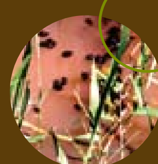
Australian Government
Australian Centre for
International Agricultural Research

Overcoming liver fluke as a constraint to ruminant production in South-East Asia



Overcoming liver fluke as a constraint to ruminant production in South-East Asia

Edited by G.D. Gray, R.S. Copland
and D.B. Copeman

Australian Centre for International
Agricultural Research 2008

The Australian Centre for International Agricultural Research (ACIAR) was established in June 1982 by an Act of the Australian Parliament. Its primary mandate is to help identify agricultural problems in developing countries and to commission collaborative research between Australian and developing-country researchers in fields where Australia has special competence.

Where trade names are used this constitutes neither endorsement of nor discrimination against any product by the Centre.

ACIAR MONOGRAPH SERIES

This series contains the results of original research supported by ACIAR, or material deemed relevant to ACIAR's research and development objectives. The series is distributed internationally, with an emphasis on developing countries.

Published by the Australian Centre for International Agricultural Research (ACIAR)
GPO Box 1571, Canberra ACT 2601, Australia
Telephone: 61 2 6217 0500
aciar@aciar.gov.au

Gray G.D., Copland R.S. and Copeman D.B. (eds) 2008. Overcoming liver fluke as a constraint to ruminant production in South-East Asia. ACIAR Monograph No. 133, pp. 155

ISBN 978 1 921434 68 6 (print)
ISBN 978 1 921434 70 9 (online)

Technical editing by Scribbly Gum Publications Pty Ltd, Canberra
Design by MA@D Communication
Printing by Bluestar Print

FOREWORD

Liver fluke is an important internal parasite of ruminants. It debilitates livestock from a wide range of economically important species including cattle, buffalo, sheep and goats. Liver fluke disease attracts less attention than diseases caused by other parasites and infections that have more obvious symptoms and cause high levels of mortality. However, its ability to markedly limit production in many environments is generally underestimated. In the humid tropics, the focus of research reported in this monograph, liver fluke causes lost production through slower growth, fewer calves, less milk, reduced draught power, and the loss of livers as a food source.

Liver fluke disease is caused by *Fasciola gigantica* in the low-altitude tropics, and by the related parasite *Fasciola hepatica* in temperate zones and in the high-altitude tropics. Many of the concepts of genetics, immunology, epidemiology and control of liver fluke disease have been transported from temperate to tropical systems and from *F. hepatica* to *F. gigantica* and are not necessarily appropriate. The results of research reported in this monograph seek to address this gap in knowledge. A wide range of research is reported from more basic science such as genetics and host resistance to more applied subjects such as epidemiology and extension methodologies. The outcome of any research should ultimately be to equip farmers with cost-effective control options. This is a challenge in some environments where production per se is not necessarily an incentive and signs of the disease may not be obvious. Effective control of liver fluke disease in the tropics needs to use locally available resources, be economically sustainable and be adaptable to changing circumstances by local specialists.

Much of the research reported has been undertaken with support from the Australian Centre for International Agricultural Research (ACIAR) and researchers are acknowledged through authorship and citation in each of the eight chapters. ACIAR provided the finances, project management support and networking among Australian and Asian scientists and is proud to have contributed to this complex research program along with partner national institutions and other donors.

Bruce Copeman.

Of the many scientists who have contributed to parasite research in the tropics, and to have led projects supported by ACIAR, Bruce Copeman requires special mention. Bruce laid the foundation for this monograph through the development of projects, supervision of students and publications of his extensive research on liver fluke and other parasites of significance in the tropics. His untimely death in 2005, after several years of sickness during which he 'tidied up' (to use his own words) many theses and scientific publications, did not allow him to complete all his writing, including this monograph. I would like to join with all staff at ACIAR and his colleagues in science in dedicating this volume to him.

Peter Core
Chief Executive Officer
ACIAR

CONTENTS

ACKNOWLEDGMENTS

Most of the work reported in this monograph is the result of research partnerships among many Australian and South-East Asian institutions. The major and most recent collaborative projects, and the lead scientists at the time the projects were initiated, are indicated in the following tables. To all those who contributed to these projects, and related projects throughout the region, the editors and staff at ACIAR would like to acknowledge their endeavours and cooperation.

Photos were taken by ACIAR project leaders or from the ACIAR photo collection, unless otherwise credited.

AS1/1997/27 Genetic and immunological characterisation of high resistance to internal parasites in Indonesian thin tail sheep

Collaborating institutions	Lead scientists
Centre for Advanced Technologies in Animal Genetics and Reproduction (Reprogen), Faculty of Veterinary Science, University of Sydney, Australia	Professor Herman Raadsma
Animal Biotechnology Research Laboratories, School of Biomedical Sciences, Monash University, Clayton, Australia	Dr David Piedrafita
Research Institute for Veterinary Science (BBalitvet), Indonesian Agency for Agricultural Research and Development, Bogor, Indonesia	Drh. S. Endah Estuningsih Drh. S.Widjajanti
Research Institute for Animal Sciences (Balitnak), Bogor, Indonesia	Dr Subandriyo
Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia	Dr E. Trimargawati

AH/2002/099 Development of a model for the control of fasciolosis in cattle and buffaloes in the Kingdom of Cambodia

Collaborating institutions	Lead scientists
School of Biomedical Sciences, James Cook University, Townsville	Dr Lee Skerratt
Department of Animal Health and Production, Cambodia	Dr Suon Sothoeun

AS1/1996/160 Control of fasciolosis in cattle and buffaloes in Indonesia, Philippines and Cambodia	
Collaborating institutions	**Lead scientists**
Graduate School of Tropical Veterinary Sciences, James Cook University, Townsville, Australia	Associate Professor Bruce Copeman
Research Institute for Veterinary Science (BBalitvet), Indonesian Agency for Agricultural Research and Development, Bogor, Indonesia	Drh. Darminto
Department of Animal Health and Production, Cambodia	Dr Suon Sothoeun
Philippine Carabao Centre, University of Southern Mindanao, Kabacan, Cotabato Province, Philippines	Professor Cornelio Subsuban
College of Veterinary Medicine, University of Southern Mindanao, Kabacan, Cotabato Province, Philippines	Dr Elsa Gonzaga

AUTHORS AND CONTACT INFORMATION

Don Cameron	Senior Lecturer in the School of Natural and Rural Systems Management, University of Queensland, Gatton Qld 4343, Australia	donald.cameron@uq.edu.au
Rod Campbell	School of Public Health, Tropical Medicine and Rehabilitation Sciences, James Cook University, Townsville Qld 4811, Australia	rsfc@ozemail.com.au
Dianne Clery	Department of Biochemistry and Molecular Biology, Monash University, Vic 3168, Australia	
Bruce Copeman (deceased)	School of Public Health, Tropical Medicine and Rehabilitation Sciences, James Cook University, Townsville Qld 4811, Australia	
Richard (Dick) Copland	School of Animal Studies, University of Queensland (Gatton), Qld 4343, Australia	R.Copland@uq.edu.au
Estuningsih Endah	Research Institute for Veterinary Science, Bogor, Indonesia	para@indo.net.id
Bruce Frank	School of Natural and Rural Systems Management, University of Queensland, Brisbane Qld 4072, Australia	b.frank@mailbox.uq.edu.au
Karen Fullard	Centre for Advanced Technologies in Animal Genetics and Reproduction, University of Sydney, NSW 2570, Australia	karenf@camden.usyd.edu.au
Wardis Girsang	Faculty of Agriculture, Pattimura University, Ambon Maluku, Indonesia	girsangwardis@yahoo.com
Douglas Gray	Research Program Manager, ACIAR, GPO Box 1571, Canberra ACT 2601, Australia	gray@aciar.gov.au
Deanne Hansen	Department of Biochemistry and Molecular Biology, Monash University, Vic 3168, Australia	
Judith Intong	College of Agriculture Central Mindanao University, Bukidnon, the Philippines	jsdintong@yahoo.com.ph
Nicola Kingsford	Centre for Advanced Technologies in Animal Genetics and Reproduction, University of Sydney, NSW 2570, Australia	nicolak@camden.usyd.edu.au
Endang Margawati	Research Institute for Veterinary Science, Bogor, Indonesia	para@indo.net.id
Martindah Eny	Research Institute for Veterinary Science, Bogor, Indonesia	para@indo.net.id
Els Meeusen	Department of Physiology, Monash University, Vic 3800, Australia	Els.Meeusen@med.monash.edu.au

Elizabeth Molina	College of Veterinary Medicine, University of Southern Mindanao, Kabacan, Cotabato Province, Philippines	bebeth18@hotmail.com
Sutijono Partoutomo	Research Institute for Veterinary Science, Bogor, Indonesia	para@indo.net.id
David Piedrafita	Department of Biochemistry and Molecular Biology, Monash University, Vic 3168, Australia	David.Piedrafita@med.monash.edu.au
Jill Pleasance	Department of Rheumatology, King's College London, London SE19RT, UK	jpleasance@gmail.com
Rhoda Prowse	Department of Biochemistry and Molecular Biology, Monash University, Vic 3168, Australia	
Herman Raadsma	Centre for Advanced Technologies in Animal Genetics and Reproduction, University of Sydney, NSW 2570, Australia	raadsma@camden.usyd.edu.au
Lee Skerratt	School of Public Health, Tropical Medicine and Rehabilitation Sciences, James Cook University, Townsville Qld 4811, Australia	lee.skerratt@jcu.edu.au
Terry Spithill	School of Animal and Veterinary Sciences, Charles Sturt University, Wagga Wagga NSW 2678, Australia	terry.spithill@csu.edu.au
Subandriyo	Research Institute for Animal Science, Ciawi, Bogor, Indonesia	balitnak@indo.net.id
Suhardono	Research Institute for Veterinary Science, Bogor, Indonesia	para@indo.net.id
Suharyanta	Research Institute for Veterinary Science, Bogor, Indonesia	para@indo.net.id
Suon Sothoeun	National Veterinary Diagnostic Laboratory, Department of Animal Health and Production, Phnom Penh, Cambodia	sothoeundahp@online.com.kh
S Widjayanti	Research Institute for Veterinary Science, Bogor, Indonesia	para@indo.net.id

GLOSSARY AND ACRONYMS

ACIAR	Australian Centre for International Agricultural Research
ANOVA	Analysis of variance
Carabaos	Domesticated subspecies of the water buffalo (*Bubalus bubalis*)
Cercaria	Free-swimming trematode larva that infects the vertebrate host
CMU	Central Mindanao University
Echinostomes	Trematode flukes from the genus *Echinostoma*
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization (of the United Nations)
FCS	*Fasciola* control strategy
FEC	Faecal egg count
FMD	Foot-and-mouth disease
Furcocercous cercaria	A free-swimming, digenetic trematode larva with a forked tail
GIS	Geographic information system
IMPROVE	Instrument for measuring progress of value to everyone model
IS	Immune sera
ITT	Indonesian thin tail sheep
KASA	Analysis of changes in knowledge, attitudes, skills, and aspirations
LOD	Logarithm of odds
Metacercaria	Cyst formed from free-swimming cercaria; the cysts attach to vegetation and are ingested by the host
MHC	Multiple histocompatibility complex
Miracidium	Free-living trematode larva that emerges from eggs and infects the molluscan host
NEJ	Newly encysted juvenile liver fluke
NGO	Non-government organisation
OIE	World Organisation for Animal Health
Paddy	Rice field
PAR	Participatory action research
PCR	Polymerase chain reaction
PCV	Packed cell volume
PLC	Peritoneal lavage cell
QTL analysis	Quantitative trait loci analysis
Rediae	Larval trematode in the molluscan host
Sarwah	Rice field
SCI	Social capital index
Sporocyst	A fluid filled germinal sac in the molluscan host producing more sporocyts or rediae (see above)
Strigeid cercaria	Cercaria of the family Strigeidae
TOT	Transfer of technology
Toxocarosis	Infection with the nematode *Toxocara vitulorum*
WWE	Whole worm extract
Xiphidiocercariae	Aquatic larval stage of some trematodes

Overview

G.D. Gray and R.S. Copland

Overview

G.D Gray and R.S. Copland

Introduction

This overview chapter describes the context of the research reported in the monograph and summarises the main findings as presented in each of the eight chapters. The monograph is not intended to be a comprehensive review. Other publications (e.g. Dalton 1999) cover all aspects of the biology of both host and parasite and there is a wealth of publications on both *Fasciola hepatica* and *Fasciola gigantica* available in the formal and semi-formal scientific literature. The reader is directed to general sources such as PubMed (www.pubmedcentral.nih.gov) to capture as much of the relevant literature as possible.

The monograph is also not intended to be a series of project reports. Much past activity, including training, extension efforts and contribution to national disease control policy is not reported here. For such details ACIAR should be contacted directly. Instead this monograph attempts to describe a scientific story of multiple projects linking researchers in Australia and South-East Asia through projects financed through a single funding body, ACIAR (the publisher of this volume). It tells of developing and making full use of the capacity of researchers and research institutions, mainly in Indonesia, the Philippines and Cambodia.

As will become evident through the eight chapters, the research combined studies on both parasite and host. The projects were based on the paradigm that an effective control program must be constructed on a platform of sound epidemiological knowledge. Epidemiology, as an integrating discipline, takes knowledge from many sources to both understand and make predictions about the pattern of parasites and the disease they cause.

Fasciolosis is an endemic disease of tropical livestock and is often described as a 'disease of production'. Fasciolosis rarely affects animals dramatically in the sense that they die or are obviously sick from it. Its most obvious symptoms of lethargy and anaemia can be caused by many factors, including other bloodsucking parasites such as *Haemonchus, Mecistocirrus* and *Trypanosoma* that cause blood loss or haemolysis. Furthermore, the disease is largely controlled in temperate and developed countries through regulated grazing and occasional drug use. Until recently, little attention has been given to research on effective control programs. This is because fasciolosis is:

- endemic to most tropical countries and does not affect trade
- causes mainly chronic symptoms with few deaths
- important mostly in developing countries with low levels of animal health inputs.

So, in many ways fasciolosis is a 'neglected disease'.

This problem has been recognised by several agencies with a mandate to investigate animal health problems in the tropics, including the Food and Agriculture Organization (FAO). A landmark publication by that organisation (FAO 1994) brought together the best possible science on the biology of *Fasciola* with knowledge of farming systems in tropical developing countries. Prepared by Dr Joe Boray (on whose research much of the advice was based) and the technical staff at FAO, it recommended that *Fasciola* be controlled by using an effective dewormer, reducing the population of snails acting as intermediate hosts and managing farms and grazing effectively. To our knowledge there has been no systematic research that has followed up on the implementation of these recommendations. In most countries in South-East Asia, systematic control of fasciolosis is not implemented. In the Philippines, as is partly

ILRI/Doug Gray

Beef production and other cattle products from indigenous breeds such as yellow cattle are important to the rural livelihoods of much of tropical and subtropical Asia.

documented in Chapter 4, a national program was designed and has been implemented for a few years.

So, the question needs to be asked: why is control of fasciolosis so difficult? There are several other parasitic diseases of tropical livestock for which effective control programs have been recommended and which have been implemented only sporadically, if at all. Notable among these is toxocarosis, which causes mortality of young calves, especially buffalo, and which is endemic in much of tropical Asia. It can be controlled by giving an inexpensive anthelmintic (such as pyrantel) orally in the first two weeks of life. But, as for fasciolosis, uptake of this treatment with, to the outside observer, such obvious benefits, has been very low (Roberts 1992). Likewise, the high mortalities associated with gut nematodes in goats can be controlled by improving housing, feed management and deworming (Sani et. al. 2005)[1] but these interventions are rarely practised in smallholder systems.

The chapters that follow, therefore, are not exclusively about technical aspects of fasciolosis. In particular, Chapter 5 describes research that attempts to unravel the incentives and drivers that enable farmers to make use of these technologies and benefit from them. One of these drivers is economic, and Chapter 2 attempts to elucidate the benefits and costs of control programs at the level of the whole industry and of individual farmers. Chapter 3 targets the epidemiology of fasciolosis and the complex relationships between the definitive hosts (cattle and buffalo), the intermediate host (snails), competing parasites and the farming systems in which all of these interact. This information forms the basis of any effective control program. Current recommendations for control programs, based on research from all sources, are described in Chapter 4. In Chapter 5 the difficult questions about uptake of technology and most effective extension strategies are asked and the way these have been applied recently in Cambodia is summarised. The appendix to this monograph is a comprehensive extension document that is being used for a national program in Cambodia. This program is a very tangible outcome from the research funded by ACIAR and the Government of Cambodia and will be monitored over the next few years for further signs of impact.

Chapters 6, 7 and 8 cover topics representing investment in the future: improved clinical diagnosis, more accurate epidemiological surveys and more resistant breeding animals may all be consequences of research on the pathology, genetics and immunology of *Fasciola* infections. These highly technical studies need sophisticated laboratory and animal research facilities, and an equally sophisticated framework for cooperation among Indonesian and Australian institutions. They have contributed significantly to the global understanding of the genetics and immunology of parasitism. That holy grail of all infectious disease, a vaccine, has not been studied as part of this suite of projects. There are many research projects throughout the world that may yet develop a technically effective and practical vaccine for *Fasciola*. For the developing world, however, the prospect of a vaccine must be considered as long-term.

Importance and potential impact of liver fluke in cattle and buffalo

One of the main drivers at the national and farm level of investments in liver fluke control is understanding the economic costs of infection and the relative benefits that might accrue if a control program is undertaken. For other diseases the drivers might be different: for example, there are international obligations to notify OIE and to prevent the spread of FMD, and for some livestock disease such as leptospirosis or anthrax there is the possibility of serious human infection. Humans have been infected with liver fluke but infections are quite unusual (Graczyk and Fried 2007). The research described in Chapter 1 seeks to identify how liver fluke caused by *F. gigantica* reduces the productive outputs: growth, milk, draught power and reproduction of cattle and buffalo in tropical Asia. It asks how that can be done best at a local level so that the benefits and costs of control can be demonstrated to

1 It is worth noting that all three of these neglected parasitic syndromes: fasciolosis and toxocarosis in cattle and buffalo, and nematodosis in goats and sheep, have been the target of sustained investment by ACIAR during the past two decades.

Brad Collis

Controlling liver fluke, especially in South-East Asia, provides more milk for both calves and children.

extension staff and farmers. One of these methods is to completely eliminate the parasite through continuous, suppressive use of dewormers. By reviewing all available information across Asia an approximate conclusion is drawn that in areas of high risk the annual loss for fasciolosis is AU$76–91 per head. Such a figure should be more than enough to convince both farmers and national planners that money for a control program will be well spent. Identifying these areas of high risk was investigated using GIS technology and results are reported on their application in Cambodia. Thus GIS can be considered a useful planning tool at a national level.

Epidemiology of *Fasciola gigantica* in cattle and buffalo

Although the life cycle of *F. gigantica* has been described in detail in the literature, the possibility of it varying between areas makes it risky to give regional recommendations. While the duration of the life cycle can be safely assumed in the bovine hosts, are the same snails implicated in transmission in all parts of tropical Asia? And what is the seasonal pattern of infection in the target production systems of a control program? How do cattle and buffalo shed worm eggs into waterways in each of these systems and how do they acquire infection? Collecting such data from each country in the region has multiple benefits. First there is the knowledge acquired. Second, and equally important, local scientists and extensionists develop a sound understanding of the pattern of infection in the animals for which they will take responsibility. This aspect of capacity building is highly important.

An unusual dimension to liver fluke control explored in Chapter 3 is the possibility of using poultry parasites to compete with the intermediate stage of *Fasciola* in the snail vector. The results are very promising and from a technical perspective show that allowing poultry to void their faeces into snail habitats can slow transmission.

Throughout tropical Asia there are two dominant patterns of cattle and buffalo management. At one extreme is an intensive irrigated system built around one, two or even three crops of rice per year with animals being allowed to feed on stubble on rice paddies and on rice straw during the rice growing periods. They may then graze on upland rainfed areas during the dry season. At the other extreme are grazing systems without rice where grazing is provided by forest, vegetation under plantation crops and by-products such a palm kernel cake. Although the parasite and host remain the same, the pattern of infection, and hence the ability to block transmission, are distinct.

The main conclusions from the epidemiological studies are that chemical control, reducing contamination of waterways with fluke eggs and reducing the consumption of metacercariae on stubble or cut straw will reduce transmission. This may be coupled with biological control of intermediate stages in the snail. The challenge is to integrate these options into a feasible control program.

Doug Gray

Cattle and buffalo both consume rice straw and contribute to rice production with traction and manure. This production cycle can also support a cycle of liver fluke infection.

Options for the control of liver fluke

As noted in the foregoing introduction, control programs based solely on the use of dewormers and 'effective management' of cattle and buffalo have been recommended and are readily available to researchers and extension workers. However, availability, costs and, in some cases, the quality of chemical available have restricted their use. It has become increasingly clear that options for 'effective management' need to be developed locally and that a local understanding of the science underlying the control options is vital for success. Thus, with a focus on preventing contamination of wet areas that constitute snail habitat and preventing livestock from consuming the infective metacercariae from rice straw and other vegetation, this chapter makes recommendations for two distinct production systems: irrigated rice production and extensive grazing. It is recognised that this distinction is not always clear as it is common for cattle and buffalo to be working or grazing in and around rice paddies for part of the year and, in some cases, being 'let loose in the forest' for many months during the dry season. Delta regions of the Mekong and Red River in Vietnam, and the flood plains and lakes (such as the Tonle Sap) in Cambodia, offer special problems as it is common practice to allow all ruminants to graze on the lush pasture, often contaminated by metacercariae, as the floodwaters retreat.

Biological control using the competing snail parasite *Echinostoma* to crowd out the intermediate stages of *Fasciola* in the intermediate snail host has long been considered an attractive option. It works, but requires the complication of ensuring that significant amounts of poultry faeces (the source of the competing parasite) enter the snail habitat along with *Fasciola* eggs from cattle and buffalo. As noted in the section describing recent developments in Cambodia, in that country at least this has been too complex to include in the emerging national program.

Approaches to extension for fasciolosis control

The history of extension practices has several distinct phases and is most clear-cut in pioneer regions (for example the American mid-west and Israel) or where there is rapid development of new enterprises (for example modern dairy production in the Netherlands). There have been many attempts to translate these successes to agriculture in the developing world, notably with a global program to extend the 'training and visit' system to established and emerging production systems across Asia. There is now an increasing awareness that such hierarchical systems based on a 'transfer of technology' model are effective in very limited circumstances. The demand for information needs to be already very high, and the benefits of effectively adopting new technologies and practices need to be seen quickly and be easily measured by the farmer. *Fasciola* control, along with many other problems faced by smallholders in the tropics, does not fit easily into that category. Hence the ACIAR projects invested heavily in exploring new ways of working with farmers in *Fasciola*-affected areas to understand how best to provide them with information, how best to enable them to learn about *Fasciola* control, and to identify and emphasise their incentives to adopt control measures.

The results are very much in line with other research that uses participatory approaches to involve farmers in decision-making and allow them to evaluate and modify technologies and practices on their own terms. The key findings included that new farmers and extension workers are more likely to show sustained commitment to disease control or other communal problems if engaged early in the process, even in the problem identification stage.

Resource-poor farmers are likely to need financial or resource assistance or other incentives to participate in training and adopt new practices. This is especially so when addressing 'common good' issues such as fasciolosis, when control of disease, or lack of it, affects the health of all community animals. The costs to resource-poor farmers must be negligible as they are least likely to risk their scarce resources on innovation.

Participatory extension processes are more resource intensive, taking up much more time of trainers, facilitators and participants than traditional linear methods—which may provide a single training session or even rely on passive transmission of information through leaflets, radio or television. The increased investment may well be justified if programs produce sustained positive benefits. These benefits were evident in the three studies described in Chapter 5, at least for the short period of evaluation immediately after the studies. It would be extremely interesting to return now, about five years after the studies were completed, to assess their lasting impact.

An aspect of participatory approaches to extension that is often ignored and difficult to measure is that benefits are multiplied when the process contributes to development of human and social capital in ways that facilitate subsequent community action in addressing other common problems. These may be other disease problems, in which the case the links are easy to identify. But the benefits may also extend to unrelated issues in the community such as water management, allocation of grazing and pasture, or lobbying for better roads to access new markets for their livestock products.

These approaches require well-trained and experienced staff whose skills may need to be developed over a long period. In the short and medium term, at least, such skilled staff are likely to be in high demand and available to only a few projects and locations. However, once their impact has been felt, the cascade of learning from farmer to farmer may be the most effective way of ensuring that innovation spreads throughout a farming system.

Pathology of fasciolosis in large ruminants

In parallel with the development of control options based on existing knowledge of the transmission and epidemiology of fasciolosis are several other research initiatives. These are aimed at finding new ways to diagnose infection (for clinical and survey purposes) and to modify the host through vaccination or other changes to the immune system that will increase host resistance. Confounding these studies is the realisation that cattle and buffalo respond differently to infection and that there may be significant differences between cattle breeds. The ACIAR projects have supported this important fundamental research on the pathology of infection in cattle and buffalo, and on differences in their susceptibility to infection. This chapter reports a series of studies on the pathology of fasciolosis in cattle and buffalo, and on the relationship between host and parasite.

The practical questions that these studies are looking to answer include the following.

- Are the pathological changes in large ruminants infected with *F. gigantica* comparable to those changes during *F. hepatica* infections? If similar then the likelihood of the extensive body of work on *F. hepatica* being relevant to *F. gigantica* in the tropics is increased.
- Are there differences in the infectivity and pathology of *F. gigantica* between cattle and buffalo and do these reflect differences in the production traits of growth, reproductive performance, milk yield and draught power?
- Are there better ways to diagnose and monitor *F. gigantica* infections in cattle and buffalo?
- What are the implications of differences between *F. gigantica* and *F. hepatica*, and between cattle and buffalo, for the development and implementation of control programs?

Genetics of fasciolosis in small ruminants

Experimental design with sufficient power to detect the presence of a major gene in both the segregation and linkage approach is influenced by the magnitude of the major gene effect, the gene frequency in the founding population, the nature and mode of inheritance of the major gene, and the number of progeny that can be screened (family size). A limited number of families from reciprocal matings with a large number of progeny per family provides an ideal design.

In this study the frequencies of trait alleles in the Indonesian thin tail (ITT) sheep population were unknown. Furthermore, strain differentiation in the ITT sheep (Garut and Sumatra strains) required that both populations should be represented in the founding population. This imposed an experimental requirement that multiple families would need to be generated. A minimum of eight families (two strains, reciprocal matings, repeat sampling) was planned for. A further constraint to the design was the total number of animals that could be generated and challenged at any one time. This was limited by animal house space, breeding resources, labour resources and experimental cost. A minimum target of 160 experimental animals (max. 250) for testing each year was aimed for, to be repeated over four rounds (years).

These targets were achieved, leading to a total genotyped population of 694 individuals in 10 families. The fact that the families have widely differing genotypic backgrounds increases the likelihood of capturing gene alleles that are only present in some of the populations. However, this also creates problems for quantitative trait loci (QTL) analysis, given that the power of detecting a quantitative trait locus in 10 families is lowered if only some have the segregating allele of interest.

Nevertheless, the current dataset has identified 25 QTL to a significance level greater than 99.9 out of 100, together with their broad chromosomal positions. Weaner coat colour displays a particularly strong response, and is likely to represent a single gene mutation. In addition, some of the wool traits identified are found in similar positions to those in a related sheep QTL mapping project (Reprogen, unpublished). This validates the genotyping and mapping accuracy of the dataset. Resistance traits are of importance to this project. There are 12 QTL for:

- measures of resistance to fasciolosis
- immunological indicators of resistance
- liver enzyme indicators of the response to infection.

It is interesting that one of the strongest QTL for a measure of resistance to fasciolosis falls on the same chromosome as the multiple histocompatibility complex (MHC). The MHC is thought to be involved in immune response to internal nematodes. The chromosomal positions of two of the primary infection *Haemonchus contortus* resistance QTL

Fallow rice paddies are an important source of liver fluke reinfection.

match those from previous studies reported in the literature. Again, this validates the results obtained in this study, and suggests those regions would produce valid candidates for further studies to find breeding markers.

However, to gain the maximum information out of the dataset, more powerful analysis techniques are needed to help sift through uninformative families, and this may well require a maximum-likelihood approach to analysing the data.

Immunology and assessment of resistance to fasciolosis in small ruminants

To study potential mechanisms in the peritoneum of ITT sheep that could kill the migrating stage of the parasite in vitro, a method was required to access immature parasites and to detect death of these parasites after they were incubated with effector cells.

A system to excyst viable immature parasites from the infective cyst (metacercariae) was developed in vitro together with a colorimetric system to detect damage to these parasites (MTT reduction assay). Initial experiments first identified two major immune cell types within the peritoneum during the early migration of *F. gigantica* parasites in ITT sheep: macrophages and eosinophils. These two immune cells have been shown to be pivotal to the killing of helminth parasites by releasing cytotoxic molecules onto the surface of parasites. These immune cells from the ITT host effectively killed immature parasites of *F. gigantica* but this ability was critically dependent on the presence of sera from *F. gigantica*-infected ITT sheep. The dependence on sera from *F. gigantica*-infected ITT sheep in mediating this effective killing was demonstrated: the mechanism was likely to be dependent on the anti-*F. gigantica* antibodies binding the effector cells to the parasite tegument.

The potential effector molecules mediating this cytotoxic mechanism were investigated by using a series of inhibitors and it was found that the primary cytotoxic molecules produced by these cells were superoxide radicals. To confirm this cytotoxic mechanism the abilities of merino macrophages and eosinophils from the peritoneum to mediate killing of *F. gigantica* parasites in vitro were compared. Merino peritoneal cells were not as effective at mediating killing of juvenile *F. gigantica* parasites. This effector mechanism of ITT sheep should be ineffective in mediating killing of immature *F. hepatica* parasite and this was confirmed by demonstrating that *F. hepatica* immature parasites were not susceptible to superoxide-dependent cytotoxicity. In support of this observation, a key defence enzyme (superoxide dismutase) against superoxide radicals was significantly higher in juvenile *F. hepatica* when compared with *F. gigantica* parasites and may explain why juvenile fluke of *F. gigantica* are effectively killed by peritoneal cells of ITT sheep when compared with juvenile fluke of *F. hepatica*.

In summary, in vitro studies have demonstrated the effector cells mediating killing of *F. gigantica* immature parasites include ITT sheep macrophages and eosinophils and the molecular mechanism of killing involves superoxide radicals.

The development of an immunodiagnostic test for a phenotypic indicator of parasite burdens was attempted by the combinational analysis of key parameters measured in a group of experimental sheep challenged with *F. gigantica* and *F. hepatica*. This study showed that combinations of certain biochemical and immunological parameters give an accurate estimation of infection: up to 80% of the fluke burden variation can be explained by these parameters. The ultimate aim is to develop a non-invasive parameter test for fluke burden prediction in animals of unknown parasite status that could be performed easily and routinely. In this study, the best predictive parameters required a complicated number of samples to be taken and used to predict infection rate and this remains impractical in the field. These studies need to be further pursued.

References

Dalton J.P. 1999. Fasciolosis. CAB International: Wallingford, UK.

FAO 1994. Diseases of domestic animals caused by liver flukes: epidemiology, diagnosis and control of *Fasciola*, paramphistome, *Dicroceoelium*, *Eurytrema* and schistosome infections of ruminants in developing countries. FAO: Rome.

Graczyk H.K. and Fried B. 2007. Human waterborne trematode and protozoan infections. Advances in Parasitology 64, 111–160.

Over H.J., Janse J.N and van Olm P.W. 1992. Distribution and impact of helminth diseases of livestock in developing countries. FAO: Rome.

Roberts J.A. 1992. Preventive treatment against toxocarosis in bovine calves. Veterinary Parasitology 44, 111–118.

Sani R.A., Gray G.D. and Baker R.L. (eds) 2005. Better worm control for small ruminants in Asia. ACIAR Monograph No. 113, Canberra.

2

Importance and potential impact of liver fluke in cattle and buffalo

D.B. Copeman and R.S. Copland

Importance and potential impact of liver fluke in cattle and buffalo

D.B. Copeman and R.S. Copland

Introduction

In this study the potential importance of liver fluke was estimated by considering the impacts of infection at the level of individual animals, farms or smallholdings, regions, countries and globally. Economic costs have been estimated for different reasons including the justification and creation of research or control programs, to design control programs to ensure that they are targeted correctly, and to assess the impact of control programs. The total global economic loss attributed to fasciolosis has been estimated earlier to be more than US$3 billion per year (FAO 1994).

This chapter reviews studies that may be used to estimate the losses caused by fasciolosis, with a focus on large ruminants in Asia. It is estimated that 300 million bovines are exposed to fasciolosis worldwide. The review builds on an earlier comprehensive review (Spithill et al. 1999) in which impacts on weight gain, draught performance, fertility and lactation were summarised and used to develop dollar estimates of lost productivity. These authors highlight the difficulty of such estimation, citing:

- incomplete information on all the elements of offtake (including milk, draught power, meat and asset accumulation) from cattle and buffalo production systems
- the risks of extrapolation from experiments on *Fasciola hepatica* (in cattle and sheep)
- the high variability within and between tropical production systems in breed, sex and cropping systems
- presence of other parasite and infectious diseases.

In the face of such incomplete and diverse information it is worthwhile to question the value of making broad estimates of loss. However, there is no doubt that control programs need to be justified and supported by accurate economic data as there is increasing competition for public and private investment. Also, even broad estimates will help to direct research towards new approaches to control and projects most likely to be beneficial.

The practical questions that stimulate these studies include the following.

- How does liver fluke infection caused by *Fasciola gigantica* reduce the productive outputs of cattle and buffalo in tropical Asia?
- What are the best ways to measure production losses at the local level?
- What are the benefits and costs of suppressive drenching trials as a way of measuring local impact and demonstrating production losses to extension agents and farmers?
- Can GIS methods be used to predict areas where prevalence is sufficiently high to cause significant production loss?
- Can recommendations be made on the best ways to assess impact to justify, plan, implement and monitor liver fluke control programs?

The following sections review studies that estimate the existing and potential losses from *F. gigantica* in tropical cattle and buffalo systems. The main lessons to be drawn from these studies and the gaps that need to be filled by further research are discussed.

National and global impacts

Liver fluke infection (fasciolosis) of cattle and buffalo is one of the most important parasitic diseases in Indonesia (Partoutomo et al. 1985) and its impact has been estimated in several studies. Thorbecke and van der Pluijm (1993) estimated that, of the overall losses from livestock diseases in Indonesia, losses from fasciolosis are second only to Newcastle disease. Prevalence of liver fluke in parts of Indonesia has been estimated at 25–90% in cattle and buffalo (Edney and Muchlis 1962; Soesetya 1975; Beriajaya 1979; Edney and Muchlis 1962; Soesetya 1975; Beriajaya and Soetedjo 1979), and in sheep and goats (Beriajaya and Copeman 1997). Estimates of the annual economic losses have been made by several authors (Table 2.1). The figure of A$96 million (US$65 million) (Winrock 1986) accounted only for the cost of lost meat production. The total annual cost of lost meat production, lost draught power and reduced fertility in infected cattle/buffalo has subsequently been assessed at A$176 million (US$107 million).

The losses in some other countries in Asia, including Cambodia, Vietnam and the Philippines, can be estimated where the farming practices in countries are similar to Indonesia and prevalence rates for fasciolosis are comparable. In 1997 the size of the cattle and buffalo herd in Asia was estimated at 589 million (FAO 1997) with the Indonesian herd (15 million) representing 2.5% of the Asian total. Using a conservative scenario of 10% prevalence and annual loss per infected animal of US$42, economic losses in cattle and buffalo alone exceeded US$2.4 billion in Asia. Similar calculations for the African cattle herd of 201 million (FAO 1997), where prevalence rates are similar to Asia, predict losses at US$0.84 billion, bringing total world losses to at least US$3.2 billion. The worldwide annual loss from fasciolosis is a substantial figure by any valuation and possibly greater than the earlier estimate of US$2 billion by Boray (1985).

Recent studies in Cambodia (Sothoeun 2007[1]) have confirmed the impact of fasciolosis in areas of high prevalence. Where prevalence exceeds 30%, for example, annual weight gains are reduced by 20–40 kg, pregnancy rate is lowered by 10% and there is 2.5 kg less liver available for consumption. Taking into account these losses and the costs of implementing a practical control program the net benefit per head of cattle and buffalo in these high risk zones of Cambodia is 274–326 thousand riel (A$76–91).

Sources of production losses

Weight gain

Monitoring weight gain of young animals is a useful way to assess the impacts of infection and interventions to prevent or control infection, improved nutrition or treatment with an anthelmintic. Younger growing animals are more susceptible to infection, less costly to maintain under experimental conditions and any results can be applied to production systems that are producing young growing animals for sale. A linear relationship between burden of adult *F. gigantica* and weight gain has been described in yearling zebu cattle. It was found that each fluke

South-East Asian countries rely heavily on the draught power of livestock but liver fluke infection can markedly weaken cattle and buffaloes.

1 Reproduced as an appendix to this monograph

Table 2.1 Prevalence of fasciolosis caused by *Fasciola gigantica* in several Asian countries.

Country	Range (%)	Mean (%)	Number of animals sampled	Reference
Cambodia				
	7–50	38	273	Sothoeun (unpublished)
		245	575	Sothoeun et al. (2006)
	0–57	12	1,406	Tum et al. (2004)
Overall estimate		18		(409 animals infected out of 2,254 sampled)
Indonesia				
	25–48	36		Suweta (1982)
Cattle		61		
Buffaloes		31		
		25		Soetedjo (unpublished)
Overall estimate		38		(Average of prevalence data)
Philippines				
	35–100			Tongson (1978)
Cattle Female		49	133	Molina et al. (2005)
Male		27	119	
Buffaloes Female		50	24	
Male		37	8	
Cattle		33	250	E. Abonado (unpublished)
Buffaloes		47	32	
		69	252	M.F. Guinsatao and N.B. Salcedo (unpublished)
Overall estimate		45		(318 animals infected out of 711 sampled)
Vietnam				
	23–90			Luong (unpublished), 21–30% in goats
Cattle		76%	140	Luong et al. (1999), ACIAR Meeting Balitvet Indonesia (106 infected)
Buffaloes		76	63	(47 infected)
Coastal	5.3–27.9		11,252	Luong (unpublished) (2,650 infected)
Delta	13–59.2			
Overall Vietnam		27.2%		(3,119 infected out of 11,455 sampled)
Thailand				
Beef		212		Pholpark (unpublished)
Dairy		7		
Buffaloes		7		
	0–85	12		Srihakim and Pholpark (1991)
Laos				
Cattle		9	76	Vongthilath (unpublished)
Buffaloes		21	274	
Cattle	0–81	23	107	Douangngeun (unpublished)
Buffaloes		38	243	
Overall estimate		26		(182 animals infected out of 700 sampled)
India				
Cattle		24.4	516	Sanyal and Singh (1995)
		39	977	Gupta et al. (1986)
China		27	120,000	Jiangang (unpublished)

reduced the potential annual gain by about 200 g, with infected animals achieving only about half the annual weight gain of that shown by control animals (Sewell 1966). Weight change in infected animals is also a useful parameter to monitor because most cases of fasciolosis are subclinical.

