.

NEWCASTLE disease (ND) is a major cause of mortality of village chickens in Thailand, as it is elsewhere in Asia. However, unlike many other Asian countries, Thailand has been able to achieve some protection of its village chickens with conventional vaccine. Therefore, the project in Thailand has involved not the introduction of an entirely new method of vaccination into an unvaccinated population, but the comparison of oral HRV4 vaccination with conventional vaccination. Studies were also made of the possible exploitation of the heat-resistance of V4 vaccine without using its oral infectivity. HRV4 vaccine and the traditional F vaccine were compared when administered by the intranasal route.

Trials in Thailand have followed substantially the protocols in the 'Implementation Manual' being developed by the ACIAR project, with modifications to suit Thai conditions. We present results of laboratory efficacy trials and field trials that have been obtained in Thailand over the last three years.

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Conventional Vaccination Program in Thailand

The most common practice for control of ND among village chickens in Thailand at present is intranasal inoculation of the F strain vaccine produced by the Veterinary Biologics Division of the Department of Livestock Development (DLD). Vaccination programs are organised at the provincial level following the recommendations of the DLD: a first vaccination of chickens at 3 days of age, a second at 21 days, a third at 3 months, and thereafter at intervals of 3 months. This program is not widely used by small-scale raisers although vaccine gives protection. Capturing birds, which normally range freely around the village, is a great deal of trouble, as is handling the vaccine, which must be kept packed in ice in an ice bucket. The F vaccine is perishable and must be maintained at a temperature of between 2°C and 4°C to preserve its quality.

Efficacy Trials

Three efficacy trials based on the projects suggested in the protocol were completed during 1988 and early 1989.

In the first trial, Websters HRV4 and a locally manufactured F strain given on uncooked white rice at 3–5 weeks failed to confer significant immunity when the birds were challenged at 8 weeks. Either vaccine conferred almost complete protection when given intranasally.

In the second trial, with vaccination and challenge as in the first, HRV4 on broken rice was unsuccessful, while on paddy and cooked white rice, it was only partially successful (21% and 36% survival, respectively).

In the third trial, with vaccination at 4 and 6 weeks and challenge at 9 weeks, HRV4 on either paddy or cooked white rice conferred almost 100% protection. In this trial, in-contact birds were also successfully protected, in contrast to failure in the second trial. Details of these trials have been prepared for publication (Tantaswasdi et al. 1992).

It is not yet possible to give a complete explanation for the trial differences; however, two factors may have contributed at least in part to the considerably better results obtained in the third compared with the second trial. As stated, vaccinations and challenge were one week later in the third trial, and this may have allowed for greater immunological maturity. Again, in the third trial, birds were run on the floor rather than on wire as in the first and second trials, and this would have considerably assisted the spread of the virus by cross- and reinfection.

Field Trials

Field trials commenced in May 1989 in Singburi Province, about 140 km north of Bangkok. Initially, almost 50 households with some 1900 birds in six districts of the province were involved. Subsequently, operations ceased in one district, some households withdrew from the project and others were added. As of July 1991, 48 households with about 1700 birds were involved in the project.

Birds in one group of six households in one district were vaccinated intranasally with F strain at 2–3 month intervals; the balance initially received Websters HRV4 orally on paddy rice at monthly intervals. The resuspended vaccine in 5% skim milk was mixed into the paddy rice on the farms immediately before feeding. It was the aim to confine birds before vaccination although some households failed at times to do this. Mixing and vaccination was carried out by DLD district-level officers.

Beginning in July 1990 (14 months after the trials commenced), the paddy rice used was autoclaved and, in one district, cooked white rice was substituted. At one household, administering HRV4 vaccine in the drinking water was tried.

In November 1990 (18 months after the trials commenced), a trial comparing HRV4 vaccine with the conventional F strain vaccine, both administered via the intranasal route, was conducted in the district in which operations had ceased 6 months earlier in April 1990.

Buy-back challenges based on the project protocol were undertaken at 3 or 4 monthly intervals.

Approximately 25% of trial birds were bled at monthly or two-monthly intervals for haemagglutination inhibition (HI) testing. Information on flock size, mortality, and production was collected at monthly intervals.

Buy-back challenge trials

Paddy rice: Buy-back challenge trials were undertaken on five occasions.

In the first challenge, undertaken after the field trials had been in progress for 6 months, survival percentages were 41 in HRV4 orally vaccinated birds and 86 in birds vaccinated intranasally with F strain.

In the second challenge, after 10 months, only HRV4 vaccinated birds were challenged, and 23% survived.

In the third challenge, after 14 months, 32% of HRV4 vaccinated birds survived.

In the fourth challenge, 17 months after commencement of field trials, and 3 months after introduction of autoclaved paddy rice as the vehicle for HRV4, survival percentages of birds vaccinated in this

manner three times at monthly intervals were 42% in one district and 85% in another.

In the fifth challenge, 20 months after the beginning of the field trial, and about 6 months after the introduction of HRV4 on autoclaved paddy rice, survival percentages of vaccinated birds were 50% in one district and 80% in another.

Because of the turnover in the bird population as grown birds were sold off or consumed and replacements were secured, the number of vaccinations that challenged birds had received was variable. Care was taken, however, to select birds which had been vaccinated a number of times. Details of survival percentages in each household are shown in Tables 1 to 6.

Results of the first three challenge trials indicated a less than satisfactory level of protection. Part of the reason for this may have been errors in procedure at the beginning of the project due to the inexperience of the people involved. With time, performance improved. For example, at first a plastic bag was used for mixing the vaccine and paddy rice, but it was later found that a plastic bottle was more effective and convenient for mixing. Likewise, when some householders were unable to call all their birds to eat the vaccine-treated feed, they were given wicker cages to confine their birds on vaccination days. There was also concern about the adequacy of the titre of the HRV4 vaccine when fed, this being generally at a level of $>10^8$ ELD_{50}/mL when reconstituted but dropping to lower than $10^6 ELD_{50}$/10 g when mixed with paddy rice. The severity of the challenge (close confinement with large numbers of affected birds) and stress (village free-ranging birds transported and confined under strange conditions) may, however, have resulted in lower survival rates than might have been expected in a natural field challenge. Later, paddy rice was autoclaved before adding the vaccine, and crowding during challenge trials was reduced. This seems to have helped increase the level of protection, as indicated by the results of the fourth and fifth challenge trials.

Variation in results from one household to another was probably related to husbandry practices, the health of the birds, vaccine dosage, and care in feeding the vaccine. There seems to be a 'good farmer' factor in response to vaccination.

Cooked white rice: The survival rate in buy-back challenge trials of birds vaccinated three or four times with HRV4 on cooked white rice at monthly intervals was 60%, and that of birds three months after their last vaccination was 40% (Table 7).

Drinking water: The mortality rate in an ND outbreak in a flock which had been presented HRV4 mixed in their drinking water at monthly intervals for four months was 21%.

Comparison of HRV4 and F by intranasal route: Buy-back challenge trials were done on two occasions.

The first was conducted one month after the birds' first vaccination. The survival percentages were 33% (7 of 21) in HRV4 vaccinated birds and 37% (7 of 19) in F strain vaccinated birds.

In the second, one month after the second of two vaccinations separated by an interval of one month, the survival percentages of both HRV4 and F strain vaccinated birds were the same: 92% (12 of 13), whereas 2 of 8 of the unvaccinated control chickens survived challenge.

Potency of vaccine returned to the laboratory

When vaccine mixed with cooked white rice, with autoclaved paddy rice, and with untreated paddy rice in the field was titrated in the laboratory, the titre of the vaccine mixed with cooked white rice was always high, while that of vaccine mixed with either autoclaved or untreated paddy rice was lower and varied considerably.

HRV4 vaccine withstands temperatures even higher than those encountered in the field remarkably well. The titre of HRV4 vaccine kept at a temperature of 50°C for 24 hours and then titrated did not change; however, the titre of F strain vaccine under the same conditions dropped a thousandfold.

Serological response to vaccination

Laboratory results

Results from the efficacy trials showed a correlation between HI titre levels and survival to challenge. Results varied slightly but in general, survival was related to a mean $\log_2$ HI titre of 1.5 or above.

The challenge trials showed a similar but more variable trend. There were deaths of birds with titres above 2 in the HRV4 group, but not in the F group. Interpretation of results in the HRV4 group is complicated by the fact that there were natural outbreaks of ND at the time of challenge, and these, rather than vaccination, may have been responsible for some high titres.

Surviving birds showed large increases in post-challenge titre. The longevity of this was studied in six birds from the second challenge trial. The titres of these birds, over a period 15 months, remained high: mostly between 5 and 8. These birds survived a second challenge while controls all died (Table 8).

Field results

Data on monthly HI titres from the field bleeding were much more varied and complicated, for these titres were from a group of chickens whose membership was continually changing. Mean monthly $\log_2$ HI titres,

Table 1. Results of the first challenge trial — November 1989

District (No. households)	No. of birds	Age (months)	HI[a] antibody before challenge	No. surviving/ No. challenged	
		HRV4 vaccination on paddy rice			
Bangrachan					
1	19	41/2–7,13	1.60	8/19	9/22 (40.90%)
2	2	3–41/2	1.50	1/2	
3	1	6–7	1.00	0/1	
		Intranasal F vaccination			
Muang					
1	8	5–6	2.50	6/8	19/22 (86.36%)
2	8	6, >8	2.38	8/8	
3	5	$3^1/_2$–4	1.60	5/5	
4	1	4	1.00	0/1	
		Control			
Village chickens (contact)	22	3–6	0.82	0/22	
Lab chickens (contact)	5	3–4	0.20	0/5	
Lab chickens (challenge)	5	3–4	0.20	0/5	

[a] Haemagglutination-inhibition, geometric mean titre, $\log_2$

Table 2. Results of the second challenge trial — March 1990

District (No. households)	No. of birds	Age (months)	HI[a] antibody before challenge	No. surviving/ No. challenged	
		HRV4 vaccination on paddy rice			
Inthaburi					
1	11	5–7[b]	2.55	2/11	7/31 (22.58%)
Promburi					
1	9	4– 6[c]	2.11	1/9	
Kaibangrachan					
1	11	5– 8	2.45	4/11	
		Control			
Lab chickens (contact)	25	6–7	1.60	0/25	
Lab chickens (challenge)	5	6–7	1.00	0/5	

[a] Haemagglutination-inhibition, geometric mean titre, $\log_2$
[b] New household receiving vaccine just 4 times.
[c] Consumption of vaccine was poor.

including results from flocks and individual birds that were affected by ND, fluctuated considerably in both oral HRV4 and F-strain groups. Mean HI titres in the F group always rose following 3-monthly vaccination, and in these flocks all new chicks received intranasal vaccine in between flock vaccinations. Fluctuations in the mean monthly titre for the oral HRV4 group were associated with outbreaks of ND and introduction of new flocks and young birds to the sampling program.

Table 3. Results of the third challenge trial — July 1990

District (No. households)	No. of birds	Age (months)	HI[a] antibody before challenge	No. surviving/ No. challenged	
		HRV4 vaccination on paddy rice			
Inthaburi					
1[b]	2	8–9	0.5	0/2	
2	5	8–9	1.6	2/5	7/22
3	5	8–10	2.0	2/5	31.81%
4	10	8–9	2.2	3/10	
		Control			
Lab chickens (contact)	10	31/2	1.2	0/10	
Lab chickens (challenge)	4	31/2	1.0	0/4	

[a] Haemagglutination-inhibition, geometric mean titre, $\log_2$
[b] Vaccinated 3 times.

Mean $\log_2$ HI titre increased with the number of vaccinations given in the group receiving HRV4 orally on paddy rice until the third vaccination. Figure 1 shows mean HI titres for all HRV4 paddy rice birds that did not encounter field challenge by ND, and did not come from flocks that experienced ND outbreaks. After five vaccinations, the mean HI titre was 1.31, compared with a mean titre in birds that had received no vaccinations of 0.61. Mean titres for birds vaccinated intranasally with F-strain (shown in the same figure) were higher, and fluctuated more, presumably because of the lower number of bird samples. In addition, titres in the F-vaccinated group were higher than those in the HRV4 group from the beginning of the trial, since these birds had been vaccinated prior to the trial's commencement.

HI response to HRV4 vaccination on cooked white rice was very similar to the paddy rice vaccination. The mean HI titre was 1.35 after four vaccinations.

The mean HI titre in the one flock given HRV4 in the drinking water was 1.55 after two vaccinations. None of the birds had received vaccine in their drinking water more than four times.

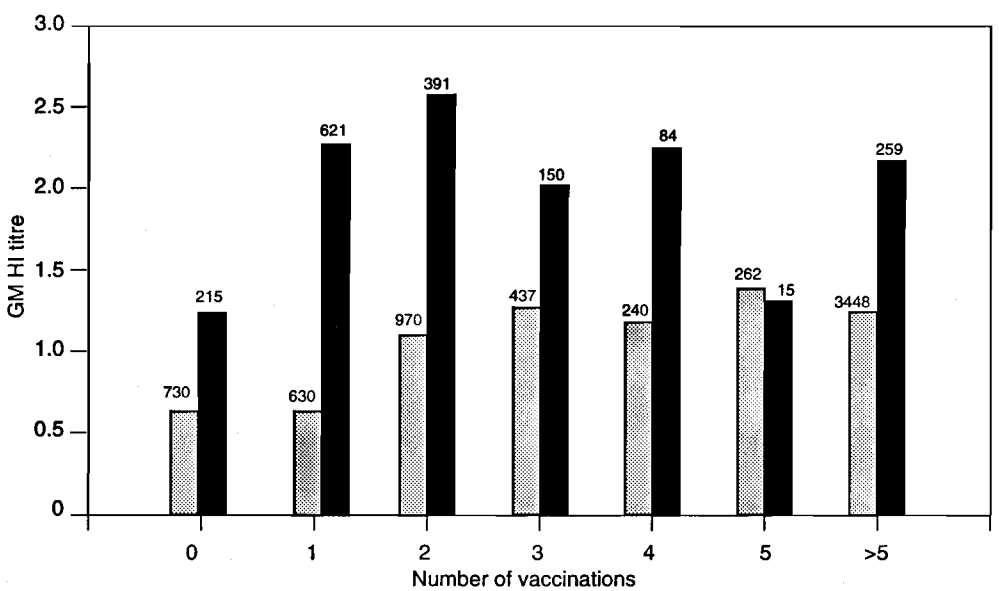

Fig. 1. GM HI titres of flocks receiving HRV4 (hatched bars) on paddy rice or intranasal F (without Newcastle disease challenge) (solid bars).

Table 4. Results of the fourth challenge trial — September 1990

District (No. households)	No. of birds	Age (months)	HI[a] antibody before challenge	No. surviving/ No. challenged	
		HRV4 vaccination on paddy rice			
Inthaburi					
1	6	4–6	1.17	3/6	
2	3	3–5	1.33	1/3	6/14
3	5	4–5	1.20	2/5	42.85%
Bangrachan					
1	2	5	3.50	2/2	6/7
2	5	4–5	3.00	4/5	85.71%
		Control			
Lab chickens (contact)	10	3	0.00	0/10	
Lab chickens (challenge)	5	3	0.40	0/5	

[a] Haemagglutination-inhibition, geometric mean titre, $\log_2$

Table 5. Results of the fifth challenge trial — January 1991

District (No. households)	No. of birds	Age (months)	HI[a] antibody before challenge	No. surviving/ No. challenged	
		HRV4 vaccination on paddy rice			
Inthaburi					
1	2	4–6	4.33	3/3	
2	2	4–5	2.00	0/2	5/10
3	5	4–5	2.60	2/5	50%
Bangrachan					
1	5	3–6	2.40	4/5	80%
		Control			
Lab chickens (contact)	5	5	1.00	0/5	
Lab chickens (challenge)	2	5	0.50	0/2	

[a] Haemagglutination-inhibition, geometric mean titre, $\log_2$

The flocks given intranasal HRV4, and those given intranasal F at the same time, developed similar patterns of mean HI titres.

Epidemiology of ND in the field

The original design for the trials was based on a concept of flocks passing from pre-epidemic condition (PE) through epidemic (EP) involving active infection or natural challenge, and then back to the pre-epidemic condition via a recovery phase (RE).

Recognition of EP conditions obviously requires evidence from the field. It was not easy to collect field or serological information to identify the EP phase. However, judgments made to identify village survey data as being related to EP conditions were based on all available sources of information: serology, laboratory reports, first-hand observations of officers, and analysis of mortality patterns. At times, the provincial and district officers sent dead or ill chickens to the laboratory, making it possible to better clarify causes of deaths.

The recovery (RE) condition following the EP condition was slightly easier to identify. Householders could usually be seen to be rebuilding bird numbers after any setback.

Improvement is needed in identifying disease trends

Table 6. Results of the sixth challenge trial — February 1991

District (No. households)	No. of birds	Age (months)	HI[a] antibody before challenge	No. surviving/ No. challenged	
		HRV4 vaccination on autoclaved paddy rice			
Inthaburi					
1	5	4–6	2.6	3/5	60%
Bangrachan					
1	4	5–6	1.25	2/4	50%
		Control			
Lab chickens (contact)	5	6	1.0	0/5	
Lab chickens (challenge)	2	6	0	0/2	

[a] Haemagglutination-inhibition, geometric mean titre, $\log_2$

Table 7. Results of the challenge trials of birds receiving HRV4 on cooked white rice

District (No. households)	No. of birds	Age (months)	HI[a] antibody before challenge	No. surviving/ No. challenged
1. One month after vaccination				
		HRV4 vaccination		
Kaibangrachan[b]	20	3–5	2.25	12/20
		Control		
Lab chickens (contact)	5	3	0.40	0/5
Lab chickens (challenge)	2	3	0.00	0/2
2. Three months after vaccination				
		HRV4 vaccination		
Kaibangrachan	20	5–7	1.65	8/20
		Control		
Lab chickens (contact)	5	6	0	0/5
Lab chickens (challenge)	2	6	0	0/2

[a] Haemagglutination-inhibition, geometric mean titre, $\log_2$
[b] Vaccinated 3–4 times.

in the field. To reach a stage where disease identification can be done confidently will require a great deal of money, time, and manpower.

Flock mortality

Flock mortality was based on all deaths from any cause, not only deaths attributable to ND. Among chicks, the average background mortality when there were no outbreaks of ND was 30% per month. Intestinal parasites, eye-worm parasites, diseases other than ND, and predation by dogs and pigs were some of the causes of death. Among growers, the average background mortality was 4.5% per month, and the causes of death were the same as those for chicks. In adults, the average background mortality was 1.68% per month, with most

Table 8. HI titres of birds surviving the second challenge trial

	HI antibody post challenge at month																
Bird no.	0[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15[b]	15[c]
1	2	12	12	12	12	10	10	10	10	11	9	10	9	10	10	9	10
2	3	12	7	7	6	5	5	6	6	6	4	5	3	5	5	1	9
3	3	14	11	10	10	8	8	9	9	9	8	7	7	7	7	7	9
4	3	14	17	11	11	10	11	9	9	10	9	10	7	8	8	8	8
5	2	14	10	10	9	8	9	8	8	9	9	9	7	8	8	8	8
6	4	12	8	7	6	6	7	7	6	7	5	6	5	6	6	5	9
	Laboratory chickens — contact control (introduced at month 3)																
1				2	2	0	2	1	1	0	1	0	0	2	2	1	Died
2				1	2	0	2	1	1	0	0	0	0	0	2	1	Died
3				1	3	1	2	1	1	0	0	0	0	0	2	1	Died
4				0	2	0	0	1	1	0	0	0	0	2	2	2	Died
5				2	0	0	1	1	1	0	0	0	0	2	2	1	Died

[a] HI titre before first challenge
[b] HI titre before second challenge
[c] HI titre 14 days after second challenge

deaths attributable to bacterial infections. When a natural outbreak of ND was suspected, samples were taken and sent to the laboratory for diagnosis. ND was diagnosed in 20 flocks during the period of the study.

