
Chapter 2

Food Pellet Vaccine for Newcastle Disease — Malaysian Studies

Development of Food Pellet Newcastle Disease Vaccine

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THE poultry industry in Malaysia and in the other Southeast Asian countries includes backyard types of operation where 20-30 birds are raised per household. About 25% of the supply of poultry meat and eggs in Malaysia is produced through small-scale and backyard operations. In the Philippines, Thailand and Indonesia, the bulk of the production of poultry meat and eggs comes from small subsistence farms. This system of poultry farming has been practiced for centuries among the rural families and will continue to form a substantial portion of poultry farming in Asia. The success of such operations depends on many factors including the prevention of diseases such as Newcastle Disease.

In Malaysia, although vaccination against Newcastle Disease is practiced, chickens that are reared in backyard operations in the rural areas are seldom vaccinated, because they are loose during the day making vaccination difficult. There is a need to develop a simple and effective method of vaccinating these chickens. A practical approach would be to incorporate the vaccine in feed which could be fed to the chickens. This paper describes the preparation of food pellets containing Newcastle Disease vaccine.

Materials and Methods

Virus

The V4 Newcastle Disease vaccine obtained from Arthur Webster Pty Ltd, Sydney, was used. Each bottle of the vaccine containing 1000 doses was reconstituted with 10 ml of sterile distilled water. The reconstituted vaccine was dispensed in 1 ml amounts in thin-walled glass ampoules which were heat-sealed and stored at -70°C.

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Selection of Heat-Stable Virus

Twenty ampoules containing the V4 Newcastle Disease virus (NDV) were thawed in an ice bath and kept in a water bath at 56 +/- 0.5°C. At various intervals two vials were removed from the water bath, the contents pooled and the infectivity determined. The infectivity was determined by inoculating 0.1 ml of the virus into 5 ten-day-old embryonated specific-pathogen-free (SPF) chicken eggs. Four days after inoculation, the eggs were chilled at 4°C for 4 hours, the allantoic fluid was harvested and the viral haemagglutinin was detected by the haemagglutination (HA) test. The HA positive allantoic fluid from virus that survived the longest period at 56 +/- 0.5°C was collected. The allantoic fluid was pooled and centrifuged for 30 min at 2500 rpm. The supernatant was collected, filtered using a 0.45 µm millipore filter, dispensed in 1 ml amounts in glass ampoules and kept at -20°C. This process was repeated several times until the virus that survived at 50°C for 9 hours was obtained. The virus was further purified by limiting dilutions in 9-10-day-old embryonating SPF eggs. A stock virus was prepared from the allantoic fluid. Sterility test was done before storing it at -70°C in 1 ml sealed ampoules. The stock virus had a titre of 10⁶EID₅₀ per 0.1 ml (EID = egg infection dose) and is designated V4-UPM.

Stability

The stability of the infectivity and the haemagglutinin of the V4-UPM-NDV and the original V4 was then tested. Ampoules containing 1 ml of the stock V4-UPM-NDV and original V4 were thawed in an ice water bath and totally immersed for a specific time in a water bath at 56 +/- 0.5°C then transferred to the ice-water bath. Samples were then assayed for infectivity using five 9-10-day-old chicken embryonating eggs per dilution. The 50% infectivity titres (EID₅₀) were calculated according to the method of Reed and Muench (1936). For haemagglutinating activity

chicken erythrocytes were used, as described by Allan and Gough (1974).

Preparation of Food Pellet Vaccine

To prepare the food pellet vaccine, 500 g of commercial pelleted chicken feed were attached to the spray housing of a Uni-Glatt Fluidised Bed Granulator, laboratory model (Glatt Benzen-Halbiegen, West Germany). Heated air at about 40°C was admitted to fluidise the pellets. Fifty millilitres of aqueous solution containing 1% polyvinylpyrrolidone (M. W. 44 000) and 10^9 EID₅₀ of the V4-UPM-NDV was sprayed from the middle of the spray housing with an atomiser at a rate of 2.5 cc/min under an air pressure of 30 psi. Upon completion of the spraying process, drying was continued for a period of 5 min at an air exhaust temperature of 40°C. After drying, the pellets were removed from the container and packed in plastic bags. The vaccine, which had a titre between 10^5 and 10^6 per 10 g of feed, was stored at 40°C.

Stability of Vaccine

Packets of pelleted V4-UPM-ND vaccine were kept at room temperature (28°C) and at 40°C for varying periods of time. Each packet contained 10 g of vaccine. At weekly intervals one packet of vaccine kept at room temperature and another at 40°C were removed and assayed for infectivity. The infectivity was assayed by mixing 10 g of the pellet vaccine with 10 ml of PBS. It was centrifuged at 1000 rpm for 30 min. The supernatant was collected and filtered using a 0.45 µm millipore filter; 0.1 ml per dilution of the supernatant was inoculated into five 9-day-old embryonated eggs and incubated at 37°C. Four days post-incubation the allantoic fluid was collected and tested for HA.

Vaccination of Chickens

Two hundred day-old chickens obtained from the university hatchery were divided into two equal groups. Chickens from group B were vaccinated at 3 and 6 weeks old. The chickens were fed with the pelleted vaccine early in the morning after starving overnight. One kilogram of vaccine was given to 100 chickens. Group A was treated as unvaccinated control.

Serology

At weekly intervals, 30 chickens from each group were bled and the sera assayed for haemagglutination-inhibition (HI) antibody titre, according to the method previously described (Spradbrow et al. 1978).

Challenge

The chickens were challenged with the velogenic viscerotropic Newcastle Disease virus designated Ipoh AF2240-220 (Chulan et al. 1982). Ten chickens from each group were infected intramuscularly by

inoculating each bird with 10^6 EID₅₀ of the virus. Contact challenge was accomplished by allowing 10 chickens from each group to mingle in the same room with unvaccinated chickens which had been challenged intramuscularly with 10^6 EID₅₀ of the virus. Chickens that were challenged either intramuscularly or by contact were kept in two separate isolation units. All chickens were observed for 14 days post-challenge.

Results

Selection of Heat-Stable Virus at 56°C

The allantoic fluids from the selected virus that survived at 56°C from 3 to 9 hours were collected and kept at -70°C in 1 ml ampoules. The allantoic fluid from 4 hours exposure time was used for subsequent experiments. Purification was done by limiting dilutions for the stock virus.