One deficiency of these types of studies is that many animals can compensate for reduced growth when the cause (e.g. liver fluke) is removed and so the total impact has not been measured accurately. Further, comparisons across studies are difficult because of differences in levels of infection, the age and sex of animals, the interaction between level of nutrition and pathogenic effect, and the difference between breeds and within breeds in resistance to infection. It is sufficient to conclude at this point that reports of weight-gain reduction need to be accompanied by descriptions of all the factors known to contribute to the effect of fasciolosis, including sex, age, breed and previous exposure.

Draught performance

Anaemia resulting from fasciolosis has been shown to reduce work output by 7–15% (Roberts et al. 1991). Combined with a further indirect reduction of 20% in potential work capacity in animals whose growth has been restricted by fluke infection, it can be concluded that liver fluke can seriously lower the work potential of both cattle and buffalo. The economic significance of this may, however, be changing rapidly in production systems where hand tractors are replacing animals as sources of draught power. In many systems, however, even if there is a gradual reduction in their use, draught power from large ruminants remains important and should not be ignored. Indeed, as the prices for fossil fuel increase, draught animal power may remain viable in more isolated areas, particularly with poorer farmers.

The cost of the reduced draught capacity caused by *F. gigantica* may be measured as the opportunity cost to a farmer caused by the longer time taken by infected animals to perform a specific task; this amounts to about 27–35% more time with buffaloes according to the conclusions of J.A. Roberts and D.B. Copeman (unpublished). As the average draught animal in Indonesia is used in land preparation for growing rice only about 23 days per year, the opportunity cost for a farmer with infected buffalo in this situation is thus the value of his labour for about seven days per year.

Further evidence that infection with *F. gigantica* adversely affects draught capacity was collected by Suhardono (2001) in Indonesia. Farmers were surveyed during the second year of a trial to measure the effects in Ongole cattle of a single treatment with triclabendazole administered in July, about six weeks after harvest of the second seasonal rice crop in the area. The survey revealed that animals treated for fasciolosis (with triclabendazole) were used twice as many days as untreated animals for preparing land for planting rice. This result suggests that farmers recognised that the treated cattle performed better than those that were untreated. Furthermore, those with untreated animals avoided the opportunity cost associated with increased time to prepare their land by hiring animals that had received treatment. Thus, where this hiring option is available, the economic cost associated with reduced work capacity in animals infected with *F. gigantica* may be the cost of hiring replacement animals for land preparation rather than the opportunity cost of a farmer's labour.

Fertility

A link has also been observed between infection with *F. gigantica,* anaemia and fertility (Suhardono 2001). There were significantly longer intercalving intervals and a lower packed cell volume in Ongole cows in Indonesia infected with *F. gigantica* than in those treated with triclabendazole each July for two years. In his study, Suhardono (2001) found that treated cows had a mean intercalving interval of 18.5 months whereas in untreated cows the interval was 31.5 months. It is thus reasonable to conclude that infection with *F. gigantica* is likely to adversely affect reproduction. Furthermore, the extent may be proportional to the degree of anaemia induced, an outcome that varies according to the level of nutrition, level of infection and breed.

Lactation

Needham (1977) found no measurable difference in weaning weight of calves (an indicator of milk output of their dams) from cows infected with

F. gigantica and those treated each 8–12 weeks with an adulticide. Such contradictory reports are to be expected due to differences between studies in factors likely to affect milk output. These include level of nutrition, size of infection and differences in resilience to infection between individuals and breeds. However, at present, too few studies have been undertaken to enable meaningful prediction of the extent to which any of these determinants affects lactation in animals infected with *F. gigantica.*

Sources of prevalence data

The first step in establishing the importance and cost of fasciolosis is to determine the prevalence of infestation. A simple approach to estimating the national impact of fasciolosis is to estimate prevalence in different systems, determine the loss caused by infestation per animal per year in each of these systems, and then multiply these to provide an overall estimate of impact.

No comprehensive, countrywide surveys of prevalence have been conducted in South or South-East Asia. Prevalence is difficult to establish because of the dependence on local physical and climatic conditions for the survival of the intermediate host snail (see Chapter 3). Prevalence may vary from 0% to 100% over a comparatively short distance (Srihakim and Pholpark 1991; Tum et al. 2004). Therefore, figures given for the national prevalence may conceal certain areas of high risk and high prevalence and hence loss. Nevertheless, national figures are presented in Table 2.1 to provide an overview.To establish prevalence, faeces need to be collected for faecal egg counts or livers examined for parasites in abattoirs. Both of these techniques are subject to inaccuracies. The faecal egg count method has a low sensitivity with many false negatives. Sothoeun et al. (2006) reported that 27% of animals with *F. gigantica* in their livers yielded negative faecal egg counts. Therefore, prevalence estimates based on faecal egg counts may underestimate true prevalence. On the other hand, prevalence data based on abattoir surveys may be inaccurate because older and sometimes sick animals are generally slaughtered (Sothoeun et al. 2006; Luong, unpublished).

The epidemiology of fasciolosis suggests that the prevalence would be higher in wetter, more fertile areas (see Chapter 3). These high-risk intensive agricultural areas are often associated with high numbers of cattle and buffaloes.

A further source of bias is that districts selected for study have often been chosen because preliminary data indicated these were areas of high risk. This is illustrated by the data from Cambodia. Sothoeun et al. (2006) found prevalence levels of 24.7%. However, a preliminary whole country study by Tum et al. (2004) found a national average prevalence of 11.6% (163 infected animals out of 1406 tested). Multiple methods were used to estimate prevalence and the level of infection at which each animal was categorised as 'infected' varied widely. Nonetheless, assuming that each infected animal lost production by an average amount determined by experimentation some national figures for the economic impact of the fasciolosis can be generated (Table 2.2).

Regional observations of production losses

Giangxi province, China

Fasciolosis is found in cattle, buffaloes and goats in all provinces of China with *Fasciola gigantica* found in 10 southern provinces. Most research has been on *Fasciola hepatica* and sometimes *F. hepatica* and *F. gigantica* were not distinguished. From 1949 to 1989 several national surveys were conducted in which major mortalities in goats, cattle and buffalo were identified. *Fasciola gigantica* is found only in south China, south of the Changjiang River. *Fasciola hepatica* and *F. gigantica* are found in varying proportions, often together in the same animals. In Guangxi *F. gigantica* was more common than *F. hepatica* and in some counties the infection rates are above 90%.

There are about 10 million cattle and buffaloes in Guangxi. They are owned by smallholder farmers who represent about 80% of the 40 million people in the province. Most of the area is mountainous and cattle and buffaloes are allowed to graze freely in hilly areas. Fasciolosis is more serious in rice-producing areas.

Table 2.2 Estimated number of cattle and buffaloes infected with *Fasciola gigantica* in various countries of Asia in areas of high or low prevalence. As an approximate indication of the total costs in each area the number of infected animals has been multiplied by the cost per infected animal derived from Sothoeun (2007, see also appendix) of between A$82 and A$98. Livestock numbers are derived from FAO (2004) and the prevalence data from Table 2.1.

Country	Total number of large ruminants (thousands)	Prevalence scenario	Total number of infected animals (thousands)	Range of total losses (millions of Australian dollars)
Cambodia	3,625	High (18.1%) Low (11.0%)	656 399	54–64 33–39
Indonesia	14,000	High (38.2%) Low (20.0%)	5,355 2,800	439–525 230–274
Philippines	5,799	High (44.8%) Low (34.0%)	2,598 1,972	213–255 162–193
Vietnam	7,050	High (27.0%) Low (15.0%))	1,903 1,057	156–186 87–104
Thailand	7,000	High (11.8%) Low (11.8%)	826 826	68–81 68–81
Laos	2,350	High (26.0%) Low (15.0%)	611 352	50–60 29–34
China	129,348	High (27.2%) Low (12.0%)	35,182 15,521	2,885–3,448 1,273–1,521
India	283,200	High (25.0%) Low (10.0%)	70,800 28,320	5,806–6,938 2,322–2,775
Total	452,372	High (26%) Low (11%)	117,932 51,248	9,670–11,557 4,202–5,022

India

In India fasciolosis is widespread and is primarily caused by *F. gigantica* although *F. hepatica* is reported in the temperate Himalayan region. Building of dams and the establishment of new irrigation systems have further widened the distribution of *Fasciola* by creating more water-covered areas suitable for propagation of its intermediate hosts, the lymnaeid snails. Thus, fasciolosis has started to appear in the semi-arid and arid regions of western India where it was hitherto non-existing.

The onset and advancement of monsoon rains have a profound effect on the incidence and seasonality of fasciolosis in India. Most of the available information on the prevalence of *F. gigantica* comes from abattoir surveys and coprological studies on animals visiting clinics, and is thus biased. It is, however, apparent that the prevalence of fasciolosis in a tropical country like India is largely determined by rainfall and production systems. A review of some of the recently conducted surveys indicate a high level of incidence in the endemic areas throughout India but in the endemic areas of the northern plains, a high 10–39% infection was recorded in cattle and buffalo. A nationwide survey in dairy animals organised by the National Dairy Development Board (NDDB) indicated two critical periods in the year: July–September and February–March (Sanyal and Singh 1995).

Lao PDR

A survey of 76 cattle and 274 buffaloes killed at the slaughterhouse of the Vientiane municipality revealed infection with *F. gigantica* in the livers of 9% of cattle and 21% of buffaloes. At the slaughterhouse of Luang Prabang only 1 of 2 cattle and 17 buffaloes examined were infected.

Fasciola gigantica, Toxocara vitulorum and strongyles are regarded as the most important parasites of ruminants in the Lao PDR. Fasciolosis has a substantial negative effect on production but is not a significant cause of mortality. It is recommended that valuable animals are treated but preventive programs that rely on chemotherapy are unlikely to be sustainable (Vongthilath, unpublished; Douangngeun, unpublished.)

Greg Hood

Growth rates and market value of livestock are reduced by liver fluke infection.

Philippines

Fasciolosis is still the leading cause of morbidity and mortality in ruminants in the Philippines. Fasciolosis research has always played second fiddle to research on the more dramatic and explosive diseases with international implications, which would account for the meagre research output. The low research priority given by the national government to fasciolosis and other economically important parasitic diseases and the lack of trained manpower for research have all contributed to a lack of qualitative and quantitative information.

Of the 252 cattle examined in Cotabato (Mindanao) in 1997, 173 (69%) were found infected with *F. gigantica* based on the presence of *Fasciola* eggs in the faeces. Cattle older than four years had the highest prevalence (82%), for those two to four years old the prevalence was 78% and for those five months to two years, prevalence was 53%. The prevalence was 72% in females and 62% in males. Barangay Bannawag had the highest prevalence proportion (92%), followed by Katidtuan (89%). Proportions of animals infected among eight villages ranged from 36% to 73%.

A study in two slaughterhouses (Mlang and Kabacan) in 1999 determined the prevalence of fasciolosis in cattle and carabaos. All animals from Mlang were negative for fasciolosis. Of 282 animals examined from Kabacan, 83 (33%) cattle and 15

(47%) carabaos were positive. There was a higher prevalence of fasciolosis in cattle and carabaos older than six years (69% and 59%, respectively). Carabaos three to six years old had a prevalence of 25% and those younger than one to three years 17% (Molina et al. 2005).

Thailand

The economic loss from fasciolosis in cattle and buffalo throughout Thailand has been assessed at not less than 100 million Baht (about US$3 million). Recent investigations have shown that the average prevalence of *F. gigantica* in cattle and buffalo in Thailand was 12%. However, the prevalence varies considerably between villages, ranging from 0% to 85%. Prevalence is high in areas surrounding dams or large ponds in which *Lymnaea auricularia rubiginosa*, the intermediate host of *F. gigantica* is found. The disease has a seasonal pattern on which control of the disease is based. All cattle and buffalo older than eight months should be treated for liver flukes each September. In addition, animals in poor condition should be treated in April to prevent severe losses, especially in high-prevalence areas or where strategic treatment was missed.

Diagnostic results of the Northeast Regional Veterinary Research and Diagnostic Center in 1998 revealed that the average prevalence proportions of infection with *F. gigantica* in beef cattle, dairy cattle and buffaloes in north-east Thailand were 22%, 7% and 7% respectively. However, the prevalence varied considerably between villages.

Humans are also infected with *F. gigantica*. From 1967 to 1990, 25 cases of human fasciolosis were reported in Thailand, of which 19 occurred in the north-east. Since then at least 10–20 new cases of human fasciolosis have been confirmed in the Khon Kaen University Hospital each year. Fasciolosis should therefore be regarded as a newly emerged zoonosis and an important public health problem (Pholpark, unpublished).

Cambodia

A national survey was conducted in Cambodia to identify zones of high prevalence for further studies into the epidemiology of this infection. Faecal samples were collected from 273 cattle and buffaloes in October and November 1998 and examined for *F. gigantica* eggs. The 14 villages in this study were in Saang district of Kandal province and in Cheung Prey district of Kompong Cham province. The proportion of animals with *Fasciola* eggs in their faeces varied greatly from village to village. All villages in Cheung Prey district had < 20% prevalence and the mean of samples from these villages was 7%. In Saang district, one of the six villages had a low prevalence, two had a medium level (20–50%), and two had a high prevalence (> 50%). The average prevalence in Saang district was 38% (Suon et al. 2006).

Vietnam

Between 1996 and 1998 faeces of 11,252 cattle or buffaloes from different regions of Vietnam were examined for eggs of *F. gigantica*. Prevalence varied from 5–28% in the coastal areas to 13–59% in the delta areas. In earlier studies age was shown to be an important determinant of infection. Prevalence increased from 16% in animals younger than three years to 37% in animals older than five years. An abattoir survey of 495 cattle and buffaloes also revealed increasing prevalence with increasing age. At three months of age, 6.8% of animals examined were infected. Prevalence in animals from four to six months old was 11%, from 7–12 months 36.4% and from 12–24 months 45.5% (Luong, unpublished).

Nepal

Among the diseases of ruminants, fasciolosis is probably the most common and perhaps one of the most important causes of livestock deterioration in Nepal. It is widespread throughout the country, affecting all species of ruminant livestock, including yaks and yakows of the Himalayas. The different local names of this disease, such as namle, mate and lew, in different regions are proof of its continued existence for many years in Nepalese animals.

Singh et al. (1973) reported an infection rate of 50–90% in animals in areas below 1800 m and estimated it caused an annual economic loss of NRs 200 million (US$20 million). Recent studies have indicated a similar prevalence of the disease but a higher estimate of economic loss (US$37 million) calculated only on decreased buffalo milk and buffalo meat production (Mahato 1993; Mahato et al. 1997).

Although infections with *F. hepatica* have been reported, infection with *F. gigantica* is the most common and widespread cause of fasciolosis throughout the country. Among the species of lymnaeid snails found in Nepal, *Lymnaea auricularia* race *rufescens*, *L. auricularia* sensu stricto and *L. viridis* have been identified as the important intermediate hosts.

The pre-monsoon rains together with rice cultivation practices promote habitat creation for *Lymnaea* spp. over a wide area. After the monsoon when rice is harvested, animals acquire infection during grazing from heavily contaminated rice fields. Rice straw, the principal food of large ruminants during the dry season (December–April), is another important source of infection with *Fasciola*, especially in stall-fed animals. Thus, it appears that there is a good case for introducing appropriate management practices to prevent animals becoming infected.

In Nepal, attempting to control the intermediate hosts using molluscicides, even during the dry season, is of limited value because of the numerous permanent habitats and the great biotic potential and aestivating ability of the lymnaeid snails. Epidemiological studies have revealed that most snails are infected by fluke eggs deposited on pasture during March–May and again in October–November. This pattern suggests that administration of anthelmintic in February and again in late August to control pasture contamination is an appropriate strategy for the control of fasciolosis in Nepal.

Suppression trials with anthelmintics

Applying anthelmintics to kill off existing infections and prevent new ones has made it possible in a few cases to measure production loss in situ. That is, infected and uninfected animals have been compared in their normal production environment. The value of such studies is that an upper limit of acceptable benefits for deworming or other control measures can be estimated without having to rely on extrapolation from experimental results. The disadvantage remains, however, that to estimate the total effect, treatment needs to be continued for at least a complete season. Even then the impacts on reproductive rate are not included. The results of the three studies described below have been important in increasing the accuracy of production loss estimates and raising awareness among scientists, policymakers and farmers, of the magnitude and extent of the liver fluke problem. These, in themselves, are important outcomes.

Philippines

A two-year suppressive treatment study begun in February 2000 compared irrigated and rainfed cropping systems. Selection of the study areas was based on the recommendation of livestock technicians. The number of animals, willingness of the animal owners and barangay officials to cooperate, accessibility of the area and whether they were from a Christian or Muslim area were all taken into account.

Takepan was chosen for the irrigated cropping system, and Colambog for the rainfed area. Both areas are in Pikit, Cotabato province and inhabited by both Muslim and Christians. Buffaloes and cattle were weighed, drenched with triclabendazole (Fasinex-Novartis) at a dose rate of 24 mg/kg body weight and 12 mg/kg body weight respectively. Two other areas, Dagupan in Kabacan and Datu Paglas in Maguindanao, serve as controls for the rainfed and irrigated areas, respectively. Data such as age, sex, body weight, reproductive performance, feeding system, draught power and function of the animals were gathered from both the treated and the control groups every four months for two years. All animals used in the study were marked with ear tags. The buffaloes are used for draught power for land preparation in lowland and in upland areas and for hauling farm products such as coconuts, rice, corn and other farm products from the field to the market. Cattle, however, were used for meat and breeding and not for draught power. Cattle and carabaos (buffaloes) graze communal areas near the dikes or river banks and canals and, from the rice planting to harvesting period, are fed with grasses by a cut and carry system or allowed to graze.

Information programs and consultations with barangay (district) officials and animal owners were conducted before the study began. Information about the project and its activities, about fasciolosis and about possible benefits from the study were

ILRI/Steve Mann

Liver fluke reduces the output of buffalo and cattle milk for consumption by calves and human consumers.

explained. Faecal egg counts were done to determine the prevalence of fasciolosis in the respective areas.

The average age at first calving was 4.0 years old in carabaos and 2.6 in cattle. The age group of one day old to three years old was more common than the older age groups in all study areas. There were more cattle (643) than carabaos (501) and more female animals (772) than male (340) and castrated animals (32). The mean live-weight gain per day was higher in treated animals in the irrigated area (0.32 kg) than in the control group (0.24 kg). However, in the rainfed area it was higher in the control group (0.35 kg) than in the treated group (0.29 kg). Generally, cattle had higher weight gain than in buffaloes in all study areas except in the control irrigated area with few cattle. The intercalving interval in buffaloes in control treated and rainfed areas was the same (19 months) but was higher in the treated irrigated areas (13 months) than in the control irrigated area.

In cattle, the intercalving interval was 16 months and 14 months in treated rainfed and irrigated areas and 15 months in control rainfed. However, data on the intercalving interval for controls in irrigated areas are not yet available. The prevalence rate for fasciolosis infection during the preliminary sampling in the study areas ranged from 33% to 95%.

Indonesia

A study by Suhardono (2001) demonstrated that the treatment of cattle reared in West Java in association with intensive production of irrigated rice with triclabendazole in July significantly reduces their level of exposure to infection with *F. gigantica* over the following 12 months. In comparison with untreated animals treated animals had improved reproductive and draught performance and higher packed cell volume (PCV) values, and yearlings had a higher weight gain. It was concluded that the timing of such annual treatment should be about six weeks after harvest of the second seasonal rice crop in an area. Furthermore, to achieve the best effect, all animals sharing common grazing should receive treatment.

Treatment in July may be regarded as strategic as it is about six weeks after the end of the period when rice is harvested, which is when most infection with *F. gigantica* occurs in this area.

Cambodia

The impact of fasciolosis on weight gain, reproductive performance, draught capacity and PCV of cattle was studied in the upper delta of the Mekong River in Cambodia where the risk of infection with *F. gigantica* is high (Tum et al. 2004). Farmers in two villages in Saang province, Prek Samrong and Preak Trang, participated in the study. Cattle in Preak Samrong were maintained free from infection with *F. gigantica* by treating them every three months for nine months with triclabendasole at 12 mg/kg body weight. No control of *F. gigantica* was practised in Preak Trang but cattle received the placebo Zanisef every three months. At the start of the study, there were 224 cattle in Preak Samrong village made up of 60 castrated males, 113 females, 49 male calves and 2 bulls. In Preak Trang village, there were 202 cattle comprising 13 castrated males, 144 females, 41 male calves and 4 bulls. Every three months all animals were weighed, faeces were collected for examination for *Fasciola* eggs, blood was collected for estimation of PCV, and condition score, skin coat, and draught strength were recorded. Weights of pregnant females were adjusted to remove the effects of pregnancy.

In Preak Samrong 52 animals were sold: 13, 26 and 13 during first, second and third periods of three months respectively. In Preak Trang, 69 cattle were sold during the same periods comprising 32, 14 and 23 animals respectively.

Mean weight gains of males and females were similar for the age group 0–0.5 years and for 0.6–1.5 years so were combined in analyses of differences between control and treated groups. Nine months after observations began treated males plus females in both the 0.5–1.5 years and 1.6–2.5 years age groups had gained significantly more weight than comparable animals in the control village (Table 2.3). During the same period weight gains of treated and control castrated males three years of age and over were similar, as were weight gains of females in this age group (Sothoeun et al. 2006).

Predicting impacts using geographical information systems

A geographic information systems (GIS) model for mapping the risk of fasciolosis in cattle and buffaloes was developed for the Kingdom of Cambodia using determinants of inundation, proximity to rivers, land use, slope, elevation, and the density of cattle and buffaloes. Determinants were subjectively weighted according to their perceived relative importance before combining them to produce a risk map of fasciolosis. The model estimates that 28% of Cambodia is potentially at risk of fasciolosis with

Table 2.3 Comparison of mean weight gains ± SE of treated and control groups over the nine months of observations in Cambodia.

	Weight gains (kg, mean ± SE)		
Groups compared	**Treated**	**Control**	**P value**
Male + female 0.5–1.5 years	99.0 ± 10.3	74.2 ± 4.9	0.02
Male + female 1.6–2.5 years	86.4 ± 4.4	73.9 ± 4.3	0.05
Castrated male 3 years and over	45.1 ± 4.9	43.1 ± 4.7	0.77
Females 3 years and over	39.3 ± 6.7	28.3 ± 4.8	0.19

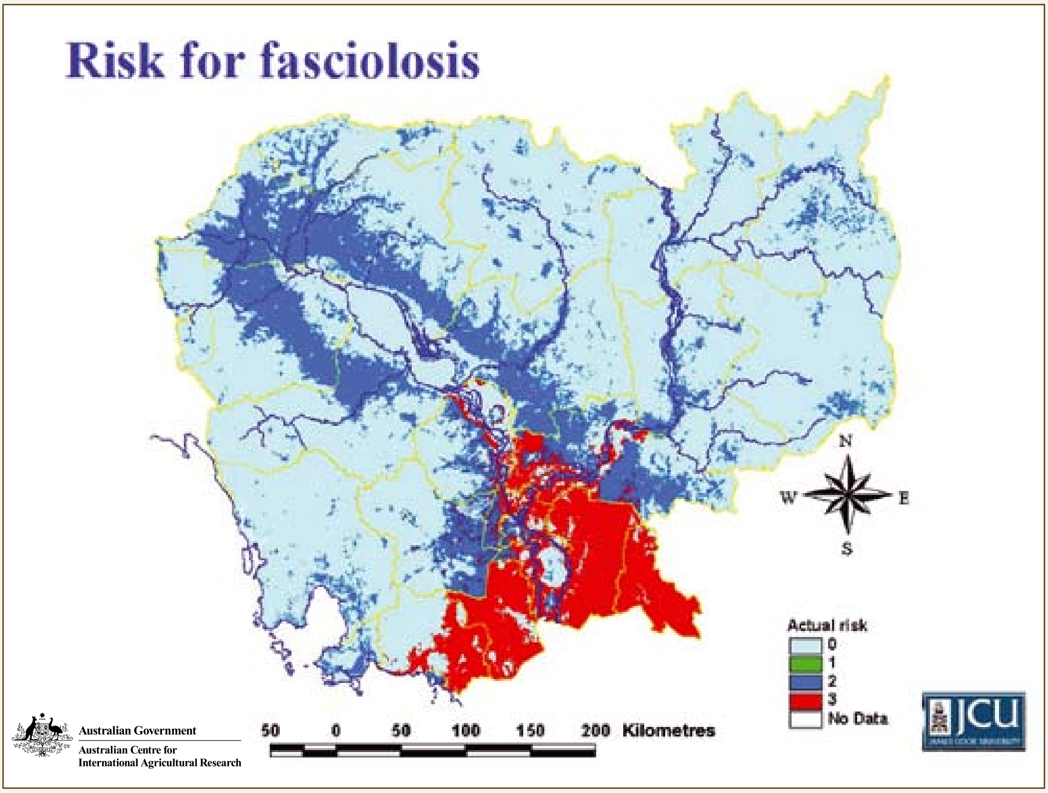


Figure 2.1 The risk of transmission of fasciolosis due to *Fasciola gigantica* (adjusted by animal density) as predicted by the GIS model developed by Tum et al. (2004) for Kampong Cham province and the four districts that were surveyed for prevalence of fasciolosis in cattle to test the model.

areas of high and moderate risk concentrated in southern and central Cambodia. The estimates of risk reflect the actual prevalence of fasciolosis in most districts surveyed, suggesting that the epidemiological determinants and weightings used to produce the model were appropriate. These results will be progressively refined as more detailed field surveys are completed to fully validate the model. A comparison between levels of risk predicted by the maps and field measurements of prevalence in 11 provinces (n = 1406) showed general agreement, which suggested that the epidemiological determinants and weightings used to produce the maps are appropriate (Tum et al. 2004). One constraint on the validity of this conclusion was that prevalence was measured at the provincial level, a very large unit, and animals were not sampled at random. An additional study sought to correct this deficiency by measuring prevalence at a more detailed scale, at district level. Faecal samples were collected from a randomly selected set of animals in four districts in one province for areas at high, moderate and no risk. This result supported the earlier conclusion that there is a good relationship between prevalence and risk predicted by the GIS model (Figure 2.1).

In Thailand (Pholpark, unpublished observations) fasciolosis is one of the most important parasitic diseases in adult cattle and buffaloes in north-east Thailand. Prevalence varies considerably between villages. It is higher in areas surrounding dams or large ponds in which *Lymnaea* (Radix) *auricularia rubiginosa*, the snail intermediate host of *F. gigantica*, is found. Strategic treatment with an effective drug is considered to be the most effective control measure. In this study, instead of performing an epidemiology survey, a GIS is being applied to define the fasciolosis risk areas and the appropriate time for strategic treatment of animals. In a pilot GIS study in Khonkaen province during 1999 the parameters used for analysis of the data were surface water, rainfall, temperature, and the boundary of the province, district or subdistrict. From results of the pilot study the high *Fasciola* risk areas and the time for strategic treatment were defined quite accurately when compared with the results of the epidemiological study that had been conducted from 1982 to 1984 (Srikitjakarn et al. 1988). However, the parameters used were considered too imprecise. To improve their predictive precision, seven parameters are now being used for analysis of data in the current GIS field trial in Kalasin province. They are:

- temperature
- rainfall
- surface water
- boundary of river, brook or irrigation canal
- slope class
- boundary between wet lands and grazing areas
- the boundary of the province, district, or sub-district.

Conclusions

It is evident from these studies that comprehensive data on the impact of fasciolosis over several years are difficult and expensive to obtain. This is due mostly to the long-term and chronic nature of the disease, its multiple effects on productivity and the difficulty of making an accurate diagnosis. There is also a natural bias in many studies, including those reported here, to focus on areas where the problems are known and not undertake a systematic survey with a wider statistical base. Nonetheless there is no doubt that the disease is widespread throughout most of tropical Asia with hotspots in areas susceptible to seasonal flooding and irrigated rice production. The exact figures will always be open to discussion but a consensus view would be that the impacts are relatively high and often unrecognised.

The challenge is how to use the information. Two pathways are suggested by the data reported here. One is to make use of increasingly accessible technologies based on GIS mapping to make predictions about places and systems that can be targeted for control programs. As more data are

Doug Gray

Animals infected by liver fluke produce fewer offspring and the interval between litters is longer.

collected the predictive models for such programs will become more accurate and useful to planners and extension services. The second pathway is to develop relatively simple ways to demonstrate to farmers and extension workers the impacts of fasciolosis and the benefits of control. Computer models and reports are not sufficient to convince them that their time and resources should be used for fluke control when there are so many other competing demands. The use of suppressive drenching with monitoring of the impacts by farmers and extension workers themselves may provide the required stimulus.

References

Beriajaya and Copeman D.B. 1997. An estimate of seasonality and intensity of infection with gastrointestinal nematodes in sheep and goats in West Java. Jurnal Ilmu Ternak dan Veteriner 2, 270–276.

Beriajaya and Soetedjo R. 1979. Annual report, Lembaga Penelitian Penyakit Hewan. Research Institute for Veterinary Science: Bogor, Indonesia.

Boray J.C. 1985. Flukes of domestic animals. Pp. 179–218 in ‘Parasites, pests and predators’, ed. by S.M. Gaafar, W.E. Howard and R.E. Marsh. Elsevier: New York.

Dalton J.P. 1999. Fasciolosis. CAB International: Wallingford, UK.

Edney J.M. and Muchlis A. 1962. Fascioliasis in Indonesian livestock. Communications Veterinariae 6, 49–62.

FAO 1994. Diseases of domestic animals caused by liver flukes: epidemiology, diagnosis and control of *Fasciola*, paramphistome, *Dicroceoelium*, *Eurytrema* and schistosome infections of ruminants in developing countries. FAO: Rome.

FAO 1997. FAOSTAT online database, at: <http://faostat.fao.org>. Accessed 3 April 2008.

Gupta R.P., Yadav C.L. and Ruprah N.S. 1986. The epidemiology of bovine fasciolosis (*Fasciola gigantica*) in Haryana state. Indian Veterinary Journal 63, 187–190.

Alice Kenney

Many smallholders rely on large ruminants to transport farm produce. Liver fluke infection can reduce their pulling power.

Kumar P. and Pachauri S.P. 1989. Efficacy of albendazole against *Fasciola gigantica* infection in buffaloes with particular reference to milk production. Journal of Veterinary Parasitology 3, 35–39.

Luong To Thu and Phan Dich Lan 1999. Fasciolosis in cattle and buffalo in Vietnam. In ‘Control of fasciolosis in Indonesia, Cambodia and the Philippines. Proceedings of the Second Annual Fasciolosis Control Coordination Meeting (ACIAR Project AS1/96/160)’, Davao, Southern Mindanao.

Mahato S.N. 1993. Epidemiology and pathogenesis of fasciolosis in eastern Nepal. Ph.D. Thesis, University of Edinburgh.

Mahato S.N., Harrison L.J.S. and Hammond J.A. 1997. Epidemiological basis of the control of fasciolosis in Nepal. Bulletin of Veterinary Science and Animal Husbandry Nepal 25, 22–26.

Molina E.C., Gonzaga E.A. and Lumbayo L.A. 2005. Prevalence of infection with *Fasciola gigantica* and its relationship to carcase and liver weights, fluke and egg counts in slaughter cattle and buffaloes in South Mindanao, Philippines. Tropical Animal Health and Production 37, 215–221.

Needham A.J.E. 1977. Observations on the economics of treatment of *Fasciola gigantica* infestation in cattle in Rhodesia. Rhodesian Veterinary Journal 8, 14–20.

Partoutomo S., Ronohardjo P., Wilson A.J. and Stevenson P. 1985. Review of diseases in Indonesia affecting draught power in domestic animals. Pp. 140–146 in 'Draught animal power for production', ed. by J.W. Copland. ACIAR: Canberra.

Roberts J.A., Bakrie B., Copeman D.B. and Teleni E. 1991. An assessment of the work output of buffalo infected with *Fasciola gigantica*. ACIAR: Canberra.

Sanyal P.K. and Singh D.K. 1995. Administration of fenbendazole in urea molasses block to dairy buffaloes in India. Tropical Animal Health and Production 27, 186–190.

Sewell M.M.H. 1966. The pathogenesis of fascioliasis. Veterinary Record 78, 98–105.

Simpson J.R., Kunkle W., Courtney C.H. and Shearer J.K. 1985. Economic analysis of controlling liver fluke. Agri-Practice 6, 20–24.

Singh N.B., Basnyat B.M., Eichenberger G. and Bommeli W. 1973. Report on preparatory phase of parasite control project. HMG/SATA: Kathmandu, Nepal.

Soesetya R.H.B. 1975. The prevalence of *Fasciola gigantica* infection in cattle in East Jawa, Indonesia. Malaysian Veterinary Journal 6, 5–8.

Sothoeun S. 2007. Fasciolosis of cattle and buffaloes and its control measures. Technical implementation procedure, Ministry of Agriculture, Forestry and Fisheries, Kingdom of Cambodia. (Reproduced as an appendix to this monograph.)

Sothoeun S., Davun H. and Copeman B. 2006. Abattoir study on *Fasciola gigantica* in Cambodian cattle. Tropical Animal Health and Production 38, 113–115.

Soun S., Hol D., Siek S., McLean M.and Copeman B. 2006. Seasonal differences in the incidence of infection with *Fasciola gigantica* in cambodian cattle. Tropical Animal Health and Production 38, 23–28.Spithill T.W., Smooker P.M. and Copeman B. 1999. *Fasciola gigantica*: epidemiology, control, immunology and molecular biology. Pp. 465–520 in 'Fasciolosis', ed. by J.P. Dalton. CAB International: Wallingford, UK.

Srihakim S. and Pholpark M. 1991. Problem of fascioliasis in animal husbandry in Thailand. Southeast Asian Journal of Tropical Medicine and Public Health 22 Supplement, 352–355.

Srikitjakarn L., Pholpark M., Leidl K., Loehr K.F. and Hoerchner F. 1988. The epidemiology and control of bovine fascioliasis in Northeast Thailand. Thai Journal of Veterinary Medicine 18, 9–22.

Suhardono D. 2001. Epidemiology and control of fasciolosis by *Fasciola gigantica* in ongole cattle in West Java. Ph.D. Thesis, Tropical Veterinary Science, James Cook University of North Queensland: Townsville Australia.

Suweta I.G.P. 1982. Economic loss due to liver fluke infestation in cattle implicative to interactions in the environmental conditions of the agricultural ecosystem in Bali. [Gangguan ekonomi cacing hati pada sapi implikasi interaksi dalam lingkungan hidup pada edosistem pertanian di pulau Bali]. Universitas Padjadjaran, Ph.D. Thesis: Bandung, Indonesia:

Thorbecke E., van der Pluijm T. 1993. Rural Indonesia: Socioeconomic development in a changing environment. NYU Press: New York.

Tongson M.S. 1978. A national fascioliasis control program for the Philippines (a professorial lecture). Pp 106–117 in 'Proceedings of the Annual Convention of the Veterinary Practitioners Association of the Philippines'.

Tum S., Puotinen M.L. and Copeman D.B. 2004. A geographic information systems model for mapping risk of fasciolosis in cattle and buffaloes in Cambodia. Veterinary Parasitology 12, 141–149.

3

Epidemiology of *Fasciola gigantica* in cattle and buffalo

Suhardono and D.B. Copeman

Epidemiology of *Fasciola gigantica* in cattle and buffalo

Suhardono and D.B. Copeman

3

Introduction

The patterns of infection of liver fluke are determined by the interaction of environmental factors, the biology of the parasite and the production system in which cattle and buffalo are expected to perform. Given the essential relationship between the parasite *Fasciola* and the water-dwelling snails of the genus *Lymnaea* that act as intermediate hosts, it is no surprise that liver fluke disease is most common in rice production systems (or areas providing a suitable habitat for the intermediate host), which may be contaminated with parasite-containing cattle faeces. These systems are not static, with gradual transition from single to double, to sometimes triple rice crops per year, and increasing reliance on machinery to replace large ruminants as a source of draught power. Nonetheless is it is still common for cattle and buffalo to be allowed to graze on recently harvested rice paddies, and straw to be cut and carried to stall-fed animals. Cattle faeces, possibly containing parasite eggs, are also used as fertiliser in many rice production systems.

A series of experiments and surveys from ACIAR projects are described which, when taken with other published work from other parts of Asia, identify opportunities for intervention and control. The results underscore the paradigm that has dominated modern integrated control of parasites: that control can only occur when the life cycle, environmental interactions and production systems are well understood.

The practical questions that stimulated these studies include the following.

- What is the seasonal pattern of liver fluke infection and how best can it be assessed in smallholder farming systems?
- What is the role of snails in the transmission of *Fasciola gigantica* in each of the production systems studied?
- What are the sources of infection for snails, and for large ruminants by metacercariae, and can these sources be eliminated?
- What opportunities are available for biological control through competition with the intermediate stages of *F. gigantica?*
- How do sources and patterns of infection differ between rice-based systems and more extensive grazing systems?

The parasite

Fasciola gigantica is one of the most important parasites of cattle and buffalo in the humid tropical areas of the world. In some regions it may also be important in sheep, goats and other domestic animals. Wild herbivores are susceptible, but laboratory animals are not readily infected. Human infection may be more common in endemic areas than the occasional case reports suggest. Hammond and Sewell (1975) proposed that *F. gigantica* is better adapted to cattle than sheep in that it is more infective and lives longer in cattle.

The intermediate hosts of *F. gigantica* are tropical aquatic snails which thrive in clear, stagnant or slow-moving water with high oxygen content and abundant aquatic vegetation (Kendall 1954). Such ecological situations are typically found at the fringes of rivers or lakes where the water levels are stable, and in irrigated rice fields throughout the humid tropics. *Fasciola gigantica* is transmitted worldwide by snails not readily distinguishable on morphological grounds or on grounds of their ecological requirements from the single superspecies *Lymnaea auricularia sensu lat.* (Kendall 1965). The life cycle of *F. gigantica* (Figure 3.1) may also be completed in some other species of snail including *Lymnaea stagnalis*, *Lymnaea pergera*, *Lymnaea tomentosa*, *Lymnaea truncatula*, and *Lymnaea palustris*. However, as *F. gigantica* does not normally occur outside the range of *L. auricularia sensu lat.*, it may be concluded that the contribution of other snails to the endemicity of *F. gigantica* is minor. In lowland tropical areas *L. auricularia* breeds throughout the year in favourable habitats (Chartier et al. 1990). The population of these snails in rivers may be negatively related to rainfall, reflecting the disruption of the habitat of the snails by flooding and also their dispersion by floodwater, as they spend up to 70% of their time floating at the surface (Widjajanti 1989). Once water levels stabilise, the population level of snails increases and is most numerous while these conditions persist. The duration and timing of this favourable period for snails may be only a few months at the end of the wet season in closed water bodies, or persist throughout the dry season in slow-moving rivers (Mzembe and Chaudhry 1979; Chartier et al. 1990; Tembely et al. 1995).