Mortality in orally vaccinated village flocks was, on average, substantially lower than that of the F flock except in July and August 1989. Natural outbreaks of ND occurred. The mortality of the F village flocks rose in June and July 1990, and in February and June 1991, because a number of birds were attacked and killed by dogs or suffered unknown diseases. Since the number of F village flocks was small, these deaths caused the percentage of mortality to be high, although the total number of dead chickens was small.

Active ND infection (or natural ND challenge) was considered to be occurring sporadically for 1.64% of the 7622 flock/days of experience in the F village, while challenge conditions were suspected in the orally vaccinated village on 2.97% of the 28365 flock/days recorded.

Mortality during ND outbreaks was adjusted for the normal 'background' death rate to give mortality rates attributable to ND. These ND outbreak mortality rates for chicks, growers, and adults are summarised in Table 9. Mortality rates increased considerably during ND outbreaks in oral HRV4 flocks. In F-vaccinated flocks, there was no additional mortality attributable to ND in growers, and less than 10% in chicks and adults. Chicks in F-vaccinated flocks all received intranasal vaccine by one month of age, while in the orally vaccinated flocks, uptake of vaccine by chicks was much less reliable.

Birds vaccinated with HRV4 in the drinking water showed less mortality attributable to ND during outbreaks than birds receiving vaccine on rice.

Protection-in-face-of-challenge in flocks fed vaccination on paddy rice

$$= \frac{MRC - MRV4}{MRC} \times 100$$

Where:

MRC = mortality rate in control flocks during outbreak (In Thailand, the mortality rate in flocks that have never been vaccinated against ND is usually above 90%.)

MRV4 = mortality rate in V4 flocks during outbreak

Protection-in-face-of-challenge in flocks fed vaccination on paddy rice

$$= \frac{90 - 33.50}{90} \times 100$$

$$= 62.8\%$$

Table 9 Outbreak mortality rates attributable to Newcastle disease, in domestic village poultry vaccinated with oral HRV4 , intranasal F-strain, or drinking water HRV4

Village chickens :	V4 group :	F group :	DW group :
Chicks	23.69	9.12	18.46
Growers	53.27	-1.07	38.91
Adults	36.33	8.71	15.46
All birds	33.58	6.04	21.30
Gr + Ad	47.68	2.08	26.05

Protection-in-face-of-challenge in flocks fed vaccination in drinking water

$$= \frac{90 - 21.30}{90} \times 100$$

$$= 76.3\%$$

Production and economics

The average monthly offtake from the HRV4 group was 4.04 birds (3.84 sold, 0.56 consumed). For the F group, the offtake averaged 2.72 birds. (2.30 sold, 0.42 consumed). Egg sales and consumption in both groups were negligible; available eggs seemed to be utilised for flock propagation.

The value of output from both groups was around 150–170 baht/month. The monthly net value of birds to the family was about 70–71 baht/month. These amounts should be regarded as very rough approximations. In reality, the quality of data varied greatly. For example, feed costs include only purchases and not home-grown feeds fed by the farmer or foods found by the birds themselves in foraging which, at some of the households, constituted a significant portion of their diet.

Discussion

Efficacy trials have shown that HRV4 vaccine is able to induce immunity effectively whether administered intranasally or orally after mixing with paddy rice, cooked white rice or drinking water. The results of the field trials differed in some areas from the results of laboratory tests because of the conditions under which they were carried out. In the field, a number of factors could not be controlled. We suggest that a 'good farmer' factor is very important.

1. Householder cooperation

The birds of householders who conscientiously cooperated in the vaccination program had better resistance than those of householders who were lax. One householder lost almost his entire flock because he had very little interest in vaccination. On the other hand, at a neighbouring household, none of the birds was lost to Newcastle disease because the householder was conscientious in vaccinating regularly.

2. Husbandry

The conditions under which chickens were raised varied greatly from one household to another. Where the birds were well cared for and healthy, resistance was better.

3. Parasitic infection

Chickens in the village tend to be infected by parasites. In particular, coccidiosis was often found. This may have reduced the level of immunity.

4. Vaccine dosage

In the village, chickens range over a large area and thus the likelihood of a bird being reimmunised by picking up excreted vaccine virus is low. An adequate amount of vaccine is therefore important for appropriate immunisation. However, the dose of vaccine received by birds varied and, in some cases, may have been inadequate to induce immunity. Some vaccine virus was lost in mixing; this was perhaps because of destruction by some substances in the paddy rice. Some virus was probably lost in the ever present dust when the vehicle was scattered on the ground. There was also variation in the way the vaccine was presented to chickens. In some households, birds were confined under wicker cages; however, the number of birds under each cage differed and so the amount of feed each bird would consume was estimated. In other households, the birds were not confined but were called and the vehicle scattered on the ground. In this case, smaller birds tended to eat too little of the vehicle or did not approach for fear of the larger birds.

In spite of these factors, both field and laboratory evaluation showed that HRV4 vaccine can be used under field conditions. The percentage of protection varied with the method and vehicle employed in administration.

In the comparative study of intranasal administration of HRV4 and F strain vaccines, both gave a high level of protection although 25% of unvaccinated control chickens survived challenge. The HRV4 vaccine is considered more practical because of its stability.

The household at which HRV4 had been administered four times in drinking water before a natural ND outbreak occurred suffered a mortality rate of 21%, indicating the level of protection was over 75%.

The households at which HRV4 was mixed with cooked white rice experienced no ND outbreaks during the field trial period; in a challenge trial, the level of protection was 60%.

Paddy rice gave the least satisfactory results. Paddy rice does not seem able to consistently and dependably act as a vehicle for the vaccine. In the tests of the recovery of virus from vaccine brought back from the field, the titres of paddy rice samples varied considerably and were sometimes very low, unlike the titres of cooked white rice samples. Nevertheless, the percentage of protection in the fourth and fifth challenge trials was about 60%, which was close to the percentage of protection-in-face-of-challenge obtained by analysis of field data.

One area of inquiry in the field tests was to determine how many vaccinations were necessary to confer protection and how long protection would last. Study of these questions, however, was hampered by the limited number of birds and difficulties in following up vaccinated birds, which were sold. On the basis of limited data, it seems that HRV4 vaccine administered four or more times mixed with drinking water or cooked white rice confers protection and that a level of protection of 40% persists as long as three months. When paddy rice is used as the vehicle, it seems that monthly vaccination is needed.

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Oral Vaccination of Village Chickens with V4 Newcastle Disease Vaccine

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Abstract

An outline of the project in the Philippines is given. The V4 vaccine was first shown to comply with Philippines requirements for veterinary biologics. Initial efficacy trials of oral vaccine were unsuccessful. Because successful efficacy trials were reported from other countries, efforts in the Philippines were then devoted to field trials. These were undertaken in five villages in La Union province. Procedures for initial organisation, vaccination, serological testing, data collection and challenge tests are described.

AROUND the middle of 1987, ACIAR officials and researchers from University of Queensland came to the Philippines and made arrangements with Philippine Government officials, particularly those of the Bureau of Animal Industry, Bureau of Agricultural Research, and College of Veterinary Medicine, University of the Philippines, to conduct village trials among the local village chickens on the efficacy of V4 Newcastle disease (ND) vaccine. The project documents were signed sometime in 1988.

The veterinary activities of the Philippine component of ACIAR project number 8717 were started in December 1988. The initial works done were quality control evaluation of the V4 ND vaccine, as required by Philippine law on newly introduced veterinary biologics. The vaccine passed the tests on purity, safety and efficacy as used in the conventional vaccination procedures. The vaccine was tried twice under laboratory conditions on commercial chickens using unhusked rice and commercially mixed poultry feeds as carriers of the vaccine by the oral method of administration. The results were unsatisfactory, probably because of the additives and preservatives and other feed components incorporated into the commercial poultry feeds which rendered the vaccine virus incapable of inducing antibody production among the test chickens.

Oral vaccination among village chickens in five barangays (villages) at Rosario, La Union province was started in March 1989. The project site is about 215 km north of Metro Manila (about 3.5 hours drive).

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Objectives

The purpose of the project was to conduct efficacy and pilot village trials with the object of demonstrating that V4 ND vaccine confers protection against indigenous velogenic strain of Newcastle disease virus (NDV).

The organised poultry farms in the Philippines use commercial ND vaccine administered in the conventional way. The results of this type of vaccination have been satisfactory. However, the same method of vaccination cannot be used among village chickens. The village chickens of rural farmer-raisers in the Philippines are left roaming freely in the community.

Field Trials of Oral Vaccinations

Materials

A total of 165 households in 5 villages (barangays) are involved in the project. One hundred and forty-five households have their chickens vaccinated and the chickens in the 20 remaining households do not receive any vaccine. At the initial survey, about 3000 birds were in the project.

The V4 vaccine produced by Arthur Webster Pty Ltd, Australia, was the biological product on trial.

Methods

Before the actual technical activities were started, the prospective farmer-cooperators were assembled and given briefings on their role, the expected benefits from

the experiment, and the importance of the project to national progress.

In February 1989, blood samples were randomly collected from the 5 barangays to determine the benchmark HI titres of the chicken population in the project site. Serology tests were done at the National Animal Disease Diagnostic Laboratory (NADDL) of the Bureau of Animal Industry at Diliman, Quezon City.

One vial of the V4 vaccine was diluted in 50 mL of a 5% solution of non-fat powdered milk (powdered skim milk). This quantity was mixed with approximately 1 kg of paddy rice (unhusked rice grain). At the start of the trial, plastic bags were used as mixing containers. These bags were not durable. Breakages and holes were frequent occurrences. Later, empty plastic containers of ice cream or salad dressing and mayonnaise were used. These containers were provided with screw-cap covers, hence there were no spillages during the mixing which is done by shaking the plastic containers as the vaccinators are walking towards the houses of the farmer–cooperators.

Collection of blood samples was done at the same visit. The chickens of the households whose production data were monitored were mostly the birds that were bled for the HI test.

The birds were caught late in the evening and kept overnight in wire cages provided for the purpose. The wire cages were supplied by the project management and were fabricated out of rolls of welded chicken wire. This chicken wire was bought in Manila and brought to the project site and some of the cooperators did the fabrication of the wire cages.

The following morning, while some members of the teams were distributing feed coated with the vaccine, other members were collecting the blood by aspirating from the radial vein of either wing of the chicken. These birds were tagged. The tag numbers and colour code were recorded together with the owner's name and address.

Serology tests

These were done at NADDL at UP Diliman Campus in Quezon City. The results were forwarded to the BAR Computer Data Bank for compilation and analysis.

Buy-back challenge tests

Some birds from both households which received and did not receive vaccination were bought from the owners and brought to NADDL compound.

Upon arrival, these were given anti stress vitamins, and a week after they were also given anti parasitic medication. On the day these were challenged, their blood samples were collected and later subjected to HI test.

Commercial White Leghorn cockerels about one month old, which were raised in isolation without any form of vaccination, were mixed with the village chickens in an enclosure about 5 metres by 3 metres. Five to ten of the commercial chickens were infected with virulent strain of the locally isolated Newcastle disease virus through their eyes or nostril. From day 1 to day 20 the chickens were observed and the results were duly recorded. All survivors after the challenge were again bled and their sera were tested for HI titres. There were three buy back trials involving a total of 59 chickens. The average survival rate is 60%.

Production Data Collection

The 145 households were given a form to fill in at the end of every month, stating the number of eggs laid, hatched, sold or eaten; the number of birds, dead, sold or eaten during the period; and expenses incurred, if any, for medication, feeds, etc.

These forms are collected by village workers and sent to the BAR Computer Data Bank for processing and evaluation.

Future Plans

The next phase is to test 10 pilot barangays wherein the farmer–owners will be taught how to administer the vaccine – indicating the procedure of mixing with paddy or cooked rice, and time of feeding.

The vaccine will be given free in the beginning, at an interval of 2 months. After 6 months, blood samples will be collected from the flock to determine any increase of HI titres. If funds are available, buy back challenge tests will be undertaken.

Implementation of a wider vaccination program against NCD would be attempted with the active participation of non-government organizations (NGO).

V4 vaccine may be produced in the Philippines so as to sustain any national ND vaccination program.

Field Trials of Heat-Adapted V4 Newcastle Disease Vaccines for Village Chickens Using a Village-Based System of Vaccine Coating of Feed. II. Field Mortality and Serological Studies

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Abstract

Field vaccination trials using heat adapted, feed delivered V4 strains of Newcastle disease (ND) were conducted at three Indonesian trial sites. The trials were designed on a village or area basis, with poultry population and serological data being collected at a household level. The vaccine was diluted with local well water, then mixed with unhusked rice grains and fed within several hours to the village poultry. A calculated dose of 10^7 EID_{50} per 10 g of grain was supplied to the estimated number of birds in each household on a monthly basis. Considerable time and manpower from local animal health staff were required to achieve a reasonable, regular vaccination coverage of the villages. At the three trial sites, initial HI titres of mixed-age grower and adult birds exhibited low to high geometric mean (GM) titres, with relatively few birds exhibiting zero titres. The GM titre for each vaccinated group did not consistently increase following monthly vaccinations. Annual and monthly field mortality data from the three sites did not demonstrate marked differences between vaccinated and control groups. One site was exposed to a pronounced field challenge from virulent ND at the end of the local dry season. The vaccines administered by this delivery system did not adequately protect the village poultry against this strong challenge. Some apparent protection was provided at another site when field mortality data for control and vaccinated groups were compared under periods of apparent challenge from ND. The overall results showed an inconsistent protection. Possible reasons for the inconsistencies are discussed.

FIELD vaccination trials using heat adapted, feed delivered V4 strains of Newcastle disease (ND) were conducted at three sites within Indonesia, as described in paper by Darminto et al. in these proceedings. The trials were designed on a village or area basis to allow for the possible transmission of the vaccine viruses between birds which are in contact, and to permit reliable extrapolation of the trial results in the event that large scale area vaccination programs are introduced using a similar practical protocol for the mixing and delivery of the vaccine to the village poultry.

This publication describes the field mortality results from the field trials. The related virological results, including buy-back challenge investigations are described in the accompanying paper (Darminto et al. these proceedings).

Materials and Methods

The field trial was replicated across three sites within Indonesia that differed in climatic, socioeconomic and poultry production characteristics. The first site was at Cinagka, near Bogor, in West Java. It has an annual rainfall of 3500 mm, with a precipitation pattern characterised by a short and non-pronounced dry period. A high human and poultry population density is present, with high contact between adjacent household poultry flocks. A large duck population also exists at this site.

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The second site was at Sei Tapung, in Riau province, Central Sumatra. This site has an annual rainfall of 3000 mm with a similar short dry period. It is a transmigration site based on a local palm oil industry. Poultry population density is lower than for the Bogor site. The third site was near Kupang in the province of NTT, Timor. It has an annual rainfall of 1500 mm, with a prolonged and severe dry period. The villages at this site are sparsely populated and there is less daily contact between poultry from adjacent households.

Whilst all poultry in the defined areas or villages were considered to comprise the trial groups, mortality and serological data for each trial group were assessed by cluster samples based on approximately 20 households randomly selected from within each area. Poultry mortality data were collected during weekly visits to these households by a local animal health official. Serological investigations were performed monthly. All poultry belonging to the monitored households were identified by individually numbered wing tags. Blood samples were collected from as many tagged mixed-aged grower and adult birds as could be captured and were tested for haemagglutination inhibition (HI) titres as described in Darminto et al. (these proceedings), using the method of Allan and Gough (1974).

Heat resistant variants of the V4 strain of ND virus, HR.V4 (Websters Pty Ltd) batch 82360 provided by the Australian Centre for International Agricultural Research (ACIAR), and RIVS2.V4 (Ronohardjo et al. 1988) were used as vaccines at the Bogor and Kupang sites. In addition to these vaccines, a variant of another ND virus strain (Ronohardjo, unpublished data) was used in the Riau site. Paddy (unhusked rice grain) rice, derived from local sources was used as the food carrier for the vaccines. Concentrated virus was initially diluted with local well water and then mixed with the paddy rice as described in the accompanying publication (Darminto et al. these proceedings). The freshly mixed vaccine and paddy rice were then distributed to the households in the villages with instructions for it to be fed as soon as practical to the poultry. This generally involved delays of up 1 to 2 hours from mixing to feeding. Ten grams of paddy rice per bird, containing 10^7 EID_{50} (50% embryo infective dose) of vaccine virus, were considered a single dose. Vaccination was performed on a monthly basis. During the course of the trial, all households within the control areas received no alternative vaccinations. Furthermore, they had not received conventional ND vaccination (using F or Kumarov strains, provided by government sponsored programs) for the previous 2 years. Periods of apparent or non-apparent challenge to the village poultry from ND virus were determined by reference to: pronounced rises in HI titres at a household level; confirmed isolation of ND virus; or increased mortalities in grower or adult birds.

Results and Discussion

Considerable time and manpower were required by the local government animal health staff to achieve a reasonable vaccination coverage of the defined areas or villages. A better coverage was achieved when the staff delivered the paddy rice containing vaccine on a door-to-door basis. This was not always practical, and if a central collection point was established in the village a person was required to visit virtually all householders the day before delivery to inform and remind people to collect the vaccine. Additional manpower was required each month to catch the poultry and collect blood samples. The most successful system, as practised in the Riau site, was the capture of the birds from the trees on long poles during the night. Capture rate of tagged birds was generally less than 50%.

Low to high HI titres were observed in mixed-aged poultry in all experimental groups from all sites at the commencement of the trial, despite the fact that no ND vaccinations had occurred for over 2 years. Shortly before selection of these trial sites, preliminary serological monitoring of poultry in other possible trial sites was carried out. These sites, despite not having recently received any ND vaccines, also consistently showed high proportions of birds with elevated titres. Thus, it was not possible to find a trial site in which the village poultry could be considered to have had limited exposure to field challenge from ND. Should such sites have been found they would probably have been atypical.

At the three trial sites, the GM of the HI titres for the vaccinated groups was not effectively elevated by successive monthly vaccinations when compared with their initial levels or with those of the control groups (Figs 1–3). A GM of the HI titres within each group, generally provided an inadequate description, as the distribution of the logarithmic transformed data remained heavily skewed or was often bimodal. Reference to the percentage distribution of zero to low (<3 $\log_2$) or of high ($>=3$ $\log_2$) HI titres for vaccinated and control groups, on a monthly basis (Darminto et al. these proceedings), and the relating of field mortalities to movements in HI titres at an individual household level. The distribution of titres in each group was influenced over time by: the capture rate; the possible effect of successive doses of vaccine; the high turnover of the village poultry population; and the effect of challenge from field strains of ND.