Heat Stability of Infectivity and Haemagglutinin

The thermostability of infectivity and haemagglutinin of V4-UPM are found to be different from that of the original V4. The HA titre of V4 decreased by 2 logarithms (base 2) during heating at 56°C within 2 hours, whereas it took 5 hours for the HA titre of V4-UPM to decrease by 2 logarithms (base 2). The haemagglutinins of V4-UPM are therefore more thermostable than the original V4.

The time required for a titre decrease by two logarithmic orders (base 10) was within 1 hour (rate constant of 0.1 1/min) for V4, and 3 hours (rate constant of 0.03/min) for V4-UPM. The less the rate constant, the more stable the virus. The difference in the rate constants of thermostability of infectivity of V4 and V4-UPM is shown in Fig. 1.

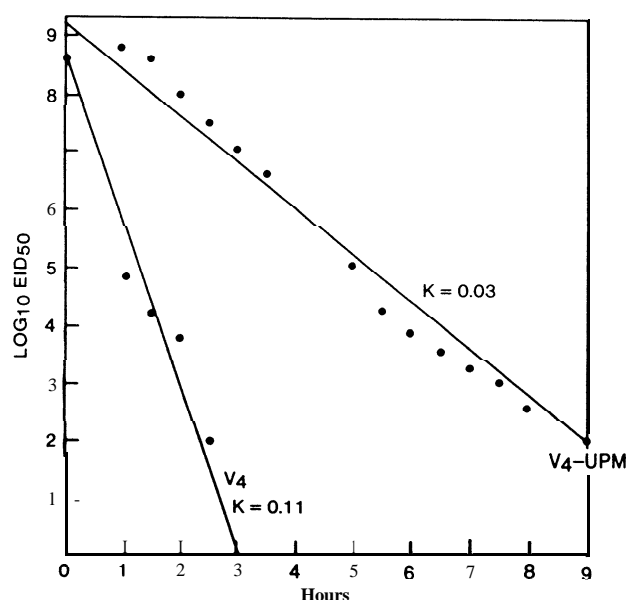


Fig. 1. Thermostability of infectivity (I) of V4 and V4-UPM Newcastle Disease virus at 56°C.

Vaccine Stability

The results of vaccine stability at 28°C and 4°C indicate that there is no significant change in titre for about 4 weeks, then the titre starts to drop from there on. However the viability lasted for about 12 weeks at room temperature.

Vaccination Trial

The HI antibody response of chickens vaccinated with oral pellet vaccine is shown in Fig. 2, and the distribution of HI antibody titres at the time of challenge is shown in Table 1. The day-old chicks started with some maternal antibody but this had dropped to zero by 3 weeks of age. There was no HI antibody response observed after the first vaccination at 3 weeks. Increase in antibody titre was only observed after the second vaccination at 6 weeks of age.

The results of challenge at 2 and 4 weeks after vaccination are shown in Table 1. About 90% of the chickens vaccinated with the food pellet Newcastle Disease vaccine were protected against challenge with the virulent NDV. All the chickens in the control group died irrespective of the route of challenge. Postmortem examination of vaccinated and nonvaccinated chickens that died from the challenge revealed haemorrhages in the proventriculus, intestine and caecal tonsils. The trachea and lungs were very congested.

Discussion

Simmons (1967) isolated an apathogenic NDV which was designated V4. Among the known properties of V4-NDV are: its immunogenicity, heat stability, avirulence and transmissibility. Numerous trials have been conducted to study the potential of the virus as a vaccine against velogenic viscerotropic

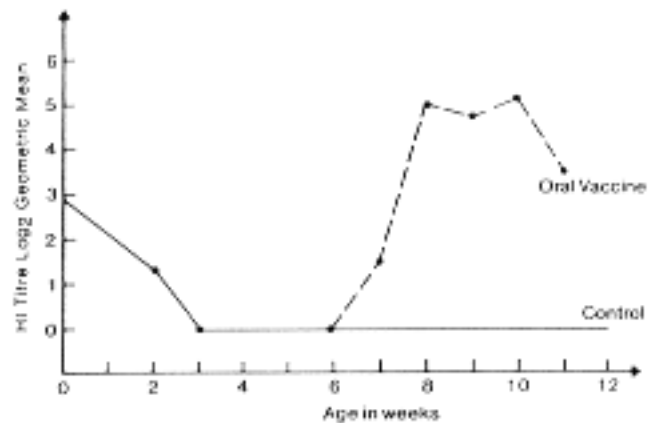


Fig. 2. HI response of chickens vaccinated with V4-Newcastle Disease oral vaccine.

Newcastle Disease virus (VVNDV). The efficacy of V4-NDV as an intranasally-administered vaccine or by spray, aerosol or drinking water method has been reported previously (Ibrahim et al. 1980, 1981). The vaccinated birds were protected against challenge with VVNDV.

However, the application of the above methods is not suitable for the backyard system of poultry-keeping where 20-30 chickens are kept per household. These chickens are let loose during the day thus making it difficult to vaccinate them. The present system of vaccinating these chickens individually either intranasally or intramuscularly is laborious and time-consuming and often not practical. A more practical approach is to incorporate the vaccine in the feed.

Exploiting the known properties of the V4 virus, its stability, immunogenicity and avirulence, an oral Newcastle Disease vaccine in the form of pelleted chicken feed was developed from a heat-stable clone of the virus. The vaccine was found to be heat-stable and could be kept at room temperature without

TABLE 1. Response of chickens to oral vaccination with heat-resistant V4-UPM Newcastle Disease virus vaccine at 3 and 6 weeks of age, and challenged with a viscerotropic velogenic strain of Newcastle Disease virus at 8 or 10 weeks of age.

Vaccine	Age (weeks) at serological testing & challenge	HI ^a antibody response		Route of challenge	Response to challenge (No. surviving/No. challenged)
		GMT ^b	% (No. tested)		
None	8	0	0(10)	IM ^c Contact	0 1 0 0 1 0
Oral	8	32	100(29)	IM Contact	11/11 10/11
None	10	0	0(20)	IM Contact	0/12 0/11
Oral	10	36	92(24)	IM Contact	8/10 10/10

^a Haemagglutination-inhibition.

^b Geometric mean titre.

^c Intramuscular.

affecting its viability. Chickens fed the vaccine were protected against in-contact or intramuscular challenge with virulent NDV and no difference was observed in mortality when challenged by both methods. According to Beard (1971) vaccines should offer greater protection if the vaccines are challenged intramuscularly (IM) or intravenously (IV) as the circulating antibodies would neutralise the IM- or IV-administered challenge virus and prevent infection in the respiratory tract. However, the challenge procedure by contact is a better method as it closely resembles the type of infection that would be expected to occur under field conditions.