Once the water level drops too fast during the dry season for the fringing aquatic vegetation to persist or the oxygen level drops too low, the habitat will be rendered unsuitable for these snails (Kendall 1954). In irrigated rice fields the population of snails is influenced by the availability of water for irrigation and the stage of growth of the crop. Snails and their eggs surviving from the previous crop may colonise recently planted rice fields or they may enter with water introduced to flood the field after planting. Their numbers then increase over the next few months before declining again a few weeks before harvest (Widjajanti 1989). Despite being aquatic, the snail hosts of *F. gigantica* are able to survive desiccation in the shade on the surface of the soil for some weeks. After one month, Widjajanti (1989) observed that mean survival time was reduced by about one-third, and egg masses by about one-half relative to hydrated controls. However, she found no adverse affect of desiccation for one month on subsequent hatchability of eggs. The eggs did not hatch until rehydrated, prompting her to propose this as a possible mechanism for survival of the population in habitats subject to periodic desiccation.

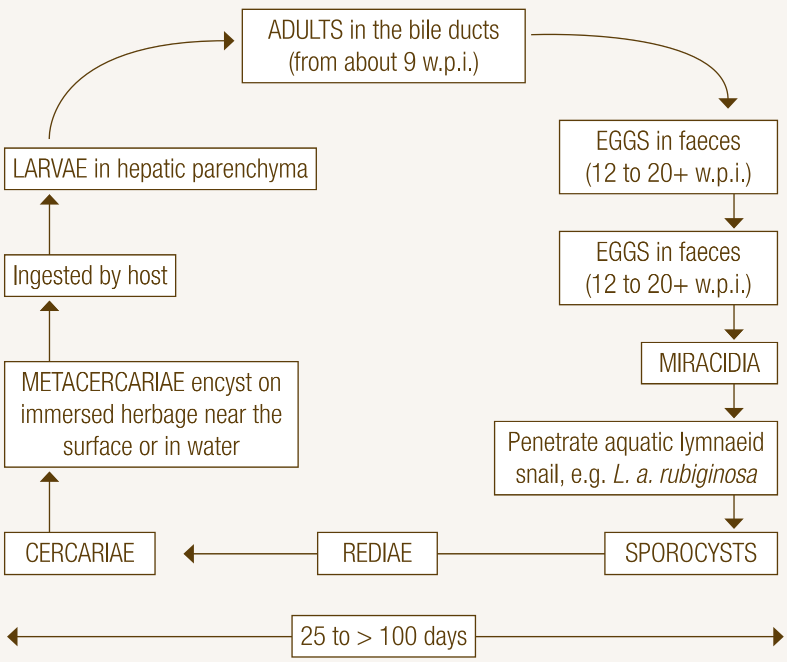


Figure 3.1 The life cycle of *Fasciola gigantica* (w.p.i. = weeks post infection).

The time taken for miracidia to develop in eggs of *F. gigantica* and for sporocysts, rediae, and cercariae to develop in snails varies with temperature. Grigoryan (1958) considered 24°C to 26°C optimal for development of miracidia, found that eggs did not survive temperatures more than 43°C and desiccation was rapidly fatal. At 27°C cercariae may develop as early as 26 days after snails are infected (al Kubaisee and Altaif 1989). Development of larvae in the snail becomes slower as the temperature drops and it eventually stops. Below 16°C only a succession of daughter redial generations are produced, but they switch to production of cercariae when the mean temperature is raised to 20°C (Dinnick and Dinnick 1964). Cercariae are shed in up to 15 waves (usually three or fewer) one to eight days apart over a period of up to 50 days (Grigoryan 1958; Da Costa et al. 1994; Dreyfuss and Rondelaud 1994). Maximum shedding at the optimum temperature range of 25°C to 27°C occurs 46–50 days after infection (Dinnick and Dinnick 1963; Asanji 1988). The number of cercariae produced per snail is usually a few hundred, but varies from fewer than one hundred to over a thousand.

After release from the snail, cercariae exist as metacercariae. About two-thirds attach to various objects near the surface to the water (Ueno and Yoshihara 1974). The remainder become floating cysts (Dreyfuss and Rondelaud 1994). Floating cysts may move with the flow of water to become a source of infection where the habitat is unsuitable for snails and therefore presumed safe from infection. The duration of survival of metacercariae is inversely related to temperature and directly to the degree of hydration. In water at 26°C, a high proportion of metacercariae remained viable for six weeks, but few were viable after 10 weeks. At 30°C and 35°C, however, few metacercariae remained viable after five and two weeks respectively. When metacercariae are stored out of water, the duration of their viability is directly related to relative humidity, and inversely to temperature and exposure to sunlight. All metacercariae stored at 21–31°C and 30–50% relative humidity were dead by day 35 (Grigoryan 1959). Desiccated metacercariae exposed to direct sunlight were all dead within eight hours. In lowland equatorial regions, therefore, aquatic habitats should be safe to graze about two months after death of snails but this period will be extended in cooler habitats for up to six months. Similarly, metacercariae which become dry on aquatic vegetation as a result of receding water levels or on hay are likely to be no longer infectious after about six weeks in lowland tropical areas but may survive up to about four months in cooler climates.

Exposure of animals to infection with *F. gigantica* from rivers and lakes may be restricted to only a few weeks each year. Infection usually occurs about two months after the habitat becomes stable (and thus favourable for snails) towards the end of the wet season and persists during the dry season for a few weeks after the habitat is no longer suitable for snails. Exposure of animals to infection from irrigated rice fields occurs when stock graze stubble, eat rice stalks after harvest, or drink water flowing from the fields. Exposure will occur throughout the year in regions where rice cropping is continuous, but only for a few weeks after harvest when rice cropping is seasonal. Infection is highest from fields fertilised with animal manure.

Newly encysted metacercariae require at least 24 hours to become infective (Boray 1969). Larval flukes develop in the hepatic parenchyma and, in cattle, enter the bile ducts about 89 days after infection (Guralp et al. 1964). The prepatent period is about 14 weeks (Grigoryan 1958; Guralp et al. 1964; Sewell 1966). The output of eggs rises for the first 12–14 weeks after eggs appear in the faeces then falls to low levels (Sewell 1966; Prasitirat et al. 1996). With the same infecting dose, faecal egg counts are up to 80% lower in buffaloes than in cattle (Prasitirat et al. 1996). Hammond and Sewell (1975) found the number of *F. gigantica* in cattle begins to fall about 28 weeks after infection. Most survived less than a year but some survive up to four years (Alicata and Swanson 1941; Hammond and Sewell 1975).

A sound understanding of relationships between the life cycle of *F. gigantica* and the system of seasonal flooding of rice paddies in South-East Asian countries (Figure 3.2) is essential for developing control strategies for fasciolosis.

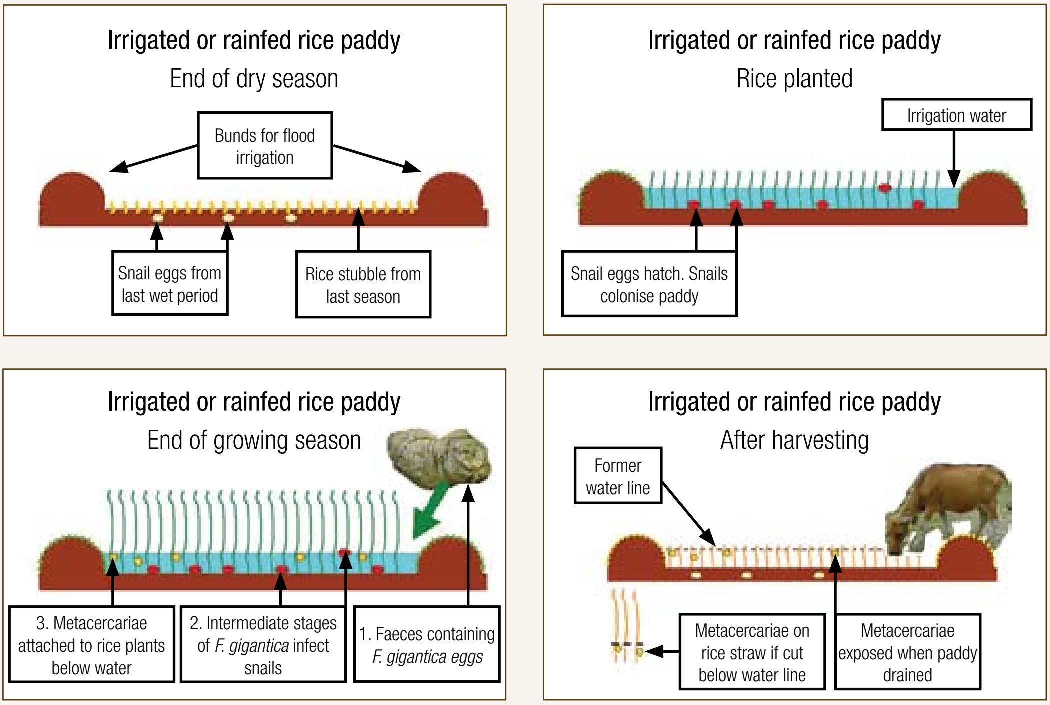


Figure 3.2 (a) The annual cycle of rice production on irrigated or rainfed rice paddy showing the development of the snail population and infective liver fluke metaceraciae.

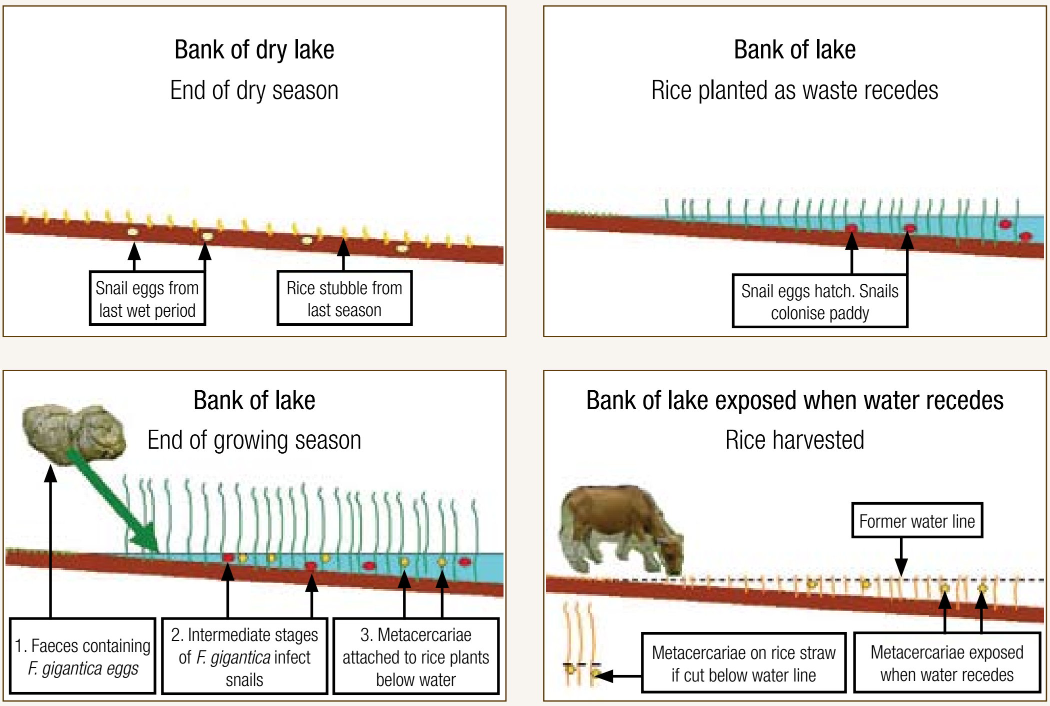


Figure 3.2 (b) The annual cycle of rice production and grazing on the banks of seasonal lakes and rivers showing the development of the snail population and Infective liver fluke metaceraciae.

Eggs in faeces

The transmission cycle of *F. gigantica* is dependent on the expulsion and survival of eggs in faeces until the eggs hatch and the resulting miracidia are released into water. Management of dung has the potential to reduce transmission, especially when cattle and buffalo dung are used to fertilise rice paddies; a common practice throughout Asia.

The projects studying eggs per se have focused on:

- reducing the numbers of eggs that survive and hatch through an examination of environmental factors that enhance and reduce survival
- improving methods to detect eggs in faeces—which had the dual purpose of improving field diagnosis of infection and evaluation of control strategies under field conditions.

Survival of eggs in dung heaps

An experiment was conducted to evaluate the impact of exposure to sun on the eggs of *F. gigantica.* Eggs were placed in dung heaps located in the shade or exposed to the sun, and examined at intervals for up to 14 weeks. Fewer viable eggs were recovered from Petri dishes than dung heaps and the rate and extent of decline in viability of eggs was greater in dung exposed to the sun than in shaded dung. This difference was attributed to the higher temperature in dung in the sun than the shade, due to the effect of direct sunlight on exposed dung and a higher rate of fermentation in exposed than shaded dung.

Dick Copland

Detecting eggs in faeces remains the commonest way to diagnose liver fluke infection.

It was concluded that strategies for storing dung which would reduce the risk it poses for infecting *L. rubiginosa* with *F. gigantica* when used as fertiliser in rice fields include:

- storing dung in the sun rather than the shade, preferably in a thin layer to heat and desiccate it
- mixing a carbohydrate with the stored dung to increase heat through fermentation (Suhardono et al. 2006a).

To further test the hypothesis that increasing the heat generation during a composting process would reduce egg development, a trial was conducted (Suhardono and G. Adiwinata, unpublished) using a microbial additive that has been adopted by some farmers in Indonesia as a means of improving the quality of manure as organic fertiliser. It is expected that the technology will also be useful for controlling fasciolosis through generation of heat, interfering with the development of eggs of *F. gigantica*.

Three types of marketed micro-organism (Stardec®, Gama 96®, and Em-4®) were used in this study. There were five heaps of faeces, each 150 cm long, 60 cm wide and 30–40 cm high, four of which were treated with microbes and one used as a non-treated control, all located in the shade. Group I was mixed with Stardec®, Group II with Gama 96®, Group III with Em-4® (remixed if the temperature was more than 50°C), and Group IV was faeces mixed with Em-4® (remixed every 24 hours). Five samples of faeces from groups I and II were collected every week for three weeks. They were then incubated in the dark at room temperature for three weeks, with a change of water every week, to promote development of *Fasciola* eggs. A similar procedure was applied to faeces in groups III and IV but samples were taken every day for four days. The temperature in the pile of faeces in Group I was 41–43°C from days 1 to 7 then decreased rapidly. In Group II, the temperature was 37–40°C. In Groups III and IV from 12 hours post incubation the temperature was 42–60°C. The hatchability of eggs in the non-treated control group was 71%. Almost no eggs developed in Groups I, III and IV from faeces collected any time after deposition. From Group II, however, a few eggs developed from the sample collected in week 1 but none in weeks two and three. From this study it can be concluded that addition of micro-organism in the process of

Christian Roth

Drying dung and using it for fuel partly breaks the liver fluke life cycle.

decomposition of faeces can kill eggs of *F. gigantica* and could be promoted as a way to reduce infection of snails with *F. gigantica* in rice fields where faeces is used as fertiliser.

Improved detection methods

One of several methods used to confirm liver fluke infection is by finding fluke eggs in the faeces. This is most effectively done using concentration techniques (e.g. Taira et al. 1983). Concentration can be achieved by both flotation and sedimentation of fluke eggs. The flotation method, which is generally used for concentrating nematode eggs, can also be used to float eggs of *Fasciola* spp.. However, the eggs become distorted, making it difficult to differentiate between eggs of *Fasciola* spp. and those of paramphistomes. Methods based on sedimentation of eggs do not have this drawback. They are also easy to perform, require little equipment and not only allow detection but also enable quantification of fluke eggs in faeces. As a consequence, techniques based on sedimentation are widely used for this purpose. Most are based on the method described by Boray and Pearson (1960). However, the sensitivity and repeatability of a technique based on this method was found to be low when used for detecting eggs of *F. gigantica* in bovine faeces. A series of experiments was conducted to find a more sensitive and reliable method consistent with the capacity of diagnostic laboratories in Asia (Suhardono et al. 2006b).

In summary, the method uses 3 g of faeces suspended in 0.05% Tween-20. The suspension is passed through three 6-cm diameter sieves in tandem to remove fibrous debris, with respective apertures of 1 mm, 450 μm, and either 266 or 200 μm. The filtrate is allowed to sediment for three minutes in a conical flask, is recovered, and then resuspended in 200 mL of 0.05% Tween-20 and once more allowed to sediment for three minutes. The sediment is washed in a sieve with an aperture of 53 μm, which retains the eggs but allows fine debris to pass through. Residue on the sieve is recovered in about 15 mL of water to which one or two drops of 1% methylene blue are added. The eggs are counted using a dissecting microscope. Use of Tween-20 instead of water as the suspending agent for faeces increased the proportion of eggs recovered threefold and reduced variability between repeated counts. This method can detect about one-third of the eggs present. It was concluded that the high proportion of *F. gigantica* eggs lost may be due to the presence of hydrophobic and covalent bonds on the eggs which bind them to debris with which they are discarded. The method is fully described in a published paper (Suhardono et al. 2006b).

Snail ecology

West Java

Fasciolidae need one intermediate host, a freshwater gastropod from the superfamily of pulmonate snail, family Lymnaeidae. In tropical regions *L. auricularia sensu lat.* serves as intermediate host of *F. gigantica* (Hubendick 1951; Kendall 1954). Besides *Fasciola* the snail also serves as an intermediate host of other trematodes (Basch and Lie 1965; Boray 1985; Estuningsih 1991). *Lymnaea rubiginosa* is a fully freshwater snail (Van Benthem Jutting 1954) and serves as intermediate host of *F. gigantica* in Indonesia (Boray 1980; Muchlis 1985). Other species of *Lymnaea* have been found in Indonesia (Boray 1980) but have proven to be totally resistant to *F. gigantica* infection. The distribution of *L. rubiginosa* in Indonesia is widely spread and the biology of the snail is well described by Widjajanti (1990). Little is known of the population dynamic of this snail in the area where agricultural activities are conducted intensively. Since it is known that the life cycle of the liver fluke is heavily dependent on *L. rubiginosa* then it is clear that the distribution of the disease will be determined by that of the snail. The aim of this study is to determine the population dynamics of the snail *L. rubiginosa* in the rice paddy environment and its infection with fluke trematodes.

Manure used as fertiliser contains liver fluke eggs, and flooding of rice paddies before planting allows snail populations to build up.

A field study was conducted in five villages in the subdistrict of Surade in West Java. In summary the findings were that the population dynamics of *L. rubiginosa* in the paddy fields vary with the cropping practices of wet paddy and that the population was very high during the wet season. More snails were found close to human habitation and more of these were infected with trematode larvae. Passive migration in streams formed by heavy rain is the most important way that snails are disseminated into new habitats such as paddy fields. In the dry season most snails die from lack of water, with the surviving snails being mainly in streams, rivers or water springs. There is no sign of snail aestivation during droughts. Infection with *F. gigantica* in snails occurred throughout the year with the peak in May, October and February. Infection with non-*Fasciola,* mainly echinostome, tended to occur more in the dry season than in the wet.

A more detailed study was conducted in West Java to catalogue all the trematodes that use *L. rubiginosa* as a first intermediate host and to identify their definitive hosts (Estuningsih and Copeman 1996). Trematode larvae in 3,253 *L. rubiginosa* were collected from irrigated rice fields at five sites around Bogor, and another 2,875 from Surade. Four types of cercariae were found in snails from the Bogor area: echinostome, strigeid, *Trichobilharzia* sp. and xiphidiocercariae; whereas in snails from Surade there were xiphidiocercariae and cercariae of *F. gigantica*, *Schistosoma* sp. and echinostomes.

The larval echinostomes found in *L. rubiginosa* from the Bogor area, and adult echinostomes in domestic ducks and chickens which grazed harvested rice fields in this area were both identified as *Echinostoma revolutum.* Since no echinostome was found in 24 rats, 11 lizards or 35 frogs caught in the vicinity of the Bogor rice fields, it was concluded that domestic ducks and chickens were the main definitive hosts for *E. revolutum* in the area. The implication here is that the prevalence of cercaria of *F. gigantica* in the snail population is a very poor indicator of the prevalence in the definitive hosts of interest.

Cambodia

The snail ecology study in Cambodia was conducted from April 1999 to March 2001 in two communes of Saang district, Kandal province: Knong Preak village of Preak Koy commune and Preak Kseow village of Rokar Kpos commune.

The snail density in the two collection sites varied markedly throughout the year. In Preak Kseow village snail density rose from 0.29 snails/m² in November 1999 to 14 snails/m² in May 2000, then peaked at 19 snails/m² in June but rapidly decreased after this. Again, in November 2000, snail density increased from 0.34 snails/m² to 3.46 snails/m² in February 2001. Preak Kseow village had a six-month period of snail density at a level of greater than 5/m². Snail density was greater than 5/m² from February to July inclusive. Knong Preak had a snail density of greater than 5/m² for only two months, December and January. Density peaked at a higher level of 27/m² in January but tailed off to a long period when no snails were found from March to October. Snail density again increased from few in November–December 2000 to a peak of 12.26 snails/m² in January 2001, then dropped immediately to zero in February.

In most of the year in Preak Kseow there was a low but constant rate of infection, below 5%, of snails with *Fasciola* larvae except for a peak in September 2000 (17.2%). The percentages of snails infected with larvae of *Fasciola* were as follows.

	1999 %	2000 %	2001 %
January		4.7	2.7
February		0.7	0.0
March		0.4	
April		0.5	
May		0.7	
June		2.7	
July		0.6	
August		0.0	
September		17.2	
October		0.0	
November	0.0	4.8	
December	2.3	0.0	

Only the months of August, October, December 2000 and January 2001 showed no infection of snails with *Fasciola* larvae.

In Knong Preak village of Preak Koy commune snail infection with larvae of *Fasciola* occurred only in December 1999 and January 2000 with infection rates 0.4% and 3.6% respectively. Infection occurred again in November–December 2000 and January 2001 with infection rates of 0.6%, 1.1% and 0.9% respectively. No infection occurred in the remaining months (Southeun, unpublished observations).

Infection of snails by the intermediate stages of *Fasciola gigantica*

The results of the study indicated that the prevalence of the larval stage of fluke infections related to the proximity to human settlement (and the accompanying animal pens). Both the size of the snail population and its infection with trematodes are inversely related to the distance from kampongs (villages). Infection with *F. gigantica* was not detected in small snails 10 mm long and most infections are in the size classes 2 and 3 (10–20 mm). The infection of snails is dominated by the disposal of ruminant faeces containing the eggs of the parasite into fresh water. This occurs particularly where faeces are applied to the paddy fields as a fertiliser, and where the drainage from cattle pens goes directly to the paddy fields. The highest prevalence of infection in snails is in paddy fields directly adjacent to cattle pens. There is also infection at the drainage level of the next lower paddy field but the level of infection was extremely low. Infection with non *F. gigantica* trematodes was higher than that with *F. gigantica* and the highest was in the fields directly adjacent to cattle pens. Based on the result of this preliminary study, further work was therefore designed in two selected snail habitats in rice field areas, one close to a kampong (< 100 m) and the other remote (500 m) and was started in the following month in May 1993 (Suhardono 2001).

Competition with *Echinostoma*

Echinostoma in other hosts

In a series of experiments to investigate further the host range of *Echinostoma*, 50 mallard ducks as well as native chickens, rats and edible frogs were examined for the presence of *E. revolutum* (Gonzaga and colleagues, unpublished observations). The study aimed to show the percentage of ducks, chickens, field rats and edible frogs with echinostomosis and which organs were commonly inhabited by the trematodes, and compared the carcass weight of infected and non-infected ducks.

Echinostoma revolutum were found in the caecum and large intestine of 24 (48%) mallard ducks, 27 (54%) native chickens and 23 (46%) rats examined. There were no *E. revolutum* found in the alimentary tract of edible frogs.

It was concluded that infection of *E. revolutum* is sufficiently common in mallard ducks, native chickens, and rats in the Kabacan region that they would be a suitable resource for biological control of infection with *F. gigantica*

Location and infectivity of metacercariae

Distribution on rice straw

The distribution of metacercariae of *F. gigantica* on fresh rice stalks was determined by cutting the lower 40 cm of stalks into lengths of 10 cm and feeding 10 kg from each level to groups of two previously uninfected merino sheep (Table 3.1). Two additional sheep were each infected with 250 metacercariae as positive controls and one uninfected sheep acted as a negative control. Seven to ten weeks later all sheep were killed and immature flukes harvested. It was found that sheep fed with the lower 10 cm of the stalks harboured 98% of the flukes recovered from animals fed rice stalks. Thirty-three and 41% respectively of the dose of metacercariae given to the two positive control sheep were recovered as

Table 3.1 Number and size of *Fasciola gigantica* recovered from *Fasciola*-naïve merino sheep infected with metacercariae of *F. gigantica* or fed portions of rice stems cut 0–10, 10–20, 20–30 or 30–40 cm from the ground.

Sheep no.	Source of infection/ distance of cut stalk from the ground	Week sheep killed	Number of *F. gigantica* recovered	Length ± SD (mm)	Width ± SD (mm)	*n*
603	Nil	6.5	0	0	0	0
611	250 mc	8	83	8.8 ± 1.5	1.9 ± 0.4	37
612	250 mc	7.5	103	6.5 ± 1.0	1.3 ± 0.3	78
628	0–10*	10.5	137	10.1 ± 2.1	2.2 ± 0.4	49
629	0–10	10.5	113	12.2 ± 2.0	2.4 ± 0.5	53
623	10–20	10.5	4	13.2 ± 1.8	2.7 ± 0.3	2
A60	10–20	10	0	0	0	0
500	20–30	10.5	1	12.5	2.5	1
98	20–30	0	0	0	0	0
102	30–40	0	0	0	0	0
708	30–40	0	0	0	0	0

* = distance in centimetres of the pieces of rice stem from ground level
mc = metacercariae of *F. gigantica*
n = number of flukes measured

larval flukes. It was concluded that rejection of the lower portion of rice stalks previously immersed in water would help control infection with *F. gigantica* in animals fed on fresh rice straw (Suhardono et al. 2006d).

Effect of temperature and humidity

The viability of the metacercariae of *F. gigantica* when stored in water at 13°C for up to 23 weeks, exposed to the sun for up to eight hours or stored at a range of temperatures and humidities for up to 10 weeks was studied (Table 3.2). Excysted metacercariae were categorised microscopically as viable (motile and undamaged), dubious (not motile and undamaged) or dead (visible necrosis). Infectivity of viable and dubious metacercariae and unselected reference metacercariae held in water at 7°C for 20 or more days was assessed by comparing numbers of flukes recovered from infected merino sheep. Mean recovery rates were respectively 54.6%, 7.2% and 37.2% for viable, dubious and unselected metacercariae. Metacercariae immersed in water remained viable longer than those allowed to desiccate. Viability was promoted by decreasing temperature and increasing humidity. Exposure to direct sun killed metacercariae within eight hours. Results indicated that in lowland Indonesian irrigated rice paddies, metacercariae immersed in water are likely to survive for less than five weeks while those that become desiccated will survive less than two weeks. This information, together with the option of exposing fresh rice stalks to direct sunlight before feeding them to livestock, can help farmers reduce infection with *F. gigantica* (Suhardono et al. 2006c).

Sources of metacercariae for infection

Rice straw is believed to be the main source of infection of cattle and buffaloes with *F. gigantica* in areas where irrigated rice is grown extensively (Ueno et al. 1975; Harrison et al. 1996). However, this conclusion has been based on largely circumstantial evidence on the ecology of the snail intermediate host and observations that prevalence of fasciolosis in draught animals is high in lowland rice irrigation areas (Edney and Muchlis 1962; Soesetya 1978; Morel and Mahato 1987; Suhardono and Estuningsih 1989). Furthermore, the possibility that water from rice fields may also be a source of metacercariae for animals and that there may be other sources has not been investigated.

The objective of this study was to identify the sources of metacercariae of *F. gigantica* infecting cattle in a lowland area of West Java where two tandem irrigated crops of rice are grown most years followed by either a period of fallow or a dryland crop.

Table 3.2 Percentage viability of metacercariae of *Fasciola gigantica* exposed to the sun or shaded while being maintained damp or dry at ambient temperature of about 30°C.

Hours exposed	Percentage viable							
	Under sunshine				Under shade			
	Damp		Dry		Damp		Dry	
	Live	Dubious	Live	Dubious	Live	Dubious	Live	Dubious
0	88	6	88	6	88	6	88	6
2	94	5	29	10	96	4	84	10
4	33	37	6	18	83	10	86	11
6	0	19	0	0	81	12	81	8
8	3	11	0	19	69	14	0	55

Water and rice stubble from recently harvested rice fields containing *L. rubiginosa* infected with *F. gigantica*, and water and forage from other habitats of *L. rubiginosa* where free-grazing cattle were observed to eat or drink were fed to merino sheep. They were then monitored, either by counting fluke numbers in the liver at necropsy or by observing *Fasciola* eggs in faeces, to determine sources of viable metacercariae. Infection with *F. gigantica* was acquired from the clear effluent water and water containing sediment from rice fields, and from rice stalks, but not from irrigation channels, the only other habitat of *L. rubiginosa* in the study area. It was concluded that the most important risk factor for cattle to become infected with *F. gigantica* was to allow them to graze in recently harvested rice fields. Fields most heavily contaminated were those flooded again after harvest and risk of infection was greatly increased by feeding fresh herbage to cattle from such fields or by allowing animals to drink from rice fields (Suhardono 2001).

Influence of production systems

Seasonal variation in Cambodia

A study was conducted in Cambodia to generate baseline data on the incidence and level of infection of *Fasciola* in cattle and buffalo. The study was conducted in six villages in four communes of Saang district of Kandal province and eight villages in four communes in Cherng Prey district of Kampong Cham province. The 373 families directly involved in the project either brought their animals for study or were interviewed. The project examined 1,790 cattle, 5,126 faecal samples, 596 cattle livers and 8,008 snails.

To understand the epidemiology of fasciolosis, the research included an abattoir study, a tracer study, an agricultural cycle study and a snail ecology study. In addition, impacts of fasciolosis on production were monitored in a suppressive treatment trial.

For the abattoir study, a total of 645 cattle were examined at the district abattoirs (Cherng Prey district of Kompong Cham province and Saang district of Kandal province) and of these 502 head (77.82%) were identified as originating from within Saang district.

Nearly all the cattle examined were described as the crossbred type, which is a cross between the imported large tall-bodied white-coated Haryana breed and the smaller local breed. A total of 644 cattle had their sex recorded. Of these, 82.9% were castrated males, 15.2% females and 1.9% bulls. The average age of cattle brought for slaughter was seven years old, with a range from two to 12 years old. Females were on average younger (5.6 years) than castrated males (7.4 years).

A total of 575 faeces samples were examined for *Fasciola* eggs, of which 142 (24.7%) were positive. Of the 596 cattle examined at the slaughterhouse, 176 (30%) had liver damage. Female cattle with both liver damage and eggs in their faeces had lower body condition than other females. There were no differences in bodyweight or condition between male cattle that had eggs in their faeces, liver damage, both, or neither of these.

There is some evidence of a seasonal infection with *Fasciola*. Positive faecal samples rose from a low of 11% in December 1998 to 34% in April 1999, 28% in May and 55% in June, then declined progressively for the remainder of 1999. These results indicate that infection was highest over the months from about November to April. Liver damage by *Fasciola* infection was high in December, January, February and March (39%, 36%, 43% and 37% respectively) and then decreased, thus providing additional evidence that infection with *Fasciola* is seasonally highest at the end and start of the year.

The average cattle liver weight was 6.2 kg (range 5.3–10.4 kg). In the 32 damaged livers examined there were means of 30.5 adult and 40.3 immature flukes per liver. The average weight of damaged liver tissue was 2.3 kg (range 0.3–10.4 kg).

A tracer study was conducted in four villages in three communes of Saang district, Kandal province: Preak Kseow village of Rokar Kpos commune, Svay Tany and Preak Run villages of Preak Koy commune and Ang village of Krang Yov commune. The study began in April 1999 and was completed at the end of March 2001. A total of 446 cattle in the target area were used (Preak Kseow village 130 head, Svay Tany 99 head, Preak Run 129 head and Ang 88 head). All were treated with triclabendazole (Fasinex) at 12 mg/kg body weight (0.1 ml/kg) to free them from any pre-existing *Fasciola* infection.

Using a prepatent period for *Fasciola* of four months, it was calculated that cattle were infected in all villages from September 1999 to March or April 2000. In Svay Tany village infection began in September (6%) and reached a peak in April (50%). Between May and July no cattle were infected, then some were infected from August of the following year. In Preak Kseow village incidence rose from 9% in September to the peak of 87% in April. No infection occurred from May to July, however, it began again in August. In Preak Run village incidence rose from 3% in September to 37% in December, dropped to 12% in February, rose again in March and reached a peak in April (57.2%). Once again the infection began from the following August. In Ang village, only small percentages of cattle were infected with *Fasciola* (1.6–4.0%). Infection occurred from September to March intermittently.

Tracer studies thus confirmed the pattern of infection with *Fasciola* suggested by the abattoir study, that incidence of infection is highest from October to April. It also indicated that April was a peak month for infection and that little infection occurred from May to August (Southeun et al., unpublished).

Seasonal variation in Indonesia

There are reports on prevalence proportions from most countries where infection with *F. gigantica* is endemic. Few studies, however, have attempted to define monthly or seasonal incidence rates even though this information provides a logical basis for rational decisions on control. Fasciolosis in Indonesia mainly occurs in cattle and buffaloes and is widespread in the main islands (Adiwinata 1955; Kraneveld 1924; Soesetya 1975; Nurdin and Salsabila 1979; Rivai 1979; Soemardi and Rubino 1979; Tarmudji and Ginting 1983; Tarmudji et al. 1983). In Indonesia old reports recognised the presence of more than one species of *Fasciola* (Krijgsman 1933). However, Mukhlis (1985) concluded only one species of liver fluke occurred in indigenous cattle and it was *F. gigantica*. The recent work of Kurniasih et al. (1996) supported this conclusion, but from molecular studies introduced the possibility of their being more than one subspecies of *F. gigantica*.

To complete their life cycle liver flukes use lymnaeid snails as their intermediate host. On the island of Java, there is only one species of snail from the

ILRI/Steve Mann

Seasonal water sources are focal points for grazing and the continual cycling of liver fluke infection through snails.

family Lymnaeidae, *L. javanica,* which is synonymous with *L. rubiginosa* (van Benthem Jutting 1956). *Lymnaea rubiginosa* is an aquatic snail which spends most of its time floating at the surface of water (Widjajanti 1989). It thrives in the clear, slowly moving shallow water of irrigated rice fields.

In most countries of South-East Asia the main reason for raising cattle in rice-cropping communities is to provide a source of power during the processing of land before it's cultivated (Jarrige 1980). They also provide manure which is used as fertiliser in rice fields and an alternative source of income if the crop fails. The management of these animals is closely integrated with cropping practices. Farmers mostly feed their cattle with crop residues as most do not have enough land to grow special fodder for their animals.

Two seasonal studies—Monthly fluctuations of faecal *Fasciola* egg counts were monitored in 60 Ongole cattle each four weeks for 13 months in five villages in the subdistrict of Surade, West Java. Faecal egg counts of *F. gigantica* were also estimated for additional groups of 30 cattle selected each month from the same villages. It was concluded that estimates of prevalence of infection with *F. gigantica* are likely to be conservative when based on faecal egg counts. It was proposed that the sudden drop in apparent prevalence during December followed by gradual recovery during the following few months was a consequence of immune stimulation (induced by an influx of metacercariae acquired by animals from rice stubble at the beginning of the harvest season in January) which either causes loss of adult worms or suppression of their egg output (Figures 3.3 and 3.4).

The incidence and time of infection with *F. gigantica* in Ongole cattle in a rice-cropping environment in West Java was undertaken using 30 Ongole tracer cattle each four weeks for 16 months in 1993–94. It was found that over 80% of infection was acquired from January to June which coincided with the period of harvest of irrigated rice. It is also concluded that the main source of infection with *F. gigantica* in cattle is fresh rice straw, especially that from fields within about 200 m of a cattle pen.

In parallel with the green revolution of Indonesia, rice stubble has become abundant. This has been due mainly to high-yield varieties of rice being introduced, a relatively short time from planting to harvest and an increase in irrigation (Heytens 1991). Because more rice stubble is available, it has become a major dietary component for cattle. Irrigated rice is increasingly producing two crops per year and this also serves to maintain a high population of *L. rubiginosa*. The close relationship between rice cultivation and cattle management and the suitability of irrigated rice fields as a habitat for *L. rubiginosa* causes the pattern of liver

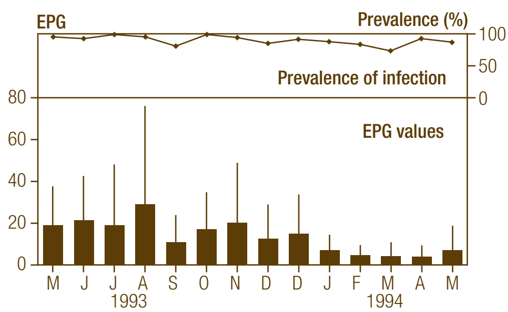


Figure 3.3. Prevalence of *Fasciola gigantica eggs* and mean egg counts per gram of faeces (EPG) ± SD of each batch of 30 Ongole cattle chosen each four weeks to become tracers, before they were treated with triclabendazole.

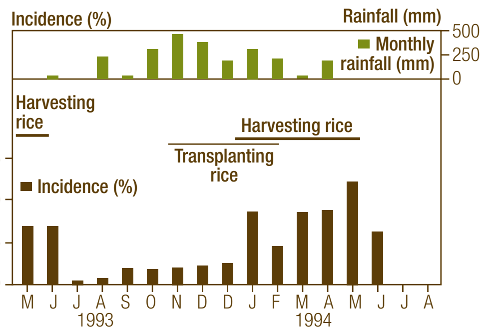


Figure 3.4. Incidence of *Fasciola gigantica* infection as detected by eggs in faeces in relation to monthly rainfall and the seasonal activities for rice production.

Table 3.3 Key research finding and their implications for control.