Monthly GM HI titres for the vaccinated groups at the Bogor site demonstrated a sharp rise after 11 months (Fig. 1). These rises, which occurred in May, corresponded to the transition from the wet to dry season. In contrast, the control group did not show a similar rise. The rises in the vaccinated groups were due to sudden increases in the percentage of high titres (>5 $\log_2$), suggesting challenge from virulent ND virus. Monthly field mortality

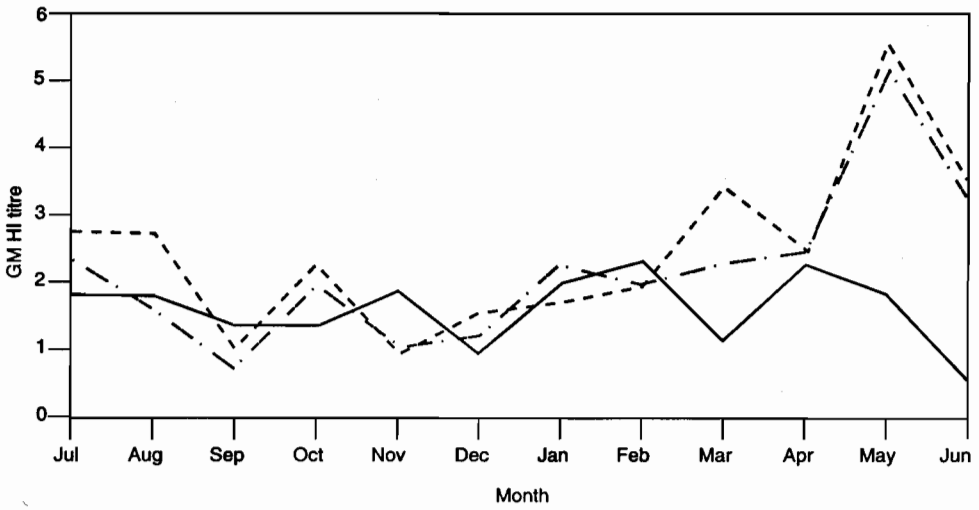

Fig. 1. Monthly geometric mean HI titres of vaccinated and control village poultry at Bogor:
–·–·–·– = HR.V4 - - - - - - - = RIVS2.V4 ———— = control

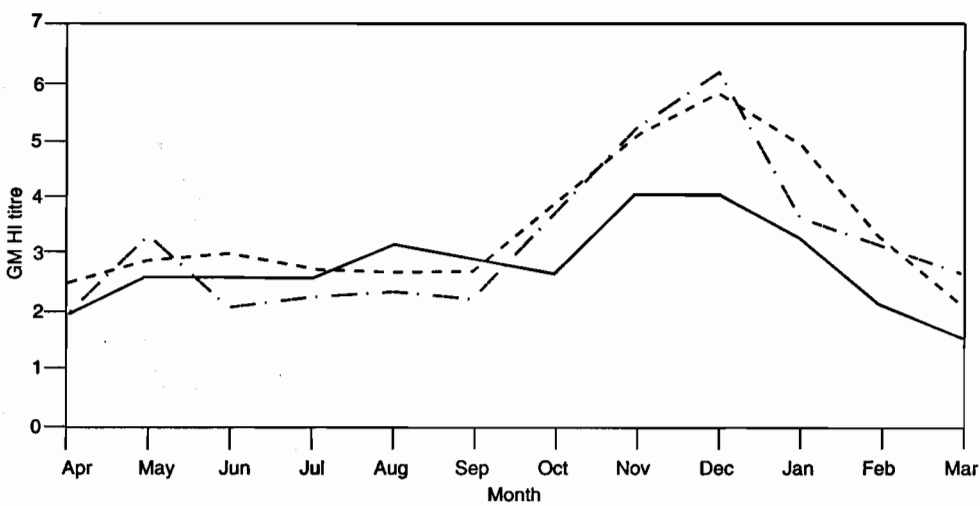

Fig. 2. Monthly geometric mean HI titres of vaccinated and control village poultry at Kupang.
–·–·–·– = HR.V4 - - - - - - - = RIVS2.V4 ———— = control

data, however, did not indicate any periods with greatly increased mortalities. Annual field mortality data from this site did not show marked differences between vaccinated and control groups (Table 1). However, separation of observation intervals into those of apparent or non-apparent challenge from ND virus, and comparison of the corresponding mortality rates between groups, did show markedly greater mortalities in control chickens whilst under apparent challenge (Table 2). The notable feature of the control group at this site was the consistently high resistance shown in the buy-back challenge trials (Darminto et al. these proceedings) and the presence of a high proportion of low to mid-range titres. This suggests exposure to, and inferred protection from, circulating field strains of low virulence ND virus. The presence, at this trial site, of a sizeable duck population, providing a probable reservoir of ND, may be a significant factor.

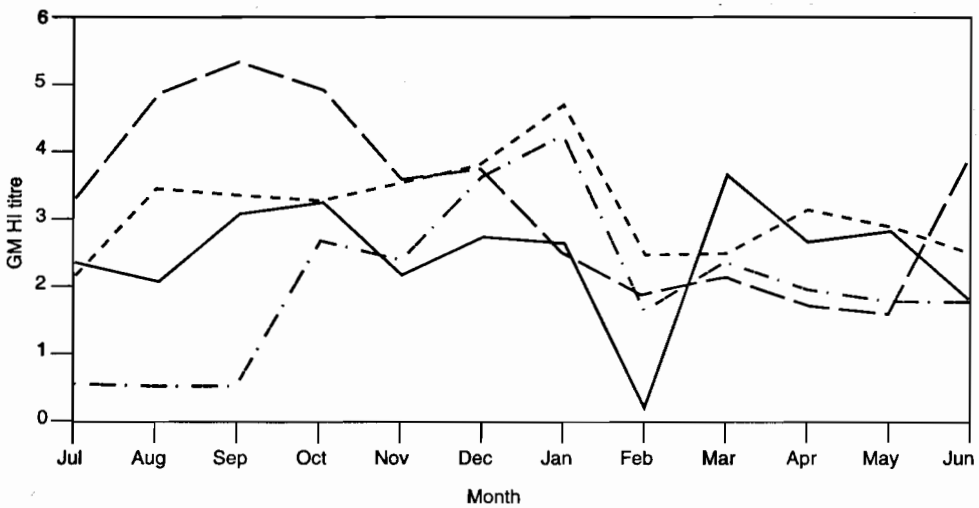

Fig. 3. Monthly geometric mean HI titres of vaccinated and control village poultry at Riau.
– – – – – = RIVS3 - - - - - - - = RIVS2.V4 –·–·–·– = HR.V4 ———— = control

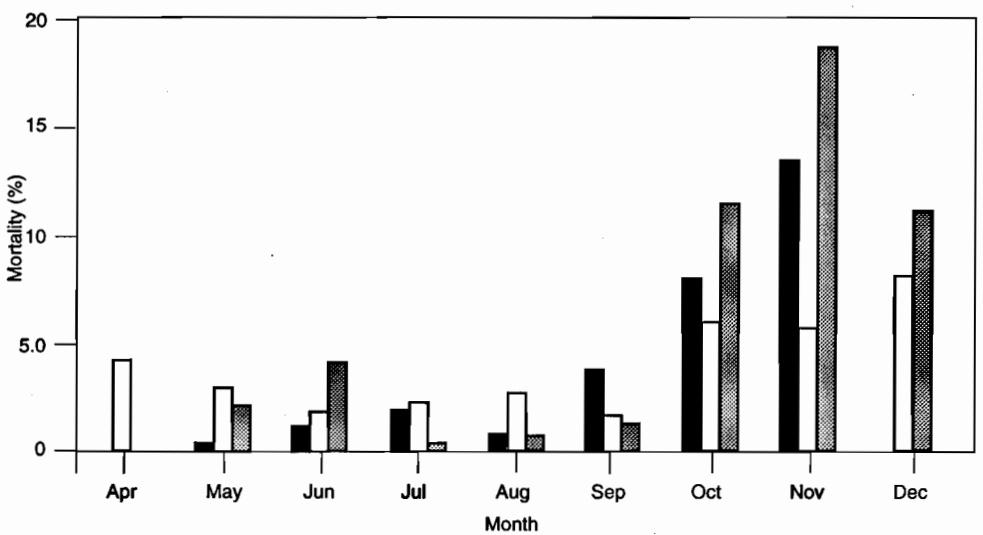

Fig. 4. Combined monthly percentage mortalities for all ages of vaccinated and control village poultry at Kupang. Mortality data for December and beyond are incomplete.

An important aspect of the Kupang site was the spread of a field challenge as evident from the rise in HI titres (Fig. 2) and in mortalities (Fig. 4), after 7 to 8 months, during October to December, coinciding to the transition from the prolonged dry to the wet season. Monitoring of the delivery of the vaccine at this site revealed that consistent coverage each month was difficult to achieve. This raises some doubts about the practicality of large scale vaccination schemes under some village conditions. While over 90% of householders whose birds were monitored for the mortality studies vaccinated their chickens regularly each month, about 60% of the other householders in the vaccinated groups did so. This would have reduced, but not eliminated, the risk of challenge to any individually monitored flock in the village. Despite this risk reduction, as a result of area vaccination, a high proportion of vaccinated households suffered significant consecutive monthly losses of chickens, growers and

Table 1. Annual percentage mortality rates in vaccinated or control groups of village poultry in the three trial sites

	Chickens	Growers	Adults
Bogor			
Control	22.7	7.1	1.4
RIVS2.V4	19.3	8.1	4.3
HR.V4	26.7	8.6	1.3
*Kupang**			
Control	25.6	8.6	5.0
RIVS2.V4	23.6	8.0	8.3
HR.V4	23.1	23.7	8.3
Riau			
Control	20.1	7.7	1.2
RIVS2.V4	9.3	7.1	2.8
RIVS3.V4	12.4	8.4	1.6
HR.V4	24.1	6.9	2.9

* Results do not include mortalities from December to February.

Table 2. Percentage mortality rates in vaccinated or control village poultry in Bogor under periods of apparent or non-apparent challenge from Newcastle disease

	No apparent challenge	Apparent challenge
Chickens		
Control	12.2	66.3
RIVS2.V4	17.3	32.6
HR.V4	26.1	49.1
Growers		
Control	12.2	17.3
RIVS2.V4	7.2	12.6
HR.V4	7.8	14.8
Adults		
Control	1.0	3.5
RIVS2.V4	3.6	1.9

adults. Some vaccinated households experienced total loss of growers or adults. This led to their disenchantment with the vaccine. Marked losses also occurred in the control group. Overall mortalities from March to early December for control and vaccinated groups are represented in Table 1. However, the figures do not represent the full mortalities from the field challenge, especially for the control group, in which significant mortalities were still occurring in December, as field mortality data for December to February has not been received from this trial site.

The annual mortalities for the Riau site showed no differences for growers and adults (Table 1). Chicken mortalities, however, appeared to be lower in the RIVS2.V4 and RIVS3.V4 groups (Table 1). Comparative mortalities between the groups for apparent or non-apparent challenge periods have not been calculated. The monthly mortalities showed a weak rise for the period August to November, especially in the HR.V4 group. This corresponded to a period of sudden rise in the HI titres for this group.

The field mortality data from these trials and the interpretation of the mortalities in conjunction with the field serological results show that vaccination with heat adapted V4 strains, using this feed-delivery system on an area basis, produces variable results. When these results are interpreted with those of the related buy-back challenge from these sites (Darminto et al. these proceedings) and the recent laboratory efficacy trials using the same feed-delivery system on genotypic 'village' poultry (Darminto and Daniels these proceedings) one can conclude that the V4 strains provide excellent protection when administered directly but when mixed in an aqueous solution with paddy rice, protection of poultry is limited. Endogenous viricidal compounds on the surface or from inner layers of the rice grain, which can be released upon mixing with water, may provide an explanation (R. Cumming, pers. comm.). These compounds, can limit the recovery of the V4 strain once mixed with paddy rice to times as short as several minutes; on other occasions virus can be recovered after several hours (R. Cumming, pers. comm.). Our trial was designed to adequately test a delivery system that was practical for a government sponsored village implementation program. Trials using feed-based delivery systems for V4 strains that relied on the field staff to distribute and feed the vaccine to the poultry within minutes of mixing are far from practical and do not allow for reliable extrapolation of results. The results from our trial strongly indicate that further work is needed to develop a reliable oral delivery system for the heat-adapted V4 strains that can be incorporated with locally available feed in a manner that is practical for extensive administration at the village or district level. This means that adequate virus must survive, once mixed with the food carrier, for at least 18 hours so that the vaccine can be distributed to the village householders in the late afternoon for feeding to their poultry early the following morning before the poultry disperse to forage.

Acknowledgments

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Australian International Development Assistance Bureau. The assistance of Dr Bruce Christie, Drh Maria, Drh Maks, Boy and the staff of the provincial Dinas Peternakan is gratefully acknowledged.

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Ronohardjo, P., Darminto, and Dirma, M.I. 1988. Oral vaccination against Newcastle disease in kampong chickens in Indonesia. In: Poultry Diseases, Proc. 112 the Asian/Pacific Poultry Health Conference, Surfers Paradise, Australia, 473–480.Table 1

A Simple Delivery System for Oral, Heat Stable Newcastle Disease Vaccines for Village Poultry

P.W. Daniels* and Darminto†

Abstract

V4 vaccine, delivered as simple eye drops or mouth drops, produced a high degree of protection against contact challenge with virulent Newcastle disease virus. A simple system delivering to individual birds a defined, adequate dose of heat-stable vaccine that is non-pathogenic but protective, and administered by smallholder farmers without direct government supervision, would offer substantial benefits over the current conventional system. Dosing from dropper bottles offers a simple solution for vaccinating village chickens against ND as an adjunct to other extension programs aimed at increasing production.

VILLAGE chickens kept under extensive management systems comprise a significant source of animal protein and financial benefit for people in Southeast Asian countries (Copland 1987). For example, the village chicken populations of Indonesia, Malaysia, and Thailand have been estimated at 174 (Anon 1989), 6.5 (Oh 1987) and 40 million birds (Danvivatanaporn 1987), respectively. Mortalities from viscerotropic velogenic Newcastle disease virus (VVNDV) infections substantially reduce the benefits from village chicken production, and are a constraint to even modest intensification of the production system (Ronohardjo 1984; Anon 1986).

Protection of village flocks in several countries has proved to be difficult by conventional vaccination procedures (Ronohardjo 1984; Hussein 1987; Pariyakanok 1987). Indonesia has a program to vaccinate village chickens every 3 months. Chickens under 4 weeks of age are vaccinated by eyedrop with strain F, while birds over 4 weeks of age are vaccinated intramuscularly with live Komarov strain, both vaccines being produced by the government vaccine laboratory (Moerad 1987). Vaccinations are given by officers of the Department of Animal Husbandry in each province. Problems include inadequate supplies of vaccines, lack of refrigerated storage facilities leading to variable decreases in potency, impractical methods of application, and inadequate transportation for field staff to carry out the work (Anon 1986; Moerad 1987).

Since such problems are common to many countries in the region and elsewhere, the potential benefits of a heat-stable, food-delivered vaccine have been identified (Spradbrow and Samuel 1987; Ideris et al. 1990). The strain V4 of Newcastle disease virus (NDV) isolated in Australia (Simmons 1967) has been shown to be nonpathogenic (Hall et al. 1967; French et al. 1967; Westbury et al. 1984; Hamid et al. 1990) and extremely effective in protecting commercial chickens against Southeast Asian strains of VVNDV (Spradbrow et al. 1978, 1980; Ibrahim et al. 1980; Bell et al. 1991). Infectivity of strain V4 of NDV has been shown to be substantially resistant to heat treatment (Kim and Spradbrow 1978; Westbury 1979). Vaccination experiments using a heat-adapted V4 strain of NDV fed mixed in feed showed good levels of protection (Ideris et al. 1987).

A food-delivered system of vaccination was proposed as it potentially solves two of the problems of conventional village chicken vaccination programs: the need for trained staff to administer injectable products; and the difficulty of catching individual birds for administration of vaccine. In Malaysia, a system of mixing vaccine with either pelleted feed or wheat grain at central vaccine preparation units, sealing the coated feed in plastic bags labelled with instructions, and distributing it to farmers for their use has produced good results (Ideris et al. 1987).

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In Indonesia, researchers initially feared that the cost of purchase of a carrier grain and the logistics of distributing vaccine-coated grain to all rural areas may be prohibitive. If the village chicken population of 174 million were to be vaccinated at least 4 times annually, as recommended in the conventional vaccination program, with 10 grams of grain per vaccination, then 7000 tonnes of grain would be needed per year. This product would then have to be distributed throughout an archipelago of 13,677 islands spanning a length of 5600 km and a breadth of 1600 km and with a land area of 1.8 m square kilometres. Initial research in Indonesia has therefore been directed towards developing a cheaper system, involving delivery of concentrated vaccine to villages for dilution in local water then mixing with available local foodstuffs, particularly unhusked rice (Ronohardjo et al. 1988).

Early results with this system were promising, with 76% to 97% protection being obtained in laboratory studies and 30% to 70% protection in field trials in villages (Ronohardjo et al. 1989). However, disappointing results were subsequently obtained during outbreaks of Newcastle disease (ND) when substantial mortalities occurred in vaccinated villages, and where buy-back challenge tests of birds vaccinated in villages have resulted in variable protection rates (Darminto, unpublished data). It has been suspected that vaccine virus applied to grain may sometimes become inactivated through reactions with the grain itself (Cumming, pers. comm.; Ronohardjo, unpublished data). Other potential problems include possible inactivation of virus on grain by environmental factors, an inadequate dosage through birds not eating enough coated grain, and poor vaccination coverage in the flock due to lack of enthusiasm for the procedures on the part of farmers and their advisers.

For some field situations it may therefore be appropriate to reassess the system of delivery of vaccine to reduce the effects of these variables. The factors favouring a vaccine based on the strain V4 are its heat stability, low pathogenicity, proven immunogenicity leading to protection when given in adequate doses, and the possibility that it may spread to neighbouring birds thereby helping to confirm protection on those inadequately vaccinated (Samuel and Spradbrow 1989). None of these properties depends on a food-delivery system, which has been advocated as a means of protecting birds that cannot be caught for government officers applying conventional vaccines.

One of the aims of an improved ND vaccination system is to encourage increased production and associated improved husbandry inputs by small farmers. Chickens protected against ND will be more valuable, and consequently the semi-feral proportion of the chicken population should decrease as farmers increase their level of care. If farmers adopt the simple practice of penning the birds at night, they are available for either food-delivered vaccination or individually applied vaccination.

Packaging the vaccine for individual bird application would allow effective vaccination without the complications introduced by attempting to coat vaccine on grain in the village, and trying to ensure that the targeted birds eat sufficient coated grain to receive an adequate dose of vaccine. A suitable, easily taught system could be based on conventional eye-drop application or, alternatively, the oral delivery of a few drops of vaccine. Application of V4 strain of NDV by mouth to the palatine cleft has protected birds against challenge with pathogenic strains of NDV (French et al. 1969; Spradbrow et al. 1978), and application of V4 as a commercial vaccine in drinking water is effective in protecting chickens against challenge with VVNDV (Ibrahim et al. 1981). Therefore, a small laboratory trial was conducted to confirm that the heat-adapted strain of V4 delivered by mouth drop could protect village chickens against challenge with an Indonesian strain of VVNDV.

As part of a broader study (Darminto and Daniels 1991, this volume), 70 village chickens were purchased at a day old, individually identified and reared in positive pressure plastic isolator tents. Vaccination began at 7 weeks, when haemagglutination inhibition (HI) serological testing (Ronohardjo et al. 1988) indicated maternal antibodies to NDV were undetectable. A heat-adapted strain of V4 NVD[1] stored freeze-dried at –20°C was reconstituted in the recommended volume of sterile distilled water to give a titre of $10^{8.7}$ EID_{50}/0.1 mL. Twenty chickens in one isolator were vaccinated by eyedrop with a dose of 10^7 EID_{50}, while 20 in another group were vaccinated with 10^7 EID_{50} in 0.1 mL of sterile distilled water *per os*. A third group of birds was kept uninfected as controls. The experimental chickens were revaccinated using the same doses and procedures 3 weeks later.

Serum samples were collected weekly from all chickens. At three weeks after the second application of vaccine, the birds were challenged with ITA strain of VVNDV (Parede and Young 1990) by contact with 10 unvaccinated, sero-negative chickens that had been infected with challenge virus by eyedrop.