The present study demonstrates the potential of the heat-stable clone V4 Newcastle Disease virus as a lentogenic food pellet Newcastle Disease vaccine. It is immunogenic and being heat-stable, it has advantages over other live vaccines whose potency deteriorates in a hot tropical climate. The ease of administering the vaccine makes it a good candidate for chickens kept under backyard conditions.

However, further studies on this heat-resistant variant are warranted. For example, it is not known exactly how selection is best applied, to what degree of heat resistance selection can be achieved, and whether the heat-resistant trait is stable when virus is passaged several times in eggs or chickens.

Vaccination of Village Chickens with Food Pellet Newcastle Disease Vaccine

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THE poultry industry forms a major component of the livestock industry in Malaysia. It includes the backyard-type operation where 20-30 birds are raised per household. About 20-25% of the supply of poultry meat and eggs in this country is produced through the small-scale and backyard operations. In the Philippines, Thailand and Indonesia, the bulk of the production of poultry meat and eggs comes from small subsistence farms rather than large commercial operations. This system of poultry farming has been practiced for centuries among the rural families and it is essentially a no-cash operation. The birds have unlimited freedom during the day to scavenge for themselves whatever food they can find. They are also given kitchen leftovers and occasionally some grains. They return in the evening to be confined in a small shed in the backyard. This traditional system of poultry production will continue to form a substantial portion of the poultry farming in Asia.

Newcastle Disease is a major disease of poultry in Malaysia and in other parts of Southeast Asia. In these countries the disease is controlled through a vaccination program. Without vaccination against Newcastle Disease, poultry rearing would almost be impossible. Although vaccination against the disease is being practiced in Malaysia, chickens that are reared in backyard operations are seldom vaccinated as they are let loose during the day making it difficult to vaccinate them. On occasions in which prior arrangement has been made, farmers may confine the chickens in the shed to enable the authorities to vaccinate them. This procedure is

usually time-consuming and laborious as the vaccinator has to travel from one house to another. The veterinary authorities also provide vaccination programs where the farmers can take their chickens to the veterinary centre to be vaccinated. The problems encountered in transporting these chickens normally discourage the farmers from making use of this facility.

There is, therefore, a need to develop a simple and effective method of vaccinating these chickens. One way to overcome the problem of vaccination would be to incorporate a vaccine virus in feed which could be fed to chickens.

A Feed Pellet Vaccine

Our approach was to isolate an avirulent Newcastle Disease virus which is immunogenic, heat-resistant and spreads readily between chickens, and to incorporate the virus in the feed that is offered to the chickens. While testing the Australian V4 Newcastle Disease vaccine for the Australian poultry industry a heat-resistant variant of the V4-NDV designated V4-UPM was isolated. Previous studies have also shown that the virus is immunogenic and highly transmissible. The V4-UPM-NDV was used as the vaccine virus for preparation of an oral vaccine in the form of food pellets. With assistance from the Australian Centre for International Agricultural Research (ACIAR), a 3-year project was initiated to develop a pelleted food vaccine which can be used for village chickens.

The oral vaccine was prepared by spraying the heat-resistant V4-UPM onto feed pellets. The potency of the vaccine is not affected when added to the pelleted feed. The vaccine is heat-stable and can be kept at room temperature without affecting the viability of the vaccine. Chickens fed with the vaccine were protected against challenge with the virulent Newcastle Disease virus. A similar vaccine regime has been applied to village chickens reared under simulated village conditions at the university.

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Vaccinated chickens when challenged with the virulent virus proved to be resistant.

Because of the encouraging results, field trials involving 15 villages in three states in Peninsular Malaysia were conducted. The vaccine was distributed to the farmers who were instructed to feed the chickens with the pelleted vaccine early in the morning. The calculated dose for each chicken was 10 g of the pelleted vaccine containing an equivalent of 10^6 EID₅₀ of the vaccine virus. Each chicken was given two doses of the vaccine at intervals of 3 weeks. The efficacy of the vaccine was evaluated either by monitoring the incidence of Newcastle Disease in the vaccinated flocks or by challenging the vaccinated chickens with the virulent Newcastle Disease virus 3 weeks after the second vaccination.

Results

Results from the field trial showed that 60% of

the vaccinated chickens were protected when challenged at the laboratory with the virulent Newcastle Disease viruses. There was no incidence of Newcastle Disease in the flocks where the trial was conducted for a period of at least 1 year. The trial was still in progress at the time of the workshop.

The present study demonstrates the potential of the oral Newcastle Disease vaccine for the village chickens. Among the advantages of the oral vaccine are the ease and simplicity in administering the vaccine and the time and labour saved. The vaccine is readily accepted by the farmers as they have the opportunity to administer the vaccine themselves. The vaccine is also able to prevent 100% mortality which normally occurs during an outbreak of Newcastle Disease in the village. The oral vaccine will provide a means of controlling Newcastle Disease in village chickens in Malaysia and possibly in other countries.

Field Trials of Newcastle Disease Food Pellet Vaccine

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THREE main types of poultry farms exist in Malaysia, as well as in other Southeast Asian countries. They are: the commercial, smallholder, and backyard types. Backyard poultry farming has been practiced for centuries among rural families (Leong and Jalaludin 1982), mainly because the method does not require any capital or labour input. They are the indigenous chickens that scavenge whatever food they can find. Some farmers provide small sheds in their backyards, which may or may not be used for confining the birds at night; otherwise the chickens rest on tree tops or under the house. These indigenous chickens provide a valuable source of animal protein to the farm family.

Disease is the main constraint to this type of farming. The chickens are slow-growing, thus disease control is very important. However, due to the unconfined type of management, disease control is rarely, if ever, carried out. The main losses are due to Newcastle Disease which can result in 90-100% mortality. Techniques commonly employed for the vaccination of chickens in the control of Newcastle Disease, such as intranasal, intraocular, intramuscular and in drinking water, are not suitable for this type of unconfined management. Handling of birds would be very laborious, therefore a simple method of introducing vaccine virus via food to these numerous small populations of indigenous chickens was carried out in 94 villages in Malaysia.

Materials and Methods

Field Survey

Three states in Peninsular Malaysia were selected for the field trials. They were Selangor, Negeri

Sembilan and Kelantan. Initially, about 300 questionnaires were prepared and distributed to the farmers to find out the status of village chicken rearing and their interest in joining the project. The survey was carried out with the cooperation of extension officers from Universiti Pertanian Malaysia, Serdang, and the staff of State Veterinary Departments. At the same time discussions were held at various village centres to explain the importance of Newcastle Disease and its control, and to introduce the food pellet vaccine.