Key finding	Implication for control programs
Fluke eggs are susceptible to heat and exposure to the sun.	Dung could be composted or exposed to sun before being spread as fertiliser. This can supplement the containment of dung to prevent contamination of waterways and rice paddies.
Laboratory methods for counting fluke eggs can be improved.	Diagnosis of suspected cases and in field survey can be improved. Evaluation of interventions in the field can be made more accurate.
Composting and heat induced by additives can reduce infectivity of eggs.	Different methods of composting need to be evaluated for their impact on fluke egg survival.
The role of *Lymnea rubiginosa* is confirmed in areas of high fluke prevalence in Indonesia.	Knowing which species and their distribution is an important aspect of identifying areas of potential problems.
Many parasite species found in *Lymnea*, including *Echinostoma* in Java.	Many species use *Lymnea* as an intermediate host, therefore accurate identification of cercariae is essential.
Echinostoma found in a range of warm- blooded vertebrates in the Philippines.	*Echinostoma* may be very widespread and has the potential to interfere with transmission of *Fasciola.*
Distribution of metacercaria is limited to the bottom 10 cm of rice straw.	Cutting rice straw higher than 10 cm would prevent infection being carried with fodder to stalled animals.
Exposure of metacercariae on rice straw to sun reduces their viability.	Leaving contaminate rice straw in the sun before feeding could reduce exposure to infection.
Prepatent periods in Ongole cattle confirmed to be 18 weeks (4 months).	This conforms to expectations but needs to be confirmed if local experimental studies are to be conducted.
Pattern of infection in Cambodia consistent with 4-month prepatent period for infection.	Once established that *Lymnea* is the intermediate host and *Fasciola* the cause of disease similar control principles can be applied across the region.
In Cambodia the highest level of infection occurs between October and April, with no new infections between May and August.	Timing of chemical treatments and interventions to prevent infection can be focused on periods of non-transmission and transmission, as appropriate.
Similar patterns in Indonesia with tracer studies provide a clearer picture.	
Infection is intimately associated with the cycle of rice planting and harvesting in Indonesia.	Changes in rice production system need to take account of implications for liver fluke infection and control programs.

fluke disease in cattle to vary directly with the intensity of cropping of irrigated rice (Edney and Muchlis 1962).

No information is documented on the time of acquisition of and source of infection with *F. gigantica* by cattle in Indonesia. As prevalence is highest in rice-growing areas, this study was undertaken to determine the time of acquisition of *F. gigantica* in Ongole cattle raised in a farming area dominated by the cultivation of irrigated rice.

Implications for control programs

The main findings present in this chapter have been tabulated (Table 3.3) and their implications for potential control programs listed.

References

Adiwinata R.T. 1955. Helminth parasites in mammals and poultry in Indonesia. (Cacing-cacing yang berparasit pada hewan menyusui dan unggas di Indonesia). Hemerazoa 62, 229–247.

Alicata J.E. and Swanson L.E. 1941. Observations on the longevity of the liver fluke, *F. gigantica* in cattle. American Journal of Veterinary Research 3, 417–419.

al Kubaisee R.Y. and Altaif K.I. 1989. Comparative study of sheep and buffalo isolates of *F. gigantica* in the intermediate host *Lymnaea auricularia*. Research in Veterinary Science 47, 273–274.

Asanji M.F. 1988. The snail intermediate host of *F. gigantica* and the behaviour of miracidia in host selection. Bulletin of Animal Health and Production in Africa 36, 245–250.

Basch P.F. and Lie K.J. 1965. Multiple infection of larval trematodes in one snail. Medical Journal of Malaya 20, 59.

Boray J.C. 1969. Experimental fascioliasis in Australia. Advances in Parasitology 60, 114–124.

Boray J.C. 1982. Molluscan hosts and trematodes in the Pasific Basin. Pp. 81–106 in 'Biology and control of endoparasites' ed. by L.E.A. Symons, A.D. Donald and J.K. Dineen. Academic Press: New York.

Chartier C., Ngota A., Lonu L. and Cabaret J. 1990. Dynamics of *Lymnaea natalensis* populations in the Bunia area (Ituri, Haut-Zaire). Annales de Parasitologie Humaine et Comparee 65, 177–182.

Da Costa C., Dreyfuss G., Rakotondravao, Rondelaud D. and Da Costa C. 1994. Several observations concerning cercarial sheddings of *Fasciola gigantica* from *Lymnaea natalensis*. Parasite 1, 39–44.

Dinnick J.A. and Dinnick N.N. 1963. Effect of the seasonal variations of temperature on the development of *F. gigantica* in the snail host in the Kenya highlands. Bulletin of Epizootic Diseases of Africa 11, 197–207.

Dinnick J.A. and Dinnick N.N. 1964. The influence of temperature on the succession of redial and cercarial generations of *Fasciola gigantica*. Parasitology 54, 59–65.

Dreyfuss G. and Rondelaud D. 1994. Comparative study of cercarial shedding by *Lymnaea tomentosa* Pfeiffer infected with either *F. gigantica* Cobbold or *F. hepatica* Linne. Bulletin de la Societe Francaise de Parasitologie 12, 43–54.

Edney J.M. and Muchlis A. 1962. Fascioliasis in Indonesian livestock. Communications Veterinariae 6, 49–62.

Estuningsih S.E. 1991. Studies on trematodes infecting *Lymnaea rubiginosa* in West Java. M.Sc Thesis. Tropical Veterinary Sciences and Agriculture, James Cook University of North Queensland: Townsville, Australia.

Estuningsih S.E. and Copeman D.B. 1996.Trematode larvae in *Lymnaea rubiginosa* and their definitive host in irrigated rice fields in West Java. Jurnal Ilmu Ternak dan Veteriner 1, 200–205.

Grigoryan G.A. 1958. Experimental study of *F. gigantica* infestation in sheep. Trudy Armyansk Inst Zhivotnovod. i Vet 3, 155–168.

Grigoryan G.A. 1959. Exsperimental'nye dannye po prodolzhitol'nosti sokhranoniya adolescariev *Fasciola hepatica* (Data from experiments to find the survival span of metacercariae of *Fasciola gigantica*). Veterinariya 36, 35–37.

Guralp N., Ozcan C. and Simms B.T. 1964. *Fasciola gigantica* and fascioliasis in Turkey. American Journal of Veterinary Research 25, 196–210.

Hammond J.A. and Sewell M.M.H. 1975. Experimental infections of cattle with *F. gigantica*: numbers of parasites recovered after varying periods of infection. Tropical Animal Health and Production 7, 105–113.

Harrison L.J.S., Hammond, J.A. and Sewell, M.M.H. 1996. Studies on helminthosis at the Centre for Tropical Veterinary Medicine. Tropical Animal Health and Production 28, 23–39.

Heytens, P. 1991. Rice production system. In 'Rice policy in Indonesia', ed. by S. Pearson, W. Falcon, P. Heytens, E. Monke and R. Naylor, Cornell University Press: Ithaca, London.

Hubendick, B. 1951. Recent lymnaeidae. Almquist & Wicksells: Stockholm.Jarrige R. 1980. Herbivores in agricultural ecosystem. Pp 763–823 in 'Digestive physiology and metabolism in ruminants'. ed. by Y. Ruckebusch and P. Thivend, AVI Publishing Company: Westport, CT, USA.

Kendall S.B. 1954. Fascioliasis in Pakistan. Annals of Tropical Medicine and Parasitology 48, 307–313.

Kendall S.B. 1965. Relationships between the species of *Fasciola* and their molluscan hosts. Advances in Parasitology 3, 59–98.

Kraneveld F.C. 1924. Bijdrage tot de therapie der distomatosis in Nederlandsch-Indie. Nederlandsch Indie Bladen von Diergeneskunde 36, 3–63.

Krijgsman B.J. 1933. The parasite worms of mammals and birds in the Nederlandsch-Indie. (De in zoogdieren en vogels parasiteerende wormen van Nederlandsch-Indie). Nederlandsch-Indi Bladen von Diergeneskunde 22, 366–379.

Kurniasih, Barker S.C. and Cribb T.H. 1996. Phylogeny of *Fasciola* species (Digenea) in internal transcribed spacer ribosomal RNA from Indonesian domestic animals. (Abstract). In 'Pasean 1996. Parasites and Southeast Asia—now'. Bali, September 1996. (Program and Abstract. p. 30).

Morel A.M. and Mahato S.N. 1987. Epidemiology of fascioliasis in the Koshi hills of Nepal. Tropical Animal Health and Production 19, 33–38.

Mukhlis A. 1985.The identity of liver fluke (*Fasciola* sp.) and its life cycle in Indonesia. (Identitas cacing hati (*Fasciola* sp.) dan daur hidupnya di Indonesia). Ph.D. Thesis. Institut Pertanian: Bogor, Indonesia.

Mzembe S.A.T. and Chaudhry M.A. 1979. The epidemiology of fascioliasis in Malawi: I. The epidemiology of the intermediate host. Tropical Animal Health and Production 11, 246–250.

Nurdin M.S. and Salsabila A. 1979. Trematodes in slaughtered animals in West Sumatra. Veterinary Parasitology 5, 82 (Abstract).

Prasitirat P., Thammasart S., Chompoochan T., Nithiuthai S. and Taira S. 1996. The dynamics of antibody titres and faecal egg output in cattle and buffalo following infection with 500 and 1,000 *Fasciola gigantica* metacercariae. Thai Journal of Veterinary Medicine 26, 85–89.

Rivai M. 1979. Losses caused by *Fasciola hepatica* in infected cattle and buffaloes slaughtered at Padang Panjang abattoir. Veterinary Parasitology 5, 81 (Abstract).

Sewell M.M.H. 1966. The pathogenesis of fascioliasis. Veterinary Record 78, 98–105.

Soemardi A.W. and Rubino 1979. Distribution of *Fasciola hepatica* in cattle and buffalo in Timor. Veterinary Parasitology 5, 81 (Abstract).

Soesetya R.H.B. 1975. The prevalence of *Fasciola gigantica* infection in cattle in East Java, Indonesia. Malaysian Veterinary Journal 6, 5–8.

Suhardono D. 2001. Epidemiology and control of fasciolosis by *Fasciola gigantica* in ongole cattle in West Java. Ph.D. Thesis, Tropical Veterinary Science, James Cook University of North Queensland: Townsville Australia.

Suhardono and Estuningsih, S.E. 1989. Contamination rate of metacercariae in rice straws in the endemic area of fasciolosis. (Tingkat kontaminasi jerami padi oleh metacercariae di daerah endemik fascioliasis). In Proceedings Pertemuan Ilmiah Ruminansia. Ruminansia Besar. Pusat Penelitian dan Pengembangan Peternakan, Badan Penelitian dan Pengembangan Pertanian: Bogor, Indonesia.

Suhardono, Roberts J.A and Copeman D.B. 2006a. The longevity of eggs of *F. gigantica* in bovine faeces. Tropical Animal Health and Production 38, 379–382.

Suhardono, Roberts J.A and Copeman D.B. 2006b. Optimisation of the procedure for counting the eggs of *Fascioloa gigantica* in bovine faeces. Tropical Animal Health and Production 38, 383–387.

Suhardono, Roberts J.A and Copeman D.B. 2006c. The effect of temperature and humidity on longevity of metacercariae of *F. gigantica*. Tropical Animal Health and Production 38, 371–387.

Suhardono, Roberts J.A and Copeman D.B. 2006d. Distribution of metacercariae of *F. gigantica* on rice straw. Tropical Animal Health and Production 38, 117–119.Taira N., Suzuki K. and Boray J.C. 1983. Detection and quantitation of *Fasciola* eggs in cattle faeces using the beads technique. Japan Journal of Parasitology 32, 279–286.

Tarmudji and Ginting Ng. 1983. The rate of liver destruction due to fasciolosis in female Friesian Holstein in the district of Malang, East Java: A histopathological examination. (Derajat kerusakan hati akibat fascioliasis pada sapi-sapi Friesian Holstein betina di kabupaten Malang, Jawa Timur: Suatu tinjauan histopatologik). Penyakit Hewan 15, 83–85.

Tarmudji, Iskandar T. and Hamid H. 1983. Fasciolosis in cattle in East Java between 1979 and 1982, based on histopathological examination. (Kasus fascioliasis pada sapi-sapi di Jawa Timur, berdasarkan hasil pemeriksaan patologik antara 1979–1982. Penyakit Hewan 15, 91–94.

Tembely S., Coulibaly E., Dembele K., Kayentao O. and Kouyate B. 1995. Intermediate host populations and seasonal transmission of *Fasciola gigantica* to calves in central Mali, with observations on nematode populations. Preventive Veterinary Medicine 22, 127–136.

Ueno H. and Yoshihara S. 1974. Vertical distribution of *Fasciola gigantica* metacercariae on stem of rice plant grown in a pot. National Institute of Animal Health Quarterly 14, 54–60.

van Benthem Jutting W.S.S. 1950. Systematic studies on the non-marine mollusca of the Indo-Australian archipelago. II. Critical revision of the Javanese pulmonate land-snails of the families Helicarionidae, Pleurodontidae, Fruticicolidae & Streptaxidae. Treubia 20, 381–505.

Widjajanti S. 1989. Studies on the biology of *Lymnaea rubiginosa*. M.Sc. Thesis, Tropical Veterinary Science and Agriculture. James Cook University of North Queensland: Townsville, Australia.

4

Options for the control of liver fluke

R.S. Copland and L.F. Skerratt

Options for the control of liver fluke

R.S. Copland and L.F. Skerratt

Introduction

Much of the literature on the control of *Fasciola gigantica* is based on successes and failures of methods used in temperate countries to control *Fasciola hepatica*. These include strategic anthelmintic treatment, grazing management, application of molluscicides, and fencing off or draining swampy areas. The relevance of these approaches to control of *F. gigantica* in the tropics must be questioned and control options developed on the basis of a sound understanding of the transmission of *F. gigantica* in tropical production systems. Relatively little is known about the epidemiology of *F. gigantica* and this hampers the development of control strategies for tropical production systems in areas where infection with *F. gigantica* is endemic. These are often areas where irrigated rice is cultivated intensively. Although regional recommendations have been made for some developing countries (FAO 1994), in most areas of tropical Asia there are no routinely used control programs for fasciolosis (Spithill et al. 1999).

To be appropriate for the low-input, smallholder-based systems of most of tropical Asia, control measures for *F. gigantica* should be low-cost, readily available and applicable with little disruption to existing agricultural practices. Snail control using molluscicides, feeding management and biological control are alternatives that may complement the use of anthelmintics. Realistic control options will only be effective if there is a sound understanding of when and how transmission of the infective stages of *F. gigantica* is taking place—and widespread adoption of measures to control infection with *F. gigantica* will ultimately depend on farmers being convinced that the benefits from control justify the costs of implementing them.

Chemotherapy remains an important tool for control and a review of the literature on the use of a wide range of anthelmintics suggests that efficacy against *F. hepatica* is a strong indicator of efficacy against *F. gigantica* (Spithill et al. 1999). There is less certainty about efficacy in host species other than cattle and in field situations where low-quality feed may affect the biological activity of a chemical given orally. Only triclabendazole and clorsulon have high efficacy against both mature and immature *F. gigantica*. There is evidence that dose rates for buffalo need to be double those for cattle and that feed with high roughage content increases drug effectiveness (Sanyal and Gupta 1996a). Some ACIAR project trials have sought to verify the effectiveness of triclabendazole in a range of situations and in cattle, buffalo and goats.

Other options for control of *F. gigantica* have been reviewed by Roberts and Suhardono (1996) and include grazing management, predator/competitor snails, predation by fish or ducks and breeding resistant livestock. Like the use of anthelmintics none of these have been adopted widely; in most cases because of a lack of knowledge on their effectiveness. This chapter will describe some experiments conducted by the ACIAR projects and consider their potential.

It is highly likely that recommendations made for pastoral areas will vary from those made for agricultural areas where the main source of infection is residual water and vegetation in irrigated rice fields, and fresh rice stalks after harvest. At the end of the chapter two separate sets of recommendations are given: one for pastoral areas, the other for irrigated rice-based systems.

The practical questions that stimulated these studies include the following.

- What are the existing technologies available for liver fluke control?
- To what extent can the strategies developed for the control of *F. hepatica* be transferred to the control of *F. gigantica* and are these of equal value in cattle and buffalo?
- What are the challenges and opportunities for control presented by the association of *F. gigantica* with paddy rice production and seasonal flooding of grazing lands?
- To what extent can biological control—competition in the snail from other parasites—contribute to control?
- Can an integrated approach to liver fluke control be developed that combines all locally available technologies?

Previous recommendations for control

Recommendations for the control of diseases caused by *F. gigantica* and *F. hepatica* were prepared for many tropical and subtropical regions on the basis of temperature and rainfall patterns (FAO 1994). These patterns, even in the absence of specific information on the ecology of the snail intermediate hosts, timing of ingestion of metacercariae or the shedding of eggs, were used to predict when anthelmintic treatment was most likely to be effective. Within Asia, recommendations were made for:

- Pakistan
- north-eastern mountains, northern plains and south-east coasts of India
- central Bangladesh
- north-eastern, central and southern China
- northern Vietnam
- Luzon in the Philippines
- western highlands of Malaysia
- Java in Indonesia.

The overarching general recommendations were to:

- strategically apply anthelmintics and eliminate the parasite from the hosts at the most convenient time for effective prevention of pasture contamination
- reduce the number of intermediate host snails through drainage and other agricultural practices
- reduce the chances of infection by efficient farm and grazing management.

The chart for Java is reproduced as Figure 4.1. Note that three treatments are recommended and further note that in irrigated rice production systems:

> *A treatment should be carried out before harvest if animals are grazed on the harvested rice fields and about two months after each harvest, when a high level of infection may occur.*

These sound recommendations do not specify which practices are most likely to lead to a reduction in snail numbers (the second general recommendation), or which efficient farm and grazing management practices are likely to be technically effective and which are environmentally, economically and socially acceptable.

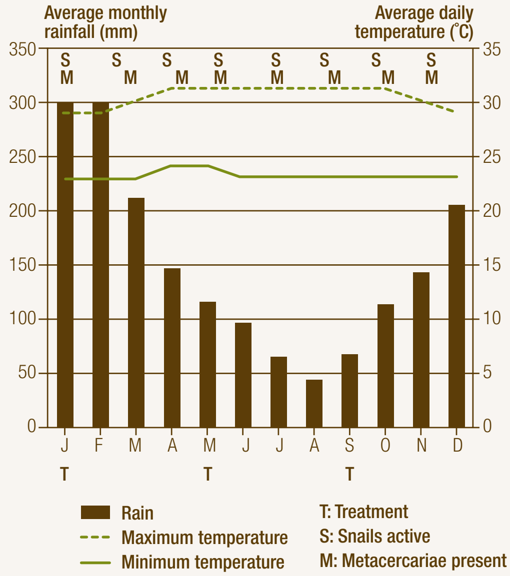


Figure 4.1 Program for *Fasciola* control in Java (from FAO 1994).

Opportunities for control

As for all helminths, control options for *F. gigantica* are based on interrupting the life cycle of the parasite and several biological and chemotherapeutic options available have been summarised diagrammatically (Figure 4.2). Each of these, if fully effective, would completely prevent transmission. In practice of course, it is a combination of approaches that will lead to effective control.

Exposure of animals to infection with *F. gigantica* from rivers and lakes may be restricted to only a few weeks each year. Infection usually occurs about two months after the habitat becomes stable (and thus favourable for snails) towards the end of the wet season and persists during the dry season for a few weeks after the habitat is no longer suitable for snails. Exposure of animals to infection from irrigated rice fields occurs when stock graze stubble, eat rice stalks after harvest, or drink water flowing from the fields. Exposure will occur throughout the year in regions where rice cropping is continuous, but only for a few weeks after harvest when rice cropping is seasonal. Infection is highest from fields fertilised with animal manure.

Chemical treatment of infected cattle and buffalo

Most reports are of studies designed to determine the efficacy of a particular drug against *F. gigantica* rather than strategies for its use in the field. They show that drugs effective against *F. hepatica* have similar activity against *F. gigantica* (Spithill et al. 1999). Because of their relatively low therapeutic index when compared with more modern alternatives, use of carbon tetrachloride, tetrachlorodifluroethane, hetol and hexachloroethane for fasciolosis can no longer be justified. All modern dewormers have high efficacy against adult flukes, whereas only triclabendazole and clorsulon also have high efficacy against both mature and immature *F. gigantica*.

The general strategy for control using anthelmintics proposed by FAO recommended treatment at the

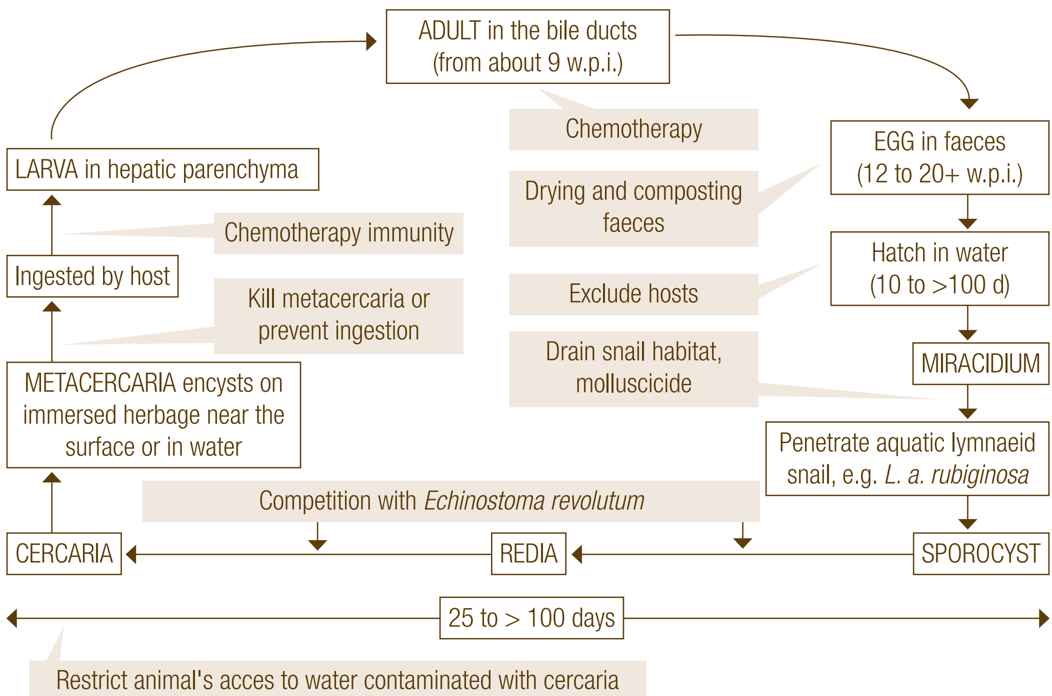


Figure 4.2 Life cycle of *Fasciola gigantica* showing control options (in shaded boxes) described in the text of this chapter (w.p.i. = weeks post infection).

end of a period of ecologically reduced activity of the parasite and snail intermediate host. This is followed by treatment one to two months after the expected peak of infection in hosts; and an additional treatment in heavily infected areas or where infection may be acquired throughout the year. While this strategy is theoretically sound, it is unlikely to be widely adopted due to the high cost of two to three treatments with anthelmintic and a lack of detailed knowledge to identify the correct timing of the treatments. Nonetheless, these recommendations for the use of an effective dewormer are very close to those detailed at the end of this chapter.

If the source of infection is herbage and water at the fringes of rivers and streams in pastoral areas, most authors in the Northern Hemisphere have recommended treating with an anthelmintic about September and again in about February to treat infection acquired from about July to December (for example, Schillhorn van Veen 1980; Morel and Mahato 1987 Rai et al. 1996). In Thailand it has been recommended that all cattle and buffalo older than eight months be treated in September (end of rainy season) and again in April (beginning of rainy season) in areas with high prevalence, especially stock in poor condition (Srikitjakarn et al. 1988; Srihakim and Pholpark 1991). To achieve control in a similar habitat in Malawi, Mzembe and Chaudry (1979) recommended treatment in January, April and September. As with the recommendations of FAO (1994) such strategies may be effective but are not widely used, possibly because of the cost but also because of lack of information on benefits that would justify the outlay for anthelmintic.

Recommendations made for pastoral areas do not apply to agricultural areas where the main source of infection is residual water and vegetation in irrigated rice fields and fresh rice stalks after harvest. When an anthelmintic is used for control in rice-growing areas, it would seem logical to treat animals with anthelmintic about three months before planting the rice crop so that stored and fresh faeces, used as fertiliser in the young crop, would be free from eggs of *F. gigantica* and thus the snails would not become infected. Animals grazing the stubble after harvest would then not be exposed to reinfection. On

ILRI/Steve Mann

Liver fluke is less of a problem in upland rice fields as there is no flooding and no build up of snail numbers.

the other hand, the advice of Traore (1989) to treat animals before grazing rice stubble does not seem logical as it will not affect the rate of acquisition of new infection by grazing stock. It might be better to treat six weeks after harvest if the field remains dry (this is sufficient time for metacercariae on stubble to die) and use triclabendazole or clorsulon as they are effective against immature and mature flukes.

This strategy was followed in West Java where infection is largely confined to the period from January to June when rice from the two annual crops is harvested. Good control has been achieved with a single treatment with triclabendazole in July, applied six weeks after the last of the seasonal rice harvest. Had only an adulticide been available for use, it would have been necessary to treat in July and September to achieve a similar level of control. The success of this strategy for a single treatment with triclabendazole in July also relied on there being a period of no or little natural transmission during the following few months. This enabled the generation of snails in the first seasonal rice crop, planted from September onwards, to remain virtually free from infection and thus break the annual cycle of transmission. A high level of control was achieved, with more than 80% of animals still with no detectable fluke eggs in their faeces 12 months after treatment.

ILRI/Steve Mann

Rice straw is an important part of the base diet of cattle and buffalo: but the base of the stalks carries infective metacercariae.

In areas where irrigation allows continuous or asynchronous cropping or rice throughout the year, there would be the opportunity for continuous exposure of animals to infection with *F. gigantica.* In such situations, in the Philippines for example, Tongson (1978) recommended anthelmintic treatment each three months. However, it should be possible to extend this period to four months if all farmers who share grazing on their newly harvested rice fields treat their animals with an anthelmintic that is effective against immature and adult flukes. Such a program would ensure that faeces of animals would remain virtually free from fluke eggs (due to the 14–16 weeks prepatent period of *F. gigantica*) with the consequence that snails would remain fluke-free and the rice crops would not be infective for grazing stock. The high cost of anthelmintic and cooperative effort required make it unlikely that such a program would be sustainable over many years. However, even if maintained for one year, it should substantially reduce the level of infection in the group of participating animals to levels that could then be kept low with grazing management or other biological means.

It is apparent that the strategic use of anthelmintic could provide an effective way to control infection with *F. gigantica* in both pastoral and agricultural settings. Furthermore, the minimal usage of anthelmintics against *F. gigantica* to date has ensured that their efficacy has remained high with no reports of development of resistant strains. Anthelmintics are, however, unlikely to achieve a high degree of acceptance in control of *F. gigantica* until more information is available on the costs and resultant benefits associated with their use. Tailoring of rational regional strategies for control is also dependent on there being sufficient knowledge of the pertaining agricultural cycle, social structures and determinants of infection with *F. gigantica*. This information, too, is rarely available.

National chemical control program in the Philippines

In the Philippines the national fasciolosis control program aims to arrest the rapid decline of the ruminant population through the eradication of fasciolosis, which is regarded as the leading cause of morbidity, low reproductive performance, and mortality among the ruminants in the country.

The control program covers 16 provinces that are found to be highly endemic for fasciolosis. These target provinces have a total ruminant population of over two million of which 35% are cattle and the remainder buffalo. The target provinces are spread throughout the country. It has four components:

- education/information campaign
- chemotherapeutic control
- disease monitoring/surveillance
- program analysis/evaluation.

The program uses drugs as the main pillar of control. Treatment of the susceptible animals was carried out every three months for three years. The efficacy of drugs to rid infected animals of their fluke burden was evaluated with the treated animals in the control areas being examined monthly. The results of treatment in cattle showed a reduction in prevalence from 48% to:

- 0.4% in the 1st year
- 0.8% in the 2nd year
- 0% by the middle of the 2nd year up to the 3rd year.

In carabaos, prevalence was reduced from 55% to:

- 6% in the 1st year
- 2% in the 2nd year
- 1% in the 3rd year.

It would appear that a greater efficacy was obtained in cattle than in carabaos. It can be concluded that such mass treatment programs can work when there is access to sufficient funds, chemicals of good quality and skilled technical support.

Suhardono

Microscopic image of metacercaria (cysts which are formed from free swimming *Fasciola* and attach to vegetation). Cutting rice straw above the water line prevents metacercaria being ingested by stall-fed livestock.

Systematic use of anthelmintics

Efficacy of treatment is relatively easy to determine if measured solely by the numbers of fluke eggs shed, or, if slaughter is a possibility, counting of mature and immature flukes in the liver. Of greater practical importance are the effects on productivity and, as described in detail in Chapter 2, these include growth, reproductive performance, milk production (for human consumption or for suckling calves) and draught power. In the Philippines one such attempt has been made to assess the total impact of systematic control of fasciolosis by drenching frequently to, as far as possible, suppress all existing and incoming infections. As many parameters of production as possible are then measured over a two-year period. The results are not conclusive but worth describing here, at least to highlight the complexity of such trials and the commitment required to undertake them. The trials were conducted by scientists of the College of Veterinary Medicine of the University of Southern Mindanao.

The study was conducted in irrigated and rainfed cropping areas in Mindanao the southernmost region of the Philippines. One irrigated and one rainfed area served as experimental and the remaining two areas as control (Gonzaga et al., unpublished).

The two-year suppressive treatment study began in February 2000 in areas representing an irrigated and a rainfed cropping system. Buffaloes and cattle are weighed, drenched with triclabendazole at a dose rate of 24 mg/kg body weight and 12 mg/kg body weight respectively. Two other areas served as

Peter Horne

Identifying when and where snails are present in the grazing system is essential for the design of a liver fluke control program. Removal of snails by ducks limits liver fluke transmission.

controls. Data on age, sex, body weight, reproductive performance, feeding system, draught power and uses of the animals were gathered from both the treated and the control groups every four months for two years. Mostly, buffaloes are used for draught power for land preparation in lowland and upland areas and for hauling farm products to market. Cattle were sold for meat, or kept for breeding. Cattle and carabaos have communal grazing areas and from planting to harvesting period of rice the animals are fed with grasses by a cut and carry system or they are allowed to graze near the dikes or river banks and canals.

In total there were 1,144 animals in the trial (in itself an indication of the magnitude of effort required). There were 772 males, 340 female and 32 castrated animals. Three hundred of the females were buffaloes and 468 were cattle. Of the 372 males, 198 were buffaloes and 174 cattle. The age of the animals varied from one-day old to more than six-years old.

The live-weight gain in buffaloes in treated irrigated and rainfed areas was 279 kg/day and 219 g/day respectively and of cattle in the same areas 361 g/day and 358 g/day. Thus in both areas cattle were growing faster. Buffalo in the control, untreated areas grew at the same rate as their treated counterparts: 231 g/day and 357 g/day, respectively. Thus treatment of fluke infection did not affect growth rate in buffaloes. In contrast the live-weight gain in untreated cattle in the same areas was 67 g/day and 343 kg/day in treated areas. Deworming substantially improved cattle growth. Emerging from these data are possible differences in impacts between buffaloes and cattle.

Intercalving interval could not be measured accurately over the course of a two-year study as insufficient buffalo or cattle cows had more than one calf during the study period. However, there was some indication that cattle and buffaloes that received treatment (triclabendazole) had improved intercalving intervals over untreated ones. The reproductive performance in treated animals in irrigated and rainfed areas is better as evidenced by more animals becoming pregnant and more calves being born than in non-treated animals in irrigated and rainfed areas.

Factors that made the study difficult to implement and interpret include:

- most animals not turning up regularly for treatment
- the loss of 11% of the animals due to accidental death, being slaughtered or stolen.

It was therefore concluded that fasciolosis infection is still present in animals in irrigated and rainfed areas. This is despite suppressive treatment because of insufficient concern by animal owners. It is clear that in view of the gestation period in cattle and buffalo a two-year trial is too short to assess the significance of treatment on intercalving intervals.

Triclabendazole in Philippine carabaos

To confirm the efficacy of triclabendazole in Philippine carabaos a study was conducted to evaluate the efficacy of triclabendazole (Fasinex®-240) administered in carabaos infected naturally with *F. gigantica.* The study aimed to determine the effect of triclabendazole at 24 mg/kg body weight and 40 mg/kg body weight, the presence of mature and immature flukes in the liver four months post treatment by post-mortem examination, and differences in liver weight between treated and control carabaos.

Six naturally infected carabaos regardless of sex and place of origin were used in the study. Two infected carabaos (treatment A) were drenched with triclabendazole at a dose rate of 24 mg/kg body weight and the other two carabaos (treatment B) were drenched at 40 mg/kg body weight on August 22, 2001. The remaining two (treatment C) were controls and given a placebo (fresh milk). Faecal samples, body weight, and PCV (packed cell volume) were collected before drenching. Faecal samples were collected 23 days after treatment and 14 days thereafter for four months. The carabaos were weighed, slaughtered and the liver and carcass weights taken. The liver was examined for the presence of mature or immature flukes.

The number of eggs per gram of faeces was reduced by 92% at nine weeks and 95% at 11 weeks in carabaos drenched with triclabendazole at a dose rate of 24 mg/kg body weight. In carabaos drenched at 40 mg/kg body weight egg number reduction was 49% at nine weeks and 82.6% at 11 weeks. The PCV values, carcass weight and liver weights did not vary significantly ($P > 0.05$) with treatment. However, mature and immature flukes were found in animals dosed with triclabendazole at 24 mg/kg body weight and in the control group.

These results support earlier work in India with swamp buffalo (Sanyal and Gupta 1996a,b) and in Indonesia (Suhardono et al. 1991) in which the efficacy of triclabendazole against natural infections with *F. gigantica* was evaluated in a field study using 102 Indonesian cattle. When cattle were treated with triclabendazole at a dose rate of 12 mg/kg orally every eight weeks for one year the faecal egg count in treated animals was reduced below that of controls.

Medicated pellets

Triclabendazole can also be incorporated into feed, including feed block and pellets. Single-dose studies on the pharmacokinetics and efficacy of triclabendazole against induced bovine and bubaline fasciolosis revealed poor anthelmintic uptake in buffaloes at the recommended dose for cattle of 12.0 mg/kg body weight. Flukicidal activity in buffaloes required 24.0 mg/kg. Similar differences between cattle and buffaloes were also observed with continuous dosing of triclabendazole. Based on the efficacy trials with sustained low-level administration, two different dose rates were used in feed pellets so as to deliver triclabendazole daily at 0.5 and 1.5 mg/kg body weight respectively, in cattle and buffaloes infected with immature *F. gigantica*. It was concluded that such medicated pellets could be used at epidemiologically strategic times to control fasciolosis in cattle and buffaloes (Sanyal and Gupta 1996a, b).

Snail control

Molluscicides have mainly been applied or recommended for use in dams to control the snails that are intermediate hosts of *F. gigantica.* This may be because the more extensive habitats such as rivers and irrigated rice fields make the cost prohibitive and also because of the adverse effects of some molluscicides on non-target animals and plants in the habitat. Dinnick and Dinnick (1963) recommended use of molluscicide in highland areas of Kenya at intervals shorter than the minimum period for development of egg to cercaria (69 to more than 100 days, depending on the temperature).

A strategic approach was also recommended by Mzembe and Chaudhry (1981), with molluscicide being applied just before cercarial shedding began in June, and repeated in September. Preston and Castelino (1977), on the other hand, applied molluscicide by aerial and hand spraying to eliminate snails from a dam and control fasciolosis over a period of two years. The unpublished finding by Suhardono, Roberts and Copeman that snails with the highest prevalence of infection with *F. gigantica* are those in irrigated rice fields adjacent to a cattle pen or village, opens the possibility for selective use of molluscicide in such areas. Two applications, five and 10 weeks after the rice crop is planted should prevent cercarial shedding in lowland tropical areas, whereas only one treatment seven weeks after planting may be effective in cooler regions where larval development in the snail is slower.

Another possibility that has not been trialled, which may be more applicable in pastoral areas than rice fields, utilises the molluscicidal properties of eucalyptus leaves (Harrison et al. 1996). These authors postulated that the leaves falling from such trees growing around the periphery of habitats of snails might provide a sustainable means of snail control.

Free-ranging ducks or geese, which eat snails, have also been proposed as a possible means for biological control of *F. gigantica* (Touratier 1988; Rai et al. 1996) but the degree to which control is likely to be achieved has not been measured. Effective control would need enough ducks to be present to eat most snails in a habitat before they shed cercariae. This may be achievable along limited stretches of the shores of lakes and streams where stock drink but large numbers of birds are unlikely to be acceptable in irrigated rice fields prior to harvest. Furthermore, the common practice of allowing large flocks of ducks to glean recently harvested rice fields (and eat the snails) is not likely to reduce the availability of metacercariae for animals that graze the stubble, as they encyst prior to harvest.

Grazing and feed management

In areas where the main source of infection is irrigated rice fields after harvest, managing feeding in a way that prevents stock accessing viable metacercariae and using biological control of infection with *F. gigantica* in snails are particularly relevant strategies. The effectiveness of storage of rice stalks as hay at room temperature in Indonesia (about 28°C) for one month to kill metacercariae was confirmed by Suhardono et al. (2006a). They also showed that metacercariae were killed by exposure to sunshine for eight hours. The bottom third of rice stalks (the portion previously immersed where metacercariae encyst) can thus be made safe to eat by exposure to sunlight, or avoided by feeding only the top two-thirds of the stalk, shown to be safe as fresh fodder. Another recommendation from their work was that high-risk rice fields adjacent to villages or cattle pens should not be grazed until they have been dry for at least six weeks after harvest, by which time most metacercariae should be dead.

Biological control

The possibility of successful biological control of fasciolosis by echinostome flukes was also demonstrated by Suhardono et al. (2006b). The ability of larval echinostomes to aggressively displace other larval flukes from their snail hosts and parasitic castration of snails by larval echinostomes is well documented (Lie 1973; Estuningsih 1991). However, previous workers were unable to devise a practical way to apply this strategy in the field. This was achieved by adding faeces from five to 10 ducks naturally infected with *Echinostoma revolutum* to bovine faeces used as fertiliser in rice fields, or by locating the duck pen over the effluent drain from a cattle pen before it entered an adjacent field. To maximise the competition between miracidia of *F. gigantica* and *E. revolutum* for snails it is important to ensure the duck and bovine faeces enter the rice field at the same time and place. This strategy was found to almost eliminate metacercariae from rice fields close to cattle pens or villages that would otherwise constitute the greatest potential source of infection for stock. However, there has been resistance in West Java to application of this novel means of control because village ducks may be concurrently infected with schistosomes, the cercariae of which cause dermatitis when they penetrate the skin of rice-field workers.

Conclusions and recommendations

Based on the above options a series of recommendations can be used as a starting point to convince farmers that control of fasciolosis is worth their investment of resources. On a technical basis these recommendations should work, based on the evidence summarised here. Adoption of these recommendations is the next challenge.

Controlling infection from irrigated rice fields

Prevent infection by:

- avoiding grazing, drinking or feeding rice stems from fields fertilised with animal dung for six weeks after the field dries out after harvest
- feeding only the top two-thirds of fresh rice stalks
- exposing rice stalks to the sun for three days or store dry for five weeks before feeding to stock.

Kill eggs of *F. gigantica* in dung used as fertiliser by:

- storing cattle dung in the sun for at least one month before using it as fertiliser in rice fields
- mixing herbage with dung to promote fermentation and increase the temperature above 45°C

Promote competition between *Echinostoma* and *F. gigantica* larvae in snails by:

- adding dung from village ducks or chickens (naturally infected with *E. revolutum*) and cattle dung together as fertiliser in rice fields
- placing a pen with 5–10 village ducks or chickens (naturally infected with *E. revolutum*) over the effluent drain from a cattle pen before it enters a rice field
- ensuring cattle, duck or chicken dung enters the rice field at the same place and time.