The group mean titres (log_2) in response to vaccination were a little higher after eyedrop application (4.4) than after oral dosing (3.4). The survival of challenge was 17 of 17 birds for those vaccinated by eyedrop and 14 of 17 for those vaccinated *per os*. These results show that village chickens can be vaccinated successfully by a simple oral application of V4 NDV in liquid form, consistent with earlier observations (French et al. 1969).

[1] Arthur Webster Pty Ltd, Sydney, Australia

It remains to devise a simple and robust delivery system for heat-adapted V4 strain ND vaccines for use in the field in villages where the numbers of chickens per flock are small. One solution may be to use small plastic squeeze bottles, as already used for the delivery of eye drop vaccines. The optimal number of doses per bottle must be determined, and appropriate diluents and stabilisers must be chosen to avoid the need for refrigeration close to the point of use. Further work must be done with farmers to ensure the method of use is readily accepted and easily learned. Indonesia already has a system of encouraging farmers to form 'farmer groups' for the purposes of adopting new technologies. Involvement of farmer groups would optimise the use of multiple dose dropper bottles as well as providing mutual support and encouragement.

In further considering the problem of restraint of individual birds, it is evident that the owners of even semi-feral village chickens must be able to catch the birds to benefit from sales or home consumption. Capture is most easily accomplished after dark, when roosting birds are more easily handled. Strategies observed by the authors include climbing into the trees or roosting places to catch birds, or more easily, nudging birds in high roosts with a long bamboo pole thereby inducing each chicken to step onto the pole. Such birds can then be brought slowly to the ground and restrained.

Any successful vaccination system will require effort on the part of the owner. Food-delivered systems require farmer training in the management of the birds to ensure good coverage and adequate dosage (Ibrahim et al. 1987). It may require no more effort by owners to restrain birds, or at least valuable breeding stock, for individual vaccination, if the vaccine could be effectively given by the farmer himself. Spread of vaccine virus to in-contact chickens may be more efficient from birds receiving a high titre of vaccine rather that a sub-optimal dose (Darminto and Daniels 1991, this volume).

If individual bird application of vaccine in the extensive system of husbandry requires that the vaccine be given in the evening by the farmer without direct supervision, as proposed, the method of delivery must be simple. The system must be both technically and conceptually suitable for the training of farmers by extension workers. It has yet to be established whether farmers would find the concept of preventing 'sleeping sickness' of chickens by eye drop vaccination more appealing than giving the medicine by mouth but, in practice, they may find oral drops easier to administer effectively. Extension trials would be appropriate.

Acknowledgments

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Newcastle Disease and Village Chickens in Other Parts of Asia and Africa

Newcastle Disease in Village Chickens in North, West and Central Africa

J.G. Bell*

Abstract

Throughout Africa, Newcastle disease (ND) is reported to be the single greatest pathological constraint in village chickens. The results of prevalence studies in Morocco, Mauritania, Benin and Cameroon are reviewed. In each case, unvaccinated chickens were found to be seropositive and, where virus isolation was attempted, velogenic ND virus was found. The results indicate a high prevalence of the virus throughout the regions studied.

A vaccination trial undertaken in villages in Morocco showed that vaccination with live or inactivated vaccines could largely protect against the mortality seen in the control village.

AT a Centre Technique Agricole international seminar on small-holder rural poultry production held in Thessaloniki, Greece, in October 1990 — at which a majority of countries in Africa were represented — all countries reported Newcastle disease as being the single greatest pathological constraint.

In this communication, the results of prevalence studies in Morocco, Mauritania, Benin and Cameroon are presented and the results of a vaccination trial in Morocco summarised. Although these results have already been presented in Europe (Bell 1991) they are repeated here in order to permit comparison of the situations in Africa and Asia and discussion with our Asian colleagues.

Prevalence Studies

Village chickens flocks in six different regions of Morocco were studied for the presence of ND (Bell and Mouloudi 1988). Three of the regions contained commercial poultry farming and three were isolated mountainous regions with no commercial poultry farming. Serum samples and tracheal swabs were taken from 100 chickens in each region. Antibodies against ND virus (NDV) were found in chickens from every region. Forty-one isolates of NDV were obtained, including some from chickens in every region. Two virus isolates from each region were characterised and all were found to be velogenic. Thus, village chicken flocks throughout Morocco harbour a reservoir of virulent NDV, independently of commercial farms.

A similar survey was undertaken in Mauritania (Bell et al. 1990b). Serum samples and tracheal swabs were taken from 80 chickens in village poultry flocks in each of three different regions. Antibodies against NDV were detected in 4.6% of chickens. Six isolates of NDV were made, of which four formed plaques on chicken fibroblast monolayers, indicating virulence.

In Cameroon (Agbede et al. 1991), blood samples were taken from 60 chickens in each of three regions, comprising equatorial forest in the east, a mountainous region in the west, and a savanna region in the north. Seropositivities for NDV were 52%, 48% and 47% respectively, with an overall mean of 49%.

In Benin (C.A.A.M. Chrysostome et al. unpublished data), seropositivities for NDV of 56%, 75% and 69% were obtained in village chickens in three ecologically different zones in the south, centre and north of the country, respectively. In both Cameroon and Benin, a wide range of titres was observed.

The seropositivity in the absence of vaccination and the virus isolations show that NDV is present in village poultry in all four countries studied. That these results are typical for Africa as a whole was suggested at the recent meeting in Greece. While other pathogens are present, in the face of the high mortality caused by ND they are much less significant. In addition, the non-intensive rearing conditions mean that their effects are less marked than they would be in intensive farms.

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Vaccination Trial

All the poultry in each of four distinct Moroccan villages were vaccinated against ND using Hitchner B1 and inactivated vaccines (Bell et al. 1990a). Poultry in a fifth village were monitored as controls. Mortality in the poultry was followed for 20 weeks after the first vaccination and blood samples were taken every 4 weeks from chickens for estimation of antibodies against NDV. Sixty-three percent of the chicken population and 60% of the turkey population in the control village died during the 20 weeks of observation. Necropsied birds showed lesions consistent with ND. Mortality did not exceed 22% in the vaccinated villages.

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The Productivity and Nutrition of Village Chickens in Sri Lanka

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Abstract

About 15% of the national egg production in Sri Lanka is derived from village chickens. Observations were made on the production characteristics of village chickens and the feed resource base available in the village. About half the families in the four villages that were studied kept village chickens. The average flock size was about 10 birds. The hen day production was 30% and the hatching rate was 67%. The mortality rate was 65% in chicks to 70 days of age. Household refuse comprised more than 70% of the crop content of village hens. It is suggested that productivity could be increased at small cost by reducing the mean age of the hen flock, by better control of laying sites and by giving young chicks preferential access to household scraps.

THE total poultry population in Sri Lanka is about 9 million birds (Livestock Census and Statistics 1989) consisting of intensively reared improved exotic birds and extensively kept native village chickens. The number under village poultry systems has been estimated at a little over 2.5 million (Fonseka 1987). This number has remained static for several years. At present, village poultry contributes about 15% of national egg production; its share of the poultry meat supply is not known.

The village chicken found today is a descendant of the jungle fowl (*Gallus gallus*) of Southeast Asia. Although there has been an introduction of exotic genotypes to the country at various times, their impact in upgrading the village chicken has been minimal. Therefore, some of the distinct features of their ancestors still remain to give them a separate identity as 'village chickens'.

The rearing of village chickens is prevalent in rural areas, where the social and economic standards of the people are generally lower than in the urban areas. Further, unemployment is high and female labour is often underutilised. Therefore, poultry keeping helps to supplement incomes and the nutritional status of those families. The rearing of village chickens requires little or no inputs, hence it remained less affected by the constraints applicable to intensive farming. This is very important to developing countries like Sri Lanka where most of the poultry feed ingredients are imported.

Although village chickens make a large contribution to the social, cultural, nutritional and economic needs of rural farmers, the industry has been overlooked in the past. Basic information on productivity and nutrition of these birds and their contribution to rural life had not been assessed. The study reported here therefore sought to investigate the production charactristics and feed resource base for village chickens. Some aspects of the socio-economic background of farming families were also studied. It was hoped that the study would primarily help to fill the present information gap and also provide the foundation needed for any future development programs seeking to optimise village chicken production.

Materials and Methods

Four villages close to the Veterinary Research Institute, Peradeniya, were selected for the study. The village chicken production system was surveyed in 34 families for one year. During the year, one family which was already keeping some hybrid hens opted for small-scale intensive egg production, six families sold out, the flock of one family died and one householder became ill. The production data were derived from the remaining 25 flocks. Each family was given a data sheet to record inputs and production from their flocks. The information was collected during fortnightly visits to the household. The

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scavenging activities of each of 15 hens were recorded in the field during a full day. The next day those hens were collected from the field late in the morning while scavenging, and taken immediately to the laboratory where blood was collected for calcium and phosphorus determinations, after which they were killed with thiopentone sodium. The contents of the crop and gizzard were collected, weighed and identified visually. The relative contribution of each item of the diet was determined by weighing. Full-day collections of refuse from individual households were made on 14 occasions, weighed, identified visually and analysed.

Proximate composition analyses were done by the standard AOAC (1970) procedures. Calcium in feed and crop samples was determined by the method of Rowe (1983). Calcium in plasma was determined with a diagnostic kit (Hoffman, La Roche and Co. Ltd.; Basle, Switzerland). Plasma phosphorus was measured by a modification of the method of Fiske and Subba Row (1925).

Results

Socioeconomic background

Approximately half of the families in the four villages in which the study was conducted kept village chickens. Twenty-nine of the households keeping records had primary education, four had secondary education and there was one graduate. None had knowledge of poultry keeping, nor did they normally keep written records of their flocks. Nine were tradesmen, two were employed in retail business, three were students, ten were labourers and ten were unemployed.

Twenty-two percent of families kept chickens only for eggs for family consumption. The remainder of the families consumed some of the eggs and sold the rest. Eggs were sold only to neighbours at prices 10–20% higher than the larger commercial eggs. The difference in price is attributed to better flavour, colour and nutritive value. One family ate their own chickens, but the others bought or sold birds for consumption for festive occasions. The prices paid were also higher than those paid for intensively reared birds. Usually, the women received the income from the flocks, and used it mainly for food and school requisites.

Husbandry

All households reared their own replacement chickens and had a simple night shelter on the ground for them. This was made from local materials such as bamboo slats, wattle and mud, and palm leaves. Only one household had a nest in the night shelter; all of the others had a nest in the family house where it doubled for laying and for incubating eggs. The birds were released for 11.7±0.5 hours each day, virtually all the daylight hours. No water was provided, the chickens being dependent on domestic slops and puddles. No special provision was made for feeding, and the household refuse was disposed of out the door as it became available. Refuse was usually disposed of twice a day, once before 0830 h and at a second time either in late morning or late afternoon. The birds, presumably familiar with the routine of household food preparation, were usually gathered around the doorway prepared for disposal. Three families provided a small quantity of commercial starter ration for their young chicks.

Flock composition

The birds appeared to be typical village chickens with mixed coloured plumage. The average flock size was:

chicks (0–8 weeks)	2.4 ± 4.8
pullets (8 weeks to laying)	1.4 ± 2.0
cockerels (8 weeks to maturity)	2.3 ± 1.5
laying hens	4.0 ± 2.1

Setting of eggs

Eggs for setting were collected in the household over several days, then all placed simultaneously under a broody hen. No selection was exercised over the source or the quality of the eggs. An odd number of eggs was regarded as favourable and the number of eggs in 80% of the sets was odd. The mean size of a set was 9.4±3.0 (n = 66).

Hatching of eggs

The mean hatching rate was 67.0±32% (n = 66). The hatchability was not affected by the season, or by the number of eggs in the set. Broody hens were sometimes lent to neighbours to incubate and to raise the brood. The mean weight of the chicks at hatching was 27 g.

Raising of chicks

The hens stayed with the brood for up to three months, by which time the young growers had separated themselves. The growth rate was very variable, body weights ranging from 41 to 100 g at 20 days, and at 70 days 142 to 492 g, with a mean of 313±163 g. The mortality rate was high with some 65% dead within 70 days (Fig. 1). Losses were attributed to predators. There was no correlation between brood size, season of hatching and survival to eight weeks.

Egg laying

The mean age at first lay was 211±36 days (n = 50). When the pullets weighed 1160±227 g (n = 28). The mean weight

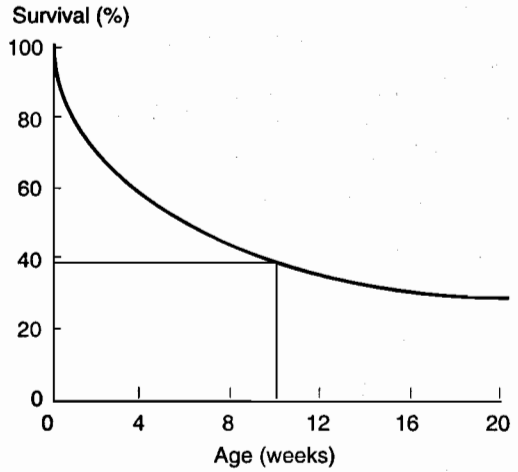

Fig. 1. Survival of chicks in scavenging flocks

of laying hens was 1259±209 g. Mature cocks weighed 1778±310 g. The laying pattern was variable, some birds laying every other day, others laying for two or three days then resting for one or two days. The mean length of the laying period was 34±13 days, and the batch size was about 20 eggs. The mean egg weight was 48±3 g (n = 76). If the hen was not given a set of eggs to incubate, she resumed laying after about three weeks. If she was incubating eggs, then there were three weeks incubating, up to twelve weeks with the chicks, then a further two weeks before laying recommenced. Some eggs were laid away from the homestead and were lost to neighbours or predators. The hen day production for all birds was 30% and, on a monthly basis, did not vary significantly during the 12 months of the study. The hen day production for the different flocks ranged from 11 to 57%. There were no significant relationships between hen day production percentage and family size or flock size.

Activities of hens

The only times that hens rested during the day were around noon on cloudless days and during heavy rain, when they sheltered under trees. Three of the 15 hens whose activities were recorded laid eggs, all in mid morning. Twelve of the 16 mated and those which did mate did so an average of four times in the day. Sixty-four percent of the matings took place in the morning. There was no relationship betwen mating and the presence of an active ovary. More than 90% of the day was spent in scavenging — walking, scratching, leaf turning and pecking. Cattle and goat pen areas were favoured scavenging areas. All hen activities were individual, birds being together only when feeding on household waste, in the early morning and evening, and when mating. They drank an average of 3.3±2.2 times a day.

Household refuse

The quantity of household refuse accumulated in a day averaged 460±210 g from 14 collections. Thirty-six percent of the refuse was cooked rice, 30% coconut residue, 8% broken rice and 26% sundry (vegetable trimmings, bread, dried fish and scraps). The compositions of the major components and of pooled refuse are presented in Table 1.

The feed intake

More than 70% of the crop contents, averaged over 15 hens, was household refuse, being cooked rice (28%), coconut residue (16%), vegetable waste (9%), broken rice (4%) and sundry household materials (15% bread, egg shell, cooking waste and dried fish). The contribution from the environment was mainly grass shoots (13%), 8% small metazoans (earth worms, snails, ants and flies), and paddy rice (7%) a proportion of which would have come from the household refuse. The mean composition of the contents of crops is set out in Table 1. The gizzard contained large amounts of insoluble grits. Most birds had a substantial accumulation of yellow abdominal fat and a moderate layer of yellow subcutaneous fat.

The apportionment of output

The egg consumption averaged 4.1/person/month, ranging

Table 1. The average compositions of the major feed components, and of the feed intake, of scavenging hens

Component	Dry matter %	Crude protein %	Ether extract %	Crude fibre %	Ash %	Ca (mg/g)	P (mg/g)
Household refuse	43.2	10.3	7.2	2.2	1.4	0.8	4.0
Cooked rice	30.0	6.5	1.0	0.6	1.0	0.3	1.3
Coconut residue	24.1	6.9	38.1	8.9	1.1	1.1	6.0
Broken rice	85.9	9.0	1.3	1.5	3.2	0.5	1.4
Crop content	34.4	9.4	9.2	5.4	16.0	0.8[a]	0.9

[a] Does not include one value of 27 mg/g from a crop containing a snail.

from 0.7 to 14 between the households, being 71% of total egg production. All but one family sold some eggs, the average annual income from eggs being Rs. 250±264, with a range from – to Rs. 1075. Sixty-one percent of families sold birds, yielding average income from sale of birds of Rs. 224±400, ranging up to Rs. 1905. The total annual income from the flocks averaged Rs. 475 ±530, ranging from 0 to Rs. 2399. The daily wage for a casual labourer is approximately Rs. 55 (US$2 = 43 SL Rupees).

Plasma measurements

Calcium in plasma was 11.0±3.2 mg/100 ml, including a value of 18.9 in a hen with an egg undergoing shell deposition in the oviduct. Phosphorus levels averaged 2.5±0.5 mg/100 ml.

Discussion

The production data observed were similar to those recorded for scavenging chickens in other studies. The data from Thailand (Janviriyasopak et al 1989) may reflect the emphasis on meat production, the flock size (30) being larger than that of the present study, the growth rate of chicks slightly higher (4.2 g/day), the mortality rate (30% to 12 weeks) much lower and the egg production (15% hen day production) about half. The consumption of birds was much higher at 13/flock/year. In Indonesia (Kingston 1980; Kingston and Cresswell 1982), the mortality rate of chicks (69% to 6 weeks) was even higher than in the present study, growth rate was slightly better and egg production was lower (20% hen day production).

The per capita consumption of eggs in Sri Lanka is 4 eggs/month (Food Balance Sheet 1989) and half the participating families have not reached that level. If they had consumed all of the eggs produced by their chickens, all but three families would have reached the national per capita consumption level. Nevertheless, the small cash flow generated by the sale of eggs may be more important than the eggs. The average incomes from the sale of eggs and birds are about equal, but that from eggs is more evenly distributed among the families.

The scavenging village chicken has cultural, social, nutritional, economic and sanitary functions in the life of the community. The feed resource base for the chickens in the traditional husbandry system described has no alternative use and, if village chickens were not present, other scavengers, particularly dogs and crows, would have performed that function with no associated productivity.

No inputs of significance were provided to the flocks described, and as subsistence farmers are unable or unwilling to pay for inputs it may be possible to increase productivity by more efficient use of the existing factors of production. The householders are reluctant to part with their hens, so one cause of low productivity could be the lower egg-laying capacity of older hens. More frequent turnovers of hens might also improve income if that is important, and some flocks might be more efficiently utilised for meat production for the festive season market, rather than their existing low efficiency egg production. The mortality rate of 65% in the rearing of young chicks represented eggs hatched which could have been sold or eaten, and laying time lost while the hen incubated the eggs and reared the batch of chicks. Further, only a fraction of the feed consumed by the chicks which died returned to the scavenging pool. Although predators were blamed for the losses, the very wide range of growth rates for chicks, and the low protein diet, probably mean that malnutrition and associated weakness are major causes of losses, both directly and, by increasing vulnerability to predation, indirectly. If the young chicks were given preferential access to the household waste by using a simple creep feeder made from local materials, then a substantial proportion of the wastage might be averted. The mortality rate of young chicks was steady (Fig. 1) and there was no evidence of a disease outbreak in any age group, so disease is unlikely to have been a primary cause of the high mortality rate. No analysis of the causes of the wide range of hen day productivities was possible, but one likely cause is hens laying away from the nest because of inadequate provision of nesting space, or bad habits. Further, as the chickens are fed around 0800 h and the hens lay around mid morning, there could be an advantage in providing laying nests in the night pen, feeding the hens with the household waste in the pen, and then releasing them after laying time. The inputs required to implement any of the above measures are information and a little labour. As nearly all heads of households were literate, the former can be provided and the latter is not particularly demanding.