The other method of vaccine introduction involved bringing about 200 farmers from the extension areas to the university's experimental simulated village chicken unit. The method of food pellet vaccine administration was demonstrated and explained. Mass media also played a role in introducing the vaccine.

Vaccine

The food pellet vaccine was prepared according to the method described earlier (Aini et al. 1986). The amount of vaccine was administered according to the number of birds in the group, with the dose calculated at 10^6 EID₅₀ per 10 g of food pellet vaccine. The amount received per bird would depend on the amount of the food pellet vaccine consumed by the bird.

Vaccination

For the first batch of farmers in each state, the birds were tagged and bled individually to record the initial status of antibody to Newcastle Disease, before the vaccine was administered. A second vaccination was carried out 3 weeks later, followed by monthly intervals. For each visit, the vaccine was distributed to the farmers and they were instructed to feed the chickens with food pellet vaccine the next morning. Birds from the second batch of farmers from all the states were not tagged or bled. The vaccine was given according to the schedule

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mentioned above. The efficacy of the vaccine in these birds was evaluated by challenge experiment as well as monitoring the incidence of Newcastle Disease in the vaccinated flocks.

Challenge

Three weeks after the second vaccination only chickens that were free of Newcastle Disease antibody at the time of the first vaccination were selected for challenge. Fifty birds from Kelantan and 100 birds from Selangor states were bought and brought back to the university for challenge. Sixty of these vaccinated birds were tagged and bled and had zero HI titre before vaccination. Another 90 village chickens from nonvaccinated areas were bought for nonvaccinated controls. They were tagged and bled before challenge to ensure that they were free of Newcastle Disease antibody titre.

The vaccinated birds were divided into three groups: A, B, and C consisting of 20 birds per group. In-contact challenge was obtained by mixing the vaccinated birds with 10 control birds which were given VVNDV strain AF-2240 intranasally at a dose of 10^5 EID₅₀ per bird. Another 20 unvaccinated birds per group acted as in-contact controls.

Results

Field Survey

More than 200 farmers responded to the questionnaires and all of them indicated their interest in joining the project. However, due to technical and personnel restrictions, not all the farmers could be chosen. Farmers were chosen according to areas and number of birds. The first batch of 18 farmers from four subdistricts in Selangor State were selected in February 1986. These included five farmers from the aborigines settlement. Later, the number of participating farmers in Selangor increased to 45 (total of 1200 chickens). They were the additions from the original four subdistricts as well as another subdistrict. However, five farmers from the aborigines settlement were later dropped from the project due

to difficulties in contacting them. Also two other farmers were dropped from the project because the farmers sold the birds during the outbreak of pullorum disease and infectious coryza.

The trials in Kelantan started in March 1986 with nine farmers from three districts. Two farmers from one district were later dropped from the project because the birds were sold, due to an outbreak of Newcastle Disease in commercial farms in the same district. Two other farmers from another district were also dropped from the project because of an outbreak of Newcastle Disease. The young chickens less than 3 months of age died and adults that survived were either slaughtered or sold. It is interesting to note these two farmers are neighbours and the birds were reared under the house in an overcrowded condition. Also, the last vaccination was given 4 months before the outbreak, due to technical problems. In November 1986, two islands in Tumpat district, Kelantan, joined the project, starting with a total of 23 farmers. By February, 1987, nine more farmers joined the project, making the total of 37 farmers for Kelantan State (total of 1800 chickens).

Two districts in Negeri Sembilan joined the project in May 1986 with a total of 19 farmers (total of 500 chickens). Therefore up to February 1987 there were 94 farmers participating in the project.

Number of Birds

The number of birds per household ranged from 10 to 100. The approximate number of birds involved in the field trials is 3500.

Serology

The chickens which had zero titre at the time of vaccination had a range of 0-6 log₂ titre 3 weeks after the second vaccination, that is, on the day of challenge. Twenty-five percent of those birds had zero titre and 10% below 3 log₂.

Challenge Experiment

The results of the challenge experiment are as shown in Table 1. Sixty-five percent from group B, 60% from group A and 55% from group C of the

TABLE 1. Results of challenge.

Group	Vacc. status	No. chickens	Challenge method	No. died	% survival
A	vaccinated	20	I/C	8	60
	nonvaccinated	10	I/N	10	0
B		20	I/C	20	0
	vaccinated	20	I/C	7	65
	nonvaccinated	10	I/N	10	0
C		20	I/C	20	0
	vaccinated	20	I/C	9	55
	nonvaccinated	10	I/N	10	0
		20	I/C	20	0

vaccinated birds survived the challenge, whereas all the nonvaccinated control died.

Disease Incidence

There is no report of the incidence of Newcastle Disease in the flock of birds where the trials were conducted except for the two cases mentioned earlier. The field trial had been going on for a year up to February 1987. There have been reports of Newcastle Disease outbreaks, however, in the neighbouring farms which did not practice vaccination.

Discussion

The results mentioned earlier cover only a 1-year period since the field trial started, and observation work to monitor the incidence of Newcastle Disease in those villages is still in progress. The serology results indicate that the chickens which had zero titre at the time of the first vaccination increased the titre ranging from 0 to 6 log₂ at the time of the challenge. The challenge results of about 60% protection when the birds were experimentally challenged with virulent NDV are encouraging, considering the simplicity in giving the vaccine and the time and labour saved. The vaccine is also readily accepted by the farmers as they can administer the vaccine themselves as easily as they feed the chickens. This is also seen by the increase in the demand for the vaccine. The vaccine would also be able to prevent 100% mortality which frequently occurs during an outbreak of Newcastle Disease in susceptible birds.

This less-than-100% protection is probably due to several constraints observed during the period of this field trial. The most important aspect is the amount of vaccine virus taken per bird. This varies greatly from chicken to chicken mainly because the adults and the young chicks usually run together. Therefore the adults take more vaccine than the young ones; and male chickens which are more

dominant would take more than females. Thus, there is the possibility of underdose in some birds as indicated from the low serology results in 35% of the birds. Secondly, few farmers did not give the vaccine as instructed as they wanted to save the vaccine till the birds became sick. There is the need, therefore, to improve the information and extension services to ensure that the farmers fully understand the importance of giving the vaccine before the disease occurs and not during an outbreak.