Drenching large ruminants with dewormers remains a mainstay of liver fluke control programs.

Regular mass treatments of cattle and buffalo have met with some success in the Philippines.

Make best use of deworming chemicals by:

- using them in conjunction with biological methods of control
- treating with triclabendazole five weeks after the last rice fields in an area have been harvested, in areas with seasonal rice cropping (no irrigated rice over the dry season)
- treating with triclabendazole (12 mg/kg for cattle, 24 mg/kg for buffalo) each four months (or each three months if available drug is only adulticidal, such as albendazole) in irrigated areas with continuous rice cropping
- treating all animals that share common grazing.

Controlling infection from lakes, swamps or streams

Prevent infection by:

- avoiding water consumption, grazing or harvesting of fringing herbage from water bodies when snails are shedding cercariae. Shedding usually occurs from about two months after the end of the wet season and may persist for a couple of months. The duration of the danger period for infection depends on the persistence of emergent vegetation and snails in the water body.

Make best use of deworming chemicals by:

- treating with triclabendazole (12 mg/kg for cattle, 24 mg/kg for buffalo) at the beginning of the wet season (or with a drug that is only adulticidal at the beginning and end of the wet season).

Recent developments in Cambodia

Based on much of the above research the Government of Cambodia has made a concentrated effort to develop a control program for liver fluke. From 2004 to 2007 a comprehensive training and extension program was undertaken that combined further evaluation of the control program by scientists, extensionists and farmers, and specific training in the implementation of control measures. A complete description of the program is appended to this monograph. In addition, a GIS model predicting the prevalence of fasciolosis was validated in Kampong Cham and was generated using specific knowledge from epidemiological studies in Kandal province and general knowledge on the epidemiology of fasciolosis. However, it still needs some validation in other areas especially at fine geographical scales (Tum et al. 2004, 2007).

The options in the program included control of drinking water, grazing management and use of drugs, very much in line with the recommendations above, with the addition of a new pour-on formulation of triclabendazole (Genesis®) which was imported for the evaluation. The third option, biological control, was modified to include only aspects of dung management and did not include the use of poultry to contribute parasites that compete with *Fasciola* in snails.

The methodology of the evaluation was to implement a two-year control program between July 2004 and June 2006 in two villages with several hundred large ruminants and compare the impacts of the program with similar villages in which no control program was implemented. This study was integrated with control of other major endemic diseases of cattle and buffalo, namely haemorrhagic septicaemia (HS), foot-and-mouth disease (FMD) and toxocarosis (infection with the nematode *Toxocara vitulorum*) to provide an incentive to farmers in villages where the control program for fasciolosis was not carried out and to avoid the confounding effects that these diseases may have had on the study. From 2006 until 2007 the acceptance and adoption of control measures by farmers were assessed.

The control program contained a training component for extension workers and an extension package for extension workers to deliver to farmers. As anticipated the farmers participating in the control program increased their knowledge of the disease and its control. The methods of control were readily accepted, adopted by farmers and were still used by farmers one year after the control program ceased and financial assistance in terms of free drugs was no longer available (Suon et al., unpublished). The benefits of control well exceeded costs in areas where the prevalence of fasciolosis was high > 60% and would result in substantial profits for farmers per cow per year in the order of US$50–100 at the time of the study (Suon et al., unpublished). Parasite prevalence, as indicated by faecal egg counts, remained around 40% in the village with no control programs and was reduced to 2–5% in the village with control. The control program resulted in about a 30 kg per annum increase in bodyweight and 10% more births during the study. Farmers were also pleased with the health and condition of their animals.

The results from this study are currently being analysed and a national control program for fasciolosis is being developed. The strategy uses the results of this research work and discussions arising from the National Workshop on the Control of Fasciolosis held in Phnom Penh in July 2006. There are two main components to the strategy:

1. Use of the geographical information system (GIS) predictive model for fasciolosis in Cambodia to target moderate and high risk areas (i.e. provinces, districts and villages) initially. There will need to be some additional validation of the model in provinces and districts identified as moderate to high risk.
2. Plan and implement a control program within each province, district and village identified as moderate to high risk by the GIS model.

References

Boray J.C. 1991. Current status of the control of trematode infections in livestock in developing countries. Draft working paper for expert consultation on helminths infections of livestock in developing countries. FAO: Rome.

Dinnick J.A. and Dinnick N.N. 1963. Effect of the seasonal variations of temperature on the development of *Fasciola gigantica* in the snail host in the Kenya highlands. Bulletin of Epizootic Diseases of Africa 11, 197–207.

Estuningsih S.E. 1991. Studies on trematodes infecting *Lymnaea rubiginosa* in West Java. M.Sc Thesis. Tropical Veterinary Sciences and Agriculture, James Cook University of North Queensland: Townsville, Australia.

FAO 1994. Diseases of domestic animals caused by liver flukes: epidemiology, diagnosis and control of *Fasciola*, paramphistome, *Dicroceoelium*, *Eurytrema* and schistosome infections of ruminants in developing countries. FAO: Rome.

Harrison L.J.S., Hammond J.A. and Sewell M.M.H. 1996.Studies on helminthosis at the Centre for Tropical Veterinary Medicine (CTVM). Tropical Animal Health and Production 28, 23–39.

Lie K.J. 1973. Larval trematode antagonism: principles and possible application as a control method. Experimental Parasitology 33, 343–349.

Morel A.M. and Mahato S.N. 1987.Epidemiology of fascioliasis in the Koshi hills of Nepal. Tropical Animal Health and Production 19, 33–38. Mzembe S.A.T. and Chaudhry M.A. 1979. The epidemiology of fascioliasis in Malawi: I. The epidemiology of the intermediate host. Tropical Animal Health and Production 11, 246–250.

Preston J.M. and Castelino J.B. 1977. A study of the epidemiology of bovine fascioliasis in Kenya and its control using n-tritylmorpholine. British Veterinary Journal 155, 600–608.

Rai R.B., Senai S., Ahlawat S.P.S. and Kumar B.V. 1996.Studies on the control of fascioliasis in Andaman and Nicobar Islands. Indian Veterinary Journal 73, 822–825.

Roberts J.A. and Suhardono 1996. Approaches to the control of fasciolosis in ruminants. International Journal for Parasitology 26, 971–981.

Sanyal P.K. and Gupta S.C. 1996a. The efficacy and pharmacokinetics of long-term low-level intraruminal administration of triclabendazole in buffalo with induced fasciolosis. Veterinary Research Communications 20, 461–468.

Sanyal P.K. and Gupta S.C. 1996b. Efficacy and pharmacokinetics of triclabendazole in buffalo with induced fasciolosis. Veterinary Parasitology 63, 75–82.

Schillhorn van Veen T.W. 1980. Fascioliasis (*Fasciola gigantica*) in West Africa: a review. Veterinary Bulletin 50, 529–533.

Spithill T.W., Smooker P.M. and Copeman B. 1999. *Fasciola gigantica*: epidemiology, control, immunology and molecular biology. Pp. 465–520 in 'Fasciolosis', ed. by J.P. Dalton. CAB International: Wallingford, UK.

Srihakim S. and Pholpark M. 1991. Problem of fascioliasis in animal husbandry in Thailand. Southeast Asian Journal of Tropical Medicine and Public Health 22 Suppl, 352–355.

Srikitjakarn L., Pholpark M., Leidl K., Loehr K.F. and Hoerchner F. 1988. The epidemiology and control of bovine fascioliasis in Northeast Thailand. Thai Journal of Veterinary Medicine 18, 9–22.

Suhardono, Roberts J.A. and Copeman D.B.C. 2006a. The effect of temperature and humidity on longevity of metacercariae of *Fasciola gigantica*. Tropical Animal Health and Production 38, 371–377.

Suhardono, Roberts J.A. and Copeman D.B.C. 2006b. Biological control of *Fasciola gigantica* with *Echinostoma revolutum.* Veterinary Parasitology 140, 166–170.

Suhardono, Widjajanti S., Stevenson P. and Carmichael I.H. 1991. Control of *Fasciola gigantica* with triclabendazole in Indonesian cattle. Tropical Animal Health and Production 23, 217–20.

Tongson M.S. 1978. A national fascioliasis control program for the Philippines (a professorial lecture). Pp 106–117 in 'Proceedings of the Annual Convention of the Veterinary Practitioners Association of the Philippines'.

Touratier L. 1988. Note on the role of nitroxinil in an integrated campaign against *Fasciola gigantica* infection of cattle in Madagascar. Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux 41, 181–189.

Traore A. 1989. Incidence and control of fascioliasis around Niono, central Mali. ILCA Bulletin, 18–19.

Tum S., Puotinen M.L. and Copeman D.B. 2004. A geographic information systems model for mapping risk of fasciolosis in cattle and buffaloes in Cambodia. Veterinary Parasitology 12, 141–149.

Tum S.M.L. Puotinen M.L., Skerratt L.F., Chan B. and Sothoeun S. 2007. Validation of a geographic information system model for mapping the risk of fasciolosis in cattle and buffaloes in Cambodia. Veterinary Parasitology 143, 364–367.

5

Approaches to extension for fasciolosis control

D. Cameron, E. Martindah, W. Girsang,
J. Intong and B. Frank

Approaches to extension for fasciolosis control

D. Cameron, E. Martindah, W. Girsang, J. Intong and B. Frank

5

Introduction

Disease control has been attempted by countless extension efforts with a wide range of outcomes: complete success for some infectious diseases, for example, foot-and-mouth disease in some developed countries, to complete failure; for example, Newcastle disease in poultry in most developing countries. Control of most diseases lies somewhere between these extremes with the success of extension programs being largely dependent on the quantity and quality of resources being supplied from national or international public sources. It is rare, possibly only in the control of diseases of intensive poultry and pig farming, that private investment has resulted in effective disease control.

Control of liver fluke disease, therefore, is similar to that for a wide range of similar diseases endemic in Asia and for which effective technical options for control have been developed. Examples of these are Newcastle disease in poultry, classical swine fever in pigs and *Toxocara* in large ruminants. What seems to be lacking, without substantial public investment, are the incentives for private farmers to invest their time and resources in implementing effective technical options. The ACIAR projects understood this constraint and were underpinned by the development of scientific understanding of fasciolosis and better technologies. Accordingly, significant programs in Indonesia and the Philippines were developed to introduce new approaches to extension and technology adoption, based on modern theories and the practice of participatory extension.

The practical questions that stimulated these studies include the following.

- What are the existing recommendations for liver fluke control in Asia and to what extent have they been implemented and with what degree of success?
- Are there alternative approaches to traditional extension systems and do these have application to liver fluke control in Asia?
- Among the new approaches which have been shown to be useful and what are the reasons for any successes and failures?
- How can proven technologies be integrated with practical extension methods to develop a successful model for liver fluke control?

This chapter describes three studies, two in Indonesia and one in the Philippines, which have laid the groundwork for implementation.

Fasciolosis control strategies in draught cattle in West Java, Indonesia[1]

Introduction

The focus of this study was to develop effective extension of six liver fluke control options to farmer groups in the province of West Java, Indonesia. These options were thought to be complementary to existing farming practices in irrigated rice cropping systems, and are aimed at interrupting the parasite's life cycle and reducing infection levels of grazing animals and their grazing areas. In summary they are:

- Feed only the top two-thirds of freshly cut rice stalks to stock.
- Dry thoroughly in sunlight the lower third of rice stalks before feeding them to cattle.

1 Principal findings from a thesis submitted for the degree of Doctor of Philosophy at The University of Queensland by Wardis Girsang.

- Prevent animals grazing in rice fields adjacent to a village or cattle pen for a month after harvest.
- Mix cattle dung with avian dung, to sterilise *Fasciola* eggs, before using it to fertilise rice fields.
- Treat cattle with effective anthelmintic in July or August each year, about six weeks after harvesting the second season rice crop.
- Compost cattle dung at temperatures above 40°C before using it as a natural fertiliser on the rice field.

The six options were released as an extension package (Fasciola Control Strategy—FCS) by research scientists in early 1996. However, the extension did not produce sustained long-term results. Evidence of discontinued adoption of FCS came from field observations in 2001. A very low proportion of farmers knew about the strategies and, of these, relatively few still practised them. Consequently, key components of the FCS were being jeopardised by continuation of management practices, including location of cattle pens beside rice fields, and continuous grazing of rice fields after harvest, that would promote spread of the disease. The prevalence of fasciolosis was still high. This evidence from farmers, field observation and faecal tests in the laboratory, proved that most farmers either did not adopt, or had adopted but subsequently discontinued, FCS.

This study, therefore, aimed to address three research questions:

1. What were the main constraints limiting sustained adoption of the six fasciolosis control strategies?
2. How can these constraints be overcome to enable the FCS to be sustained?
3. Can an extension model be developed to facilitate adoption of technological innovation? How could such a model be implemented by research organisations?

Research approach

In contrast to the top–down, linear transfer of technology (TOT) approach, a systemic, participatory learning approach was proposed as an alternative way to overcome these constraints. A participatory action research (PAR) methodology was employed to address the constraints and nurture and promulgate innovation adoption. In early 2001 a semi-structured questionnaire was used with face-to-face, in-depth

Working with women, often in separate groups, is essential in capturing their ideas and gaining their cooperation for liver fluke control.

interviews for 24 households to identify constraints limiting sustained adoption (Girsang et al. 2001).

The main intrinsic constraints identified were lack of time, labour, cash, and group collective action. The main extrinsic constraint was lack of continuous facilitation, and the principal innovation constraint was the prohibitive cost of the technology. It was found that these constraints were interdependent and needed to be addressed simultaneously.

To overcome these constraints, facilitation through a continuous participatory learning process was used on the farms from early 2001 to August 2003 in three phases. In the preparation phase, workshops were conducted collaboratively by researchers, policymakers, extension agents and farmer group leaders at district, subdistrict and village level to enhance conceptual knowledge and skills about the FCS. Then, farmers were helped to identify their own needs, including the FCS technology options.

In the implementation phase, the participatory learning for the FCS was conducted through monthly discussion meetings in three farmer groups in three villages. The first farmer group was in Kadaleman village which had 35 farmers with 63 cattle. The second group was in Wanasari village which had 67 farmers with 114 cattle, while the third was in Jagamukti village with 30 farmers with 60 cattle. The main issue at the implementation stage was a gap between the knowledge of the resource-poor farmers and their access to material/financial resources needed to address the prohibitive cost of the FCS technology. Facilitation of a learning process is not a sufficient condition for sustaining the innovation adoption process. Provision of productive material and financial incentives was implemented to reduce the prohibitive costs of the technology for resource-poor farmers. Thus facilitation is critical to enable producers to overcome existing and emerging problems.

Farmers and facilitators then discussed the medium term outcomes of the participatory learning process. About 57 farmer households from Kadaleman, Wanasari and Jagamukti (with facilitation), and Cidahu village (without facilitation), were selected purposively then interviewed intensively. Field observation showed that innovation adoption tended to be more sustained in farmer groups where there was continuous facilitation. The medium-term outcomes of the FCS technologies have been identified in terms of human, social and financial capital improvement.

Human capital outcomes

Human capital was enhanced through participatory learning in terms of the number of farmers in the group who gained knowledge and continuously practised the FCS technology set. Although the number of farmers who gained knowledge about the cattle manure control strategy increased considerably, only a small proportion adopted the practice.

The proportion of farmers who knew about the compost strategy increased significantly from 25% in 2001 to 80% in 2003, and was reflected in an increase in adoption from 13% to 30% of farmers. The number of farmers who understood the benefits of the strategy of mixing cattle dung and chicken manure increased significantly from 17% in 2001 to 80% in 2003, and adoption increased from 17% in 2001 to around 40% in 2003.

The feeding/grazing and drug control strategies followed the same pattern, where the number of farmers with knowledge about the FCS increased considerably, but most of them did not adopt the FCS continuously. Furthermore, farmer groups that discontinued facilitation tended to reject the new technology. None of the farmers in Cidahu village practised drug control in 2003, probably because there had not been continuous facilitation. The number who practised drug control in Kadaleman village also decreased significantly, from 45% in 2001 to 10% in 2003, when the drug incentive (cost subsidy) was terminated.

In contrast, in the presence of continuous facilitation, the proportion of farmers practising drug control increased from 29% in 2001 to 65% in 2003 in Wanasari and Jagamukti villages. This implies that, as a complementary activity to facilitating participatory learning, provision of material and financial incentives was one way to encourage sustained adoption.

Social capital outcomes

Participatory learning processes facilitated social capital improvement. This was measured by five indicators:

- level of involvement in the farmer organisations
- group networks
- levels of trust and reciprocity
- group performance
- group collective action.

Farmers are involved in many social, economic and religious organisations but farmer group activities were high on the list for most farmers. The participatory learning process strengthened group relationships with external organisations (research and extension agents), and enhanced trust. Trust and networking are part of the social capital needed to sustain group collective action. The social capital index (SCI) was used to measure group collective action. Farmers in three of the villages scored high on some of the SCI indicators as a result of voluntary contributions of time and material resources by some members to group activities which helped to maintain trust and sustain collective action.

Farmers scored lower on SCI for indicators of collective farm management. Farmers preferred to work individually on their own small area of rice land. They considered that working collectively on a larger public area of rice land could lead to a conflict of interests among the members of the farmers' group. It is argued that the participatory learning process facilitates social capital, which is turn enhances collective action. In Kadaleman, Jagamukti and Wanasari villages, group leaders perceived that their group performance improved from a low to medium level. They observed that their groups were better able to prepare and implement plans for enhancing productivity or group capital formation for collective action.

Financial capital outcomes

Participatory learning processes enhanced financial capital improvement. Farmers perceived that their cattle were fatter if they followed the FCS recommendations. Participatory learning helped reduce the prevalence of fasciolosis from 74% in 2001 to 39% in 2002. The FCS contributed approximately 700,000 rupiah/animal pair/year to household income, similar to the A$63/animal/year (Chapter 2 this volume). Net income was found to have increased by 40% after adopting the FCS, but twice the labour and three times the input costs were needed. As they have low levels of resources, farmers tend to be risk averse, seek to avoid incurring extra costs, and therefore may be slow to adopt the FCS even when benefits are clearly evident. This is likely to be more pronounced when the expected benefit materialises in the future, whereas the prohibitive costs need to be paid now.

Participating methodology

Participatory action research (PAR) methodology was applied to address the limitations of the conventional transfer of technology approach. The role of researcher and extension agents (professionals) shifted from regulator to facilitator. PAR methodology enhanced farmer group participation to modify the FCS technology set, so that the innovation adoption process was more sustained and able to produce continuous improvement and innovation.

However, the participatory learning process and action facilitation needs to be combined with incentives of material and financial subsidies through a direct and transparent delivery system. Facilitating enhanced participation is a necessary but not a sufficient condition to sustain adoption. A continuous intervention of material and financial incentives is needed to help resource-poor farmers. In contrast to the conventional survey approach, the specific contribution of the PAR methodology is that the participatory learning process enabled producers to modify and recreate knowledge and technology according to local conditions; for example, farmers modified the cattle pen and chicken coop from single to multi function.

Implications for policy and practice

Farmers

The participatory learning approach is suitable for empowering farmer groups who have low formal education and lack resources to address existing and emerging problems, recreate knowledge, and modify and develop new practices. Farmer groups were enabled to identify their needs, to develop and implement plans, to observe and evaluate their actions and modify or develop new plans. The learning cycle of plan, act, observe and reflect is suitable for enhancing farmer knowledge and skill. The other implication was that participatory learning involves farmers and their household members and thereby promotes community or social learning. A participatory learning approach enhances the producer's productivity as a necessary condition to facilitate empowerment.

Agricultural extension managers

The role of extension agents needs to change from technology transfer to participative action learning. In the former, the role of the extension worker was to fulfil the external organisation needs, while the latter will focus on facilitating producers to address their own needs. Extension agents have an important role to link within and between farmer groups and to external organisations, so they need capability and competency as facilitators who promote change. This implies changing their role to address farmer needs as well as institutional or government programs.

ILRI/Steve Mann

Changing the way rice straw is harvested, by cutting higher than 20 cm off the ground, requires changes in the long-term practices of small farmers.

Policymakers

Intervention should be accompanied by a continuous, facilitated learning process. Policymakers and researchers need to learn to appreciate producer needs rather than focus solely on institutional needs, and also be able to acknowledge producer knowledge and experience before designing research, development and education programs. This will also enhance the efficiency and effectiveness of donor-aided programs by developing transparency and reducing transaction costs. The material/financial incentives need to be delivered transparently and directly to farmer groups. In the long term, participatory learning is expected to enhance the role of the farmer groups and reduce the role of the local government to control the local resources.

Extension strategies in Yogyakarta province, Indonesia[2]

This study describes the process undertaken to explore the application of participative extension methods to facilitate the adoption of a parasite control program in Yogyakarta special province, Java, Indonesia. Fasciolosis, caused by the parasitic liver fluke *Fasciola gigantica* causes significant production and economic loss in bovines in Indonesia and many other tropical rice farming areas. The research project grew out of the apparent failure of an earlier conventional 'transfer of technology' approach to establish, within indigenous farming practice, a suite of relatively simple, low-cost, control measures developed by research parasitologists within Balitvet (Research Institute of Veterinary Science, Indonesia) to complement existing practices.

The initial program of fasciolosis control strategies began in 1996 in Surade subdistrict, West Java, with

2 Principal findings from a thesis submitted for the degree of Doctor of Philosophy at The University of Queensland by Eny Martindah.

a conventional linear 'transfer of technology' (TOT) extension model, and was controlled by researchers. Subsequent investigations in the target area revealed that by 2000 the program had not produced any long-lasting outcomes and had effectively failed. Apparent reasons included a lack of active learning associated with a lack of a sense of program ownership by farmers and extension workers. Exploration of alternative, participatory approaches was therefore deemed justified.

The aim of this study was to revise the fasciolosis extension program used in Surade, for conduct in Yogyakarta special province, using an approach informed by PAR, to give farmers and other relevant stakeholders a greater sense of involvement, and addressed the following four research questions.

1. What were the constraints limiting adoption of a sustainable level of biological control of fasciolosis in intensive rice production areas in Indonesia?
2. Does a participatory approach that includes all stakeholders produce more sustained adoption and outcomes by effectively addressing the identified constraints?
3. What are the key elements and their interrelationships in a participatory extension process required to deliver sets of technological innovations?
4. What general principles emerge that will allow agricultural and/or extension research institutions in Indonesia to promote better implementation of technical research findings?

The first two questions are specific to the fasciolosis program, while the last two are aimed at using learning from it to identify principles of effective extension.

The overarching research philosophy is qualitative in that the objectives relate to gaining insights and understanding, rather than to proving or disproving particular hypotheses.

A three-phase study framework was implemented during the period January 2000 to November 2003. The phases were:

- planning and design activities
- initiating participatory research
- promoting the FCS more widely.

The study involved five farmer groups (180 farmers) in three districts within Yogyakarta special province, but, for various reasons, one group withdrew before completion. The study sites were selected by purposive sampling based on known fasciolosis exposure status. A total of 28 stakeholders from government institutions were directly involved, including eight extension agents. A participant observer made first-hand observation of activities and interactions, sometimes participated in the activities of the subjects under investigations, and acted as a facilitator during the learning process of the complex technology adoption. Throughout the study into the FCS implementation process, all relevant stakeholders were encouraged to plan, act, observe and then think critically before refining plans for the next set of activities.

Introductory participatory workshops that engaged players from policy, research, extension and farming domains, resulted in planning for a program to support the implementation of FCS.

In the second phase, the extent to which FCS flourished or otherwise in a situation was found to be associated with the emergence of locally adapted technologies combined with supportive technical services personnel. The implementation process of FCS has helped to answer the four research questions. FCS implementation depended on farmers' perceptions of the practices with their decisions on adoption being influenced by social, technical, environmental and economic factors. The constraints limiting adoption of a sustainable level of biological control of fasciolosis (addressing research question 1) include a lack of:

- problem awareness
- knowledge of innovation principles
- stakeholders' involvement in the program.

Also limiting adoption was the dynamic interplay occurring between participants.

To address research question 2 and 3, and deliver sets of technological innovations, these key elements and their interrelationship must be addressed in a participatory extension process that is includes and respects all stakeholders.

The evident impacts of the fasciolosis control program included social capital development through networking and knowledge building. Thus, relationships of trust as well as friendship between the livestock owners, researchers, and technical services personnel developed. Human capital formation, in the form of increases in skill and knowledge in all stakeholder groups, was fostered through the facilitation of an action learning process and the communication it encouraged. As an example, the farmers were able to describe the key concepts of the control program, and 14 months after the intervention ended were still actively implementing FCS with limited extension input. The implication is that farmers are more likely to adopt the recommended practices if their knowledge of fasciolosis and the long-term benefits from adopting control strategies for sustainable control, is enhanced in ways that involve their active involvement.

Activities in the fasciolosis control program confirmed that PAR encourages joint collaboration between individual stakeholders, as well as between organisations and institutions, in mutual action that represents a positive change from the conventional hierarchical approach (research question 4). An overall improvement in productive efficiency in the target villages is likely to occur, and the enhancements to human and social capital may act as a catalyst in the future for implementing other extension programs. Since the extension agents and veterinarians from this study are each individually responsible for numerous other groups, the overall experience is expected to be transferable and adaptable to a range of different subjects and situations. There is already evidence of this occurring. Furthermore, although the skills learnt by relevant stakeholders and extension agents were fostered within the specific FCS context, they form a set that is generally and widely applicable to PAR implementation in many situations.

Findings indicate the desirability of implementing the principles of PAR widely in extension strategies, but challenges have been identified that need to be addressed. The high initial financial and resource costs need to be seen to be outweighed by the potentially more effective transmission and learning and more sustained adoption of technical innovations. Ownership by the stakeholders needs to be supported on an ongoing basis with locally available resources. As an example, policymakers will need to be prepared to provide appropriate levels of financial, technical, and managerial resources in appropriate monetary and human resource terms, not only in the establishment phases. At the same time, research institutions may need to provide some limited follow-on assistance, such as intermittent technical support (including advisor visits) to enhance the prospect of sustainability and to consolidate achievements (Martindah et al. 2005).

PAR as a research approach

PAR is a form of action research in which practitioners act as both subjects and co-researchers (Argyris and Schön 1989); that is, it is a methodology rather than a series of techniques. Taking action is seen as central to the work (Marshall and Rosmann 1999). The aim of PAR is to foster a more collaborative research process to achieve goals in three areas of improvement: practice, the understanding of the practice by its practitioners, and the improvement of the situation in which the practice takes place (Carr and Kemmis 1986). The goal of involvement is no less important than improvement (Dickens and Watkins 1999), as involvement means collaboration within a mutually acceptable ethical framework governing the collection, use and release of data, and is considered vital to research. It is one critical aspect that distinguishes action research from other forms of social research (Peters and Robinson 1984).

Action research consists of cycles of planning, acting, observing, and reflecting or evaluating, and then taking further action. PAR incorporates these same steps, but is more emancipatory, in that all participants have a commitment to conduct research for themselves and reflect on its nature (McTaggart 1991).

PAR combines *research* to understand the problem situation with *action* to improve it (Cohen and Manion 1989). It is useful in poorly understood situations where a lot of variables are interacting and there are no clear answers. PAR means that all relevant stakeholders do what researchers usually do. It is a way that people can participate together to find a suitable solution to a common, complex problem, both for the advancement of science and for the improvement of human welfare (Whyte 1991).

Local people are empowered to become involved in the research process and to implement potential solutions or take action. The common important principles (Pretty 1994 p. 42) are as follows.

- **A defined methodology and systemic learning process**—the focus is on cumulative learning by all participants and, given the nature of these approaches as systems of inquiry, their use has to be participative.
- **Multiple perspectives**—a central objective is to seek diversity, rather than characterise complexity in terms of average values. The assumption is that different individuals and groups evaluate situations differently, which leads to different actions. All views of activity or purpose are heavy with interpretation, bias and prejudice, and this implies that there are multiple possible descriptions of any real-world activity.
- **Group inquiry processes**—all involve the recognition that complexity of the world will only be revealed through group inquiry. This implies three possible mixes of investigators, namely those from different disciplines, from different sectors, and from outsiders and insiders (local people).
- **Context specific**—the approaches are flexible enough to be adapted to suit each new set of conditions and actors, and so there are multiple variants.
- **Facilitating experts and stakeholders**—the methodology is concerned with the transformation of existing activities to try to bring about changes which people in the situation regard as improvements. The role of 'expert' is best thought of as helping people in their situation carry out their own study and so achieve something. These facilitating experts may be stakeholders themselves.
- **Leading to sustained action**—the inquiry process leads to debate about change, including confronting the constructions of other people, and this debate changes the perceptions of the actors and their readiness to contemplate action. This leads to more sophisticated and informed constructions of the world. The debate and/or analysis not only defines changes that would bring about improvement but also seeks to motivate people to take action to implement the defined changes. Action is agreed, so implementable changes will represent an accommodation between the different conflicting views. This action includes local institution building or strengthening, so increasing the capacity of people to initiate action on their own. Diagrammatic representation of the three phases of PAR employed in this study is shown in Figures 5.1–5.3.

In-field evaluation of PAR by stakeholders

A SWOT analysis was carried out to obtain an understanding of stakeholders' perspectives on the PAR approach. This was important as it served as a review or evaluation of the program and process, and was expected to identify the measures necessary to ensure success in employing PAR when developing extension strategies in conjunction with farmers and technical services. The results are presented in summary tabular form in Table 5.1. Each group of stakeholders (participants in the meeting: farmer representatives, technical services personnel and

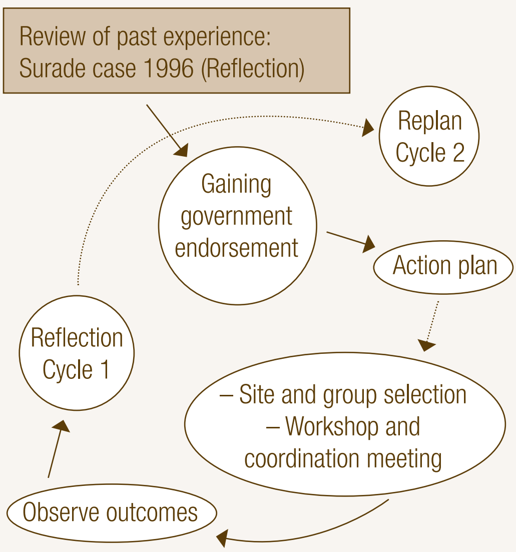


Figure 5.1 Phase 1: The cycle of action and reflection in the planning of the liver fluke extension program in Surade, Indonesia.

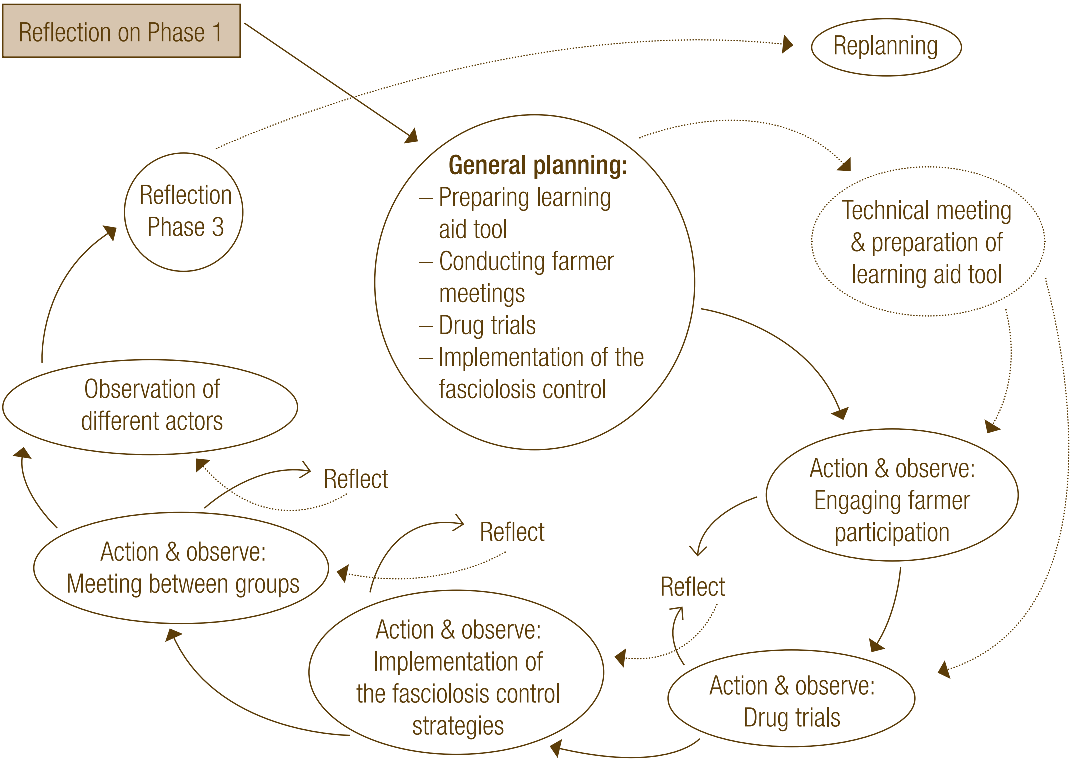


Figure 5.2 Phase 2: general planning of activities among district officials and village participants in the control program.

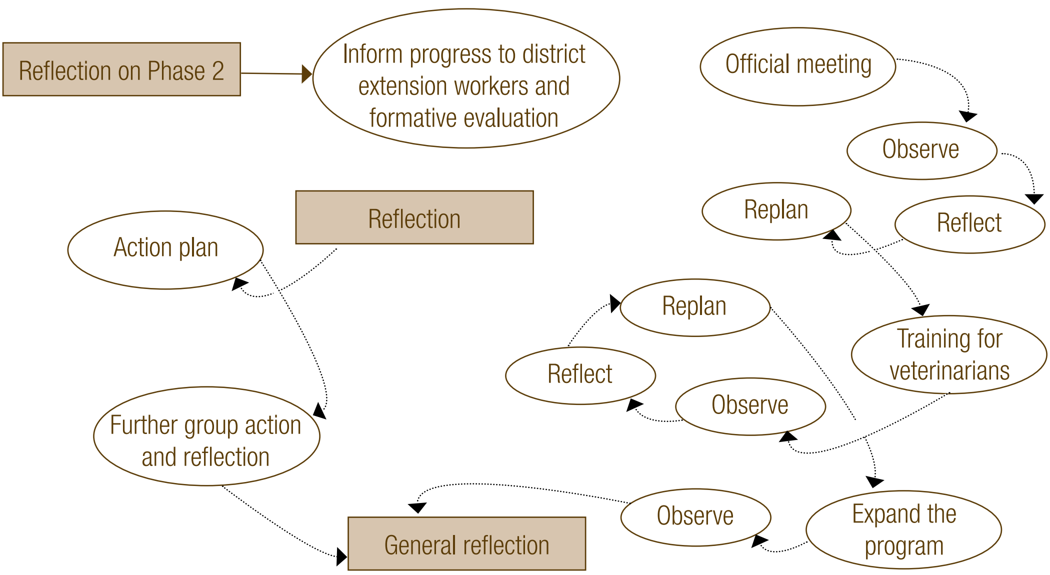


Figure 5.3 Phase 3: review of district and village activities, modification of activities, and planning for expansion to other districts.

Table 5.1 Stakeholders reflection of PAR approach to implement fasciolosis control strategies, based on a SWOT analysis.

Farmer representatives	Technical services personnel	Policymakers
Strengths: 1. Increasing the information flow from research to extension agents and farmers. 2. Building relationship between researchers, extension agents and farmers. 3. Breaking down culture silence of farmers. Increasing knowledge and learning process.	**Strengths:** 1. Accelerating the information flow from researchers to extension agents and farmers. 2. Building relationships between researchers, extension agents and farmers. 3. Increasing knowledge and skills of extension agents and farmers in conducting research processes. 4. Breaking down a culture of silence among farmers. 5. Enhancing the role of technical services personnel in the research process.	**Strengths:** 1. Continually enhancing the institutional learning. 2. Accelerating two-way communication between farmers, researchers and policymakers. 3. Breaking down communication barriers between stakeholders.
Weaknesses: 1. Requiring active participation of farmers. 2. More time consuming and costly. 3. Requiring common motivation.	**Weaknesses:** 1. Requiring a more specific skill and attitudes. 2. Requiring active participation of stakeholders and change in extension strategy. 3. More time consuming and operationally costly. 4. Requiring an understanding of the nature of innovation.	**Weaknesses:** 1. Increasing operational costs. 2. Requiring more specific skills and attitudes. 3. More complicated process and needs a change in extension strategy.
Opportunities: 1. Resulting in more appropriate technology (by refusing and adapting). 2. Developing a sense of ownership to the research results. 3. Facilitating the adoption of research results. 4. Facilitating learning process. 5. Encouraging solidarity amongst farmers.	**Opportunities:** 1. Encouraging technical services personnel to employ PAR approach. 2. Facilitating learning process. 3. Encouraging collaboration among the technical services personnel. 4. Developing a sense of ownership of the innovation.	**Opportunities:** 1. Developing a sense of ownership of the research results. 2. Encouraging the involvement of various stakeholders in the extension activity. 3. Encouraging collaboration of different institutions.
Threats: 1. Culture of silence among farmers. 2. Visit frequency of technical services personnel.	**Threats:** 1. Culture of silence among farmers. 2. Discontinuation of operational budget. 3. Dependency of farmers upon government assistance.	**Threats:** 1. Culture of silence among farmers. 2. Dependency of farmers upon government assistance. 3. Discontinuation of operational budget.

policymakers) had its own SWOT assessment of the PAR approach. The table captures their positive but realistic views of the strengths, weaknesses, opportunities and threats relating to PAR, and support its continued application in similar research–development–extension situations, as discussed briefly below.

Contribution of PAR to fasciolosis control

This study has made a substantial original contribution in the following areas.

- **Providing an innovative way of doing extension research**. This study gave the opportunity for researchers to develop their research capacity, particularly in the field of extension methodology and to gain complementary skills in developed extension strategies using the PAR approach.
- **Providing a validated basis for implementation of the PAR approach.** The process of participatory action research provided useful learning experiences for participants, and may be viewed as a way to orient people towards commitment to ongoing learning.
- **Produced practical outcomes for the benefit of controlling fasciolosis.** The group members proved to be highly cooperative in complying internally with good group consensus, and externally with good cooperation among the technical services personnel, when facilitating activities at the group level contributed to the success of the program.
- **Developed principles for general participatory approaches to extension**. The outcome from the study of developing extension strategies, in conjunction with technical services and farmers, to control fasciolosis in Yogyakarta special province, has had positive impacts at the district level as well as at the community level. Although the skills learnt by government (policymakers) and extension agents during this study were in a context specific to fasciolosis control, many skills are generic and could be applied to a wide range of rural extension situations. However, this outcome has been less pronounced at the provincial level. Since the introduction of the decentralisation scheme, the district level has autonomy to decide policy and develop the region, including the control of animal disease. The interest shown by all associated with the field work of this study, and its positive results, should mean that future agricultural research in Yogyakarta special province will be warmly received.