The study was designed to interfere as little as possible with the existing husbandry system. No birds were vaccinated for Newcastle disease (ND), which can cause mortalities approaching 100% (Fonseka 1987). As all of the chickens were penned every night they could be controlled for an organised vaccination program. With the threat of ND removed, consideration could be given to increasing productivity by providing supplementary inputs. The analysis of crop contents is similar to that quoted by Prawirokusumo (1988), but the higher ether extract and lower crude fibre in the present study allows more scope in the choice of supplements. The proximate analysis of feed and crop content, and the presence of substantial abdominal fat in all hens, indicates that the availability of protein was a constraint on production. Protein to supplement the diet is available in local by-products including fish meal, coconut oil meal and rice bran. The moderate level of fibre in the crop content (5.4%) would also allow supplementation with high fibre protein

supplement such as coconut oil meal. The levels of Ca and P in the diet were very low, as were the levels in plasma, compared with those of birds in intensive production systems (S.P. Gunaratne and A.D.N. Chandrasiri, pers. comm.). Calcium and phosphorus could be easily and cheaply provided in the form of shell grit and bone meal. Supplementation of deficient nutrients in the feed resource base would inevitably increase the production and the net output.

This study is location specific, for other locations would have different access to the by-products of cultivation and harvest, perhaps a more distinct seasonal availability of feed in the environment, a different domestic diet, and a different density of housing, among other factors. Nevertheless, some of the same simple principles can be applied in any environment to provide the information needed as a basis for increasing the welfare of the village families which keep scavenging chickens. The aggregate information could then be used to develop recommendations for improving the productivity of scavenging chickens in any particular cultural environment. Benefits from scavenging village chickens accrue to those in the community who have the greatest need and would be multiplied enormously over the poultry flocks of the developing world. Perhaps the first step would be to reduce the information void, and make poultry owners aware that there are options, and that it is possible to increase the benefits from their chickens without necessarily incurring additional costs.

Acknowledgment

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The Role of Village Chickens in the Poultry Industry in Sri Lanka

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Abstract

Up to the 1950s all eggs produced in Sri Lanka were derived from indigenous village chickens. Eggs were also imported from India. In the mid 1950s, male birds from improved breeds were issued to farmers in order to improve the performance of their offspring. The deep litter system was also introduced and popularised in the late 1950s. Devastating epidemics of Newcastle disease which discouraged most farmers from engaging in large scale poultry breeding were controlled by the introduction of vaccination.

The commercial poultry sector grew in the 1960s. There are 14 private and 3 state farms importing parent stock, and producing commercial hybrids locally. At present, of a poultry population of 9 million, about 75% are reared under intensive conditions, and the balance (25%) are what may be described as village chickens. This segment of the poultry population contributes approximately 15% towards the island's egg production. It consists of different grades of management systems ranging from absolute free range (scavenging all day, fed only household refuse), to semi-intensive (housed for part of day, and supplementary feeding). The type of bird also is variable, ranging from indigenous birds to improved crosses.

The egg is recognised as the cheapest source of animal protein in the diet of Sri Lankans. The price escalation of eggs and chicken meat, relative to other animal protein sources have been relatively low, during the past decade, and poultry meat now ranks relatively low in price. Of all the animal industries, the poultry industry has shown the highest rate of development during the past few decades.

Sri Lanka is an island nation of population 17 million and area 66 000 km^2. The estimated poultry population in 1991 is 9 million, approximately 25% of which can be categorised as village chickens. These are estimated to contribute approximately 15% of the total egg production in the country. For comparison, populations of other livestock species are: 1.8 million cattle: 0.96 million buffaloes: 0.5 million goats: 29 000 sheep; and 86 000 pigs.

Historical

Up to the 1950s, all eggs produced in Sri Lanka came from indigenous village chickens, each bird producing 40–60 eggs per year. The cost of production was practically nil, since these birds scavenged for their food, with some supplementation by household kitchen refuse. Minimal housing was provided at night, for protection from predators, and an enclosure of some type for part of the day to facilitate egg collection.

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The rest of the eggs consumed in Sri Lanka at that time were imported from India.

In 1955, the Government of Sri Lanka began to expand and improve the poultry industry. Initially, males from improved breeds were distributed to farmers. This doubled the egg production in the subsequent generation. The deep-litter system of intensive management was introduced and popularised in the mid 1950s. One of the reasons why farmers were reluctant to take to large-scale poultry farming was the heavy losses caused by devastating epidemics of Newcastle disease (ND). Vaccination against ND began in 1951, but became popular, and more effective, with the introduction of the lyophilised vaccine in 1960. During the period 1955 to 1964, poultry production increased by 85%, and it was possible to discontinue the import of eggs from India in 1963.

During this period, a group of large-scale poultry breeders emerged. Initially, they imported commercial day-old layer chicks. Later in the 1960s, they started importing parent stock, and produced commercial layer chicks locally.

The Commercial Poultry Sector

The commercial poultry sector has grown rapidly since the 1960s, substantially boosting the poultry industry. At present, there are 14 private and 3 State farms importing parent stock, and the island's incubator capacity is estimated at 2 million eggs per setting. The sector has imported parent stock from highly reputed sources of proven high performance in their countries of origin. It is noted, however, that their performance under local management conditions has been variable, and this is attributed to the genotype x environmental interaction, as these birds have been reared and selected under high resource consuming environmental conditions in their countries of origin, which most local farmers are unable to provide.

In one State farm alone, a breeding program has been carried out with the objective of producing a commercial layer adapted to local environmental and management conditions.

The broiler industry in Sri Lanka was introduced in the 1970s and grew markedly in the 1980s. The output of broiler chicks rose from 40 000 per week in 1980 to 280 000 per week in 1990.

The Role of the Egg in Human Nutrition

Protein malnutrition is a common feature among the low income earning rural and urban Sri Lankan population. The egg has many advantages over other sources of animal protein in bridging this gap. Apart from its high biological value and the absence of storage problems (unlike meat, fish, etc.), it is also the cheapest source of animal protein. The costs per gram of protein from various sources shown in Table 1 illustrate this point.

Table 1. Cost per gram (in Sri Lanka rupees) of pure protein from different sources in Sri Lanka

Protein source	Cost per g of protein
Eggs	0.33
Fresh cow's milk	0.30
Beef (moderate fat)	0.48
Chicken	0.57
Pork (lean)	0.57
Pork (moderate fat)	0.80
Fish	0.80

When one considers the increase in market price of various animal protein sources in Sri Lanka during the period 1982–89 (Table 2), it is evident that the cost of egg protein has increased by less than most other sources of animal protein.

Table 2. Increase in price of animal protein from various sources in Sri Lanka over the period 1982–89

Protein source	Increase in price (%)
Eggs	166
Beef	240
Mutton	239
Pork	197
Chicken	176
Fish (fresh)	193–254[a]
Fish (dried)	137–213[a]
Milk (fresh)	213
Pulses	151–194[a]

[a] Depending on the variety

Consumption of Poultry Products

The consumption of poultry meat and eggs in Sri Lanka is low compared with the developed countries, and even some developing countries. Nevertheless, the annual per capita consumption of poultry meat and eggs has increased from 100 g and 20 eggs in 1980 to 1 kg and 47 eggs in 1990. This increase in per capita consumption, despite the population growth over the same period, gives an index of the development of the industry. The average figure of 47 eggs is derived from a narrow segment of the 'elite' urban population, who consume around 150–200 eggs per year, and the vast majority of the rural population whose consumption is extremely low.

Consumer Preferences

The busy urban housewife, who has little time to devote to food preparation as part of her daily routine, prefers a soft broiler chicken to a village chicken. In rural areas, however, the village chicken is preferred for traditional preparations and therefore fetches a higher market price. Overall, there is a preference among Sri Lankans for the meat and eggs of free-range village chickens.

Poultry Production Systems

Over 50% of the poultry population of Sri Lanka is concentrated in the western coastal belt, approximately 50 km south, 80 km north and 15–20 km inland of the capital, Colombo. This distribution pattern is influenced by several factors. These are: (1) the proximity to potential markets in the urban and suburban areas around Colombo, and a large number of tourist hotels; (2) proximity to the feed manufacturing industry; and (3) the relative absence of religious prejudices among the population in this area.

The different poultry production systems prevalent in the country, and the types of birds and management systems involved, are shown in Tables 3 and 4. What may be described as 'village chickens' fall into the 'semi-

intensive' and the free range or 'backyard' categories. This group accounts for approximately 25% of the poultry population and contributes approximately 15% of the national egg production. While all production systems are found throughout the island, there is a concentration of larger holdings in the western coastal belt.

Table 3. Characteristics of poultry production systems in Sri Lanka

Production system	No.of birds/ holding	Source of replacement chicks
1. Intensive		
Large scale	> 5000	Private breeder Imported parents
Medium scale	1000–5000	"
Small scale	100–1000	" or locally bred hybrid
2. Semi-intensive	50–100	Locally bred hybrids
3. Free range	10–50	Self sustaining

The intensive large-scale poultry farmers (> 5000 birds) are quite independent, and have adequate resources to look after themselves. To a lesser extent, this is true of the medium-scale category (1000–5000 birds). Government efforts through the Department of Animal Production and Health are directed towards two objectives: (1) To develop and improve the intensive small-scale poultry farmer, with a view to moving towards the goal of self employment. Many of those who have taken to poultry farming under the Government's Poverty Alleviation Program fall into this category. (2) To improve the status of the semi-intensive poultry farmer, with a view to elevating him to the intensive small-scale category. This is particularly important in the urban areas, where land for free-range or semi-intensive management is limited. A noteworthy finding of many surveys has been that, while the consumption of poultry products in general, and eggs in particular, is low among the rural sector of the population, it is above the national average among those persons in the rural sector who raise poultry in their own houses. Thus, encouraging small-scale poultry raising at home also means encouraging higher consumption of poultry products, thereby elevating the nutritional status of the rural population.

Table 4. Inputs to the various types of poultry production systems in Sri Lanka

Production system	Feeding	Management	Inputs
Intensive Large > 5000	Produced in farm or custom mixed	Hired management and labour	1. Staff + 2. Labour + 3. Feed + 4. Day-old chicks + 5. Maintenance+
Intensive Medium 1000–5000	Purchased	Owner managed Hired Labour	1. – 2. Labour + 3. Feed + 4. Day-old chicks + 5. Maintenance+
Intensive Small 100–1000	Purchased	Owner/family	1. – 2. - 3. Feed + 4. Day-old chicks + 5. Minimal
Semi-intensive 50–100	Purchased + scavenging	Owner/family	1. – 2. – 3. 50% feed 4. Day-old chicks 5. –
Free Range 10–50	Scavenging	Owner/family	1. – 2. – 3. – 4. – 5. –

Poultry Feed

In the commercial poultry sector, feed accounts for 70–90% of the cost of production. With the growth of the industry from 1980 to 1990, there has been an eight-fold increase in the demand for broiler feed, and a two-fold increase in the demand for layer feed. Imported strains of birds in the commercial sector are highly sensitive to quality of feed and, for the maintenance of high production levels, feed quality is of paramount importance. To supplement what is locally available, feed ingredients are imported and, as a result, there is a drain on valuable foreign exchange. The type of bird the local breeding program is seeking to develop is one that is less sensitive to feed quality, and could produce reasonably well on poor quality feeds, and poorer management conditions in the hands of the small-scale farmers. The semi-intensive sector is relatively less dependent on the feed industry, while free-range birds are totally independent.

Research on the 'Village Chicken'

Until recently, little or no work was done on village chickens. Epidemics of ND have been a major problem in this segment of the poultry population. Among the free-range birds, vaccination coverage is estimated at only 20%. These birds may also be considered to be a reservoir of infection. Attention was focussed on the health of village chickens during field trials with the oral feed-delivered V4 ND vaccine. It is intended to investigate other causes of mortality in the near future.

Attention has also been drawn to production parameters for, and nutritional status of village chickens, and investigations are currently in progress. The overall objective is to improve the productivity of this sector, with minimal additional inputs.

The Poultry Industry in Lesotho

Lebohang Khomari*

Abstract

Rural chickens have been reared in Lesotho for centuries. Since the introduction of commercial poultry in 1972, the rural industry has been neglected. There are modestly sized layer and broiler industries, based on chickens imported from the Republic of South Africa. Newcastle disease (ND) is the major infectious disease, introduced with the commercial chickens. There is a small local capacity for the production of vaccines.

Rural chickens are now reared in the less accessible rural areas. There have been recent attempts to improve the productivity of these chickens in some areas by cross breeding with imported Plymouth Rock stocks. ND vaccines are not used in the rural chickens except at the request of owners.

BASOTHO people have been keeping village poultry for centuries. Indigenous chickens are normally reared under very inexpensive management systems. They are provided only with night shelter including nests for laying and brooding for hens. The rest of the chickens find a sleeping place anywhere near the house. During the day the chickens are left to roam and scavenge. Normally the chickens are fed on household refuse or maize when available, which could be twice or three times a day depending on the number of meals a family has, but no water is ever provided for them.

Commercial poultry rearing started only in 1972. The most prominent component of this modest industry is layer rearing, which amounts to a population of 22000. This is supported by a very good market outlet for the sale of eggs and also by protective legislation that inhibits the importation of eggs. Eggs can be imported by the Egg Circle (Board) only during times of egg deficits. Some 239 million eggs were imported in 1990.

The broiler industry is still small, despite the fact that 103.86 million carcases of poultry meat were imported in 1990. The broiler population is about 1 million. The reasons for this situation are lack of established market outlets, precarious supply of day-old chicks and stiff competition from the Republic of South Africa (RSA). All commercial chickens are imported from the RSA, which means their supply to the domestic broiler industry can never be guaranteed and as a result, neither can supplies to commercial outlets such as supermarkets. Supermarkets need not only guaranteed supplies but also properly dressed chickens that have undergone some inspection in a recognised poultry slaughterhouse. There is no embargo on poultry meat coming from RSA, as Lesotho belongs to the common customs union. This is an unfortunate situation, as our commercial farmers are subjected to unfair competition. Our farmers import day-old chicks, feedstuff and veterinary drugs from RSA, which increases their costs and consequently the cost of production is high. The commercial poultry industry has undoubtedly raised the standard of living of our community and has created jobs for many women, who make up 99% of the poultry farmers.

Realising that poultry keeping is an important contribution to the livelihood of the Basotho nation, the government sought to assist in reducing losses due to disease. A study of the status of poultry diseases was conducted, and it was established that Newcastle disease (ND) was the number one killer disease in chickens. It was also determined that the ND vaccine that was then imported was not readily available and was very costly. For these reasons, the Livestock Department requested that a small vaccine production unit be established specifically to produce ND vaccine.

Local production of ND vaccine began in 1984. The vaccine was then sold to the farmers for half the cost of the imported vaccine; currently, it is about two-thirds the cost. A cost analysis of production as compared with importation has not been undertaken. Only the La Sota

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strain is used. It is cultured in specific pathogen-free eggs that are also imported from the RSA.

Since all commercial chickens are imported, first vaccinations are done in their country of origin. Initially, after their arrival in Lesotho layers were vaccinated with La Sota vaccine at intervals of 3 months. Broilers were vaccinated at 9–10 days of age. With the introduction of oil-based ND vaccine, layers are now not revaccinated throughout the laying period unless there is an outbreak. Broilers are also not vaccinated unless there is an outbreak or they are raised in areas where there seem to be regular outbreaks. In these instances, broilers are vaccinated at day 9.

Newcastle Disease

ND was first recorded in Lesotho in 1972 following the introduction of hybrid chickens. This resulted in outbreaks of ND in indigenous chickens. Campaigns to treat all poultry with Komarov vaccine were then adopted as a policy. Strict precautions were taken, and farmers were not allowed to keep both exotic and indigenous chickens close together. Consequently, the incidence of ND declined. As the industry grew bigger, and strict and regular vaccinations of commercial chickens were instituted, vaccination of the indigenous chickens stopped. It is now done only at the request of owners.

No outbreaks of ND had been reported for a decade in indigenous chickens until this year, 1991. In August 1991, an outbreak involving both exotic and indigenous chickens was reported. This occurred in a very small area.

Rural Poultry

There are 1.6 million rural chickens. Most of them are concentrated in areas inaccessible by road. People in accessible rural areas are rearing commercial chickens.

Attempts to improve indigenous chickens to meet the increasing nutritional demands of the rural people started only this year with the introduction of Plymouth Rock cockerels and hens. Statistics on their performance are still incomplete.

Their introduction was initiated by the farmers, some of whom were keeping commercial chickens and were experiencing immense problems with unavailability of day–old chicks, and constant increases in the cost of feedstuff and veterinary drugs.

When the Plymouth Rock was introduced, some farmers tried to rear them under management systems similar to those used for commercial hybrid chickens. Their performance was poor, compared with those that were left to roam and scavenge. Their progeny have better body mass than indigenous chickens and they are resistant to diseases. During the outbreak that occurred in 1991, the Plymouth Rock indigenous chickens were not affected, even though the outbreak occurred in the area where the Plymouth Rock project is under way. Most likely this was due to passive immunity.

The Plymouth Rock cockerels and hens are vaccinated at 1 day old and at 4 weeks, after which they are distributed to the farmers to introduce them to their indigenous chickens. Their progeny are not being vaccinated. Very few diseases are encountered in indigenous chickens. These are internal and external parasites, and sporadic outbreak of fowlpox.

Conclusion

Commercial chicken production generates attractive income returns and the Livestock Department of Lesotho has favoured this enterprise over rural chicken production. However, this restricts improvements in standard of living to people who can raise security for bank loans to pay for commercial chickens, their highly priced food and the numerous veterinary products that are required to control their diseases. The less privileged members of society receive no benefits from the commercial industry. The introduction of the Plymouth Rock represents an attempt to improve utilisation of rural chickens so as to benefit the poorer people. For about 50% of the population, rural chickens supply the only source of animal protein, an ingredient that is lacking in most of the Basotho's diet. Commercial broilers of 7–8 weeks of age cost US$4.5, while the GNP is US$284. Indigenous chickens are preferred and fetch higher prices when they are available. Unfortunately, their population is low and this resource has yet to be properly exploited.

Epidemiology of Newcastle Disease and the Need to Vaccinate Local Chickens in Uganda

Mukiibi Muka George*

Abstract

Viscerotropic, velogenic strains of Newcastle disease virus are recognised as serious causes of mortality in village chickens in rural Uganda. The spread of Newcastle disease is generally attributed to the movement of infected chickens. A pilot vaccination study is being undertaken in three villages.

EPIDEMIOLOGY in our context embraces factors associated with production and transmission of Newcastle disease (ND) in the rural flocks. In Uganda, all farmers consider ND as a serious disincentive to investing in the chicken business.

The first documented evidence of ND in Uganda occurred in 1955 in and around Kampala, in the Central Region. There had been, however, earlier reports of a similar disease in the Eastern Region.

Elsewhere in Africa, cases had been reported in Kenya (Mombasa) 1935, Kenya (Nairobi) 1939, Sudan 1951, and Nigeria 1952. The first cases of ND worldwide were reported in England 1927, Java 1926 and Korea 1929.

This suggests that ND first appeared in Europe and Asia, then spread to Africa, probably through sea ports. It is also documented that the origin of the local (rural) chickens in the world was the 'jungles' of Asia.

The viscerotropic velogenic strains of ND virus are more common in the rural areas, sweeping through villages with mortalities approaching 100%. There could, however, be some less virulent strains in the rural areas that are either not easily recognised or given less attention.

In Uganda, the most virulent strain characterised so far was isolated by the author in 1986 in Entebbe. This virus produced 100% morbidity with 98% mortality. It had an ICPI of 1.75, IVPI 2.7 and MDT of 44 hours. Further characterisation by Alexander related it to PMV-1 with monoclonal binding pattern P.