Some farmers would introduce newly purchased chickens into the flock and also there would be additions of newly hatched chicks from time to time. The newly purchased chickens may not have been vaccinated against Newcastle Disease making the flock susceptible to the disease. Therefore vaccination needs to be done regularly to ensure that young chicks that may not have taken the vaccine before, or not enough vaccine, and any newly introduced chickens would receive the vaccine. This may also allow the vaccine virus to be established in the flock and be transmitted from chicken to chicken either directly or indirectly.

Other disease problems such as pullorum and infectious coryza were often encountered in the field and if they are not properly diagnosed, they would interfere with the interpretation for the monitoring of incidence of Newcastle Disease. However, so far, our study demonstrates the potential of the food pellet Newcastle Disease vaccine for the village chickens. As is already known one vaccine or one vaccination program may not be suitable for different levels of husbandry and different areas. Therefore, further work needs to be carried out to improve the vaccine as well as to find the most suitable vaccination schedules for different methods of husbandry and areas. With the reduction of Newcastle Disease outbreaks in the village chickens, the level of challenge to commercial poultry would also be reduced, and control of other diseases can be investigated.

Efficacy of Food Pellet Newcastle Disease Vaccine: Laboratory and Simulated Village Experiments

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NEWCASTLE Disease is one of the most important diseases of poultry in many parts of the world including Southeast Asia. The velogenic viscerotropic Newcastle Disease (VVND), considered as the most virulent form of the disease (Utterback and Schwartz 1973), is common in Southeast Asia, where the disease is controlled in most countries through a vaccination program. Techniques commonly employed for the vaccination of chickens include addition of vaccine virus to the drinking water, application of vaccine virus to the conjunctival sac or external nares, and intramuscular injection, also dissemination of vaccine into the air as a spray or an aerosol. Recently we reported the development of an oral Newcastle Disease vaccine in the form of pelleted chicken feed (Aini et al. 1986). Chickens fed this vaccine were protected against VVND virus. This report describes the efficacy of the vaccine administered orally, intranasally or by in-contact in commercial broilers as well as village chickens raised under simulated village conditions.

Materials and Methods

Vaccine

The food pellet V4-UPM Newcastle Disease vaccine was prepared according to the method described earlier (Aini et al. 1986). The approximate dose per chicken is 10^6 EID₅₀ of the vaccine virus in 10 g of food pellets. For the intranasal route, the wet form of the V4-UPM vaccine was used at the dosage 10^6 EID₅₀ per bird.

Vaccination

EXPERIMENT 1

Four hundred day-old chicks were bought from a

commercial farm and divided equally into groups A, B, C and D. The chickens came from parent stock which had been vaccinated against Newcastle Disease. Group A was vaccinated with the food pellet vaccine. In-contact vaccination was achieved by vaccinating 20% of chickens from group B intranasally and allowing them to mix with the rest of the group. Group C was vaccinated intranasally. Group D was treated as unvaccinated control. Groups A, B and C were vaccinated at 3 and 6 weeks of age. The four groups of chickens were kept in four different isolation units.

EXPERIMENT 2

Five hundred day-old chicks were divided into five equal groups A, B, C, D, and E. Groups A, B, C and D were vaccinated at 1, 2, 3, and 6 weeks, respectively, with the food pellet vaccine. Group E was treated as unvaccinated control. The five groups were reared in five different isolation units.

EXPERIMENT 3

Chickens were purchased and raised under simulated village conditions at the university's experimental unit. At this unit they were provided with sheds which were enclosed by fencing. Food and water were provided in the shed but the birds were free to go in and out of the sheds. They were vaccinated twice with food pellet vaccine, at 3-week intervals.

SEROLOGY

Serum samples were collected at weekly intervals up to 12 weeks. For each collection 30 random samples were taken from each group. The haemagglutination inhibition (HI) test was carried out according to the method previously described (Spradbrow et al. 1978).

Challenge

EXPERIMENT 1

Chickens were challenged intramuscularly or by contact as previously described (Ibrahim et al. 1981).

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EXPERIMENT 2

At various times after vaccination 20 chickens from each group were taken for challenge. Ten were challenged by the intramuscular route and another 10 by in-contact method.

EXPERIMENT 3

Two weeks after the second vaccination, two groups of birds of 20 each were challenged by the in-contact method. Another group of nonvaccinated controls were challenged by intranasal and in-contact methods.

Results

The HI antibody response of chickens vaccinated with V4-UPM vaccine is shown in Fig. 1, and the distribution of HI antibody titres at the time of challenge is shown in Table 1. All groups of vaccinated chickens responded positively to the three routes of vaccination. However, chickens that were vaccinated intranasally responded earlier than the other two groups. Positive responses were not observed until 1 week after the second vaccination for groups A and B.

The results of challenge at 2 and 4 weeks after the second vaccination are shown in Table 2. About 90% of the chickens from all the vaccinated groups were protected against challenge with the virulent NDV, by the in-contact method. Protection above 70% was also observed in all the vaccinated groups challenged directly using the intramuscular route. All the chickens in the unvaccinated control group died irrespective of the routes of challenge. Postmortem examination of all the birds that died during challenge revealed haemorrhages in the proventriculus, intestines, caecal tonsils and caecum. The trachea and lungs were very congested. All the lesions were more severe in the unvaccinated group than in the vaccinated groups.

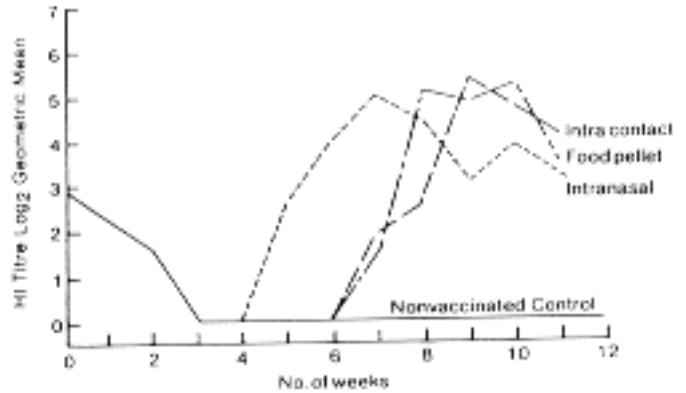


Fig. 1. HI response of vaccinated and nonvaccinated chickens.

The results of challenge from experiment 2 show that chickens vaccinated at 1, 2, 3, or 6 weeks of age were not protected when challenged with virulent Newcastle Disease. There were some differences in the onset and duration of the disease in the four vaccinated groups. Mortalities were observed as early as 3 days after challenge in groups A and B and by the seventh day all the chickens had died. In groups C and D there was some delay in the onset of the disease and most chickens died on the twelfth day after challenge.