Suhardono

After cutting, rice straw can be left in the sun to reduce the infectivity of any attached metacercariae.

Implications for research

Due to the benefit of PAR methodology, it is recommended for use in other social research, particularly in poorly understood situations, as PAR is a powerful strategy which combines *research* to understand the problem situation with *action* to implement potential solutions. The close linkage between research and action can be viewed as a learning strategy for the advancement of science and for the enhancement of human welfare. The focus is on cumulative learning by all stakeholders involved. The process of participatory action provides useful learning experiences for participants to develop a commitment to ongoing learning.

Implications for policy and practice

The implementation of FCS with PAR suggests that research and extension agencies should consider embracing the PAR approach, at least in certain circumstances, so that actors are able to learn by direct experience in the research process. It could happen if a PAR specialist or champion with high commitment and motivation is appointed to district or province level to promote the PAR approach (policy implication); and train research, development and extension people through involvement in specific projects that employ the PAR approach, where these people would be expected to pass on their experience and skill to others (practice implication). Collaborative and effective communication between research, extension and policy levels would provide a great way for improving the identification of, research into, and extension of 'new innovative practice' about local or regional problem issues. These help develop trust and confidence among partners, and enable sharing of responsibilities and vision. More importantly, if farmers are actively involved in decision-making about innovation early in the process, when agendas for research or specific issues are being set, they are likely to remain much more committed to it than if they are treated only as recipients of developed research outcomes.

Fasciolosis control strategies in Bukidnon province, Philippines

Introduction

In the Philippines the National Fasciolosis Control Unit of the Bureau of Animal Industry generally uses chemicals to control fasciolosis infection in cattle and buffaloes by giving free drenching/treatment to selected regions in the country. In the southern Philippines, as in many parts of South-East Asia, the prevalence of fasciolosis is closely associated with irrigated rice production.

The model of Bennett and Rockwell (1995) was used in this evaluation to assess the overall effects of the extension program in terms of production levels and farmers' welfare. The evaluation would enable extension workers to learn more through systematic observation and analysis of this experience.

The seven sequential steps in evaluating effectiveness of extension programs include:

- inputs and resources
- activities
- people involvement
- reactions
- changes in knowledge, attitudes, skills and aspirations (KASA)
- changes in farm practice and end results.

Using these steps, an extension program could be assessed by its process (inputs and resources, activities, people involvement, reactions and KASA) and performance (changes in farm practice and end results). Similarly, the IMPROVE (instrument for measuring progress of value to everyone) model of Claridge and Frank (1998) used the same steps in evaluating progress of an agricultural program. Bennett (1976) argued that the higher you climb the hierarchy, the clearer you could see whether the program is successful or not.

This study sought to assess performance of the fasciolosis control extension program in Bukidnon province, southern Philippines. Specifically, this study aimed to:

1. determine the changes in farm practice of farmer participants in the fasciolosis control extension program in Bukidnon province, over three cropping seasons
2. determine consequences of adoption of the fasciolosis control strategies as perceived by the farmers
3. identify farmers' preferred sources of fasciolosis information
4. determine socioeconomic factors associated with perceived consequences of adoption of fasciolosis control strategies
5. determine the extent to which fasciolosis control strategies will be continued at village level.

Methodology

An extension program was conducted in Bukidnon province to introduce the technical options of the FCS to 135 livestock raisers in 7 low-lying villages across 3 municipalities/cities. These farmers were participants of the integrated pest management program on lowland rice of the Department of Agriculture, purposely chosen because of the close association between livestock and lowland rice production. Table 5.2 reflects the distribution of participants in the study.

Before the extension program began, faecal samples of carabaos and cattle were collected. When analysed they showed 65–100% prevalence of fasciolosis in the locale of the study. Copies of the results of the faecal analysis were distributed to the relevant technician and to the chair of the committee on agriculture in each barangay for monitoring and incorporation in their local agriculture programs. In addition, a survey on the extent of awareness of fasciolosis was conducted which showed only a 11–57% knowledge level about fasciolosis among the participants.

Table 5.2 Participants of the study.

Villages	Respondents of the evaluation survey
Valencia City	
Maapag	25
Batangan	11
Sinayawan	15
Malaybalay City	
Apo Macote	16
Managok	44
Maramag	
Kalagutay	17
San Miguel	7
TOTAL	**135**

The extension program was implemented in two separate phases, each lasting for three months from March to May in 2000 and 2001 in seven villages. Although using different methods of extension, both phases discussed the effects of fasciolosis on cattle and buffaloes and on lowland rice production, the life cycle of liver fluke and the biological/management and chemical control of fasciolosis. Both phases were conducted during summer as farmers have less farm activities due to irrigation cut-off. To measure the performance of the extension program, it was evaluated after the immediate cropping season in 2000 and then the next two cropping seasons.

The first phase of the program was implemented in the summer of 2000 and included a seminar–workshop, individual extension activities, preparation of a brochure and poster, and provision of a feed supplement to livestock owners. The village hall was used as the venue for brochure and poster discussions for displaying feed supplements, specimens of snails and fluke, and storage of office supplies. The seminar–workshop determined which liver fluke control measures were preferred by farmers.

The second phase of the program was implemented in the summer of 2001 and included presentation of an eight-minute video about fasciolosis during farmers' meetings in each village. Copies of the video were given to the local extension workers. Free treatment of liver fluke-infected animals was provided by the Department of Agriculture due to the high prevalence of the disease in the seven villages.

The 135 farmers and other participants were surveyed to evaluate performance of the fasciolosis control extension program. Performance of the fasciolosis extension program was measured according to four indicators:

- changes in farm practice
- perceived consequences of adoption
- preferred extension media as sources of information
- extent to which fasciolosis control strategies will be continued.

Two semi-structured questionnaires were developed. The first included changes in farm practice during the first cropping season of year 2000 and was administered in August (after the first phase). The second questionnaire covered the second crop of 2000 and first crop of 2001 and was administered in August 2001 (after the second phase). It consisted of statements on practice changes, perceived consequences of adoption, preferred sources of information and the extent to which control strategies are to be continued at village level. Both sets of questionnaires were personally administered to farmers either at their homes or in the farm by a hired enumerator. Data on the socioeconomic profile of farmers were taken from another ACIAR study (value orientation) on the same respondents (Intong et al., unpublished). These data were used to determine factors associated with perceived consequences of adoption.

Data were analysed descriptively using rank scores and percentages. Correlation analysis was used to determine association between dependent and independent variables. The general linear model was used to test significant differences in changes in farm practice among farmers in seven villages during the three cropping seasons. Analysis of variance (ANOVA) was used to test for significant differences among farmers in seven villages, in terms of their perceived consequences of adoption and extent of continued use of fasciolosis control strategies.

The overall aim of the study was to evaluate an extension program that promotes a technology to help livestock raisers control fasciolosis in village cattle and buffaloes. In turn, performance of the program may influence its methods as well as the technology (Figure 5.4).

Results

Changes in farm practices: adoption behaviour of farmers towards fasciolosis control strategies

Data in Table 5.3 indicate that farmers have adopted two practices to control fasciolosis in their village on a trial basis; that is, preventing animals grazing and drinking in rice fields, irrigation canals and shallow creeks, and using drugs to deworm infected animals.

Results from the general linear model show that farmers in the seven villages vary significantly in their adoption behaviour towards the different fasciolosis control measures over the three cropping seasons. The data show a pattern between the three dates for several variables. It was observed that the adoption level during the first 2000 crop was followed by a lower adoption level for the second crop, then a higher figure for the first 2001

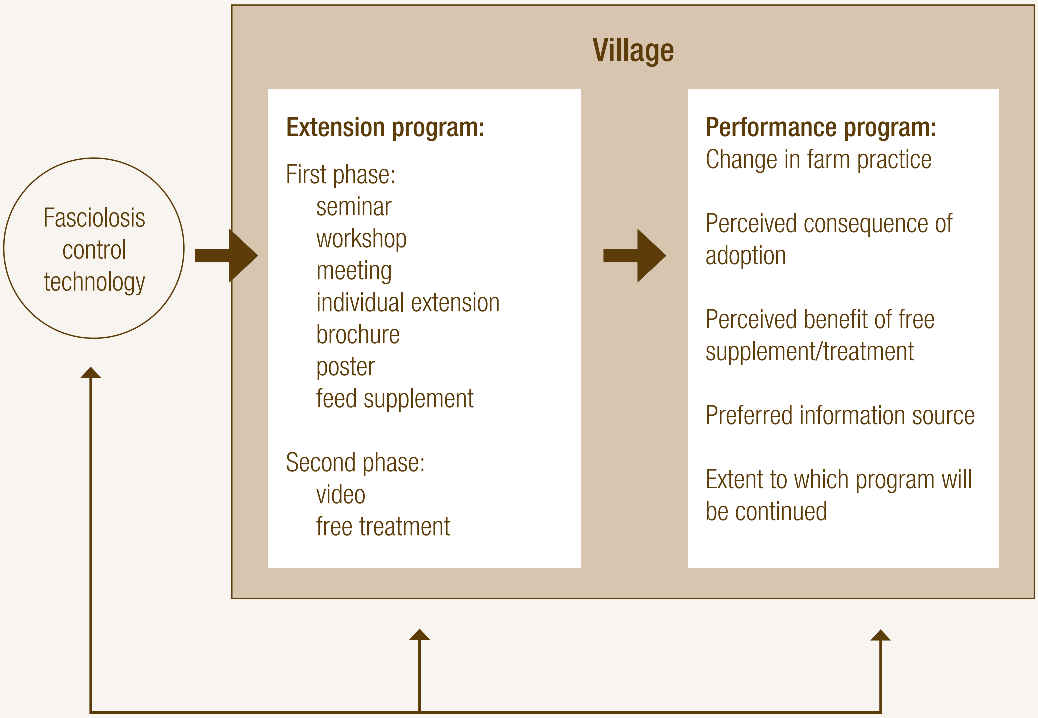


Figure 5.4 Schematic model used in the study.

Table 5.3 Extent of adoption of fasciolosis control measures among farmers in Bukidnon province over three cropping seasons.

Practices/subpractices	First crop 2000		Second crop 2000		First crop 2001		MEAN SCORE *N* = 135
	A	*(B–A)*	B	*(C–B)*	C	*(C–A)*	
Animals are prevented from grazing on newly harvested fields.	4.10	*(0.19)*	3.91	*0.33*	4.24	*0.14*	3.91
Animals are prevented from grazing along irrigation canals and river banks.	3.91	*(0.06)*	3.85	*0.44*	4.29	*0.38*	3.85
Animals are prevented from drinking water in irrigation canals, shallow creeks or rice fields.	3.87	*(0.16)*	3.71	*0.58*	4.29	*0.42*	3.71
							3.82[a]
Infected animals are dewormed using drugs.	3.66	*(0.37)*	3.29	0.93	4.22	*0.56*	**3.72[a]**
Ducks/chicken are raised to provide additional income for the family.	3.52	*0.47*	3.99	*(.12)*	3.58	*(0.08)*	3.48
Ducks/chickens are raised to eat snails in the rice fields.	3.92	*0.07*	3.52	*(.41)*	3.17	***(0.34)***	3.83
Duck/chicken manure is mixed with cattle/carabao faeces before applying to field as fertiliser.	3.04	*(0.12)*	2.92	*0.25*	3.40	*0.13*	3.04
							3.45[a]
Cut rice straw is dried and used as animal feed.	3.26	*(0.24)*	3.02	*0.31*	3.33	*0.07*	**3.20[a]**
Forage grasses are planted for animal feed.	3.11	*(0.03)*	3.07	*0.26*	3.33	*0.22*	
							3.17[a]
Animal faeces are collected and used for compost.	2.96	*(0.10)*	2.86	*0.55*	3.39	***0.45***	3.07
Compost and grasses/straws/animal wastes are used as fertiliser.	3.02	*(0.11)*	2.91	*0.48*	3.41	*0.37*	3.10
A shed/house is constructed to keep animals.	2.61	*0.03)*	2.59	*0.64*	3.23	*0.62*	3.23
							3.13[a]

[a] overall mean per practice

(GLM) F-value = 14.55*** $P < 0.0001$

Legend:	4.51–5.0	Fully adopted	A	–	First crop 2000
	3.51–4.50	Adopted on trial basis	B	–	Second crop 2000
	2.51–3.50	Thinking to adopt	C	–	First crop 2001
	1.51–2.50	Still evaluating/deciding			
	1.0–1.50	Decided not to adopt			

crop. In particular, there were more farmers who use drugs to control fasciolosis (+0.93) on a trial basis during the first crop of 2001 than during the second crop of 2000. On the other hand, there were more farmers who thought of constructing a shed for their animals (+0.64) during the first crop of 2001 compared with 2000. Similar trends were observed in these two practices during the first crop of 2001 compared with the first crop of 2000 (+0.56; +0.62).

Compared with the results of the seminar–workshop, farmers tended to revise their plan from one of drying cut stalks as animal feed to that of preventing animals from grazing and drinking on rice fields, canals and shallow creeks. On the other hand, farmers previously planned to use drugs as the last recourse in controlling fasciolosis. However, results of the evaluation show that farmers dewormed their animals on a trial basis.

Perceived consequences of adoption of fasciolosis control strategies

Data in Table 5.4 describe the consequences of adoption of the fasciolosis control strategies and the extent to which farmers perceived these effects in the villages. Findings show that farmers in the seven villages vary significantly ($P < 0.05$) in their perceptions of the consequences of adoption of the fasciolosis control program. In particular, farmers totally agreed that liver fluke-free animals worked faster, commanded a higher price and provided more income to the family than infected ones. However, they were undecided whether composting rice straw with animal manure helped them spend less on fertiliser; drying stalks provided feed for the animals and using rice straw as animal feed enabled farmers to allocate time for other livelihood activities.

Table 5.4 Perceived consequences of adoption of the fasciolosis control strategies (ANOVA).

Perceived consequences	Rating score $N = 135$
Liver fluke-free animals work faster in the farm than sick ones.	4.65±0.49
Liver fluke-free animals command a higher price when sold.	4.62±0.51
Liver fluke-free animals provide more income to the family than sick animals do.	4.60±0.53
Composting rice straw/grasses with animal faeces helps in making the environment clean.	4.32±0.60
With ducks, population of snails in the rice field has decreased.	4.32±0.65
Keeping the animals in pens/shed contributes to a clean and healthy environment.	4.20±0.67
Animals are becoming healthier.	4.12±0.57
Cutting rice stalks facilitates land preparation for the next cropping season.	4.07±0.73
Farmers are now becoming more conscious of their animals' health.	3.97±0.60
Ducks provided additional income for the farm family.	3.78±0.58
Farmers are working together to solve animal health problems.	3.61±0.61
Farmers are spending less on fertiliser as a result of composting.	3.47±0.59
Drying rice stalks provides animals with available feed.	3.35±0.55
Using rice straw to feed animals provides farmers with time for other livelihood activities.	3.31±0.56

F-value = 2.77 $*P < 0.05$
Probability: 0.01321
Totally agree; 3.51–4.50, Agree; 2.51–3.50, Undecided; 1.51–2.50, Disagree; 1.00–1.50, Totally disagree

Table 5.5 Perceived benefit from the free treatment supplied by the fasciolosis extension program.

Activity/condition change	Rating score *N* =1 35	*F*-value (GLM)
Deworming:		
Increased size of animals	4.23±0.68	3.89**
Increased weight of animals	4.24±0.68	
Improved animal strength for farm work	4.17±0.72	
Increased meat/carcass when slaughtered	3.95±0.76	
Commanded high price when sold	3.98±0.75	
Gave pride to animal owner	3.91±0.75	
Feed supplement (vitamins and minerals):		
Improved health of animals	3.32±1.14	3.60**
Increased size of animals	3.34±1.14	
Increased weight of animals	3.32±1.14	
Improved strength of animals for farm work	3.32±1.14	
Commanded high price when sold	3.33±1.15	

** $P < 0.01$

Great extent 3.51–4.50; Some extent 2.51–3.50; Little extent 1.51–2.50;
Very little extent 1.00–1.50; No extent <1.00.

Perceived benefit from free treatment provided by the fasciolosis extension program

In Table 5.5, data show the extent to which the respondents and their animals had been helped by the free treatment given through the fasciolosis extension program. Findings reveal that farmers in the seven villages vary significantly ($P < 0.01$) in their perceived benefit from the free treatment (generic name: Rafoxanide) and free feed supplement[3] provided by the program in relation to their experience before the treatment. Specifically, the farmers perceived that the free-deworming activity of the program has to some extent increased size, weight and draught power of the animal.

Results of the correlation analysis (Table 5.6) show that both farmers' source of information and religious affiliation were significantly associated ($P < 0.05$) with perceived benefits derived from free treatment of their cattle and buffaloes through the fasciolosis extension program.

Dick Copland

Fasciola eggs can survive in dung pats for several months.

3 Commercial name is Cecical, generically a vitamin–mineral combination powder containing dicalcium phosphate (97%), vitamin A (150,000 units), vitamin D3 (30,000 units), vitamin E (500 i.u.), potassium iodide (100 mg), manganese sulfate (3,500 mg), ferrous sulfate (1,500 mg), copper sulfate (1,500 mg), cobalt sulfate (30 mg) and zinc sulfate (200 mg) per 500 kg.

Table 5.6 Socioeconomic characteristics associated with perceived benefits derived from free treatment supplied by the fasciolosis control program.

Socioeconomic characteristics	Correlation coefficient	Probability
Age	–0.079	0.346
Household size	–0.062	0.468
Education	0.113	0.182
Ethnic origin	–0.023	0.786
Religion	0.169*	0.044
Other sources of income	–0.151	0.073
Length of livestock experience	0.012	0.879
Sources of information	0.184*	0.029
Membership in organisation	0.032	0.704

*$P < 0.05$

Preferred source of information on fasciolosis

Farmers were asked to rate their preferred source of information based on the extension methods used in the fasciolosis program. Data in Table 5.7 reveal that farmers considered the seminar–workshop followed by farmers' meetings and handouts as the best ways to get fasciolosis information. The least preferred fasciolosis information medium is the wall poster possibly because copies were posted in strategic places of the villages but were not given individually to farmers. Farmers preferred the CMU–ACIAR and the Department of Agriculture/LGU as agencies from which to obtain fasciolosis information.

Extent to which fasciolosis extension program may be continued at farm level

Data in Table 5.8 show that farmers would like to continue two fasciolosis control strategies: deworming, and preventing their animals from grazing on newly harvested fields. This finding is consistent with the adoption behaviour of farmers shown in Table 5.2.

Discussion

The extension program included a combination of individual, group and mass media implemented over two years to 135 Bukidnon farmers in seven villages.

Consequently, Bukidnon farmers have adopted on a trial basis two fasciolosis control strategies: preventing animals grazing in rice fields; and using drugs. They were likely to continue these practices 'to some extent'. The higher adoption value during the first crop of 2001 could be attributed to the implementation of the second phase of the extension program before this cropping season. This could also indicate a stabilising of decision as the succeeding extension program is reinforcing farmers' actions. On the other hand, the results indicate that farmers reconsider their previously preferred fasciolosis control strategies in favour of strategies that require less labour, money and time and are easy to apply. This is manifested in farmers' trial adoption of not grazing animals on newly harvested fields. Although previously the least preferred strategy because of cost, the latest survey shows that farmers adopted chemical control of liver fluke on a trial basis. However, they tend to depend on free drugs given by the government and NGOs. This difference in farmers' plan and action indicates that adoption of an innovation entails a continuous decision-making process and constant evaluation of resources (Frank 1988; Intong 1996).

Farmers see benefits in treating *Fasciola*-infected animals; but they were not sure whether compost reduced the need for fertiliser, or that dried straw provided feed or saved time. Economically, farmers considered the improved health condition of their animals as attributable to the program (Table 5.5). Although, implementation of the program was rather

Table 5.7 Respondents' preferred source of information on the control of fasciolosis in the village.

Specifics	Ranking score N = 135	Rank
Preferred information media used in fasciolosis extension program		
Seminar–workshop	1.79	1
Farmers' meeting	2.52	2
Hand-outs	3.50	3
Fasciolosis control video	3.87	4
Individual consultation	4.15	5
Poster	4.48	6
Agency that assisted most in the control of fasciolosis at the villages		
CMU–ACIAR	1.26	1
Department of Agriculture/LGU	1.78	2
Agribusiness companies	3.46	3
Village council organisation	3.49	4

Table 5.8 Likelihood of continued adoption of fasciolosis control strategies.

Fasciolosis control measures	Rating score N = 135	F-value
Deworming through drugs	4.28	7.65***
Preventing animals from grazing on newly harvested fields	3.84	
Mixing duck/chicken manure with cow/buffalo dung as fertiliser	3.35	
Mixing cow/buffalo dung with grasses/rice stalks as compost fertiliser	3.31	
Drying cut rice stalks as feed for animals	3.26	
TOTAL	3.61	

***$P < 0.0001$
Great extent 3.51–4.50; Some extent 2.51–3.50; Little extent 1.51–2.50; Very little extent 1.00–1.50; No extent <1.00.

short, there is, however, a great possibility that the positive response of farmers is the result of the recent intervention and the intermittent or irregular deworming/supplementation over a longer period. It is observed that farmers do not practise regular deworming and they also rarely give commercial feed supplements to their animals. Thus, results imply that the fasciolosis extension program has effected expected outcomes measurable by the farmers. The findings also implied that farmers had seen the fasciolosis control program as serving their self and the community's interest (Misra 1990), which may help in achieving sustainability of the program.

Fluke treatment benefited 'to some extent'; but feed supplement benefited their animals 'to little extent'. Information source and religious affiliation were associated directly with perceived benefits. As farmers nominated six sources of fasciolosis information, access to information sources may have helped them recognise the value of an extension activity. In addition, farmers' religious affiliation is supportive of livestock production.

Farmers preferred interactive activities in fasciolosis control with CMU and the Department of Agriculture as information sources. This implies that farmers want to be involved even in technology dissemination. As stakeholders, farmers need to be considered as participants of any extension program, as in the control of fasciolosis. Their contribution during the extension program could help attune the technology to the local farming system by bridging the gap between laboratory experiments and farmers' fields to achieve ecological suitability of the technology (Chamala and Mortiss 1990; Roling 1997). Consequently, this could lead to sustainability of the program in the villages.

Conclusions

The salient findings of the study are that farmers:

- have adopted control strategies that require fewer resources
- see benefits in adopting these strategies
- would like to continue adopting
- preferred interactive extension activities.

Overall, performance of any extension program depends to a great extent on how expected outcomes are effected and whether intended beneficiaries are able to feel benefited by the program. Although it is too early to call, findings of this study show some promising evaluation results. As farmers tend to reconsider their resources when deciding about an innovation, there seems to be a need to constantly monitor the process as well as the impact of any agricultural program. On the other hand, farmers tend to favour extension in which they participate. That is, in developing and evaluating programs there is a need to involve the farmers in the whole process. This not only achieves farmers' ownership of an idea, but also facilitates its institutionalisation in the farmers' local environment, which would eventually help sustain the program.

General discussion of extension approaches

These three studies have attempted to provide new insights into the design of an extension program for liver fluke control and, together, they do point very clearly to the need for a deeper understanding of the needs of the end user, and a more interactive approach to the design and implementation of extension programs. All three studies focused on *Fasciola* control but in the real world there would be other diseases of similar or even greater importance of concern to each smallholder. While the primary challenge is to design a process that leads to effective extension for liver fluke, the problem that follows is how to integrate liver fluke control with other disease control programs. The major conclusions that can be drawn from this chapter are that:

- farmers and extension workers are more likely to show sustained commitment to disease control or other communal problems if they are engaged early in the process, even in the problem-identification stage
- resource-poor farmers are likely to need financial or resource assistance or incentives, especially for addressing 'common good' issues such as fasciolosis, but this needs to be provided in ways that neither create dependence nor detrimentally affect other activities
- participatory extension processes are more resource intensive than traditional linear methods, but this is well justified if programs produce sustained positive benefits, as is evident in these three projects
- benefits are multiplied when the participatory extension process contributes to development of human and social capital in ways that facilitate subsequent community action in addressing other common problems
- extension staff may need skills development to enhance their capacity for participatory methods
- farmers learn best and most readily from other farmers.

References

Argyris C. and Schön D.A. 1989. Participatory action research and action science compared. American Behavioral Scientist 32, 612–23.

Bennett C. 1976. Analyzing impacts of extension programs. U.S. Department of Agriculture Extension Service, No. ESC 575: Washington D.C.

Bennett C. and Rockwell K.1995. Targeting outcomes of programs (TOP): an integrated approach to planning and evaluation. Unpublished manuscript.: University of Nebraska: Lincoln, NE, USA.

Carr W. and Kemmis S. 1986. Becoming critical: education, knowledge and action research. Falmer Press: London.

Chamala S. and Mortiss P. 1990. Working together for Land Care: group management skills and strategies. Australian Academic Press: Brisbane.

Claridge C.L. and Frank B.1998. IMPROVE: Instrument for measuring progress of value to everyone. National Heritage Trust, Australia.

Cohen L. and Manion L. 1989. Research methods in education, 3rd edn, Routledge: New York.

Dalal-Clayton B., Dent D. and Dubois O. 2000. Rural planning in the developing world with a special focus on natural resources: Lessons learned and potential contributions to sustainable livelihoods, an overview. Environmental Planning Issues, No. 20, International Institute for Environment and Development, and Department for International Development: London.

Dickens L. and Watkins K. 1999. Action research: rethinking Lewin, Management Learning 30, 127–40.

Frank B. 1988. Factors influencing the adoption of selected innovations in the extensive beef cattle industry of the North Queensland dry tropics. Ph.D. Thesis. Department of Geography, James Cook University of North Queensland: Townsville Australia.

Girsang W., Cameron D.C. and Frank B. 2001. Evaluation of extension fasciolosis control in rice-livestock farming systems, Surade, West Java. Proceedings, ACIAR AS1/96/160 reporting and planning meeting on control of fasciolosis in cattle and buffaloes: Sydney.

Intong J.D. 1996. The influences of farming environment on adoption behaviour: a study of maize farmers in southern Philippines. Ph.D. Thesis, Department of Agriculture. The University of Queensland: Brisbane, Australia.

Marshall C. and Rossman G.B. 1999. Design of qualitative research, 3rd edn. Sage: Thousand Oaks, California.

Martindah E., Cameron D.C. and Frank B. 2005. Participative approaches to enhance adoption of fasciolosis control strategies in cattle, in Yogyakarta province, Indonesia'. Pp. 164–173 in 'Developing entrepreneurship abilities to feed the world in a sustainable way. Proceedings International Farm Management Congress', Campinas, Brazil.

McTaggart R. 1991. Principles for participatory action research. Adult Education Quarterly 41, 168–87.

Misra D.C. 1990. Defining agricultural extension for 1990. Agricultural Situation in India 14, 5.

Mzembe S.A.T. and Chaudry M.A. 1979. The epidemiology of fascioliasis in Malawi : 1. The epidemiology in the intermediate host. Tropical Animal Health and Production 11, 246–250.

Peters M. and Robinson V. 1984. The origin and status of action research. Journal of Applied Behavioral Science 20, 113–24.

Pretty J.N. 1994. Alternative systems of inquiry for sustainable agriculture, IDS Bulletin 25, 37–48.

Pretty J.N. 1995. Regenerating agriculture: policies and practice for sustainability and self-reliance. Earthscan Publications: London.

Rogers E. (with Shoemaker F.) 1971. Communication of innovations: a cross-cultural approach. The Free Press: New York.

Roling N. 1988. Extension science: Information systems in agricultural development. Cambridge University Press: Cambridge, UK.

Uphoff N.1999. Understanding social capital: learning from the analysis and experience of participation'. Pp. 215–253 in 'Social capital: a multifaceted perspective', ed. by P. Dasgupta and I. Serageldin. World Bank: Washington DC.

Whyte W.F. (ed.) 1991. Participatory action research, Sage: Newbury Park, California.

6

Pathology of fasciolosis in large ruminants

E.C. Molina, L.F. Skerratt and R. Campbell

Pathology of fasciolosis in large ruminants

E.C. Molina, L.F. Skerratt and R. Campbell

6

Introduction

Considerable progress is being made in developing options for controlling fasciolosis based on existing knowledge of its transmission and epidemiology. In parallel with this approach, other research is being directed at finding new ways to diagnose infection (for clinical and survey purposes) and to increase host resistance through vaccination or other changes to the immune system. Confounding these studies is the realisation that cattle and buffalo respond differently to infection and that there may be significant differences between cattle breeds. To support these research initiatives the ACIAR projects have included important fundamental research on the pathology of infection in cattle and buffalo, and compared the susceptibility to infection of different animals. In this chapter a series of studies is reported on the pathology of fasciolosis in cattle and buffalo, and on the relationship between host and parasite.

The practical questions that these studies are looking to answer include the following.

- Are the pathological changes in large ruminants infected with *Fasciola gigantica* comparable to those during *F. hepatica* infections? If similar, then the likelihood of the extensive body of work on *F. hepatica* being relevant to *F. gigantica* in the tropics is increased.
- Are there differences in the infectivity and pathology of *F. gigantica* between cattle and buffalo and do these differences influence the effects of *F. gigantica* on the production traits of growth, reproductive performance, milk yield and draught power?
- Are there better ways to diagnose and monitor *F. gigantica* infections in cattle and buffalo?
- What are the implications of differences between *F. gigantica* and *F. hepatica*, and between cattle and buffalo, for the development and implementation of control programs?

Pathology in cattle and buffalo

The acquisition of *F. gigantica* infection is a continual process during the season of parasitic release from snails and the survival of metacercariae on plants, usually some months after rain or flooding. Transmission may occur over only a few weeks or months each year and severity of infection depends on environmental and management conditions. These may vary considerably between pastures adjacent to lakes or streams, irrigated rice fields or river systems, and between high and low rainfall areas. The result may also be modulated by the breed or species of the host and the infectious dose is a critical factor.

The disease process comprises three phases which in time may occur simultaneously, depending on the duration of infection:

- pre-hepatic migration, including intestinal penetration
- hepatic migration and tissue destruction
- bile duct localisation and egg production.

During the evolution of the disease, which is usually chronic in large ruminants, the host is affected in a variety of ways that are expressed in poorly defined clinical signs. Even farmers may have to be convinced that their animals are affected by showing them specimens, slide shows or the improvements in body weight, draught performance or fertility that occur after treatment. The immediate effect on an animal is tissue damage, with resultant haematological and biochemical changes and an immunological response. Weight gain, fertility and draught power are subsequently impaired, most likely as a consequence of these pathological changes.

Liver pathology

There has been little systematic research on the pathology of *F. gigantica* infection in large ruminants. Prevalence of lesions increases with age and is highest in cattle and buffaloes (carabaos) over six years of age in the Philippines. The prevalence was higher in male cattle but there was no significant difference between male and female buffaloes (Molina, unpublished). Infected livers are bigger and heavier than normal ones. In the earlier stages, pale or haemorrhagic migratory tracts are present but, as the disease advances, the liver becomes generally paler and firmer and the bile ducts more prominent from dilated and thickened walls (Figure 6.1). There may be fibrinous tags on the capsule or fibrous adhesions between organs. Hepatic and mesenteric lymph nodes are reactive and enlarged.

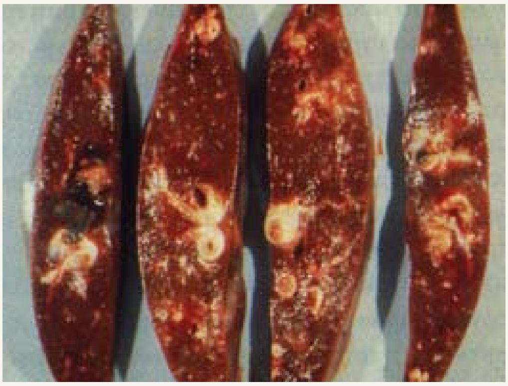


Figure 6.1 Affected livers showing bile ducts more prominent from dilated and thickened walls.

After penetration of the liver capsule, young flukes wander in the parenchyma for several weeks when their total effect is dose dependent. Histological changes occur in the following three phases which may coexist in fully developed cases.

- Necrotic and haemorrhagic tracts appear in sub-capsular and parenchymal areas.
- There are cellular reactions with infiltration by eosinophils and later macrophages and lymphocytes. Vascular damage may lead to infarction. As the developing flukes wander for several weeks in the liver parenchyma, tracts become larger.
- In the later stages, the immunological reaction of macrophage and lymphocyte infiltration merges with fibrotic healing of the necrotic areas. The portal areas also become fibrotic. As the flukes enter the bile ducts from about eight weeks after infection (Figure 6.2) and begin egg production about 12 weeks after infection, there is a catarrhal reaction. The epithelium becomes hyperplastic, the walls are infiltrated by eosinophils and mononuclear cells and the bile darkens with blood. The duct walls become fibrotic and sometimes calcified.

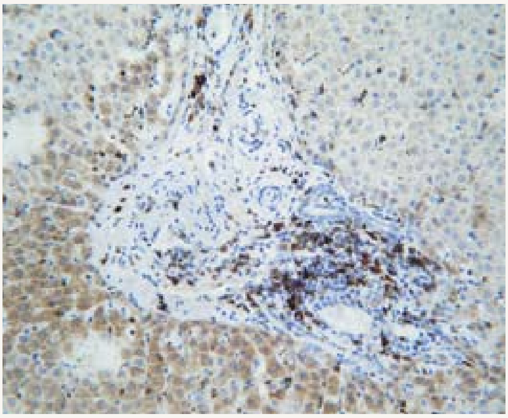


Figure 6.2 As infection progresses, the walls of the bile duct are infiltrated by eosinophils and mononuclear cells and the bile darkens with blood. The duct walls become fibrotic and sometimes calcified.

Pathophysiology

Disruption of liver structure and haemorrhaging cause significant biochemical changes although these are not as severe as in sheep in which the ratio of the mass of parasite to liver is much smaller than in cattle. Anaemia due to haemorrhage may develop when adults become established in the bile ducts. Its severity is directly proportional to the adult parasitic load in cattle though not in buffaloes, indicating that cattle are less resilient to infection (Wiedosari et al. 2006). Buffaloes maintained a PCV of 34% compared with about 30% in Bali (*Bos sondaicus*) and Ongole (*Bos indicus*) cattle after weekly infection with 15 metacercariae over 32 weeks. During the same period, buffaloes developed an eosinophilia of 24% compared with 21% in Ongoles and 12% in Bali cattle. Breed and species therefore play a significant role in the response to infection.

In the same experiment, serum hepatic enzymes were significantly increased in all infected groups, with buffaloes showing the least effect. Glutamate dehydrogenase (GLDH) ranged from 80 IU/L in Bali cattle and buffaloes to 120 IU/L in Ongoles though Bali cattle showed wider fluctuations during the experimental period. Gamma-glutamyl transpeptidase (γ-GTP) ranged from 15 IU/L in buffaloes to 30 IU/L in the cattle. Buffaloes therefore seem to suffer less dysfunction. Total serum protein and bilirubin were elevated after damage to the liver parenchyma and bile conducting system, while urinary bilirubin was similarly increased in proportion to the number of *F. gigantica* in buffaloes. In studies on sheep in Kenya, serum albumin levels declined during the migratory and bile duct phases while serum bilirubin and glutamyl transferase levels increased, more significantly in Dorper than in the Red Masai breed (Waweru and Kanyari 1999).

Immune response

At autopsy the immune response can be seen in the enlarged hepatic lymph nodes and, histologically, in the macrophage–lymphocyte reaction in the intestine, lymph nodes and liver. Knowledge of the immunity to fasciolosis is mainly drawn from studies with *F. hepatica* in sheep but, as there appear to be differences from *F gigantica,* it seems unwise to draw exact analogies at present (Spithill et al. 1999).

Functional pathology

In addition to the hepatic changes, other organs may be affected in ways that are not fully understood. One study has indicated that *F. gigantica* can reduce weight gain in the growing yearling zebu (Sewell 1966). However, variables such as level of infection, breed, species, age, sex and, in particular, level of nutrition, may influence the effect. There is evidence that the level and type of nutrition, especially protein, may give some protection against this effect (Graber 1971). Several studies have shown that adult animals are less susceptible to weight change. There is evidence that, in anaemic animals especially, draught performance is reduced. The adverse effect of anaemia on fertility reported from India (Kuma and Sharma 1991) and Indonesia (Spithill et al. 1999) is expressed in longer intercalving intervals. It is known that retarded weight gain may lead to a delay in maturity and fertility (Entwistle 1978).

Attempts have been made to determine the effect of fasciolosis on milk yield but these have produced contradictory data due probably to the complex of variables listed above which interplay in animals. The total effect of fasciolosis therefore depends on environmental, genetic and pathological factors that may vary considerably. Infectious dose is one of the most influential.

Host–parasite relationships

The extent to which infection adversely affects infected animals depends in part on their susceptibility to infection. Reports about differences in susceptibility to infection with *F. gigantica* between cattle and swamp buffaloes are limited. It is known that there are genetically determined differences by which animals express protective immune responses to parasite infections. So there may be differences in host–parasite relationships in *F. gigantica* infection between cattle and swamp buffaloes that may influence the varying levels of resilience and/or resistance between these species to the parasite. Breed differences in susceptibility to *F. gigantica* have been demonstrated between Friesian and Boran cattle (Wamae et al. 1998), between Indonesian thin tail sheep, merinos and St Croix sheep (Wiedosari and Copeman 1990), and between Red Masai and Dorper sheep (Waweru and Kanyari 1999).

E. Wiedosari et al. (pers. comm.) demonstrated that buffaloes were affected less by *F. gigantica* than were Bali and Ongole calves. In response to a trickle infection of 15 *F. gigantica* metacercariae for 32 weeks, weight gains of buffalo calves were not significantly affected but those of Bali and Ongole cattle were significantly reduced by means of 160 g and 98 g per day, respectively. The buffaloes in their study also showed less of a reduction in PCV than Bali and Ongole calves, reflecting the higher resilience of buffaloes to infection than cattle.

Between Bali and Ongole cattle, E. Wiedosari et al. (pers. comm.) observed that Ongoles were more resilient than Bali calves since their mean daily weight gain was higher than the Bali calves.

A series of studies was undertaken in the Philippines (Molina et al. 2005a,b) on naturally and artificially infected cattle and buffalo. A study of naturally infected cattle and swamp buffaloes showed that swamp buffaloes were not as severely affected by *F. gigantica* as cattle (Molina et al. 2005c). Clinico-pathological parameters that indicated a higher resilience to infection in buffalo included red blood cell (RBC) counts, PCV and haemoglobin levels. In another study, Molina et al. (2005a) observed that the numbers of mature and immature flukes and faecal egg counts in swamp buffaloes were lower than in cattle. Although this study involved naturally infected animals, these observations suggest that swamp buffaloes are likely to be more resistant to *F. gigantica* than cattle. Lower faecal egg counts in buffaloes than in cattle were also observed by Prasitirat et al. (1996) and E. Wiedosari et al. (pers. comm.) from the same infective dose of *F. gigantica*. Consistently lower fluke counts in buffaloes than in cattle were seen at 3, 7, 12 and 16 weeks post-infection. At 16 weeks post-infection *Fasciola* eggs were not observed in buffaloes whereas eggs were first observed in cattle at this time. This suggests there is a longer prepatent period in buffaloes than in cattle or suppression of fluke development in swamp buffaloes.