The socio economic importance of ND in rural Uganda can easily be appreciated if one considers the role the rural chickens play there. Rural chickens require little attention, feeding on leftovers and spending most of their time scavenging around the garden. This way they act as efficient waste-disposers, converting food leftovers into valuable animal protein. They do not require specialised housing and some roost outside on trees.

These rural chickens comprise 80% of the total poultry population of Uganda, which is about 20 million.

Almost every homestead has some chickens and they provide the cheapest source of animal protein in the form of eggs and meat. In addition, they are a ready source of income to help homesteads purchase basic requirements such as soap and paraffin, and may even help pay school fees in the rural primary schools.

The cost of a hen is 1500/–Uganda shillings (US$1.50) which can buy a bar of soap, 1 kilogram of salt and one litre of paraffin. Similarly, two hens when sold would pay school fees for a term in the rural area. Culturally, at every function in Uganda there will be a chicken either slaughtered, exchanged or given away. Thus, if a homestead has had its flock destroyed by ND then it can be in a difficult position if a visitor arrives or a cultural function is held.

The seasonal occurrence of ND in Uganda is a well-established phenomenon among the rural farmers, so much so that, just before the dry season sets in, farmers panic and start selling off their stock. This usually triggers the spread of disease. The spread of ND in the rural areas may be abetted by both the management methods and cultural attitudes of the people. These birds roam in villages in search of food and this encourages the spread of disease. Birds given as gifts may be transported long distances on foot or bicycles. If there is an outbreak in one village, the disease is thus likely to spread widely due to human influence.

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In rural Uganda, ND occurs in waves which sweep through villages in the dry months of July/August and December/January. These hot seasons may be accompanied by high winds. During such periods there is less human activity in gardens, but more movement in the form of visits. The chicken is the smallest domesticated animal and is therefore used by parents to instill in children a love for animals. Because of this, whenever children visit relatives, they are initiated in animal husbandry by giving them a bird to take home.

In some areas in the Eastern Region, there is a barter system whereby for seven chickens one may be given a goat, and seven goats may be exchanged for a local cow.

The rural chickens are part of an integrated farming system where it is estimated that 15–20 chickens produce about 2 kg of chicken manure per day which is used as fertilizer in gardens. This is an input into the soils of organic fertilizers which encourages the development of earthworms on which birds feed. The soils also get aeration through worm burrowing. In addition to this, engorged ticks fall off cattle either in the evening or early morning, so the chickens are usually introduced in Kraals where they pick up these parasites.

While all these facts about the rural chicken and ND are appreciated, little effort has been made to protect these birds in Uganda.

A pilot study was therefore undertaken to show the impact of ND vaccinations on the rural chickens.

An area distant from the large towns was selected to minimise the influence of exotic birds and in a place where the homesteads are representative of the rural people. Initially, three villages, namely Busesa, Ibalanku and Ibaako, were selected. Farmers were visited and the idea of protecting their flocks against ND was introduced by the local extension veterinary staff. Arrangements were made to provide free vaccine and basic cold chain facilities from the Vaccine Production Laboratories.

On set days, farmers were requested to keep their chickens indoors or tied up so that they could be vaccinated by the field veterinarian and his team of two poultry attendants. The route of administration of vaccine was the nostril/eye drop.

These vaccinations were first done in early May. In late May a booster dose was given and a census of chickens was undertaken in the participating homesteads.

In September, after the dry season, a census was again taken and farmers were asked general questions about their chicken flocks.

Table 1. Busesa village

Farmer no.	Total		Off Take and Loss May–Sept. 1991						Apparent total
	May 91	Sept.91	Disease	Predator	Sell	Goat barter	Slaughter	Other	
1	13	20	–	–	–	–	5	–	25
2	4	17	7	–	–	7 (1 goat)	–	–	31
3	10	20	1	1	–	–	–	–	22
4	29	34	–	8	–	–	2	3 (gift)	47
5	20	24	3	8	2	–	3	–	40
6	21	25	–	10	7	–	–	–	42
7	9	17	–	–	–	–	–	–	29
8	16	22	4	–	–	–	–	–	26
9	16	21	–	–	–	8 (1 goat)	–	–	29
10	2	9	–	2	–	–	–	–	11
11	8	17	–	3	–	–	–	–	20
12	12	9	–	4	–	–	–	–	12
13	22	18	–	7	–	12 (2 goats)	3	–	40
14	22	32	–	14	–	–	–	–	46
15	25	17	–	3	–	12 (2 goats)	–	–	34
16	25	48	3	–	2	–	–	4 (accident)	57
17	27	38	–	5	–	–	1	–	44
18	2	1	2	–	5	–	6	–	14
19	9	4	5	3	–	–	–	–	12
20	11	4	4	5	–	–	–	–	13
21	10	9	–	–	8	–	–	–	17
Totals	313	406	29	73	24	39	20	7	

Preliminary results are shown in Tables 1, 2 and 3 for the three villages involved. The counts were total, including all ages of chickens.

The impact of predators is shown to be high and, in the absence of ND, ranks highest as a problem for village chickens. The economic importance of the village chickens is shown by a barter system in which some farmers obtained goats for their chickens. Cases of disease affect individuals rather than flocks, and were bacterial and parasitic in origin.

Table 2 Ibulanka village

Farmer no.	Total		Off Take and Loss May–Sept. 1991						Apparent total
	May 91	Sept.91	Disease	Predator	Sell	Goat barter	Slaughter	Other	
22	51	77	–	–	–	–	–	–	77
23	24	36	–	3	–	4	–	–	43
24	12	3	–	6	–	5	–	1	15
25	15	34	–	–	–	–	–	–	34
26	42	48	–	8	5	17 (3 goats)	4	3 (gift)	85
27	72	52	–	12	–	16 (2 goats)	3	–	83
28	2	11	–	–	–	–	–	–	11
Totals	218	261	0	24	5	42	7	4	

Table 3. Ibaako village

Farmer no.	Total		Off Take and Loss May–Sept. 1991						Apparent total
	May 91	Sept.91	Disease	Predator	Sell	Goat barter	Slaughter	Other	
29	10	23	–	4	–	–	3	–	30
30	18	16	8	4	–	–	–	–	28
31	10	18	–	–	–	–	–	9 (thefts)	25
32	8	8	–	4	–	5 (goat)	1	–	18
34	22	32	–	6	–	–	2	–	40
35	9	15	3	5	–	–	2	–	25
36	4	–	4	–	–	–	–	–	nil
37	22	32	–	10	–	–	5	2 (gift)	49
38	11	32	–	4	–	–	–	–	36
39	28	26	4	5	–	5 (goat)	6	–	46
40	6	37	–	–	–	–	–	–	37
41	17	10	6	4	–	–	–	–	20
42	40	46	5	7	8	–	3	–	69
43	8	23	–	–	10	–	–	–	33
44	12	18	–	–	–	8 (1 goat)	–	–	26
45	6	8	–	5	–	–	–	–	13
46	10	11	–	5	6	–	–	–	22
47	5	0	5	–	–	–	–	–	nil
48	2	19	–	3	–	–	–	–	22
49	17	16	–	1	3	7 (goat)	–	–	27
50	12	26	–	3	–	–	–	–	29
51	25	28	–	10	–	–	3	–	41
52	53	48	–	6	–	16 (2 goats)	1	–	71
53	20	32	–	3	–	–	–	–	55
Total	375	324	35	89	32	74	26	14	

From these figures, a growth of about 30% was achieved excluding the loss and offtake in a period of four months. This shows the high potential of village chickens.

As for Newcastle disease, it is too early to draw conclusions of the impact of these vaccinations since this will require almost 12 months of observation.

Village Chickens and Newcastle Disease in Nigeria

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Abstract

Exotic and rural scavenger chickens are kept in both the rural and urban areas of Nigeria. Unlike the exotic birds, which number about 30 million, the local scavenger chickens numbering about 120 million are not routinely vaccinated against prevailing diseases. Prominent among these is Newcastle disease(ND). However, the exotic birds are protected with ND vaccines produced at the National Veterinary Research Institute, Vom. Recent investigations have indicated that the local poultry farmers would welcome ND vaccines capable of protecting the local chickens.

BOTH introduced chickens and rural scavenger chickens are kept in the urban and rural areas of the country. The exotic birds number 30 million while the rural scavenger chickens number 120 million (Yahaya, personal communication). About 85.5% of the rural scavenger chickens are found in the northern part of the country, the balane in the south. Kane State has the highest number (18.41%) in the north and Oyo State has 5.05% in the south.

Although little or no veterinary care is given to the rural poultry, they are present in greater numbers than the exotic breeds. They are found in villages and cities, and are kept by both the low and high income earning classes of people. They provide a cheap source of animal protein to the rural populace.

In Nigeria, a tentative diagnosis of Newcastle disease (ND) was made in April 1951, in two outbreaks on some poultry farms in eastern Nigeria (Kirby 1951). This was confirmed by laboratory tests at Vom. There were other outbreaks in parts of western Nigeria which were reported and confirmed (Hill et al, 1953). Other outbreaks of ND reported from eleven different parts of Nigeria indicated the extent of the disease. Some details of the incidence of ND and other poultry diseases in Nigeria are given in Table 1.

Currently, ND is viewed as one of the most serious fatal poultry diseases of economic importance in Nigeria among the exotic and local chickens (Fatunmbi and Adene 1979). Between 1981 and 1989 confirmed outbreaks of ND ranged from 11–82 (Annual Report, National Veterinary Research Institute, Vom, 1981–1989).

Vaccination

Since the first reported case of ND in Nigeria, the National Veterinary Services have adopted several prophylactic measures, the most significant of which is vaccination. ND vaccination was introduced into Nigeria even before the disease was recognised (Hill et al 1953). Following outbreaks of the disease in the Congo, the Komarov vaccine (Komarov and Goldsmith 1946) was imported from South Africa to protect the local flock and this was, for a long time, the only vaccine in use. Local production of the vaccine in Vom in 1953 was as a result of the difficulties encountered with the imported Komarov vaccine. This was due to the poor transport network between and within the country.

In an attempt to find a mild vaccine devoid of the disadvantages of the mesogenic strain, an intraocular vaccine strain obtained from Israel was introduced into Nigeria. This was followed in 1964 by the production of the La Sota vaccine, the seed virus of which was obtained from Holland. A comprehensive policy of immunisation was established using the three vaccines now produced at the National Veterinary Research Institute, Vom. These vaccines are used mainly for the exotic birds.

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Virulence

Velogenic strains of ND virus have mostly been found in the wild birds in Nigeria (Onunkwo and Momoh 1981; Omorodion and Olabode 1989; Adu 1987). However, lentogenic and mesogenic strains have also been documented. These birds serve as reservoirs and a source of dissemination of the virus to susceptible birds. More work needs to be carried out.

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Table 1. Poultry disease outbreaks in Nigeria. Comparing the situation in 1977–78 and in 1982.

Disease	No. of outbreaks	No. of birds involved	No. dead (%)	% of total mortality	No. of outbreaks	No. of birds involved	No. dead (%)	% of total mortality
	1977–1978				1982			
Newcastle disease	21	49391	26348 (52.7)	77.6	17	62621	27789 (28.3)	74.7
Gumboro disease	38	62123	7202 (11.5)	21.1	21	28488	4962 (17.4)	20.8
Fowlpox	19	5272 (4.8)	258	0.8	3	5700	37 (0.64)	0.20
Marek's disease	4	5900	130 (2.2)	0.4	–	–	–	–
Chronic respiratory disease (CRD)	–	–	–	–	2	2000	18	0.08
Fowl typhoid	–	–	–	–	5	9432	808 (8.6)	3.40
Coccidiosis	–	–	–	–	6	7000	128 (1.8)	0.54
Aspergillosis	–	–	–	–	1	10000	54 (5.4)	0.23
Avian leucosis	–	–	–	–	2	8500	17 (0.2)	0.07
Total	82	123226	33938	100	57	133743	23813	100

Source: Federal Livestock Department, Kaduna.

Village Chickens and Newcastle Disease in Bangladesh

Mohd. Asadullah*

Abstract

Newcastle disease is an important disease to the 80% of the population of Bangladesh who live in villages and raise chickens. The control of Newcastle disease depends on conventional vaccines and the provision of efficient cold chains.

EIGHTY percent of the people of Bangladesh live in villages. Most of the villagers like to raise chickens in their backyard. Chickens are generally kept as scavengers, and play an important role in the rural economy of Bangladesh. Villagers sell chickens and eggs in the village market to meet their income requirements. Nowadays, women and landless rural people are trying to acquire technology for small-scale poultry farming. Poultry farming has so far benefitted only higher income groups. Therefore, the Bangladesh Rural Advancement Committee (BRAC) has established a project in which landless women are provided with one cockerel and 10 chicks. Another non-government organisation (NGO) has come forward to train landless people and distressed women in the villages to raise chickens scientifically.

However, there are many constraints in the development of poultry farming and raising chickens in the villages, such as non-availability of balanced food, the occurrence of diseases, etc. The main diseases encountered are Newcastle disease (ND), fowlpox and fowl cholera. As well as diseases, heavy rains and sudden floods can destroy entire poultry populations in low lying areas.

Occurrence of Newcastle Disease

ND, which is popularly known in Bangladesh as Ranikhet disease, is endemic in this country and epidemics are reported throughout the year, with a peak during the months of November-February. A survey conducted by the Department of Microbiology and Hygiene of Bangladesh Agricultural University, Mymensing, indicated that field infections are caused by velogenic strains.

Velogenic forms of the disease are characterised by very high mortalities, reaching 90% or more. The typical signs are increased respiratory rates, prolonged gasping, respiration with outstretched neck and head, partially opened beak, nasal discharge, profuse diarrhoea, temperature elevation by 1-2°C followed later by subnormal temperature, and nervous signs such as paralysis, tremors or torticollis. This is the usual form of the disease existing in Bangladesh.

Vaccines in Use

The selection of the type (live or inactivated) and the strain (lentogenic or mesogenic) of vaccine depends mostly on availability and the requirement of immunisation which, in turn, is determined on the basis of the type of field virus occurring in the region.

Considering all these points, the following types of vaccines have so far been developed in Bangladesh against ND:

1. Live lentogenic vaccine:- These are prepared with 'F' strain
2. Live mesogenic vaccine:- These are prepared with Mukteswar strain.

Current Control Program

The current program of vaccination against ND in Bangladesh includes eye drop administration of a live lentogenic vaccine of 'F' strain in baby chicks from

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day-old onwards to 7 days of age, followed by a live mesogenic vaccine of 'M' strain administered intramuscularly (I.M.) at 8 weeks of age, or in adult birds, and then usually at 6 month intervals.

In the case of broilers, the birds gain weight very quickly, and a single application of lentogenic vaccine during the first two weeks of life may not be sufficient considering the risk and the cost of such a flock. It is therefore necessary to revaccinate birds at 6-7 weeks of age with a mesogenic strain, particularly when disposal of birds is delayed.

Freeze dried vaccine of ND was introduced to Bangladesh in 1964, with the assistance of an FAO expert, Dr M.S.El. Sabban. Since then, this vaccine has been in routine use in the mass vaccination program of the Livestock Directorate. Its production is gradually being increased (Table 1).

All batches of vaccines are subjected to classical tests such as chick titration, safety testing and challenge. A virulent field virus is used in challenge testing. The requirement for all clean eggs and chicks for production of vaccine, chick embryo titration, chick titration, and safety testing are met by the Central Government poultry farm.

With the help of the Asian Development Bank (ADB), the Directorate of Livestock Services, Bangladesh, has established cold rooms in every district. A cold chain is maintained to supply vaccine to district cold rooms, and freezevans are used to carry vaccines to the most remote parts of the country.

To control ND in Bangladesh, mass vaccination efforts are being strengthened every year, particularly through the help of non-government organisations. For example, BRAC (Bangladesh Rural Advancement Committee) is training unemployed people to become poultry workers. The training includes preventive and curative aspects, along with rearing management. The vaccines are distributed to them by BRAC, free of cost, from government stocks. They are regularly supervised at village level. The poultry workers or vaccinators of both government and NGOs are provided with thermos flasks to maintain the cold chain; other inputs provided include a carrying bag and a glass syringe.

Given the efforts described in this paper, it is expected that ND will be totally controlled in Bangladesh in the near future.

Table 1. Production and distribution of Newcastle disease vaccine (million doses) in Bangladesh during the years 1986–1991.

Year	Newcastle disease vaccine for adults (M.strain)		Newcastle disease vaccine for chicks (F strain)	
	Production	Distribution	Production	Distribution
1986–87	58.29	64.29	26.54	25.99
1987–88	106.97	77.39	35.13	29.10
1988–89	99.57	104.27	44.53	45.01
1989–90	125.67	116.21	43.26	43.36
1990–91	134.37	128.03	55.37	55.59
Total	524.87	490.19	204.83	204.05

Present Status of Poultry in Nepal

Upendra Mishra*

Abstract

Nepal, a Himalayan Kingdom with diverse climatic zones, has a long history of traditional poultry keeping. Improved breeding commenced in 1953.

Poultry are a valuable source of dietary protein and rural cash income. The country has commercial, smallholder and backyard types of farms, with a total population of 12 million birds, including ducks. Some 92 000 t of poultry meat and 35.5 million eggs were produced during 1990, contributing 1.6% of Agricultural GDP.

Ninety percent of poultry are raised as free-ranging birds with no significant investment from villagers. Village chickens called 'Shankini' are slow growing and susceptible to disease. Most poultry diseases are present in the country, but Newcastle disease (ND) in particular causes great economic losses, estimated at NRs. 74.77 million in 1990.

Movements of poultry increase during the hatching and festival seasons. The country is self reliant in feed products, but improved genetic materials are imported.

The Department of Livestock Services and the Nepal Agricultural Research Council are the agencies responsible for disease diagnosis, research, vaccine production and control activities, with a network of different units throughout the country. Strict legislation to control disease is lacking.

ND is common throughout the country, with higher incidence of outbreaks in summer. Current locally manufactured vaccines (F_1 and R_2B) have limitations and are used in only 10% of birds. Outbreaks still occur in vaccinated flocks.

The food pellet NDV4 strain vaccine is under trial at Pakhribas Agricultural Centre. Results have been encouraging, but further trials are needed in different agro-ecological zones of Nepal.

NEPAL is a developing, land-locked, agrobased country, situated in the mid Himalayan zone, with a land area of 147 181 sq.km, and ranging in altitude between 300 and 8000 m. Altitude and topographical differences result in varied climates. Nepal has three main ecological belts viz. Terai 17% (300–1000 m altitude), Hill 68% (1000-3000 m) and mountain 15% (3000–8000 m) with a population more than 19 million. The average population density is 129 persons/sq.km.

Nepal has the highest animal population density in the world (36 per sq.km.) with an average livestock holding of 2.09 per house. The roles of livestock in socio economic development and as a source of supplementary cash income, self employment, livelihood and existence are crucial, contributing 18.2% of the agricultural gross domestic product (AGDP), which accounts for 58.2% of the total GDP of Nepal.

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Poultry Production

Nepal has a long history of poultry keeping. Improved poultry keeping commenced in 1953. In 1959, 1700 improved birds (200 cocks and 1500 hens) were brought from New Jersey, USA, for cross breeding purposes. Today, most of the popular poultry breeds are imported from abroad.

Poultry form a valuable source of protein in the diet and of rural cash income, as well as a source of manure to the average farmer. Village free-range poultry still play a dominant role in meat and egg production. About 90% of the birds are raised in the rural countryside with no

significant investment in feeding and housing. The rural poultry survive on grass seeds, grass tops, grain residues, insects and kitchen wastes. Nepal is self reliant in poultry feed production, and has commercial, small holder and backyard types of farms for meat and egg production. About 92 000 t of poultry meat and 35.5 million eggs are produced each year.