For the simulated village experiment, 63% protection was obtained for group A and 65% for group B, when challenged with virulent Newcastle Disease virus, whereas all the nonvaccinated birds died.

Discussion

The efficacy of the V4 strain of Newcastle Disease virus as intranasal, aerosol, spray and drinking water vaccines has already been reported (Ibrahim

TABLE 1. Distribution of HI antibody titres at time of challenge in chickens vaccinated at 21 and 42 days old via oral, in-contact and intranasal routes, and HI antibody titres of unvaccinated controls.

Age at challenge	Group	Method of vaccination	No. chickens tested	HI distribution (log ₂)							Geometric mean	% HI	% Immune to challenge	
				0	1	2	3	4	5	6				7
8 weeks	1	oral-feed	29				3	6	12	3	5	5.0	100	90.9
	2	in contact	30	14	0	1	0	3	8	1	3	2.7	50	80
	3	intranasal	18	0	1	0	3	5	6	2	1	4.4	94	90
	4	control	10	9	1	0	0	0	0	0	0	0	0	23.5
10 weeks	1	oral feed	24	0	0	2	2	5	3	4	8	5.2	92	100
	2	in contact	28	0	0	2	6	7	5	0	8	4.7	93	100
	3	intranasal	15	0	0	2	4	5	2	1	1	3.9	87	90
	4	control	20	0	0	0	0	0	0	0	0	0	0	0

TABLE 2. Response of chickens to oral, intranasal and contact vaccination with heat-resistant V4-UPM Newcastle Disease virus vaccine at 3 and 6 weeks of age and challenge with a viscerotropic velogenic strain of Newcastle Disease virus at 8 or 10 weeks of age.

Vaccine	Age at serological testing and challenge (weeks)	HI antibody response		Route of challenge	Response to challenge (No. survivors/ No. challenged)
		GMT	% (No. tested)		
None	8	0	0(10)	IM	0/10
				Contact	4/17
Oral	8	32	100(29)	"	11/11
Intranasal	8	21	94(18)	"	10/11
					8/10
Contact	8	7	50(30)	"	9/10
					3/10
None	10	0	0(20)	"	8/10
					0/12
Oral	10	36	92(24)	"	0/11
					8/10
Intranasal	10	15	87(15)	"	10/10
					9/10
Contact	10	26	93(28)	"	9/10
					8/11
					10/10

et al. 1980, 1981). Chickens vaccinated by any of these methods are protected against intranasal challenge or natural infection with virulent NDV. The application of Newcastle Disease vaccine via drinking water, spray or aerosol is commonly employed for mass Newcastle Disease vaccination programs. The principal advantage of these methods is the saving of labour and the simplicity of administration. These techniques are usually employed to vaccinate chickens reared under intensive or semi-intensive systems of poultry production. Such techniques may not be suitable for the vaccination of chickens reared under the backyard system of poultry keeping where each household rears about 10-20 chickens which are free to roam during the day.

We recently developed an oral vaccine whereby the vaccine virus is incorporated in commercial chicken feed (Aini et al. 1986). Chickens vaccinated with this vaccine were protected against the VVNDV. The present study confirms the finding that the food pellet Newcastle Disease vaccine stimulates a satisfactory immune response and protects the chickens against mortality and morbidity. There was no significant difference between antibody levels of groups of chickens that were vaccinated intranasally or orally and both groups were equally protected against virulent NDV. The present study also demonstrates that a single oral vaccination is not sufficient to confer immunity in vaccinated chickens as a high percentage of the chickens died from the challenge. In our previous findings (Ibrahim et al. 1981) there was no

difference in terms of HI antibody response between chickens vaccinated intranasally at 21 days old and chickens vaccinated intranasally at 21 and 35 days old. The vaccinated chickens in both groups were immune when challenged at 49 days old and 77 days old with virulent Newcastle Disease virus. The present study indicates that one oral vaccination is not sufficient and that the immune response needs to be boosted with at least one other oral vaccination. However the ease of administering the vaccine should solve the problem of revaccinating the chickens at regular intervals.

The vaccination under simulated village conditions gave about 60% protection. This may be due to the variation in the amount of vaccine taken per bird as the adults and young birds were kept together, compared to laboratory trials where all birds were of the same age.

Another property of the clone V4-NDV that was exploited in this study is its transmissibility. The high transmissibility of the V4-NDV between infected and in-contact chickens has been reported by several workers (Kim and Spradbrow 1978b; Ibrahim et al. 1981). Chickens acquiring simulated natural infection with the V4-NDV at 8 weeks of age developed high levels of immunity when challenged 3, 5, 10 and 21 weeks later with the virulent NDV. In the present study chickens placed in contact with intranasally vaccinated chickens developed immunity to Newcastle Disease.

Poultry meat and eggs are important sources of protein and income for the people in rural areas of Southeast Asia. The VVNDV continues to be a

threat to the chickens reared under backyard operations. It is essential to the farmers that their chickens be protected against Newcastle Disease as the loss of even one chicken is important. Since the objective of vaccinating chickens in the rural areas

is to prevent mortality during an outbreak, the food pellet vaccine appears to be effective. A similar vaccine regime may be applied to chickens kept in villages. However, this aspect needs to be studied further.

Velogenic Viscerotropic Newcastle Disease Virus

May C. Lai and A. Latif Ibrahim *

CONSIDERABLE work has been done over the years on production and improvement of Newcastle Disease vaccines and on the control of disease. The focus here is on Newcastle Disease control and the use of a vaccine incorporated with pelleted chicken feed.

In Malaysia, the Newcastle Disease virus that is responsible for the high morbidity and mortality of chickens is the velogenic viscerotropic Newcastle Disease virus or VVNDV. The properties of an isolate used as a challenge virus in most of our experiments on Newcastle Disease are presented here. This Newcastle Disease virus isolate has been designated AF2240 and it was isolated from a local field outbreak in the 1960s.

Morphology and Characterisation of AF2240

AF2240 is a paramyxovirus and is pleomorphic in nature. Under negative contrast electron microscopy, intact virions had diameters ranging from 150 to 600 nm and spikes 10 to 12 nm long were present at the periphery. These spikes were interpreted as the glycoproteins. Tubular structures, 17 nm in diameter, were interpreted as the nucleocapsids and they had serrated edges and a central core of 4-5 nm in diameter. The morphology of AF2240 observed was similar to that of other Newcastle Disease virus isolates described (Horne et al, 1960; Waterson 1962, 1964; Compans and Choppin 1967; Hosaka and Shimizu 1968; Kingbury and Darlington 1968; Cheville et al. 1972; 1972a).