These differences in resistance and resilience to *F. gigantica* between cattle and swamp buffaloes may result from varying host–parasite relationships during infection with *F. gigantica* between these animals. An investigation on their immune responses during infection shows that there are differences between cattle and swamp buffaloes in some aspects of their immune responses. The IgG2 response was much higher in buffaloes than in cattle (Molina, unpublished data). Also, the number of T cells in the liver of buffaloes increased, whereas those in the liver of cattle showed a decreasing trend after an initial increase at week three post-infection (Molina and Skerratt 2005). Buffaloes showed increased serum IL-8 levels while cattle did not (Molina 2005). IgG2 was considered to be associated with resistance to *F. gigantica* in Bali cattle as these animals showed increased IgG2 levels from week four of infection and their GLDH levels and egg counts declined after weeks 24 and 26, respectively (E. Wiedosari et al. 2006). This response was also considered to be a protective response against *F. hepatica* in cattle vaccinated with *F. hepatica* cathepsin L and haemoglobin (Mulcahy et al. 1998). The increasing T cell response in the liver of buffaloes was considered to be related to the suppressed development or delayed migration of flukes in buffaloes (Molina and Skerratt 2005). Molina (2005) has suggested that increased serum IL-8 levels in buffaloes may be associated with a higher level of innate resistance against the flukes in buffaloes since, in other infections, this cytokine is a manifestation of an innate immune mechanism through its ability to enhance the phagocytic ability and oxidative burst of neutrophils.

Cattle and swamp buffaloes manifest similarities as well as differences in their immune responses during infection with *F. gigantica*. Differences in the clinico-pathological and parasitological manifestations during infection were also observed. These varying responses to *F. gigantica* infection represent differences in host–parasite relationships in *F. gigantica* infection between cattle and swamp buffaloes and may be linked to the observed varying levels of resistance and resilience to infection between these hosts. Genetically determined differences in species resistance may also play a role in influencing the differences in their resilience and resistance to *F. gigantica*.

References

Entwistle K.W. 1978. Reproduction. Pp. 172–273 in ‘Beef cattle production in the tropics’, ed. by R.M. Murray and K.W. Entwistle. James Cook University: Townsville, Australia.

Graber M. 1971. Role du facteur alimentaire dans la distomatose bovine et ovine a *Fasciola gigantica*. Bulletin of Epizootic Diseases in Africa 19, 45–60.

Kuma S. and Sharma M.C. 1991. Infertility in rural cows in relation to fascioliasis. Indian Journal of Animal Sciences 61, 838–840.

Molina E.C. 2005. Serum interferon-gamma and interleukins-6 and -8 during infection with *F. gigantica* in cattle and buffaloes. Journal of Veterinary Science 6, 135–139.

Molina E.C., Gonzaga E.A. and Lumbao L.A. 2005a. Prevalence of infection with *Fasciola gigantica* and its relationship to carcase and liver weights, and fluke and egg counts in slaughter cattle and buffaloes in southern Mindanao, Philippines. Tropical Animal Health and Production 37, 215–21.

Molina E.C., Gonzaga E.A., Sinolinding E.O., Lumbao L.A., Peralta A.A. and Barraca A.P. 2005c. Differences in susceptibility between cattle and swamp buffaloes to infection with *Fasciola gigantica*. Tropical Animal Health and Production. 37, 611–6.

Molina E.C., Sinolinding E.O and Peralta A.A. 2005b. Clinicopathological indications of resilience of swamp buffaloes to infection with *F. gigantica*. Tropical Animal Health and Production 37, 451–455.

Molina E.C. and Skerratt L.F. 2005. Cellular and humoral responses in liver of cattle and buffaloes infected with a single dose of *Fasciola gigantica*. Veterinary Parasitology 131, 157–163.

Mulcahy G., O'Connor F., McGonigle S., Dowd A., Clery D.G., Andrews S.J. and Dalton J.P. 1998. Correlation of specific antibody titre and avidity with protection in cattle immunised against *Fasciola hepatica*. Vaccine 16, 932–939.

Prasitirat P., Thammasart S., Chompoochan T. Nithiuthai S. and Taira S. 1996. The dynamics of antibody titres and faecal egg output in cattle and buffalo following infection with 500 and 1,000 *Fasciola gigantica* metacercariae. Thai Journal of Veterinary Medicine 26, 85–89.

Sewell M.M.H. 1966. The pathogenesis of fascioliasis. Veterinary Record 78, 98–105.

Spithill T.W., Smooker P.M. and Copeman B. 1999. *Fasciola gigantica*: epidemiology, control, immunology and molecular biology. Pp. 465–520 in 'Fasciolosis', ed. by J.P. Dalton. CAB International: Wallingford, UK.

Wamae L.W., Hammond J.A., Harrison L.J.S. and Onyango-Abuje J.A. 1998. Comparison of production losses caused by chronic *Fasciola gigantica* infection in yearling Friesian and Boran cattle. Tropical Animal Health and Production 30, 23–30.

Waweru J.G. and Kanyari P.W.N. 1999. A comparison of serum biochemical changes in two breeds of sheep (Red Masai and Dorper) experimentally infected with *Fasciola gigantica*. Onderstepoort Journal of Veterinary Research 66, 47–49.

Wiedosari E. and Copeman D.B. 1990. High resistance to experimental infection with *Fasciola gigantica* in Javanese thin-tailed sheep. Veterinary Parasitology 37, 101–11.

Wiedosari E., Hayakawa H. and Copeman B. 2006. Host differences in response to trickle infection with *Fasciola gigantica* in buffalo, Ongole and Bali calves. Tropical Animal Health and Production 38, 43–53.

Genetics of fasciolosis in small ruminants

H.W. Raadsma, K.J. Fullard, N.M Kingsford, E.T. Margawati, S.E. Estuningsih, S. Widjayanti, Subandriyo, T.W. Spithill and D. Piedrafita

Genetics of fasciolosis in small ruminants

H.W. Raadsma, K.J. Fullard, N.M Kingsford, E.T. Margawati,
S.E. Estuningsih, S. Widjayanti, Subandriyo, T.W. Spithill and D. Piedrafita

Introduction

The quest for genetic approaches for the control of disease in livestock continued throughout the twentieth century. Emphasis was first on selection of resistant breeds, but with technological advances in genetics, there was an increased focus on quantitative approaches within breeds. Most recently, molecular techniques were used to identify resistance in individuals that could be used for selective breeding (Gray et al. 1995). This pattern has been followed for resistance to liver fluke but from a base of very limited knowledge on how livestock develop resistance to infection, and if there is any genetic component to that resistance. The problems associated with investigating these problems in tropical large ruminants are especially severe, given the lack of basic information on the epidemiology of disease, and relative lack of expertise and facilities to undertake large-scale genetic experiments.

The discovery, albeit based on limited data, of evidence for a single gene of resistance to *F. gigantica* in Indonesian thin tail (ITT) sheep (Roberts et al. 1997) stimulated significant interest in investigating the genetics of resistance to *F. gigantica* in smaller livestock that could be experimented on more easily. This interest was further increased by the availability of new genetic and statistical technologies that enhanced the possibility of detecting genes of major effect. While many animals may still be needed, the prospect of detecting major genes for a wide range of disease and production characteristics is an enticing prospect.

In this chapter the results of a major ACIAR project are summarised. The project sought to capitalise on the wide variation in production and disease traits between merino and ITT sheep. Crosses of ITT and merino offer an excellent opportunity for resistance and other genes to segregate. The practical questions that both stimulated these studies and emerge from their results include the following.

- Is there a single gene for resistance to *F. gigantica* segregating in ITT sheep and will it be of sufficient magnitude to offer a further option for control of the disease? The consequent question is whether there is an equivalent gene in the cattle of buffalo genome.
- Which method, if any, is most effective for detecting a resistance gene of large magnitude?
- Are there also genes that confer high levels of resistance to the bloodsucking nematode parasite *Haemonchus* in the ITT sheep, which also seriously constrains the production of sheep and goats in the tropics?
- In the absence of any single major genes, are there other candidate genes which, together, confer high levels of resistance? Where are these located and how can they be detected?

Linkage mapping

Linkage mapping is a way to order genetic markers along the genome, with the number of breakpoints occurring during meiotic recombination as an indication of the distance between the markers. Genetic maps have been used to identify the position of genes on chromosomes, identify the position of anonymous loci exhibiting phenotypic segregation and are increasingly being used to help translate information across species. That is, if the position and function of genes are well known in a species such as humans, genetic maps can be used to indicate homologous positions and gene blocks in other species such as sheep. Information painfully gleaned over the course of decades in model species, does not have to be regathered in new species.

Genetic maps are widespread among livestock but are most comprehensive in terms of marker density in cattle. Human–cattle comparative maps are available in the public domain. Sheep maps lag behind cattle in the number of high-quality maps available, and in their marker density. The first sheep genetic maps appeared in the early 1990s, but the first comprehensive map, including all chromosomes, was published by an international sheep mapping consortium in 1995. It was based on a standardised international mapping flock. This map has been improved upon in recent years. The latest published map appeared in 2001, but further updates are readily available on the Australian sheep gene mapping website (<http://rubens.its.unimelb.edu.au/~jillm/jill.htm>). The latest sheep map (v4.2) comprises 1,251 markers spanning approximately 3,600 centiMorgan (cM). The international mapping flock continues to be used, with nine three-generation families totalling 127 full-sibs.

To conduct a QTL (quantitative trait loci) study to show the position of anonymous genes controlling traits of interest to sheep breeding a genetic map has to be constructed using the flocks that have been phenotyped. This will have the added advantage of adding information to the published sheep map, in that many more individuals were used in this study than in the international mapping flock. The full methodologies of this approach have been described by Raadsma et al. (2000) and Margawati et al. (2002).

Creation of resource population

By the end of the breeding cycles, a large genetic resource was available consisting of three generation, half-sib families. The F1 generation included representation from merino and two ITT populations. A total of 10 families was created (genotyped $n = 694$). Average number of genotyped animals per drop was 49.6. Four of the families were deemed phenotypically interesting by preliminary segregation analysis, and these were augmented by a second drop (mean augmented family size $n = 101.75$). The second drop of two of the augmented families diverged from the standard merino backcross design, in being a mixture of F2, ITT backcross, and merino backcross animals, to provide greater analytical power to identify recessive genes (Table 7.1).

Map construction

A low-density marker screen with an average of 30 cM intervals was planned in the construction of a genetic map of the autosomal chromosomes. Initially, 258 microsatellite markers were selected on the basis of map location (Maddox et al. 2001) and PIC scores from the Australian sheep gene mapping resource[1]. Of these, 134 were discarded as they could not be successfully amplified using a labelled M13-tailed primer approach, or were not informative over a sufficient number of families. In general, markers that were informative over at least two of the four largest families were favoured. Twelve additional markers were added to supplement areas of low marker coverage (Figure 7.1).

Genotype scoring was carried out independently by two individuals, with mismatches being called on a third reading, or removed. Mapping was carried out using the CarthaGene software[2] employing pre-existing knowledge of linkage group assignments.

Results and discussion

A total of 136 markers were used, with means of 97 informative markers per family, and 7.13 informative families per locus. Mean map distance between markers was 33.12 cM (range: 1.9–190 cM, median: 25.15 cM), although this includes 19 gaps with low logarithm of odds (LOD) scores, tending to inflate estimates of distance. Mean map distance based on the Australian sheep gene map V4.3 (Maddox et al. 2001; <http://rubens.its.unimelb.edu.au/~jillm/jill.htm>) was 29 cM (range: 2.2–74.2 cM, median: 26.2 cM). The planned genome coverage was thus achieved. The mean number of phase-known informative individuals per marker was 376.91. This should be contrasted with the mean number of 98 phase-known informative individuals used in the construction of the Australian sheep gene map. Incorporation of the additional information into the Australian map will strengthen it, firming marker orders and distances. The improved map could become a stable reference for sheep genetic comparisons worldwide (Table 7.2). The table compares the new ITT map with the Australian sheep gene male map for marker positions. Inter-marker

1 <http://rubens.its.unimelb.edu.au/~jillm/jill.htm>

2 <http://www.inra.fr/bia/T/CartaGene/index.html>

Table 7.1 The numbers of breeding animals and the contribution of each of the pure breeds (M, Merino; S, Sumatra; G, Garut) available in the years of the project.

	Merino, ITT, F1	**Merino backcross, F2 and ITT backcross**
Bought	270 Merino 28 Sumatra 18 Garut	
1997	6 MM 1 SM 9 GM	
1998	1 MM 3 SS 2 MS 4 MG 6 SM	
1999	29 MM 16 SS 17 MS 7 MG 32 SM 18 GM	184 GM (Sires 1261, 1263, 1265)
2000	5 MM 33 SM	58 MSM (Sire 1258) 77 MGM (Sire 1267) 142 SMM (Sire 1273, 1262)
2001	24 MM 18 SS 12 GG	54 MSM (Sire 1630) 69 MGM (Sire 1578) 51 SMM (Sire 1348) 4 GMM (Sire 1263) 67 F2 and ITT backcross (Sire 1263)
2002	56 MM 35 SS 27 GG	138 SMM (Sires 1273, 1262) 2 GMM (1261) 78 F2 and ITT backcross (Sire 1261)
2003	35 MM 27 SS	7 GMM (Sire 1263) 41 F2 and ITT backcross (Sire 1263)
Total	**423**	**972**

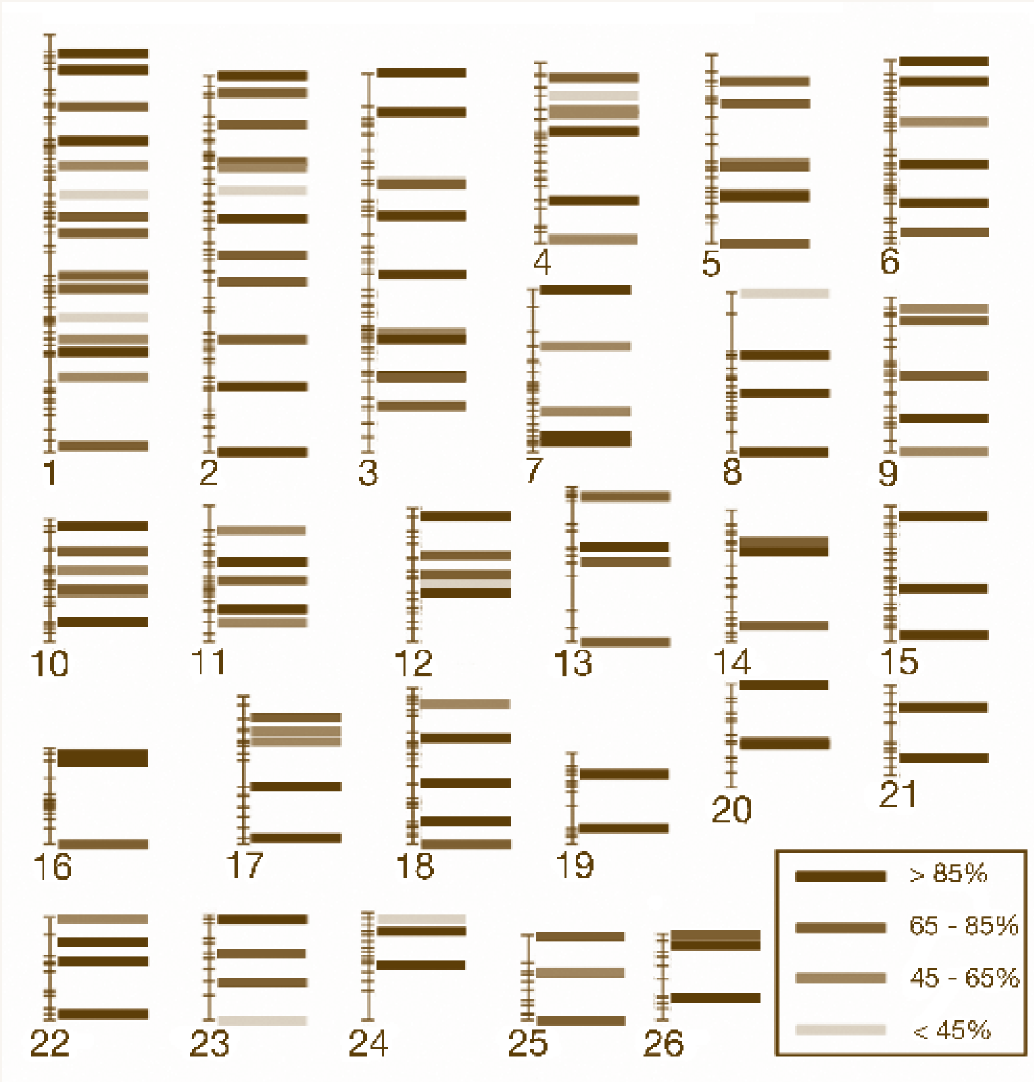


Figure 7.1 Marker coverage based on Maddox et al. (2001) map. Bars indicate the positions of markers, and depth of shading indicates the informativeness of the marker.

Table 7.2 Comparison of marker positions between Maddox male map v3.4, and new ITT map. Solid lines indicate gaps in ITT map, where marker distances taken from Maddox map.

Chr.	Lab No.	Marker	Maddox Male cM	ITT Male cM	Meioses	2 point LOD
1	2084	MCM46	24.4	24.4	394	59.4
	2002	EPCDV21	36.5	28.9	471	17.2
	278	OARHH51	71.5	60.7	410	19.5
	1160	BM6465	101.3	90.5	502	11.3
	1158	BM4129	123.9	124.5	304	6.7
	610	BMS482	148.8	157.3	192	6.7
	14	BM6438	165.3	182.3	326	19.9
	26	MAF64	182.5	202	493	10.7
	612	CSSM04	220.6	239.2	432	46.2
	20	INRA011	228.9	250.8	481	10
	216	BM6506	254	276.4	154	3.3
	614	URB038	270	293.4	277	27.8
	1136	BMS4045	282.4	298.8	356	9.4
	1138	BMS1789	300.8	322.7	211	1.2
	298	MCM357	362.2	386.7	377	
2	2024	LS42	0	0	338	12.5
	828	CSRD65	20.4	22.3	341	16.1
	302	MCM147	41.1	46.6	473	7.8
	306	MCM505	68.6	86.6	322	15.2
	616	BMS1341	75.7	92.8	170	0
	242	FCB128	99.9	117	197	17.1
	46	TGLA10	115.9	134.8	464	13.4
	618	BM81124	150.2	165.5	394	21.3
	44	HH30	172.4	186	440	2.3
	1140	BMS1126	220.2	233.9	316	2.4
	312	MCM554	254.3	268	472	0
	42	FCB11	328.5	342.2	435	
3	622	BMS1350	0	0	507	18.1
	1512	ILSTS28	31.1	31.9	559	0.9
	714	BMS710	82.6	83.4	288	14.8
	60	TGLA67	84.8	87.8	408	17.7
	2090	INRA131	108.6	102.4	309	0.5
	224	BM827	152.6	146.4	427	18.4
	628	ILSTS42	158	160.5	246	0
	1142	BMS1617	211.3	213.8	258	3
	832	VH130	217.3	213.8	333	8.2
	630	BM8230	251.9	250.2	281	19.9
	634	BMS1248	253.4	250.2	515	11.6
	632	BMS772	276	270.8	110	
4	1144	BMS1788	13.6	13.6	436	21.5
	324	MCM218	31.3	28.6	224	23.8
	326	MCM2	44.1	40.9	333	10.6
	636	BMS1237		45.2	110	15.1
	1178	MCM144	62.2	54.4	482	0.9
	248	HH35	124.8	117	560	15.3
	328	MCM73	152.2	145.5	364	
5	1488	MCM380	32.5	32.5	296	8.1
	640	TGLA303	55.8	43.8	232	0
	644	BMS2258	101.2	89.2	197	4.2
	1146	BMS792	105.9	98.1	339	9.3
	266	TGLA137	116.9	113.2	201	32.6
	1174	MCM527	127	119.4	416	6.2
	646	BMS1247	169.2	159.4	355	
6	338	CP125	4.9	4.9	508	21.6
	2096	MCM204	31.3	28.2	414	5
	88	HH55	64.9	66.5	219	9.4
	84	BM4621	103.1	95.8	597	16.8
	344	CSRD93	138.5	131.1	565	14.2
	346	MCM214	166.3	147.4	165	
7	652	BM3033	0	0	432	1.4
	838	RNS5 (BRN	49.9	49.9	320	0.4
	812	BMS1620	113.2	113.2	285	23.7
	840	BMS2721	130	131.3	588	55.3
	656	MCM185	137.4	143.2	451	
8	96	BM1227	0	0	142	0.3
	358	UWCA9	52.7	52.7	454	29.4
	356	KD101	68.6	68.5	422	0.5
	842	BMS1967	131.5	131.4	506	

Chr.	Lab No.	Marker	Maddox Male cM	ITT Male cM	Meioses	2 point LOD
9	662	ETH225	7.2	7.2	236	17
	220	BM757	16.2	16.9	366	4.3
	1170	BL1009	68	58.7	347	9.5
	106	BM4513	107.8	95.1	584	6.5
	360	RJH1	134.7	126	159	
10	846	SRCRS25	4.8	4.8	202	11.8
	848	AGLA226	31	21.5	420	21.5
	250	HH41	49.9	36.1	293	30.2
	366	ILSTS56	59.5	51.4	422	19.9
	666	BMS585	63.7	60	254	20.3
	124	TGLA441	87.9	78.7	530	
11	128	HEL10	29.5	29.5	333	11.8
	1534	CSSME70	53.7	58.2	495	34.7
	668	BM17132	67.3	71.5	385	39.7
	1518	MCM120	90.9	87	506	10.7
	376	ETH3	97.1	105.4	177	
12	136	HUJ614	11.3	11.3	479	14.9
	380	TGLA53	47.5	38.9	342	15.7
	1200	CSSM03	60.2	59.4	375	14.7
	1164	BM8225	67	66.7	147	16.7
	2008	MCMA52	77.7	83.5	563	
13	386	IL2RA	9.4	9.4	243	7
	388	MCM152	49.2	38.6	438	27.3
	230	HUJ616	63.7	54.4	389	0.4
	856	BMS2319	126.3	117	352	
14	390	CSRD70	31.1	31.1	513	93.3
	1148	BMS2213	39.5	36	590	2.1
	860	LS30	104.1	100.6	523	
15	404	BR3510	32.1	32.1	511	0.3
	864	Z27076	89.6	89.6	544	10.9
	1166	BM848	130.8	129.6	551	
16	406	RM106	6.7	6.7	410	47.1
	680	BM1225	20	20.2	607	0
	412	MCM150	86.8	87	356	
17	1212	VH98	26.7	26.7	361	23
	1490	AGLA299	36.5	37.5	272	33.8
	1524	VH116	47.8	42.6	355	11.5
	1202	BM7136	83.8	74.4	461	6.4
	170	TGLA322	137.1	116.5	412	
18	1216	BM1117A	14.2	14.2	323	14.9
	174	VH54	43.1	39.5	460	9.8
	252	HH47	79.2	68.9	329	12.6
	684	CSSM18	116.8	94.9	408	15.5
	2102	TMR1		117.1	308	
19	426	CSSM06	24.7	24.7	545	9.1
	1150	BMS875	72	64.4	455	
20	696	INRA132	17	17	550	3.1
	430	CSRD26	60.6	74	225	22.6
	262	MHC1		76.4	402	
21	196	VH110	30.7	30.7	474	7.5
	190	BMC1206	63.1	71.1	438	
22	698	BMS651	0	0	192	9
	702	BMS907	21.3	20	449	15.5
	214	BM1314	44	43.4	414	2.5
	200	MAF36	88.2	95.6	497	
23	1186	BL6	15.9	15.9	357	4.7
	226	CSSM31	55.7	51.8	332	20.4
	1538	MCM136	81.9	74	483	9.2
	818	URB031	109	89.7	86	
24	256	JMP29	5.1	5.1	140	7.9
	1156	BMS744	20.8	20.5	529	25.7
	704	BM737	37.9	47.4	571	
25	888	MCM200	0	0	356	1.7
	1558	MCMA7	35.8	35.8	329	2.5
	210	RBP3	78.8	81.7	356	
26	890	BMS2168	0	0	373	14.1
	708	BMS629	10.9	1.9	143	0.6
	822	JMP23	62.3	53.3	602	

distances with low two-point LOD support were taken directly from the Australian sheep gene map, and these gaps are indicated with a line.

Conclusion

The map constructed for this project shows great similarity with the established sheep map. LOD scores are high, apart from 19 instances generally explained by large intervals between markers. This confirms that the DNA genotyping was accurate, and that genome coverage is extensive. The map is sufficiently detailed to allow a low resolution QTL scan to be carried out successfully. Furthermore, the map will be employed in updating the established map, which will prove invaluable in comparative studies across species.

Segregation analysis

The presence of a putative major gene for resistance to internal parasites can be confirmed by several approaches. A relatively simple approach is to use segregation analysis in appropriate resource families. Under this approach, the distribution of animal phenotypes is examined for conformity to an expected distribution consistent with a population distribution in which a major gene is segregating. A suitable resource population usually consists of a three-generation pedigree with the contrast (expression) of the major gene in the third generation. This is schematically shown below where the putative resistance allele (R) is dominant over alternative susceptible (S) alleles and expressed in a susceptible (S) background. Bimodality of phenotypic expression in the 3rd generation backcross is indicative of the presence of a major gene, and can be evaluated statistically through a number of procedures of which maximum likelihood analyses provides the most power.

Foundation lines—	RR	x ↓	SS
F1-max heterozygosity		RS	
Backcross for expression R allele		x ↓	SS
Progeny to be screened	RS	&	SS

Each independent backcross family (excluding the second drop of two families, having a mixture of genotypes) was submitted to segregation analysis. A mixture model maximum likelihood approach was employed to determine which of two contrasting models was the more likely:

(a) that a single mean and variance explained the observed phenotypic distribution

(b) that a mixture of two means and variances, caused by underlying major allelic differences affecting the trait, explained the observed distribution.

Each family was explored incorporating a sex effect, a sex-QTL interaction effect and, where appropriate, a drop effect, a drop by QTL interaction, and a drop and sex by QTL interaction to see whether any of these distributions were conformed to more closely. Tested phenotypes include:

- fluke count
- *Haemonchus contortus* first challenge FEC count (Log (x + 100) transformed)
- *H. contortus* second challenge FEC count (Log (x + 100) transformed)
- carcass weight
- coat colour.

Results and discussion

The results are summarised in Table 7.3.

Fluke count

Families 1261, 1262 and 1273 displayed significant evidence for a gene affecting the liver fluke number phenotype. In family 1261, this was evident only in males due to a significant gene-by-sex interaction. Similarly, segregation was evident in family 1262 following consideration of drop by QTL interactions (the QTL was more evident in one drop than in another), and in 1273 allowing both drop and sex by QTL interactions (Figure 7.2).

H. contortus FEC

Given the many sheep displaying a trait count of zero, a common phenomenon when measuring faecal egg count, a skewed phenotype distribution is inevitable. The maximum likelihood segregation analysis is not appropriate here, as significant deviations from a normal distribution will be found in all cases. We are therefore unable to search for a gene affecting initial parasite infection using segregation analysis. However, by removing all cases of zero counts, and analysing all sheep displaying

Table 7.3 Evidence for major genes within families for selected resistance and production traits.

	Segregation Analyses: Major Traits									
	1258	**1261**	**1265**	**1267**	**1348**	**1578**	**1630**	**1262**	**1263**	**1273**
Log Hc1 Mean 4,6,8 WO Zero										
P Value for Presence of a QTL	NS	**0.0366**	NS	NS	NS	**0.0117**	NS	NS	**0.0049**	NS
With Interaction		Sex							Drop	Sex
Mean	1.2706	3.1313	2.5496	0.8878	2.8861	2.7162	3.1498	1.4246	3.1734	1.5294
QTL Effect	0.0000	-1.7363	-1.0442	-0.0042	-0.0456	0.7093	0.0169	0.0207	-1.3057	0.0000
Log Hc2 Mean 4,6,8 WO Zero										
P Value for significance	NS	NS	NS	**0.0320**	**0.0399**	NS	NS	**0.0192**	**0.0239**	NS
With Interaction				Sex	Sex			Sex and Drop	Drop	Drop
Mean	1.6958	2.9775	2.8239	0.9331	1.8775	1.7553	2.0560	1.3513	3.0189	1.8710
QTL Effect	0.0154	-0.1254	-0.0090	0.8432	0.6084	1.1529	0.0444	0.7937	0.0031	-0.9478
Fluke Count										
P Value for significance	NS	**0.0035**	NS	NS	NS	NS	NS	**0.0003**	NS	**0.0000**
With Interaction		Sex	Sex					Drop	Drop	Sex and Drop
Mean	45.3179	16.8156	22.6153	35.8160	45.2065	39.4132	40.3762	35.3843	19.1924	29.4556
QTL Effect	-0.0108	6.5906	0.0001	5.0079	0.4752	-3.6149	-1.5176	23.0971	20.3888	26.5913
Carcass Weight										
P Value for significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
With Interaction						Sex				
Mean	7.0085	9.1048	9.7926	8.6077	10.5060	10.4906	12.7347	11.3369	10.3584	9.6741
QTL Effect	3.6160	4.9441	0.3816	3.0571	0.0465	-2.0473	-3.5774	-1.9383	-0.0870	-0.2797
Colour(1-6)										
P Value for significance	NS	**0.0000**	NS	NS	**0.0005**	NS	**0.0018**	NS	**0.0364**	**0.0000**
With Interaction		Sex			Sex				Sex and Drop	Sex and drop
Mean	3.3017	3.9497	3.9374	3.2242	4.1428	2.9527	2.8685	3.3161	4.8738	3.0361
QTL Effect	0.0215	1.9482	-0.0350	0.0279	-1.2337	0.1538	1.3777	-0.0630	-1.6187	-0.1375

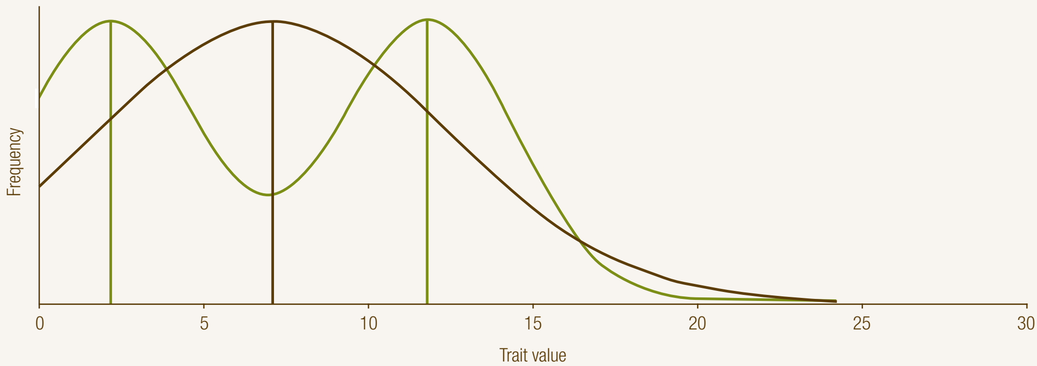


Figure 7.2. Bimodal segregation curve for fluke number in sire family 1261 males only, versus expected curve assuming no QTL.

a FEC greater than zero (log transformed), it is possible to analyse the likelihood of a gene affecting the resistance of sheep to parasite reproduction, once infection has occurred. This is likely to reflect differences in female parasite egg production, or adult parasite killing. Families 1261, 1578 and 1263 show a significant bimodal distribution for the first challenge, suggesting an underlying major gene effect. Again, significant interactions were evident. Family 1263 also showed significant evidence for resistance to secondary challenge, together with families 1267, 1348 and 1262. There is strong evidence that sire 1263 has one or more genes segregating within it that confer resistance against *H. contortus*.

Carcass weight and colour

There is no evidence for segregation for the trait of carcass weight in any of the families. This is not unexpected—if carcass weight is dominant in the merino population, then all of the 3rd generation progeny would contain at least one dominant allele, and no differences in carcass weight would be evident. In other words, the experimental design is not optimal for recognising genes inherited from the merino. However, QTL analysis could well uncover concealed trait variation. In contrast, there appears to be strong evidence for a major gene influencing fleece colour, as shown in families 1261, 1348, 1630, 1263 and 1273. Discovery of such a gene could have large implications for the merino breeding industry, where breeding focus is on removing all colour from fleeces.

Conclusion

The segregation analyses prove that there are major genes segregating for the two main traits investigated in this project, namely resistance to *H. contortus* infection, and resistance to *F. gigantica* infection, in at least some of the 10 families. This suggests that further QTL analysis will be successful in pinpointing a chromosomal region containing a gene or genes that strongly influence the outcome of infection. The same outcome might be expected for fleece colour, which could have major implications for future breeding programs.

QTL analysis

A weakness with segregation analysis is the inability to confirm the presence of a major gene if there is overlapping expression through background genes and/or non-genetic factors. A design with significantly greater power to detect the presence of major genes is through linkage analyses. The segregation of a major gene can be confirmed through significant linkage with polymorphic markers located in close proximity to the major gene. When the chromosomal location of the major gene is unknown, many polymorphic markers can be selected to give coverage across the genome. The same design used in segregation analysis can be used, with the added complexity that all animals across the three generations require genotyping with a panel of polymorphic markers. The major additional advantage is that this approach provides evidence for a possible chromosomal location, and can be used as a foundation in a positional cloning strategy to evaluate positional candidate genes in a chromosomal region containing the major gene.

Preliminary QTL analysis was undertaken for a range of parasite resistance, morphology and production traits, using the half-sib analysis servlet of QTL Express software (Seaton et al. 2002). QTL Express employs regression analysis as described in Haley and Knott (1992). Genotype probabilities for each animal were calculated at 4 cM intervals along each of the 26 autosomes. A chromosome-wide threshold for statistical significance was calculated for each chromosome, based on a permutation test of 1,000 iterations. A combined analysis of all 10 families was undertaken adjusting for age, sex and year of birth as fixed effects. Chromosome-wide significance to a level of $P < 0.05$ was an initial indicator for the retention of results.

Results and discussion

QTL analysis was carried out across 51 traits. Examples of QTL output are shown in Figure 7.3a and 7.3b). Both outputs demonstrate a significance level of $P < 0.01$. However, weaner coat colour shows a significantly stronger QTL peak, in fact, showing genome-wide significance. All QTL results are summarized in Table 7.4.

Conclusion

Experimental design with sufficient power to detect the presence of a major gene in both the segregation and linkage approach is influenced by the magnitude of the major gene effect, the gene frequency in the founding population, the nature and mode of inheritance of the major gene, and the number of progeny that can be screened (family size). A limited number of families from reciprocal matings with many progeny per family provides an ideal design.

In this study the frequencies of trait alleles in the ITT population were unknown. Furthermore, strain differentiation in the ITT (Garut and Sumatra strains) required that both populations should be represented in the founding population. This imposed an experimental requirement that multiple families would need to be generated. A minimum of eight families (two strains, reciprocal matings, repeat sampling) was planned for. A further constraint to the design was the total number of animals which could be generated and challenged at any one time. This was limited by animal house space, breeding resources, labour resources, and experimental cost. A minimum target of 160 experimental animals (maximum 250) for testing each year was aimed for, to be repeated over four rounds (years).

These targets were achieved, leading to a total genotyped population of 694 individuals in

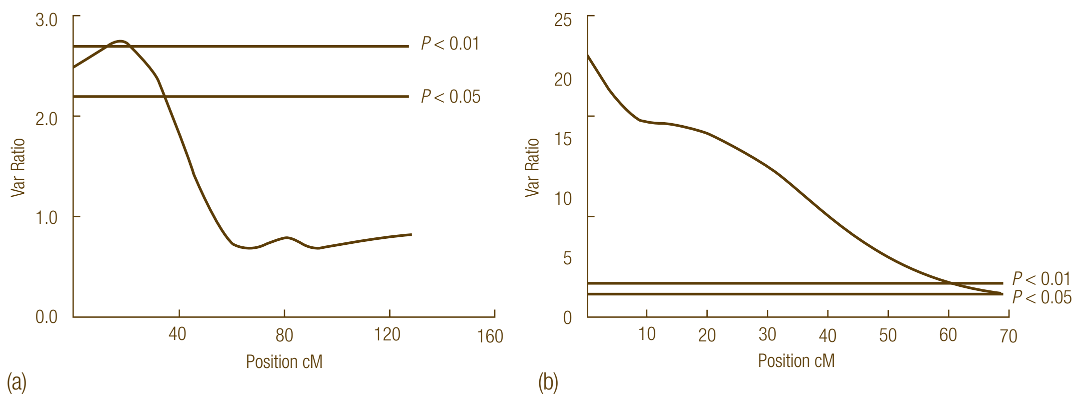


Figure 7.3. Examples of QTL Express output: (a) mean eosinophil count as a proportion of the total leukocyte count in weeks 2, 4 and 6; (b) weaner coat colour are shown with significance thresholds after chromosome-wide significance testing using all families.

Table 7.4 Summary results of QTL scan for selected resistance and production traits across all 10 families.

Experiment	QTL	p<= 0.05	p<= 0.01
***Fasciola gigantica* challenge**	Fluke Number	2	0
	Fluke Wet Weight	2	0
	Fluke Wet Weight/Fluke Number	1	1
	Liver Score	2	0
	Liver Weight	3	1
	Eosinophil Volume (Early)	3	2
	Eosinophil Smear (Early)	3	0
	Neutrophil Smear (Early)	0	1
	Lymphocyte Smear (Early)	0	2
	Eosinophil Volume (Late)	3	1
	Eosinophil Smear (Late)	0	0
	Neutrophil Smear (Late)	3	0
	Lymphocyte Smear (Late)	0	1
	GLDH Week 10	2	0
	AST Week 10	4	0
	GGT Week 10	1	0
	Albumin Week 10	2	0
	Globulin Week 10	2	0
	Albumin/Globulin Week 10	3	2
	Total Protein Week 10	1	0
	GLDH Week 14	2	0
	AST Week 14	1	0
	GGT Week 14	0	0
	Albumin Week 14	1	1
	Globulin Week 14	2	0
	Albumin/Globulin Week 14	2	0
	Total Protein Week 14	3	0
	Delta Weight	0	0
	Delta PCV	0	0
***Haemonchus contortus* 1st Challenge**	FEC 1st Challenge All Transformed Data	1	0
	FEC 1st Challenge Binary (with or without FEC)	1	0
	FEC 1st Challenge (Zero Values excluded)	1	0
	Delta Weight 1st Challenge	3	1
	Delta PCV 1st Challenge	2	1
***Haemonchus contortus* 2nd Challenge**	FEC 2nd Challenge All Transformed Data	1	0
	FEC 2nd Challenge Binary (with or without FEC)	1	0
	FEC 2nd Challenge (Zero Values excluded)	2	1
	Delta Weight 2nd Challenge	1	0
	Delta PCV 2nd Challenge	0	0
Production Traits	Weaner Earlength	6	1
	Weaner Taillength	3	1
	Weaner Fleece Colour	0	1
	Hoggett Fleece Colour	0	1
	Hoggett Fleece Yield	2	0
	Hoggett Fleece Total Weight	2	1
	Hoggett Clean Fleece Weight	0	1
	Hoggett Wool Micron	0	1
	Hoggett Kemp	1	1
	Hoggett Style	1	2
	Carcass Weight	4	0
	Carcass Circumference	2	0
	Mean	1.61	0.49

10 families. That the families have widely differing genotypic backgrounds increases the likelihood of capturing gene alleles that are only present in some of the populations. However, this also creates problems for QTL analysis, given that the power of detecting a QTL in 10 families is lowered when only some have the segregating allele of interest.