Explosive growth of population and increasing tourism has accelerated demand for poultry meat and eggs. Poultry meat currently costs around 70 N rupees/kg and eggs around 2.5 N rupees each.

Poultry disease situation and control measures

Village chickens called 'Shankini' are slow-growing and present problems in disease control. Most of the well-known poultry diseases occur in Nepal. Of the viral diseases, Newcastle disease (ND) is a major cause of high mortality (90%). It occurs throughout the country, with highest incidence in summer. Almost all pathotypes of ND virus are present in the country. Outbreaks in vaccinated flocks are also noted. The disease causes great economic loss by mortality and morbidity, estimated at 75 million N rupees per year.

Present vaccines have limitations and are used in only 10% of poultry. Village chickens are left unvaccinated due to lack of trained technicians at farm level and the unavailability of vaccine.

Day-old birds are vaccinated with F_1 strain by the nasal or eye instillation method in the hatchery. In the few hatcheries where Marek's disease vaccine is administered, F_1 strain is given between 4 to 10 days of Marek's vaccination.

Poultry Movement and Trade Patterns

Commercial poultry birds, meat and eggs are generally transported to remote areas by aircraft, mule or porters, while in easily accessible areas they are moved by truck, bus, rickshaw, bicycle and porters. Movements of young chicks increase in the hatching season (September-March) and of other birds for meat and eggs during festival times, especially 'Dashera & Tihar' in the months of October and November.

Legislation for Disease Control

Legal provisions for the control of disease are made under the Infectious Disease Act 1963 but specific laws to cover, for example, quarantine, slaughter house management and meat inspection, and cruelty are lacking at present. A proposal for these acts has been submitted to the parliament.

Status of Newcastle Disease

Past record of Newcastle disease

Clinical, pathological and serological studies have revealed the presence of ND in Nepal from early times. It is universally accepted by all those connected with the poultry industry that it is constantly present and widespread in distribution, affecting all poultry.

Present ND situation

As already noted, ND causes large economic losses in Nepal due to mortality and morbidity (meat & egg production). It affects all age groups of chickens.

Only commercial poultry units, which constitute 10% of the total poultry population, have been taking advantage of the current vaccination program in easily accessible urban areas. The existing type of ND vaccine has limitations for use in rural conditions, and has, as well, led to several cases of post-vaccination reaction and reoccurrence of the disease in vaccinated flocks.

The disease affects mainly domestic fowls. Ducks and geese develop a symptomless infection with production of antibodies. Various wild bird species are also affected during outbreaks.

Resources Available for Newcastle Disease Control

Agencies involved in ND control

The Department of Livestock Services and some private hatcheries are involved in the control of ND in Nepal. Lentogenic strain F_1 and mesogenic strain R_2B vaccines are produced locally and distributed throughout the country by different veterinary hospitals. Some private farms also procure ND vaccine from other countries.

Organisation of veterinary services

Nepal has a network of Livestock Service Sections (75), checkposts (24), dispensaries (4), Livestock Service Centres (749), Regional Laboratory (2) and Central Level Units (5) under the DLS & NARC's CADRD, PAC and LAC (see Fig. 1)

Diagnostic facilities

Diagnostic facilities are available only at the Central Animal Disease Research Division (CADRD), and at Tripureswor and Pakhribash Agricultural Centres of the Nepal Agricultural Research Council (NARC). Both microbiological and serological facilities are available inside the country. Antigens and hyperimmune serums are obtained from India. Procurement of enzyme

substrates, monoclonal antibodies, SPF chicks and embryonated eggs, poses problems.

Epidemiological surveillance and reporting system

Epidemiological surveillance is carried out by CADRD and outbreaks of disease are generally reported by DLSS and private farmers directly to CADRD in the centre, PAC, LAC and regional laboratories. Samples are received from throughout the country for diagnosis. There is a well-developed communication network for notification of disease outbreaks.

Vaccine supply

The Biological Products Division (BPD) of DLS produces various vaccines which are distributed with minimal charge through district livestock service sections (DLSS) and to private farmers throughout the country. Some hatcheries also procure poultry vaccine from abroad.

Vaccine storage and distribution facilities

All poultry vaccines are stored at –20°C centrally and in the freezing chambers of refrigerators at district level. Vaccines are distributed to the regions by refrigerated van, from where DLSS distribute them to farmers in thermos flasks packed in ice.

Control Policy

DLS of HMG/N has an ND control policy based on vaccination of poultry. There is no mass slaughter policy in the event of outbreaks. Hatchery owners and private

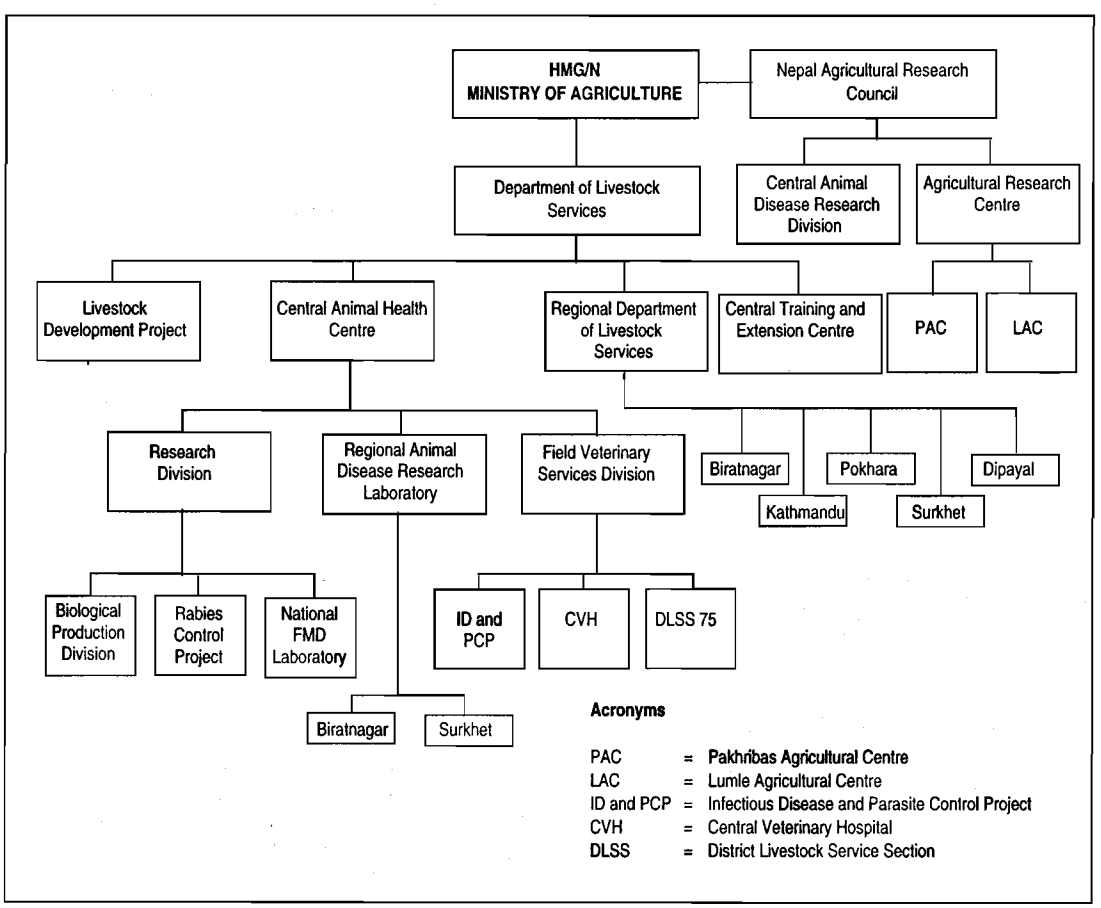

Fig. 1. Organisation of veterinary services in Nepal

farmers are not subsidised, thus they do not slaughter infected birds. Other hygienic measures are maintained by the owners themselves. There is no insurance system.

Research into Newcastle Disease Virus

Since the establishment of NARC, the CADRD has commenced epidemiological studies, virus isolation activities, sero-investigations, and studies of the efficacy of different types of vaccines available. Recently, food pellet NDV4 vaccine has been tested at PAC with encouraging results. Further trials are needed in different agro-ecological zones of Nepal.

Newcastle Disease in Myanmar

Kyaw Zaw Lwin*

Abstract

In Myanmar, 85% of poultry population occurs in villages and depends on indigenous breeds of chickens. Newcastle disease is a serious problem in these chickens. Vaccination against Newcastle disease is usually feasible only in commercial poultry operations. Simple, effective vaccines are required for village chickens. An efficacy trial of V4 food vaccine is being undertaken.

THE poultry population in Myanmar (Table 1) had been fairly constant for about a decade before declining sharply in 1988–89, owing to socio-political instability in the country. Since then, apart from the importation of a few batches of exotic breeds for state poultry farms, there have been few improvements to either commercial or small scale rural poultry farming. The 25 million chickens raised in 1990–91 produced an estimated 0.07 million tonnes of chicken meat, constituting 39% of total meat production. In Myanmar chicken meat is widely accepted by all national and religious groups, consumer preferences ranging from local, indigenous chickens to exotic broiler breeds. It is estimated that annual per capita consumption of chicken meat is 1.66 kg. Of total poultry production, 85% is derived from rural poultry farming, which is totally based on local indigenous chickens.

Unlike other livestock farming, a small flock of chickens can be raised by any household at village level. The average size of a rural poultry flock ranges from 5 to 50 birds, which are mainly kept as a source of additional income or for family consumption. Though each unit is small, for the whole country they add up to a population of birds larger than would be possible at commercial level.

At present, feed prices, like those of other commodities, are rising. Nevertheless commercial-scale intensive poultry farming remains profitable because poultry products are also increasing in price.

Poultry production in Myanmar has long been hampered by Newcastle disease (ND), the most devastating poultry disease. Each and every year, most village chicken flocks are subject to severe losses caused by ND. The severe impact of ND was first recognised in 1934. The disease is viscerotropic velogenic, and effects chickens twice each year, in March–April, the hottest period in the country, and again in August–September, the later part of the monsoon. The most susceptible age groups are birds under 3 months old and older birds over 5 or 6 months of age.

A natural balance has been achieved between host and disease, and though the country suffers annual chicken losses, they survive the sweeping effect of the disease and continue to contribute to the country's requirements. Birds are usually protected against ND by vaccination; eye drop vaccine for chicks and intramuscular injection for older birds. The former are given Weybridge F strain eye

Table 1. Chicken population in Myanmar (yearly basis)

Year	Population ('000)
1981–82	27 234
1982–83	29 037
1983–84	31 001
1984–85	32 868
1985–86	32 681
1986–87	32 382
1987–88	33 519
1988–89	33 944
1989–90	24 195
1990–91	23 188

* Livestock Breeding and Veterinary Department, Myanmar

application at about 2 weeks of age, with Komarov strain given as a booster at 8 weeks after initial vaccination with F strain vaccine. It is recommended that Komarov vaccination be repeated at 6 month intervals for layer and breeder birds. The vaccines that are widely used in the country are produced locally at the biologics production division under the Livestock Breeding and Veterinary Department. At present, vaccination is limited to birds raised in intensive commercial farms, which are usually situated at peri-urban areas of the large cities and towns. Since vaccine must be stored or transported at low temperatures, vaccination is possible only when electricity or ice is available. Therefore, the use of vaccine is out of reach of village level farmers. Those poultry farmers who live in suburban areas and who are willing to have their birds vaccinated may do so by contacting veterinarians.

The veterinarians provide ND vaccination services free of charge to farmers, apart from the cost of poultry vaccine charged for at a reasonable rate by the department. At every veterinary office in large cities and townships, vaccination against common poultry diseases is provided by departmental veterinarians. Those assigned at village level may undertake poultry vaccination depending on their locality and the feasibility of vaccination. Normally, country practitioners serve as both animal health and extension workers. They hold discussions on animal health and animal husbandry with farmers whenever they visit their households. They give advice to the farmers on disease control and they may despatch any specimen to a nearby diagnostic veterinary laboratory whenever they have doubts about the causal organism. There is a central veterinary diagnostic division in Yangon and three regional diagnostic laboratories in Mandalay, Pathein and Taunggyi, which serve to help the local veterinarians in disease control and disease investigation. Table 2 gives the numbers of ND outbreaks, and mortalities, in 1990–91, as reported by the central laboratory and the 3 regional laboratories. It is clear that most of the cases were from nearby intensive poultry farms. There are likely to be cases not reported from rural backyard poultry farms, and it is assumed that such cases may be many times higher than the reported ones.

As each veterinarian may have responsibility for up to 10 to 12 villages (see Table 3), disease control is far from satisfactory. In addition, the practical problems of vaccination at village level, such as storage, handling, and administration of vaccine are substantial. Therefore, there is a need to develop a simple, more effective method of vaccinating the chickens. The most appropriate way to overcome the problem of vaccination would be to incorporate a virus vaccine in the feed fed to chickens. If ND were brought under control, the population of village chickens would rise dramatically. It seems that the main possibility lies with the food pellet Newcastle V4 virus vaccine. Myanmar has been from the very beginning involved with the ACIAR Project, but because of circumstances beyond our control, the project has not been implemented as yet. Without implementation, which means lack of assistance being rendered in the needed areas, progress will be markedly retarded.

For the time being, arrangements have been made to carry out efficacy trials. If results are favourable, field trials will be undertaken and we would hope to be able to attend the next meeting with fruitful results. We are delighted to see more countries participating in workshops conducted by ACIAR, at which we can in turn share our experience with other participating countries. Like other countries, we are keen to see the project implemented in our country in the near future. In an experiment conducted before ACIAR involvement, it was shown (unpublished data) that V4 vaccine given by conventional routes protected against challenge with a local isolate of velogenic virus.

Table 2. Newcastle disease incidence in Myanmar in 1990–91

Source	No. of outbreaks reported	Estimated morbidity ('000 birds)
Central Diagnostic Laboratory	94	4340
Regional Laboratories	110	338
Total	204	4678

Table 3. Veterinarians assigned at various administrative levels in Myanmar

State Division	Veterinarians			Total
	State & divisional level	Township level	Village tract level	
Kachin	1	12	26	39
Kayah	1	5	14	20
Kayin	1	8	19	28
Chin	1	10	14	25
Sabaing	2	39	128	169
Tanintharyi	1	11	17	29
Bago	2	31	112	145
Magway	2	26	108	136
Mandalay	2	31	140	175
Mon	1	11	41	53
Rakhine	1	17	50	68
Yangon	2	16	69	87
Shan	2	26	84	112
Ayeyarwady	2	26	116	144
Total	21	269	938	1228

Poultry Production and Newcastle Disease in Vietnam

Tien Dung Nguyen*

Abstract

Most poultry raising in Vietnam is in the village sector, but cash returns to the villagers from this enterprise are irregular. Newcastle disease causes catastrophic losses in village chickens, infection usually entering villages through introduced birds. There is an urgent need for a vaccine suitable for use in village chickens.

ACCORDING to the General Department of Statistics, the total number of chickens in Vietnam in 1990 was 200 million. About 95–98% of the poultry population is in the household sector. The commercial raising of poultry is under the control of the Union of Poultry Enterprises (UPE), a State-run company at national level, which owns large poultry farms each having up to a hundred thousand chickens. Among other functions, the UPE has to keep the genetic poultry stock for supplying commercial chickens to farms of provincial level. While good performances are recorded in the farms of UPE, where there are no market or other impediments to development, in the household sector, performance is meagre and many obstacles limiting poultry production remain. All of these obstacles make the villagers' income from poultry production irregular. Therefore, villagers, who make up more than 80% of Vietnam's population, are not induced to take up poultry raising.

Village Poultry

Through the centuries, village chickens were selected for particular traits. Among these, hens with good maternal traits were the most important, as village chickens were, and still are, basically scavengers. As a result, they are of small size, weighing 1–2 kg at maturity.

Production system

Each village family has an average flock of 10 chickens, comprising a single cockerel and various hens that are used as reproducers (some families keep these birds for years), other males that are killed for meat at specific festivities (New Year, wedding etc.) or sold at maturity, and other hens showing poor maternal traits that are used only as layers. The number of birds kept increases in the rice harvest period (May–June and October–November) when spilt grain around the house and in the rice fields provides feed for extra chickens and ducks. The number of chickens per family varies depending on the size of the family garden, availability of food (rice, cassava, maize etc.), market opportunities and local practice.

Village chickens are kept in free range and usually have to find food for themselves. They are enclosed at night for safety, in a coop constructed with local materials (bamboo, palm leaves, straw etc.). In this coop, each hen has a box made of bamboo or wood, with straw serving as a nest for laying and brooding. After laying a dozen or so eggs the hens enter a brood phase.

The most important indigenous chickens are known as the 'ri'. They are of small body size (hen live weight: 1.0–1.2 kg; cock weight up to 1.7 kg), resistant to variations of climatic conditions, attentive to any possible dangers (predators, strange objects etc.), energetic and wild. For consumption they are also preferred to other chickens for their tasty meat. However, it is difficult to point out exactly the genetic characteristics of a pure breed of 'ri' chickens. There has never been any breeding program followed by villagers. What is seen now around the country side are crossbreed chickens.

Village chickens are generally for family consumption but for villages around large cities they are also a source of extra income for the owners. Normally, chickens are sold live at the age of 5–6 months. Cockerels are more sought after in the market as people seek them for spiritual festivities.

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Factors influencing village poultry production

Poultry raising is a common practice of Vietnamese farmers, but has received little attention because it does not yet provide a regular income to villagers. In the lowland areas, small plot sizes restrict poultry raising, whereas in intermediate and highland areas, where larger plots are available, predators and restricted markets are the limiting factors. Above all, however, poultry diseases are the main reason discouraging villagers and limiting the development of poultry production.

Newcastle Disease in Vietnam

Newcastle disease (ND) was first recognised in Vietnam in 1956. Since then, outbreaks occur yearly causing heavy losses to poultry production. The viscerotropic form of the disease is the most frequently occurring. Vietnamese villages are separated from each other like islands in the paddy fields. Once ND enters a village, all unvaccinated chickens die. The infection normally enters via newly introduced birds. This is the most common source of the infection because already infected chickens are sold at low prices in village markets. Close to big cities, ND is a significant problem, whereas in remote regions it is not so serious. It seems likely that ND in Vietnam is closely related to the movement between chickens' localities. ND also appears to be seasonal, because it occurs most often at the beginning of winter (November–March), though this is no longer true in regions where new poultry production enterprises have been established.

Control

In the past people knew that their chickens would have problems when a chicken from the market was newly introduced into the village. Precautions concerning market chickens were and still are effective measures against ND. Vaccination was carried out for the first time in 1964. The vaccine was produced in the country using a mesogenic strain from China known as H1. Later, the strain La Sota was introduced in 1968. National veterinary authorities have directed the vaccination programs by organising small vaccination campaigns involving participation of primary and secondary pupils. These activities, involving simultaneous vaccination in a district or in one or many provinces at a time, have yielded significant successes, especially when NDV strain La Sota has been used in the drop vaccination form. However, since the adoption of new government policies in 1986, these activities are no longer undertaken and ND outbreaks are again being reported around the country. A new approach is needed to meet this new situation. It can be said now that control work on ND in Vietnam has to follow the approach of other countries. Obviously, vaccination now has to be carried out by chicken owners, something that is already happening, particularly around the large cities. Generally, however, vaccination programs need cool chain conditions for long keeping and distribution of the vaccines. Furthermore, it is necessary to motivate and to educate farmers of the necessity of vaccination, and to teach them where to get and how to keep and to use the vaccine, the last being among the activities of vaccine suppliers. Some models of the new approach are under way and have shown good results. The main problem ahead is how to vaccinate all chicken flocks in a geographical area, or at least to maintain an acceptable level of immunity in that area. Past vaccination campaigns made people aware of the benefits of vaccination. The main problem now is in supplying an appropriate vaccine to farmers.