AF2240 has been found to have an intravenous pathogenicity index (IVPI) of 2.56 (Abdul Rahman et al. 1976), and intracerebral cytopathogenicity index (ICPI) of 1.9 (Lai 1985) and a mean death time (MDT) of 53 hours (Bell in press). Such values placed AF2240 close in rank with other well-documented challenge viruses such as Herts 33, Milano and G.B. Texas.

Cytopathogenicity of AF2240

Like other virulent Newcastle Disease virus isolates described, AF2240, when grown in cell cultures, caused haemadsorption, polykaryocytosis and plaque formation. As pointed out by Reeve and Poste (1971), the virulence of strain in vivo appeared to be paralleled by the ability of the same strain to destroy and damage cells when grown in vitro. Similarly, strains which are avirulent in vivo generally fail to produce cytopathic effects in vitro.

Replication of AF2240

Studies using fluorescent antibody technique and electron microscopy revealed that AF2240 replicated in the cytoplasm of infected cells. Electron microscopy has shown that the nucleocapsids first appeared in the cytoplasm around the nucleus of the infected cells, and in the later stages of infection they were found mainly near or below altered cell membrane. The altered cell membrane was more electron dense than the adjacent membrane and had a fuzzy extramembranous coat interpreted as the spikes as seen in negative contrast electron microscopy. The maturation of AF2240 was seen at the cell membrane where the outfolding altered membrane and associated nucleocapsids pinched off by budding. The altered host cell membrane then became the viral envelope enclosing the viral nucleocapsids. This release of virus by budding is in agreement with previous reports on NDV replication (Feller et al. 1969; Yunis and Donnelly 1969; Donnelly and Yunis 1971; Hecht and Summer 1974; McNulty et al. 1977).

AF2240 as a Challenge Virus

AF2240 has been used as a challenge virus in many vaccination trials and it has been shown that AF2240 can cause 100% mortality in susceptible flocks (Ibrahim et al. 1980; 1980a; 1981; Chulan et al. 1982; Ernawati and Ibrahim 1984). From post-mortem examination of infected chickens, lesions commonly observed were haemorrhages in the

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proventriculus, small intestines and caecal tonsils (Ibrahim et al. 1980; 1980a; 1981) and also in the trachea (Chulan et al. 1982). Such lesions conformed to those described for the VVNDV by McDaniel and Osborne (1973).

In an experiment in which electron microscopy was used to evaluate the damage in the trachea of nonvaccinated and vaccinated chickens challenged with AF2240, it was found that although not observed grossly, damage was present in the trachea of the vaccinated chickens. However, the degree of damage of AF2240 was very much reduced in the vaccinated flock. At the same time, none of the vaccinated chickens showed any signs of Newcastle Disease after the challenge and no mortality was recorded. On the contrary, signs of the disease were observed in the nonvaccinated flock as early as day

5 post-infection and the chickens within the first week of challenge.

Along with electron microscopic studies, virus isolation was carried out on the trachea of both the nonvaccinated and vaccinated chickens which were challenged. It was found that Newcastle Disease virus could be isolated from the trachea of all the nonvaccinated chickens from day 3 post-infection. However, in the vaccinated flock, Newcastle Disease virus was isolated from only 19% of the challenged chickens and the presence of virus was only detected between day 6 and day 9 post-infection. It should be noted here that vaccination did prevent clinical manifestation of Newcastle Disease, but it did not prevent infection. The ability to isolate Newcastle Disease virus from the vaccinated flock pointed to the possibility of these chickens becoming carriers.

Technology Transfer of Food Pellet Newcastle Disease Vaccine

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THE village poultry plays an important role in the farming systems in Southeast Asia. Village poultry not only provide a source of meat but also income to the farmers. It is of great importance to the farmers that the poultry are kept alive and healthy as the loss of even one chicken means a loss of income. Profitable poultry production in the village is only possible if the major poultry diseases are brought under control.

A number of parasitic, bacterial and viral diseases are known to infect chickens and one of the most important diseases is Newcastle Disease. This disease is known to cause high mortality among village chickens especially in villages where a vaccination program is not practiced. It is essential for the villagers to understand that the chickens should be vaccinated at all times. Although various types of Newcastle Disease vaccines have been developed and various methods of administering the vaccines have been devised, the farmers are still faced with many problems to prevent direct and indirect losses from the disease.

Techniques commonly employed to vaccinate village chickens are not very practical. These techniques include addition of vaccine to drinking water, intramuscular injection and dissemination of vaccine as spray or aerosol. Recently an effective, simple and cheap method of administering vaccine was developed. This was made possible by the isolation of a heat-resistant Newcastle Disease vaccine virus and the incorporation of the vaccine in feed pellets which can be given to chickens. Trials conducted under laboratory and simulated field conditions showed that chickens vaccinated with these vaccines were protected against the virulent Newcastle Disease. This paper describes the transfer of this technology to the farmers in the rural areas.

Transfer of Technology

The most important phase in the application of biotechnology in animal health and production is the successful introduction of the results into practice. Unlike in the commercial sector, results of research conducted at universities or research institutes generally find their way to the user groups through some types of extension education.

In the transfer of any new technology some basic principles need to be considered. These are: (1) the technology should be appropriate to the farmers based on their needs and be compatible under the real situation; and (2) the mode of transfer of new technology must be effective: a) using mass media to disseminate the research findings, b) demonstrating new technology to farmers, c) visiting by researchers and extension agents to villages where farmers would be briefed on the new technology, and d) using questionnaires to determine the interest of the farmers in the new technology; and (3) interdisciplinary teamwork.

The laboratory and simulated field trial demonstrated that the technology should be appropriate to the farmers so the vaccine could be fed to chickens. This should eliminate the problem of having to catch the chickens and to vaccinate them individually. Moreover the vaccine should be heat-stable, and should not lose its potency under the hot tropical environment. The vaccine should be effective in the village situation.

In the transfer of the technology, the mass media such as newspapers, extension bulletins, radio, and television were fully utilised. This was effective as there were enquiries regarding the vaccine not only from within the country but also from outside the country. Visits were also made to the villages where the new technology was explained to the farmers. Farmers were also invited to the university where demonstrations were held on the vaccination of chickens reared under simulated village conditions. The farmers were given the opportunity to feed the vaccine to the chickens. Questionnaires were given

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to the farmers at the end of the demonstration and among the information asked was whether they were interested in getting involved in our vaccination program against Newcastle Disease, where the farmers would be supplied with the vaccine. Most of the poultry farmers were interested in the new technology and were ready to participate in the vaccination program.

Teamwork is also essential in the transfer of new technology. In the present study three main agencies are involved in our strategies to disseminate our findings to the villages. These agencies are the Faculty of Veterinary Medicine and Animal Science, the Centre of Extension and Continuing Education, and Division of Veterinary Services, Malaysia. While most of the development of the vaccine is being done at the Faculty of Veterinary Medicine and Animal Science, the Centre for Extension and

Continuing Education assists us in disseminating the information. The Division of Veterinary Service was involved both in the selection of villages for the field trial and also in the actual field trial.

Conclusion

In order for a new technology to be accepted, it must have relative advantages to the farmers. Farmers will be interested in the new technology if it has economic value to them. The technology must also be acceptable to the farmers and not too complex. In the case of the food pellet Newcastle Disease vaccine, the technology is expected to be accepted by the farmers as it suits their lifestyle. Without spending much time, they would be able to have their chickens vaccinated against Newcastle Disease.

Production of Newcastle Disease Vaccine in Malaysia

Lo Honn Seang *

THE procedures in producing Newcastle Disease vaccine appear relatively simple. But the production of an effective and safe vaccine for practical use is not readily achieved in many countries. Besides the differences in materials used, testing methods employed and production facilities, there are even variations with regard to immunogenicity and potency among the same Newcastle Disease vaccine strains used for production.

This paper attempts to describe the strain and method of production in our institute, and highlights the problems involved. We started to produce Newcastle Disease vaccine in 1949. At present, two strains of vaccine are produced, namely lentogenic Asplin's 'F' and mesogenic Mukteswar.

Vaccine Seeds

Newcastle Disease Asplin's 'F' strain: This strain originated from Weybridge, U.K., in 1953. Prior to 1982, this uncloned vaccine seed was passaged from time to time in nonspecific-pathogen-free (non-SPF) eggs without a proper seed-lot system for production. In 1982, a new seed was cloned from the existing seed by limiting dilutions and further plaque-purified. A master seed-lot (MSL) and a working seed-lot (WSL) were produced in SPF eggs purchased from Wickham SPF Farm, U.K. Both master seed-lot and working seed-lot were characterised and tested in vitro and in vivo.

The following tests were used as markers: (a) intravenous pathogenicity index (IVPI); (b) intracerebral pathogenicity index (ICPI); (c) mean death time (MDT); (d) plaque morphology; (e) serological response and mortality in day-old chicks (maternally immune) and 3-week-old susceptible chickens by intranasal/intraocular routes of inoculation for a period of 3 weeks; (f) challenge with virulent strain of NDV for chickens (e) above.

Both MSL and WSL were also subjected to

different purity tests before being freeze-dried for storage and subsequent production.

Newcastle Disease Mukteswar strain: This strain was obtained from India in 1949. Like the Newcastle Disease Asplin's 'F' strain, there was no proper seed-lot system prior to 1982. A new seed was again cloned in 1982 with the same method as that described for Newcastle Disease Asplin's 'F' above.

Source of Eggs

Nine to ten-day-old embryonated eggs are used for production of Asplin's 'F' and Mukteswar strain of vaccine respectively. The eggs are candled and washed lightly with 70% spirit before they are drilled and inoculated via i/a route with about 10 000 EID₅₀ per 0.1 ml of the respective seed. Any dead eggs within the first 24 hours of incubation at 37°C are discarded. It is important that only clean eggs be used and they should never be washed with water. This will minimise a lot of contamination problems.

Newcastle Disease 'F' vaccine is harvested in 150-ml bottles 96 hours post-incubation and after overnight chilling at 4°C. The Mukteswar strain is harvested 48 hours post-incubation without chilling. Each bottle of vaccine is subjected to preliminary sterility tests in nutrient agar for 4 days before they are pooled.

The pooled vaccine is sealed in ampoules, frozen at -20°C for storing, or freeze-dried in vials with 5% lactose and 0.1% polyvinylpyrrolidone (PVP), M.W. 700 000 as stabiliser.

Meanwhile, samples of vaccine are subjected to various quality control tests. When the quality tests are found to meet the requirements, the vaccines are issued for use.

Test Requirements

1. In vitro

These include the following tests for purity: (a) anaerobic and aerobic bacterial cultures: (b) fungal

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culture in Sabouraud's medium; (c) mycoplasma cultures; and (d) extraneous viral contaminant detection mainly by agar gel precipitation test on postvaccination sera of chicken and passages in eggs after serum neutralisation.

2. In vivo

The test is a safety and potency test in chicken as follows:

	Treatment	Observation	Challenge	Tests
Safety test	Given undiluted vaccine via proper route	No noticeable reactions within 3 weeks	Nil	HI for ND HA for EDS AGPT for IB, CELO, Reovirus, IBD, etc Nil
Potency test	Given field dose of vaccine via normal route	No noticeable reactions within 2 weeks	Challenged with $10^{6.5}$ EID ₅₀ of virulent virus	Nil

Qualitative Assay

Both haemagglutination (HA) and egg-infectivity titration are carried out. For Newcastle Disease 'F' vaccine, the standard required titre is no less than $10^{6.5}$ EID₅₀ per bird-dose and for Mukteswar strain, the required titre is no less than 10^5 per bird-dose.

Problems

In our institute, over 126 million doses of Newcastle Disease vaccines were produced in 1986. The ability to expand this capacity is limited by the availability of rooms properly designed in accordance with the recommendation given by FAO

(Allan et al. 1978) In this connection, a new biology building is being built for this purpose. Also, an independent assay and quality control unit is to be established in the new building where more sensitive tests are to be introduced for better quality control of vaccines.

Many poultry diseases, especially those of viral origin, can be transmitted through eggs. The danger

of using contaminated embryonated eggs for vaccine production, therefore, must always be in the minds of poultry vaccine manufacturers, especially if quality control employed is not sufficiently sensitive and specific.

The use of SPF eggs in production will certainly help in eliminating this danger. But keeping an SPF flock is expensive and unless there is an economical scale of production or the volume of vaccine production justifies the establishment of an SPF flock, the final cost of Newcastle Disease vaccine may become too high.

Alternatively, killed vaccine or tissue-cultured Newcastle Disease vaccine provides an easier way to control possible poultry pathogen in vaccine and research effort therefore can be directed to this end.