Newcastle disease research

Almost all research work on ND in Vietnam is conducted in the National Institute of Veterinary Researches (NIVR), Hanoi. The NIVT was established in 1968, as part of the Ministry of Agriculture and Food Industry. It has now 150 staff members and a branch institute at Nha Trang city (Central Vietnam). ND research has been undertaken by NIVT since its foundation. Virus isolation, virulence determination, vaccine, ND immunity and immunisation schedules have been topics of study. Vaccines used against ND in Vietnam were developed in the NIVR. Current work consists of epidemiological surveillance, using some genetic markers to follow NDV circulation. Other studies indicated that the number of HN proteins per virion varied depending on NDV strain. This greatly influences the results of HI tests if a specific strain is not used in every laboratory.

While research to develop commercial poultry enterprises is essential, as mentioned above, up to 98% of poultry production is in the village chicken sector meaning that the main national resource lies in this sector as, we believe, is the case in other countries of this region. NIVR work is now being reorientated to protect this resource. In addition, the relation between wild and/or migrating birds and ND occurrence in scavenging chickens is still obscure, not to mention the increasing commercial activities in the region demanding tough measures for controlling ND. Against this background, we see the ACIAR project to develop a heat-resistant vaccine as being very significant.

The Poultry Industry in Kenya with Particular Reference to the Newcastle Disease Problem

J.T. Musiime*

Abstract

The total poultry population in Kenya is about 23 million, 70% of which comprise scavenger chickens, the rest improved (European) breeds. The agricultural sector contributes about 25% to the annual gross national product (GNP), of which 4% is from the poultry sub-sector mainly from the intensive production system. With ever increasing prices of red meat, scavenger chickens have become the main source of animal protein, in the form of meat and eggs, for the rural human population which comprises about 80% of the country's total population.

Newcastle disease (ND) is the most important poultry disease in the country followed, in the descending order, by fowl typhoid and fowlpox. The literature on the ecology and epidemiology of ND in Kenya and the nature of the causative virus strains is sparce.

However, the limited information available shows that the disease is widely distributed throughout the country, and occurs mainly during the cold and dry periods in the year, peaking in June-July.

The disease causes 80-90% mortality in both improved and scavenger chickens wherever there are outbreaks. Losses due to ND mortality, around the Nairobi area mainly among the exotic chickens, were estimated at US$0.6 million in 1989.

The disease is controlled by vaccination of chickens under the intensive sysstem and occasionally scavenger chickens in some foci of outbreaks, using F strain vaccine. However, this method has not been entirely successful, especially among scavenger chickens. An alternative method is needed.

KENYA covers an area of 583 000 square kilometres and is bordered on the east by Somalia, on the north by Ethiopia, on the north-west by Sudan, on the west by Uganda and on the south by Tanzania. It has a 400 kilometre coastline on the south-east.

Lying between 3°N and 5°S, 34°E, the country lies within the equatorial zone. It is almost bisected by the equator and the 38°E longitude.

The population, according to the 1989 census, is 24 million, with an annual growth rate of 3.8%, which has come down from 4.0%. About 80% of the population live in rural areas.

Kenya is mainly an agricultural country. However, only about 20% of the country is suited to crop production. The remainder is either semi-arid or arid and supports mainly cattle production under the pastoral system.

The agricultural sector contributes about 25% to the annual gross national product (GNP) of which 4% is from the poultry sub-sector, mainly the intensive system under which European (exotic) chickens are kept. The country's livestock population is estimated at 12.1 million cattle, 8.5 million goats, 7.3 million sheep, and 23 million poultry. About 70% of the poultry population is comprised of scavenger chickens; the balance is made up of improved (European) breeds.

The Importance of Scavenger Chickens

The prices of red meat, milk and poultry products (eggs and meat) from the intensive system have been gradually increasing in the recent past, especially in the urban areas. This has led to most people not being able to consume these animal products regularly because of their high cost. Most people, especially in the rural areas, depend mainly on eggs and poultry meat from scavenger chickens as their source of animal protein. Normally, the price of a whole

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scavenger chicken (average dressed weight is approximately one kilogram) would be the same as the price for half a kilogram of a dressed broiler chicken. Moreover, the eggs produced by the scavenger chickens are much cheaper than the eggs produced by intensively raised exotic chickens. This differential is influenced by a system of price control.

Most rural households keep a minimum of ten scavenger chickens. These would consist of one or two cocks and the rest would be hens. Each hen lays 10–15 eggs per batch and there are normally four batches a year. With a hatchability of 80%, each hen produces 8–12 chicks per batch. About 60% of these would survive predators and other causes of chick mortality to reach adulthood. The length of time it takes a chick to mature depends on, among other things, the availability of food. Usually the chickens are left to scavenge for their living and with an occasional supplement of owners' food leftovers. The chickens are rarely housed during the day. However, the chicks and their mothers are housed where the population of predators is very high.

As the chicks mature, the oldest chickens are either killed and eaten or are sold off to produce income. Besides supplying poultry meat and eggs for human consumption, the scavenger chickens are a source of petty cash. They also fulfil a social function — a visitor may be given a chicken as a gift on his departure.

Newcastle Disease in Kenya

Newcastle disease (ND) is the most important poultry disease in the country followed, in descending order, by fowl typhoid and fowlpox. Published information on disease outbreaks, number of positive cases diagnosed, epidemiology and the ecology of the disease is scanty. What information is available can be obtained from the Annual Reports of the Veterinary Department and the report of the work done by Nyaga (1982).

The disease was first encountered on the Mombasa Island (Daubney 1936) and later spread throughout the country. To date, it is still widely distributed. Mortality among the exotic breeds is usually more than 90% and 80–90% among scavenger chickens. Outbreaks are usually associated with the introduction of scavenger chickens from elsewhere. The owners of the scavenger chickens are well aware of the clinical signs of the disease and as soon as some birds start to die of the disease, the rest are quickly sold off. Most of them would be in the incubation period. This inevitably helps to spread the disease.

Intensive poultry production is concentrated in the Central Province, Nairobi area and the Rift Valley Province. The indigenous poultry flocks are concentrated in the eastern, coast, Nyanza and western provinces.

The records show that the larger number of ND outbreaks have been recorded in the Central and Rift Valley Provinces (see Table 1) and have occurred during the cold and dry periods in the year, with peaks in the June–July period. What is normally recorded is the tip of an iceberg, as the majority of the outbreaks that occur in the country, especially among the scavenger chickens, are not reported.

In the Nairobi area alone, there were 36 outbreaks recorded in 1989 and 17 in 1990. Most of these outbreaks were among exotic chickens kept on commercial farms. Losses due to mortality among exotic birds in the Nairobi area were estimated at US$0.6 million in 1989. No attempt has been made to estimate the economic losses due to ND either among the exotic or scavenger chickens in the whole country.

Control of Newcastle Disease

Vaccination against ND was introduced into Kenya in 1958. An inactivated vaccine was used. By 1965 the incidence of the disease had decreased remarkably.

Table 1. Distribution of Newcastle disease outbreaks by provinces (1957–1971)

Province	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971
Nyanza	4	1	2	3	E	1	1	E	2	2	1	6	3	2	8
Rift Valley	2	3	14	2	5	10	13	E	13	14	23	23	32	7	10
Southern	–	2	2	–	–	–	–	–	–	–	–	–	–	–	–
Central	63	16	29	22	21	6	23	E	3	8	5	9	11	6	7
Coast	E	2	7	E	2	E	E	E	E	1	8	2	E	2	
Northern	–	1	–	–	–	–	–	E	–	–	–	–	–	–	–
Western	–	–	–	–	–	–	E	E	E	1	E	11	6	1	2
Eastern	–	–	–	–	–	–	–	E	–	15	–	6	9	1	6
Nairobi	–	–	–	–	–	–	–	–	–	–	9	–	6	–	–
North Eastern	–	–	–	–	–	–	–	–	–	–	1	1	3	–	1

E = endemic

Thereafter, however, there was a dramatic increase in the disease incidence. There was again a decrease in the incidence of the disease for the period 1972–73 with the introduction of the F strain vaccine. The F strain vaccine is still in use. Broiler chicks are vaccinated at 18–21 days old, followed by a booster at 8–9 weeks. Layer chicks are similarly immunised. However, they are given a second booster at 18–22 weeks, again using the F strain vaccine. Previously, Komarov was used as the second booster for the layers, but farmers prefer the F strain vaccine, and the use of Komarov was therefore stopped in 1988.

Vaccine production recently became the responsibility of a parastatal body which charges for the vaccines it produces. In 1989, when the ND vaccine was still being produced by a government laboratory, the charge was 10 kenya cents per dose (US$ = 29 kenya shillings, 1 sh. = 100 cents). A total of 13,549,500 doses was produced in that year, with nearly all doses sold. In 1990, when the parastatal body took over, the charge was raised to KShs.2 per dose (950% increase). The farmers refused to buy the vaccine. The charge was then reduced to 30 cents per dose. Some of the total 10,460,000 doses produced in 1990 have not yet been used.

Vaccination is mainly carried out on commercial farms and in some foci of outbreaks among scavenger chickens near the urban centres. The owners of the scavenger chickens are not keen to have their birds vaccinated when there is no disease outbreak. These birds are a source of infection to other scavenger chickens and to the commercial farms.

Conclusion

Scavenger chickens in Kenya have a great potential for the provision of poultry meat, eggs and cash to the rural communities. However, their productivity is inhibited by the widespread occurrence of ND, to which they are highly susceptible. The current vaccination program has not been successful in controlling the disease. Outbreaks have occurred in peri-urban areas where vaccination has been practiced regularly. In order to control the disease effectively, studies are needed of the epidemiology of the disease and for the biological characterisation of the causative virus strains. Consequently, a vaccination program with an effective delivery system of the vaccine(s) should be developed.

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Poultry Disease in Africa and the Newcastle Disease Problem: an Overview

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Abstract

The majority of people in Africa live in rural areas and earn very low incomes. Almost every homestead has some chickens and these provide the cheapest source of animal protein in the form of eggs and meat. Chickens are also a ready source of petty cash for subsistence. Greater attention should be paid to factors that limit the productivity of scavenger chickens, especially the Newcastle disease (ND) problem, for the benefit of rural communities.

THERE is an enormous deficit between the animal protein produced in Africa and that required to feed the continent's 550 million people. The deficit is attributed to low productivity of livestock rather than low numbers.

Cattle, sheep and goat populations are 180 million, 187 million and 151 million, respectively. These figures represent 14.5%, 15%, and 29% of the world total, respectively. Their productivity is 6.8% of world beef, 0.6% of world milk for cattle, 16% of world sheep and goat meat and 11.5% of the world sheep and goat milk. In an attempt to satisfy the demand for animal protein, most of the African countries continue to import milk and meat worth millions of dollars. These imports would have been much higher if it were not for the existence of a poultry industry in most countries in the continents.

Poultry Industry in Africa

The poultry industry in Africa involves both scavenger chickens and exotic (European) breeds. The latter are kept under intensive conditions for commercial purposes.

The poultry population in the continent is 1690 million (FAO–OIE–WHO 1990), scavenger chickens far outnumbering the exotic breeds. This may be a low estimate, because while it is possible to get an accurate figure for commercial poultry, it is difficult to do the same for scavenger chickens. For the latter, the estimate made is usually based on the average chickens per household.

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The bulk of animal feed, including that for poultry, consists of food grains, especially maize. Moreover, quite a large proportion of the African people depend on food grains for their staple food, so that there is competition between them and livestock for the available food grain. To make the situation worse, the human population has been increasing while grain production has been declining. These factors have been responsible for the high costs of animal feeds, including those for poultry. Correspondingly, the prices for commercial poultry products have become quite high. For example, the price for one kilogram of a dressed broiler chicken in Nairobi is K.Shs.70.00 (1US$–29 K.shs.), much higher than European prices.

Most of the people of Africa live in rural areas and have a very low income. They cannot, therefore, afford to buy poultry products from the commercial farms. Scavenger chickens are their main source of animal protein. In the first instance, they are cheap to produce. Secondly, people find it much easier to kill a chicken for family consumption than, say, a goat, sheep or cow. The larger livestock are kept mainly for prestige or as a sign of wealth. The higher the number of animals one keeps the higher is one's status in the community and also the wealthier is one regarded.

Also, the indigenous birds provide a larger proportion of the table poultry trade. They are preferred to the exotic birds for the staple dishes, usually curries, of the lower- and middle-grade hotels and restaurants.

Live indigenous birds are on sale wherever there is a market. Large numbers are transported in large wicker baskets, on lorries, from rural to urban areas.

The Newcastle Disease Problem in Africa

Newcastle disease (ND) has a severe economic impact on the chicken industries of Africa. Countries that responded to a questionnaire we circulated have reported very high mortalities both in commercial and scavenger chickens. Mortality in the former is usually higher than in the latter. However, in scavenger chickens, mortality can approach 100% if the disease is newly introduced. For example, in 1985, nearly all the scavenger chickens in Mutare Province of Zimbabwe were killed following the introduction of ND from neighbouring Mozambique (Hargreaves, pers. comm.). Zimbabwe had previously been free of the disease for a number of years.

In most of the eastern African countries, the disease flares up in the scavenger chickens during the dry seasons, which also tend to be windy (Kombo, Msiska, Mukiibi and Wamukoya, pers. comm.). For other countries that have reported the disease to us, there has been no indication of seasonality in the disease. What seems to be clear from the reports is that the disease is still very prevalent (Table 1).

Some countries take ND very seriously, to the extent that they have had it as one of the notifiable diseases for a long time. These countries are Algeria, Angola, Botswana, Burkina Faso, Cameroon, Ghana, Kenya, Madagascar, Malawi, Mali, Mauritania, Mozambique, Niger, Nigeria, Swaziland, Tanzania, Togo, Tunisia, Uganda, Zambia and Zimbabwe (OAU/IBAR 1990).

Table 1. Reported outbreaks of newcastle disease in Africa, 1985–1991.

	1985	1986	1987	1988	1989	1990	1991
Algeria	–	–	–	3	2	x	x
Angola	2	5					
Botswana	8			38	15	x	
Burkina Faso	9					x	
Cameroon							
Chad							x
Congo						x	x
Cote D'Ivoire						x	x
Egypt	33	19	20	17	8		x
Ethiopia							x
Ghana	27	28	4	75	79	x	x
Kenya	3			3	8	x	x
Lesotho				35		x	
Madagascar	40	22	11		29	x	
Malawi		7	1			x	
Mali	1						x
Mauritius			182	1	10		
Mauritania			4				
Mozambique		2				x	x
Namibia						x	x
Niger							
Nigeria	13	6	4	3	1		x
Senegal						x	
Sudan						x	
Swaziland	1						
Togo					2		
Tunisia	2	10	28	27	79		
Uganda							
Zaire		5				x	
Zambia		9			51	x	
Zimbabwe	2					x	x

Control of Newcastle Disease in Africa

The main control method for ND is by vaccination using the conventional vaccines. Malawi has implemented vaccination with V4 thermostable strain vaccine in commercial flocks only.

Generally, routine vaccination is undertaken in the intensive farms. For scavenger chickens, vaccination is done only around the foci of disease outbreaks.

For vaccination to be an effective method for controlling ND, there is a need to study the epidemiology of the disease and the causative virus strains in each country.

The African countries themselves are aware of the limited success they have achieved in controlling the disease. They have, therefore, commissioned the Pan African Veterinary Vaccine Centre (PANVAC) to implement quality control measures for ND vaccines used on the continent. ND vaccines have thus become priority number three after rinderpest and contagious bovine pleuropneumonia vaccines. PANVAC was also recently requested by participating countries, to coordinate the pilot trials of V4 thermostable vaccine in scavenger chickens in Africa. The results obtained in Southeast Asia are therefore of much interest to PANVAC in particular, and Africa in general.

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Summary and Recommendations

Summary and Recommendations

Conclusions

1. Velogenic Newcastle disease is a problem in village chickens

In many countries Newcastle disease is the most important cause of loss in village chickens. In a very few, Newcastle disease is regarded as a less serious problem and in some countries, mostly islands, Newcastle disease does not occur.

2. One solution is a thermostable vaccine that can be taken to remote areas with minimal reliance on a cold chain

In Websters heat-stable V4 vaccine we have an extremely safe vaccine that has protected against challenge with all the velogenic strains of Newcastle disease virus against which it has been matched in four continents.

3. If village chickens can be caught, application of vaccine by conventional methods is most efficient

In some countries where chickens are well housed, the most efficient vaccination regime will be to utilize the thermostability of Websters V4 vaccine combined with individual vaccination.

4. In many areas, if village chickens are to be vaccinated, the vaccine will have to be delivered on food to unconfined, scavenging chickens

Websters V4 vaccine has been used successfully as a food vaccine in laboratory trials and in the field in several countries. Successful vaccination has been judged by serological testing, by experimental challenge and by analysis of mortality data collected in the field. However, the protection conferred can sometimes be erratic but current research offers a solution to this problem.

5. Food vaccination is to be used for village chickens over a wide area of Malaysia

The Malaysian control scheme will use vaccine mixed in bulk in ribbon blenders in central locations onto wheat grains that have received no other treatment. The pre-mixed grain will be transported to villages. We congratulate our Malaysian colleagues on rapidly reaching the implementation phase of the project, but note that this solution to the problem of oral vaccination will not be applicable in all countries. It is understood that other countries are planning implementation phases that will use other methods to prepare their food vaccines.

6. Successful implementation of this project could eventually improve the well-being of millions of villagers in many countries

Computer models indicate economic benefits will be gained with relatively modest levels of protection.

Recommendations

1. There is a need to perfect food delivery systems

Dr Rob Cumming and his group reported on methods for treating grain that reliably allow recovery of virus from grain after 18 hours and that reliably produce antibodies in chickens when fed after this time. The efficacy of this method should be established in challenge trials as a method of urgency.

2. The introduction of thermostable vaccines to further countries should commence with pilot trials

There seems to be no requirement to demonstrate the antigenicity of Websters V4 vaccine anew in further countries. Thermostable V4 could be used in these countries as a conventional vaccine without further research. However, if food vaccines are to be used, suitable methods for delivering vaccine on locally available foods must be determined.

3. Pilot trials will require team efforts

It is essential that people with diverse skills — including poultry experts, economists, epidemiologists and virologists — be included in planning and performing pilot trials and in later implementation.

4. Extension must be a vital part of any new vaccine program

Skilled extension workers are required, not only for the implementation phase of projects, but for the successful initiation of pilot village trials. Community participation is essential for the success of these programs.

5. The control of Newcastle disease should lead to further studies that will allow the most efficient exploitation of the village chicken

Village chickens have been a neglected resource because of the ravages of Newcastle disease. As Newcastle disease is controlled with thermostable vaccine, the full economic potential of the village chicken and its scavenging environment must be defined and developed. Obvious problems will be the large brooding losses that occur in all countries and the eventual need to control chicken populations to protect the scavenging environment.

6. Consideration should be given to the future funding of village chicken programs

Now is the time to consider approaches to suitable agencies for funding new pilot projects, and existing and new implementation programs. Implementation programs should allow for serological monitoring of vaccine efficacy. Funding will also be required for the future studies that will establish a science of village chicken production.

7. Future workshops will be required to monitor the progress of vaccination programs and other studies on village chickens

This workshop, and the previous workshop held in Kuala Lumpur, have assisted greatly in the exchange of information on Newcastle disease vaccines for village chickens and in co-ordinating research. With the completion of the ACIAR project in 1991, possibly APHCA would be a suitable co-ordinating body.

8. Minimal standards for safety and potency should be established for vaccines for use in village chickens

Even if vaccines for use in village chickens are not produced in specific-pathogen-free eggs, they should meet prescribed minimal standards.

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