Nevertheless, the current dataset has identified 25 QTL to a significance level greater than 99.9 out of 100, together with their broad chromosomal positions. Weaner coat colour displays a particularly strong response, and is likely to represent a single gene mutation. In addition, some of the wool traits identified are found in similar positions to those in a related sheep QTL mapping project (Reprogen, unpublished). This validates the genotyping and mapping accuracy of the dataset. Resistance traits are of importance to this project. There are 12 QTL for (a) measures of resistance to fasciolosis, (b) immunological indicators of resistance and (c) liver enzyme indicators of the response to infection. It is interesting that one of the strongest QTL for a measure of resistance to fasciolosis falls on the same chromosome as the multiple histocompatibility complex (MHC). The MHC is thought to be involved in immune response to internal nematodes. The chromosomal position of two of the primary infection *H. contortus* resistance QTL match previous studies reported in the literature. Again, this validates the results obtained in this study, and suggests those regions would produce valid candidates for further studies to find breeding markers.

However, to gain the maximum information from the dataset, more powerful analysis techniques need to be employed to help sift through uninformative families, and this may well need a maximum likelihood approach to analysing the data.

References

Gray G.D., Woolaston R.R. and Eaton B.T. (ed.) 1995. Breeding for resistance to Infectious Diseases in Small Ruminants, ACIAR Technical Monograph No. 34, Canberra.

Haley C.S. and Knott S.A. 1992. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69, 315–324.

Maddox J.F., Davies K.P., Crawford A.M., Hulme D.J., Vaiman D., Cribiu E.P., Freking B.A., Beh K.J., Cockett N.E., Kang N., Riffkin C.D., Drinkwater R., Moore S.S., Dodds K.G., Lumsden J.M., van Stijn T.C., Phua S.H., Adelson D.L., Burkin H.R., Broom J.E., Buitkamp J., Cambridge L., Cushwa W.T., Gerard E., Galloway S.M., Harrison B., Hawken R.J., Hiendleder S., Henry H.M., Medrano J.F., Paterson K.A., Schibler L., Stone R.T. and van Hest B. 2001. An enhanced linkage map of the sheep genome comprising more than 1000 loci. Genome Research 11, 1275–1289.

Margawati E.T., Tammen I., Jones M. and Raadsma W. 2002. Application of microsatellite markers in identification and parentage assignment of sheep. Berita Biologi 6, 475–479.

Raadsma H.W., Smith A., Margawati E., Spithill T.S., Piedrafita D., Estuningsih E., Suharyanta, Widjajanti S., Partoutomo S. and Subandriyo. 2000. A strategy to search for major genes in resistance to *Fasciola gigantica* in ruminants. Proceedings of the 27th International Conference on Animal Genetics.

Roberts J.A., Widjajanti S., Estuningsih E. and Hetzel D.J. 1997. Evidence for a major gene determining the resistance of Indonesian thin tail sheep against *Fasciola gigantica.* Veterinary Parasitology 68, 309–314.

Seaton G., Haley C.S., Knott S.A., Kearsey M., Visscher P.M. 2002. QTL Express: mapping quantitative trait loci in simple and complex pedigrees. Bioinformatics 18, 339–340.

8

Immunology and assessment of resistance to fasciolosis in small ruminants

D. Piedrafita, E. Estuningsih, T.W. Spithill, J. Pleasance, S. Widjajanti, Suharyanta, S. Partoutomo, N. Kingsford, D. Hansen, D. Clery, R. Prowse, E.N.T. Meeusen and H.W. Raadsma

Immunology and assessment of resistance to fasciolosis in small ruminants

8

D. Piedrafita, E. Estuningsih, T.W. Spithill, J. Pleasance, S. Widjajanti, Suharyanta, S. Partoutomo, N. Kingsford, D. Hansen, D. Clery, R. Prowse, E.N.T. Meeusen and H.W. Raadsma

Introduction

Studies on the immune responses of sheep to *Fasciola gigantica* have proceeded in parallel with studies on the genetics of resistance. This is because:

- sheep with high levels of resistance to infection (presumably genetic but little is known of the genes responsible) offer the best opportunity to compare resistant with susceptible groups of animals
- a better understanding of the mechanism underpinning resistance will lead to a more targeted approach to finding useful genes and their markers.

At the initiation of the project the following observations were made on parasite resistance in the Indonesian thin tail (ITT) sheep breed.

- ITT sheep were shown to be an unusual breed in that they are able to kill the migrating parasites of *F. gigantica*.
- Cortisol administration suppressed resistance in ITT sheep suggesting an immune response was necessary but the mechanism was unknown.
- Immune suppression does not affect resistance after four weeks infection suggesting killing of the immature migrating stage by an immunological mechanism occurs within 4–6 weeks of infection.
- Studies in ITT sheep show little liver damage occurs after infection which suggests many migrating flukes may not get to the liver.

These observations suggest that, within the first 4–6 weeks of infection, parasites are likely to be killed within the gut wall and/or peritoneum. They also suggest the recently excysted immature juvenile parasite is the primary target of the effective immune response in ITT sheep. These observations are analogous to those seen in the resistant host, the rat, to *Fasciola hepatica* infection. In this model resistance is immunological and occurs at the gut wall and peritoneal cavity (Hughes 1987). These sites are also important in the resistance to other helminth parasites, and the findings suggest the gut wall and peritoneal cavity are important sites in mediating killing of helminth parasites. We therefore decided to study the peritoneal cavity for immune mediators correlating with resistance in ITT sheep.

With this strategy in mind, it is worth noting two other observations on *Fasciola* infections:

- merino sheep are not resistant to infection with *F. gigantica* (Roberts et al. 1997)
- ITT sheep are not resistant to *F. hepatica* infection (Roberts et al. 1997).

Thus, by combining these observations with those cited earlier we have rigorous criteria that must be met when determining whether a predicted effector pathway of ITT sheep has the potential to play a critical role in the resistance of ITT sheep to *F. gigantica* infection. These are:

- a demonstration that the immature *F. gigantica* parasite can be killed by the end product of this putative immunological parasite resistance pathway in ITT sheep
- that this putative immunological resistance pathway is not as effective at mediating death of immature *F. gigantica* parasites in the susceptible merino sheep
- that this putative immunological resistance pathway in ITT sheep is ineffective in mediating death of immature *F. hepatica* parasites.

The immune responses against invading *F. gigantica* parasites within the peritoneum is likely to involve complicated processes with numerous pathways and effector mechanisms (Figure 8.1). With such a complicated system, we needed to develop strategies to focus on key end-points (or 'bottlenecks') of these pathways mediating parasite death in the peritoneum. One such strategy we have used is to focus on which cellular responses are occurring when the parasite is being killed. This strategy is based on the finding from the literature that there is a relatively finite number of immune effector cells that can kill large extracellular parasites. By investigating their role and the toxic molecules produced that can kill the parasite, we will then be able to determine the central pathway(s) directing the protective immune response(s) and identify the underlying resistance mechanism in ITT sheep. This will then allow candidate genes that control these pathways to be identified and, hence, the genetic basis of resistance in the ITT sheep.

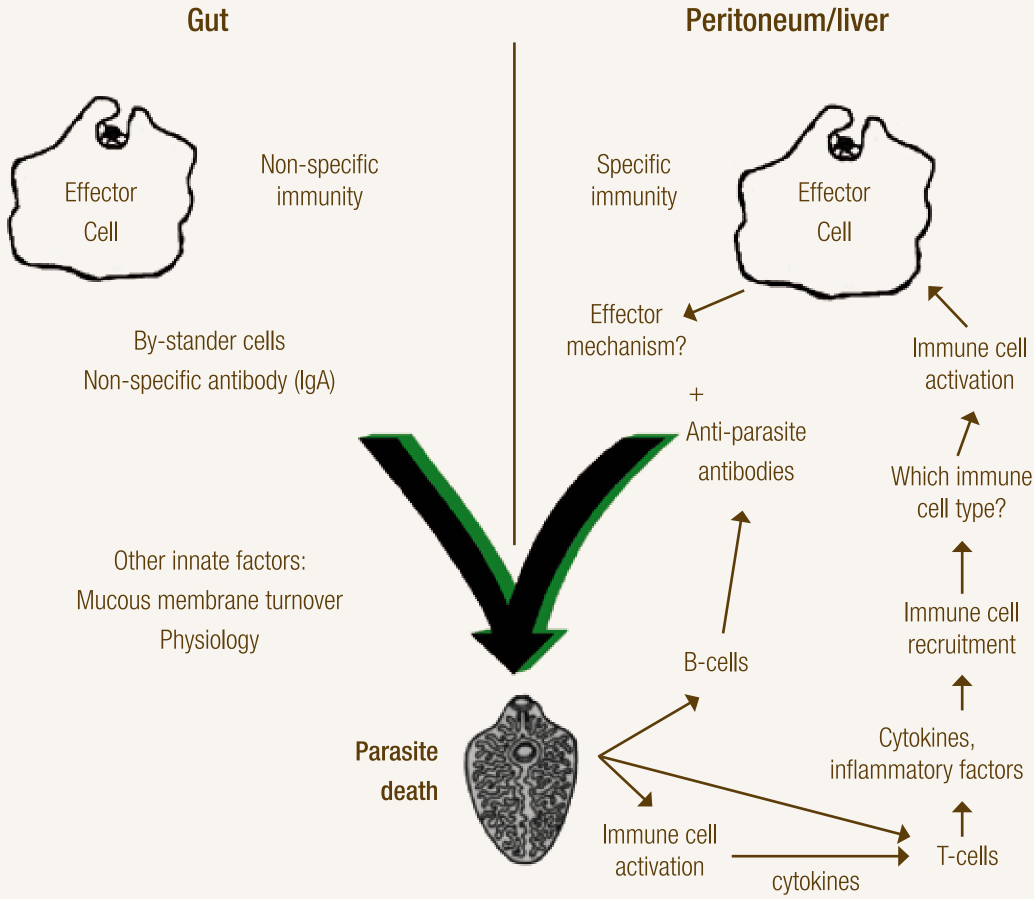


Figure 8.1 Current evidence suggests that the protective responses employed by some hosts to kill incoming *Fasciola* parasites involve two basic mechanisms. The first is at the gut wall where innate factors such as non-specific and hypersensitivity reactions of resident effector cells, natural antibodies and mucus may limit the number of parasites penetrating the gut wall after initial infection. The second involves the induction of T-cell-dependent adaptive immune responses, following parasite migration in the peritoneum and liver. This leads to parasites being killed by parasite-specific antibodies and as yet unidentified effector molecules from activated immune cells killing in these body compartments. In addition, once elicited, components of adaptive immunity may cooperate with the innate response mechanisms to limit infection and kill parasites at the level of the gut (reviewed in Spithill et al. 1999).

In vitro studies

A method to recover juvenile liver fluke from their cysts

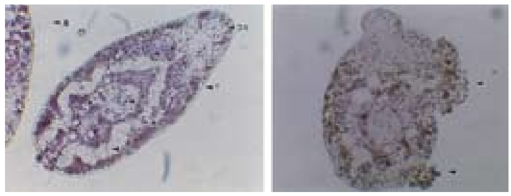
To study how the parasites are killed they need to be removed from the cyst which usually only occurs inside the animal. Newly encysted juvenile (NEJ) parasites were recovered from the cyst by artificially using chemicals to activate the excystment procedure, in effect making them think they were inside the animal. This procedure is outlined below.

Procedure for the excystment of *Fasciola gigantica* metacercariae:

1. Metacercariae were suspended in 10 mL of distilled water (dH_2O) in the presence of 1% pepsin (from porcine stomach mucosa, grade I, Sigma, USA), 0.2% HCl (BDH Chemicals, England) and incubated for 45 minutes at 37°C.
2. The metacercariae were centrifuged in a Beckman GS-6R centrifuge for one minute at 100–300 rpm and washed with dH_2O. This washing process was repeated. Metacercariae were resuspended in 10 mL of: 0.2 mM sodium hydrosulphite, 1% (w/v) $NaHCO_3$, 0.8% (w/v) NaCl, 0.2% taurocholic acid (Sigma, USA), then 0.5% HCL (50 µL) was added to the tube to generate carbon dioxide. The tube was quickly closed, mixed and sealed with parafilm around the lid and incubated for 1.5 hours at 37°C.
3. Metacercariae were then washed twice, as described above, and resuspended in 10 mL of RPMI cell culture medium (Sigma, USA) and finally suspended in 2 mL of RPMI containing 10% foetal calf serum. RPMI contained 2 µg/mL Amphotericin B (Squibb & Sons Inc., USA), 10 µg/mL Gentamycin (Sigma, USA).
4. Newly excysted juveniles, empty cyst walls, unexcysted metacercariae and debris, were transferred to an 'excystment tower' within a 24-well plate (Greiner Labortechniks, Austria) and incubated at 37°C for 1–3 hours. NEJ, which migrated through the mesh separating the excystment tower in two compartments, were collected and used in experiments.

A method for the detection of liver fluke damage and killing

A method was developed to detect parasite damage based on the ability of juvenile NEJ parasites to reduce the yellow tetrazolium dye, MTT, into a purple insoluble compound in the parasite tissues (Figure 8.2).



(a) (b)

Figure 8.2 Healthy viable juvenile flukes were able to reduce MTT into purple/blue formazan granules throughout their tissues, had clearly defined structures and were very motile (a). Non-viable flukes had either poor internal structures or lysis of the outer tegument, were unable to reduce MTT (hence no purple colour) and were not motile (b)

Killing of NEJ *Fasciola gigantica* in vitro by peritoneal lavage cells and immune sera from ITT sheep

Peritoneal lavage cells (PLCs) collected from four-week *F. gigantica*-infected ITT sheep consisted of 40–50% monocyte/macrophages, 40–50% eosinophils, 2–20% lymphocytes and less than 5% neutrophils (Figure 8.3). When NEJ of *F .gigantica* were incubated with these cells and sera from *F. gigantica*-infected ITT sheep many cells adhered to the NEJ liver fluke tegument and this resulted in a mean killing of 60% of NEJ *F. gigantica* (Figure 8.4). Killing of NEJ *F. gigantica* in vitro required both immune sera and PLCs. When NEJ *F. gigantica* were incubated in the absence of sera, or with sera from *F. gigantica*-naïve ITT sheep, a mean 85% of parasites were viable (Figure 8.4). The viability of NEJ liver fluke was also not affected by incubation with sera from *F. gigantica*-infected ITT sheep in the absence of PLCs (> 80% viable NEJ; Figure 8.4).

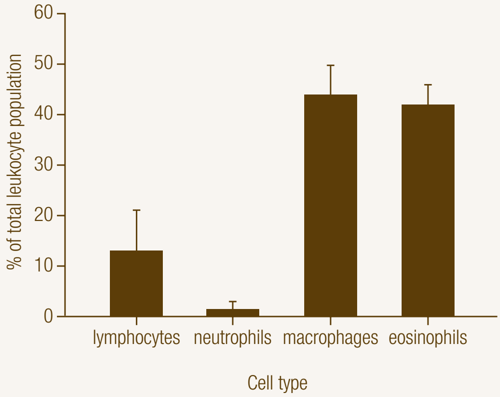


Figure 8.3 Differential cell counts of peritoneal lavage cells from *Fasciola gigantica*-infected ITT sheep. Cytospin preparations were stained using the Diff Quik® system and each cell type identified and expressed as a percentage of the total number of leukocytes counted. Values represent the mean ± SD from 10 ITT sheep.

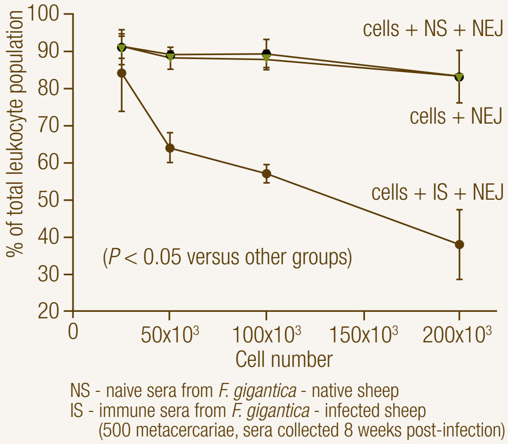


Figure 8.4. Effect of increasing numbers of peritoneal lavage cells from four-week *Fasciola gigantica*-infected ITT sheep on the killing of newly excysted juvenile (NEJ) *F. gigantica*. Each of three replicate wells containing 20–30 NEJ and cells (E:T ratio of 0.25–2 x 10^5 cells:1 NEJ) in 1 mL of media were incubated for three days and the viability of the NEJ was then determined. Results are the mean ± SD of three experiments. The means of the 'cells + IS + NEJ' group were significantly different by the Dunnett's multiple comparison test at $P < 0.05$.

Killing of NEJ *Fasciola gigantica* in vitro by PLCs requires direct cell contact

These results suggested that the mechanism of cytotoxicity to NEJ liver fluke by ITT sheep PLCs, when incubated with sera from *F. gigantica*-infected ITT sheep, necessitated intimate contact between the effector cells and the NEJ liver fluke tegument, which was mediated by the inclusion of sera from *F. gigantica*-infected ITT sheep. To test this possibility, NEJ *F. gigantica* were incubated with sera from *F. gigantica*-infected ITT sheep in wells with tissue culture inserts in which the PLCs were separated from the NEJ by a 0.45 µm filter to inhibit direct contact between NEJ liver fluke and PLCs. Separation of NEJ liver fluke from PLCs, in the presence of sera from *F. gigantica*-infected ITT sheep, resulted in a mean of 80% viable parasites, when compared with a mean of only 20% viable parasites in incubations performed in the absence of tissue culture inserts (Figure 8.5). This result suggests that killing of NEJ *F. gigantica* by ITT PLCs requires intimate contact between the PLCs and the fluke tegument.

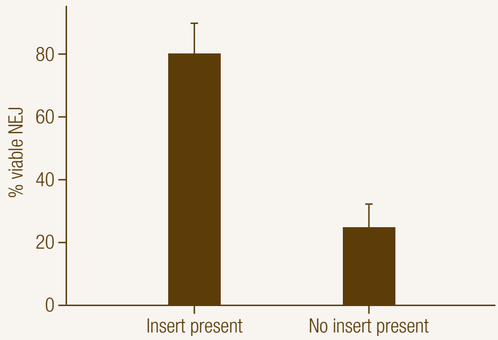


Figure 8.5 Effect of physical separation of newly excysted juvenile (NEJ) *Fasciola gigantica* and peritoneal lavage cells of *Fasciola gigantica*-infected ITT sheep on the subsequent killing of NEJ liver fluke. Each of three replicate wells containing 25–50 NEJ parasites were placed in 24-well tissue culture plates with sera from *F. gigantica*-infected ITT sheep. In those incubation wells containing the insert, the peritoneal cells were placed inside the insert at an E:T ratio of 2x10^5 cells:1 NEJ; the sheep peritoneal cells were thus separated from the NEJ parasites by a 0.45 µm membrane. After being incubated for three days, the viability of the NEJ parasites was determined. Results are from three experiments.

Identification of the cytotoxic mediator of parasite killing in vitro produced by PLCs

Cytotoxic molecules released by macrophages and eosinophils include reactive nitrogen (nitric oxide) and oxygen (superoxide, hydrogen peroxide) intermediates. Inhibition of nitric oxide production by L-NMMA did not reverse the cytotoxic effects of ITT sheep PLCs as expected (data not shown), since we have previously shown that lavage cells of ITT sheep (and other sheep breeds) do not generate detectable levels of nitric oxide under our incubation conditions in vitro. However, adding the inhibitor of superoxide radicals, superoxide dismutase (SOD), significantly reduced the killing of NEJ *F. gigantica* from a mean 65% killing to a mean 20% killing (Figure 8.6). Superoxide radicals can give rise to other reactive oxygen species including hydrogen peroxide and hydroxyl radicals. However, inhibitors of hydrogen peroxide (catalase) and the hydroxyl radical scavenger (mannitol) had no significant effect on the mean killing of NEJ *F. gigantica* incubated with ITT sheep PLCs and sera from *F. gigantica*-infected ITT sheep alone (Figure 8.6). This superoxide-mediated cytotoxicity appeared to exert its effects early in the incubation period with ITT PLCs and immune sera, as indicated by a reduction in mean parasite motility within 24 hours when compared to incubations with the addition of SOD (Figure 8.7).

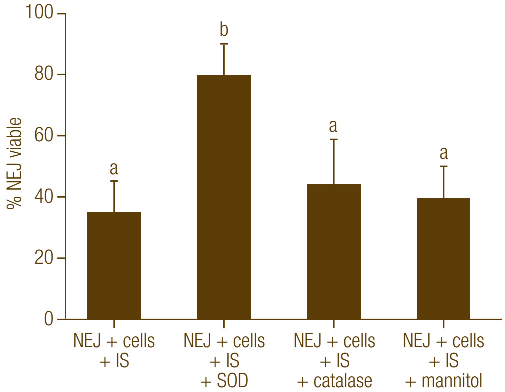


Figure 8.6 Effect of adding exogenous superoxide dismutase (SOD), catalase or mannitol to culture incubations on the viability of newly excysted juvenile (NEJ) *Fasciola gigantica* by immune sera (IS) and peritoneal lavage cells from *F. gigantica*-infected ITT sheep. Each of three replicate wells containing 20–30 NEJ and cells (E:T ratio of 2 x 10^5 cells:1 NEJ) in 1 mL of media were incubated for three days with or without exogenous SOD and the viability of the NEJ parasites was then determined. Results are the mean ± SD of three experiments. For each incubation, mean values with the same superscript (a or b) could not be significantly differentiated by the Dunnett's multiple comparison test at $P < 0.05$.

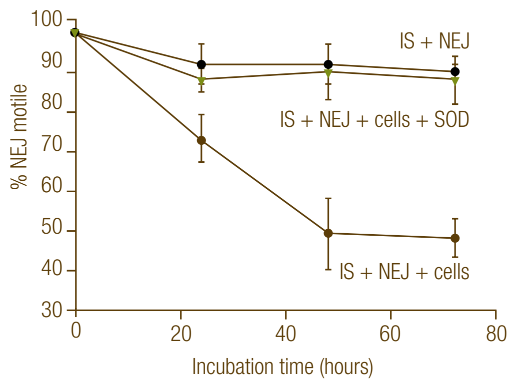


Figure 8.7 Effect of adding exogenous superoxide dismutase (SOD) to culture incubations on the motility of newly excysted juvenile (NEJ) *Fasciola gigantica* in the presence of immune sera (IS) and peritoneal lavage cells from *F. gigantica*-infected ITT sheep. Each of three replicate wells containing 20–30 NEJ and cells (E:T ratio of 2 x 10^5 cells:1 NEJ) in 1 mL of media were incubated for three days with or without exogenous SOD and the motility of the NEJ liver fluke was determined over a 72-hour period. Results are the mean ± SD of three experiments. The means of the 'IS + NEJ + cells' group were significantly different by the Dunnett's multiple comparison test at $P < 0.05$.

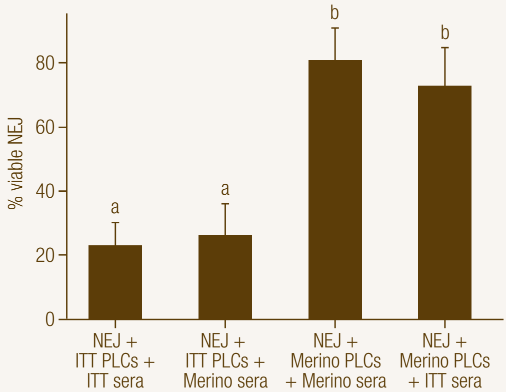


Figure 8.8 Effect of ITT or merino peritoneal cells on the killing of *Fasciola gigantica* juvenile parasites incubated with immune sera. Effect of increasing numbers of peritoneal lavage cells from four-week *F. gigantica*-infected ITT or merino sheep on the killing of newly excysted juvenile (NEJ) *F. gigantica.* Each of three replicate wells containing 20–30 NEJ and cells (E:T ratio of 0.25–2 x 10^5 cells:1 NEJ) in 1 mL of media were incubated for three days and the viability of the NEJ was then determined. Results are the mean ± SD of three experiments.

Comparative parasite killing in vitro produced by merino and ITT PLCs

In comparison with merino peritoneal cells, ITT peritoneal cells are effective in killing juvenile *F. gigantica* parasites. These results demonstrate that ITT peritoneal cells are able to kill juvenile fluke more effectively than peritoneal cells of merino sheep irrespective of the sera source (Figure 8.8).

Identification of effector cells in ITT PLCs mediating cytotoxicity in vitro

Our in vitro cytotoxic assays used PLCs that consisted of two major immune cell types, monocyte/macrophages and eosinophils, which have been shown to have important roles in helminth parasite killing in other animal models. We therefore obtained cell populations enriched for macrophages or eosinophils to test whether each cell type was capable of mediating superoxide-dependent cytotoxicity to NEJ *F. gigantica.* Resident peritoneal cell populations from *F. gigantica*-naïve ITT sheep contained greater than 90% monocyte/macrophages with no eosinophils present (Figure 8.9). Cell populations collected from ITT mammary glands infused with a soluble somatic *F. gigantica* lysate contained a mean 90% of eosinophils (Figure 8.9). When NEJ *F. gigantica* were incubated with either of these enriched cell populations and sera from *F. gigantica*-infected ITT sheep, a significant portion of parasites (> 70%, Figure 8.10) were killed which is comparable to the level of killing of NEJ parasites seen with the whole peritoneal cell population from *F. gigantica*-infected ITT sheep described above (Figure 8.4). The cytotoxicity mediated to NEJ *F. gigantica* by the monocyte/macrophage-rich or eosinophil-rich populations was also abrogated by the addition of SOD to inhibit superoxide radical formation (Figure 8.10).

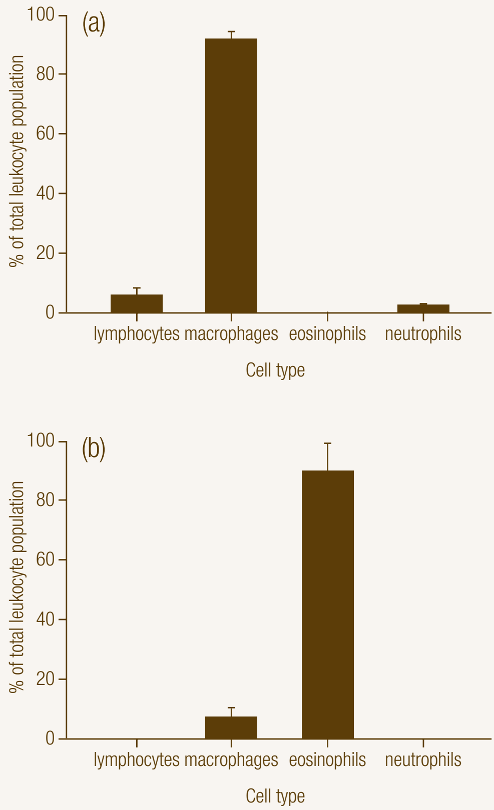


Figure 8.9 Differential cell counts of (a) peritoneal lavage cells from *Fasciola gigantica*-naïve ITT sheep and (b) mammary-elicited lavage cell samples from *F. gigantica*-infected ITT sheep. Cytospin preparations were stained using the Diff Quik® system and each cell type identified and expressed as a percentage of the total number of leukocytes counted. Values represent the mean ± SD from three separate animals.

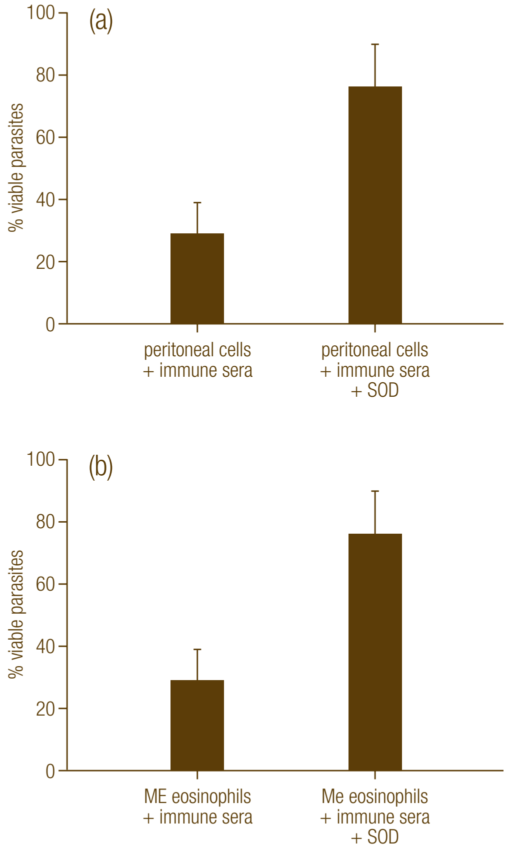


Figure 8.10 Effect of adding exogenous superoxide dismutase (SOD) to (a) culture incubations of peritoneal lavage cells of *Fasciola gigantica*-naive ITT sheep and (b) mammary elicited (ME) lavage cells from *F. gigantica*-infected ITT sheep on the subsequent killing of newly excysted juvenile (NEJ) *F. gigantica* by ITT immune sera. Each of three replicate wells containing 20–30 NEJ *F. gigantica* and cells (E:T ratio of 2 x 10^5 cells:1 NEJ) in 1 mL of media were incubated for 3 days with or without exogenous superoxide dismutase or immune sera and the viability of the NEJ liver fluke was then determined. Results are the mean ± SD of three experiments.

ITT PLCs do not kill NEJ *Fasciola hepatica* in vitro

Previous work had shown that ITT sheep are susceptible to infection with the temperate liver fluke *F. hepatica* (Roberts et al. 1997). Interestingly, we have also shown that NEJ *F. hepatica* is highly resistant to oxygen free radical-mediated killing in vitro (Piedrafita et al. 2000; 2001). These observations suggested that *F. hepatica* is resistant to the ITT effector mechanism(s) that is effective against *F. gigantica*. To see if there are inherent differences between the susceptibility of *F. hepatica* and *F. gigantica* to killing by ITT effector mechanisms, we directly compared the killing of NEJ *F. hepatica* and NEJ *F. gigantica* in vitro by incubating PLCs isolated from the same *F. gigantica*-naïve ITT sheep with each parasite in the presence of homologous immune sera. Only NEJ of *F. gigantica* were susceptible to killing by PLCs of *F. gigantica*-naïve ITT sheep: no cytotoxic effect was observed against NEJ *F. hepatica* (Figure 8.11). Extended incubations (10 days) of PLCs with NEJ of *F. hepatica* did not result in an increase in parasite killing (data not shown). Incubations of NEJ of *F. hepatica* or *F. gigantica* with heterologous sera from *F. gigantica*-infected animals or *F. hepatica*-infected animals, respectively, also resulted in killing of *F. gigantica* NEJ but not *F. hepatica* NEJ (Table 8.1).

Table 8.1 Comparative susceptibility of newly excysted juvenile (NEJ) *Fasciola gigantica* (Fg) and *Fasciola hepatica* (Fh) incubated with heterologous *Fasciola*-immune sera (IS) to killing by peritoneal lavage cells of *F. gigantica*-naive ITT sheep. Each of three replicate wells containing 20–30 NEJ *F. gigantica* or *F. hepatica* and cells (E:T ratio of 2 x 10^5 cells:1 NEJ) in 1 mL of media were incubated for three days with heterologous immune sera and the viability of the NEJ liver fluke was then determined at 72 hours. Results are the mean ± SD of three experiments.

Incubation	% viable NEJ	
	F. gigantica	*F. hepatica*
NEJ liver fluke + immune sera	80 ± 7	87 ± 10
NEJ liver fluke + cells + immune sera	42 ± 10	90 ± 7

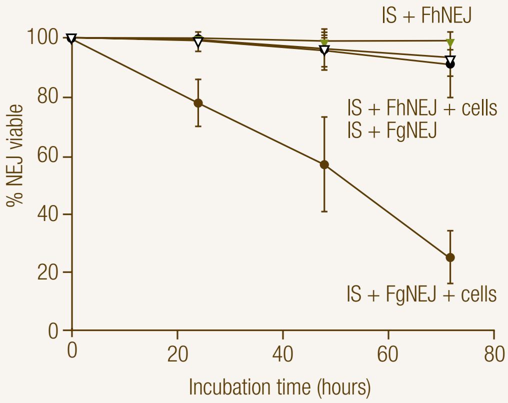


Figure. 8.11 Comparative susceptibility of newly excysted juvenile (NEJ) *Fasciola gigantica* (Fg) and *Fasciola hepatica* (Fh) incubated with homologous *Fasciola*-immune sera (IS) to killing by peritoneal lavage cells of *F. gigantica*-naive ITT sheep. Each of 10 replicate wells containing 4 NEJ and cells (E:T ratio of 2 x 10^5 cells:1 NEJ) in 0.2 mL of media were incubated for 3 days and the viability of the NEJ liver fluke was then determined at 24, 48 and 72 hours. Results are the mean ± SD of 5 experiments. The means of the 'IS + FgNEJ + cells' group were significantly different by the Dunnett's multiple comparison test at $P < 0.05$, to incubations with IS + FgNEJ.

Antioxidant defence enzyme levels in somatic extracts of NEJ and adult *Fasciola*

The dramatic difference in susceptibility to killing by superoxide exhibited by NEJ of *F. gigantica* and *F. hepatica* could result from differences in the specific activity of defence enzymes such as SOD, catalase, glutathione *S*- transferase (GST) or glutathione peroxidase (GSH-Px). We therefore compared the levels of some defence enzymes in whole worm somatic extracts (WWE) of NEJ *F. hepatica* and *F. gigantica*. For comparison, enzyme levels were also determined in extracts of adult flukes. To ensure that meaningful comparisons could be obtained, WWEs were prepared from 5000 NEJ of *F. hepatica* and *F. gigantica* excysted on the same day and the assays for activity were performed immediately under identical conditions. Adult *F. hepatica* WWE was prepared from parasites collected from cattle whereas adult *F. gigantica* WWE was prepared from parasites collected from ITT sheep: in both cases, parasites were snap frozen on dry ice and WWE were subsequently prepared and assayed under identical conditions. Due to the limited amount of sample, only GST and SOD activity levels were measured in NEJ WWE, not GSH-Px or catalase, as we had shown hydrogen peroxide was not mediating killing of the parasite and GST is a general defence enzyme that acts against most species of free radicals.

GST and SOD activity was detected in WWE from two separate batches of NEJ of each liver fluke species (Table 8.2). GST expression levels were similar in the two parasites. The mean SOD activity was approximately 33% greater in WWE of NEJ *F. hepatica* than in NEJ *F. gigantica.* GST, SOD and GSH-Px activity were measured in adult *F. hepatica* and *F. gigantica* (Table 8.2). Adult WWE of *F. hepatica* had significantly higher levels ($P < 0.001$) of both GST and GSH-Px compared with adult WWE of *F. gigantica* whereas similar SOD levels were measured in WWE of both *Fasciola* spp. Catalase was not detected in either *Fasciola* spp. WWE was as observed previously in WWE of *F. hepatica* (Piedrafita *et al.* 2000).

Discussion and conclusions from in vitro studies

To study potential mechanisms in the peritoneum of ITT sheep that could kill the migrating stage of the parasite in vitro, we initially needed a way to access immature parasites and then to detect death of these parasites after they were incubated with our effector cells. We first developed a system to excyst viable immature parasites from the infective cyst (metacercariae) in vitro and proceeded to develop a colorimetric system to detect damage to these parasites (MTT-reduction assay). Initial experiments first identified two major immune cell types within the peritoneum during the early migration of *F. gigantica* parasites in ITT sheep: macrophages and eosinophils. These two immune cells have been shown to be pivotally involved in the killing of helminth parasites by releasing cytotoxic molecules onto the surface of parasites. We were then able to demonstrate these immune cells from the ITT host could indeed effectively kill immature parasites of *F. gigantica* but this ability was critically dependent on the presence of sera from *F. gigantica*-infected ITT sheep. We subsequently investigated the dependence on sera from *F. gigantica*-infected ITT sheep in mediating this effective killing and demonstrated the mechanism

Table 8.2 Antioxidant defence enzyme levels in whole worm extracts of adult and newly excysted juvenile (NEJ) *Fasciola hepatica* and *Fasciola gigantica*. Superoxide dismutase activity is expressed as U/mg protein. Significant differences ($P <$ 001) for anti-oxidant defence enzyme activities between adult *F. hepatica* and *F. gigantica* WWE were calculated using the unpaired alternative t-test. Significantly higher levels of GST and GSH-Px were present in adult *F. hepatica* WWE than in adult *F. gigantica* WWE.

Enzyme activity (nmol/min/mg protein)	NEJ parasite[a]		Adult parasite[b]	
	F. gigantica	*F. hepatica*	*F. gigantica*	*F. hepatica*
SOD	33, 32	44, 46	43 ± 8	43 ± 9
GST	512, 491	486, 503	2924 ± 616	9165 ± 1247
GSH-Px	na	na	294 ± 8	535 ± 39
CAT	na	na	bd	bd

SOD, superoxide dismutase; GST, glutathione *S*-transferase; GSH-Px, glutathione peroxidase; CAT, catalase; bd, below detectable limit of assay; na, not assayed.

[a] Values represent the mean enzyme activity of three determinations from each of two separate preparations of 5000 NEJ.

[b] Values represent the mean ± SD from separate preparations of 20–50 parasites collected from three donor sheep infected with 250 metacercariae (*F. gigantica*) or 20–50 parasites collected from three infected cattle at the abattoir (*F. hepatica*).

was likely to be dependent on the anti-*F. gigantica* antibodies binding the effector cells to the parasite tegument. Next we investigated the potential effector molecules, which might be mediating this cytotoxic mechanism. By using a series of inhibitors we were able to determine the primary cytotoxic molecules produced by these cells were superoxide radicals. To fulfil our second criterion and indeed confirm this cytotoxic mechanism could play a pivotal role in the putative immunological parasite resistance pathway in ITT sheep, we directly compared the ability of merino macrophages and eosinophils from the peritoneum to mediate killing of *F. gigantica* parasites in vitro. We demonstrated that merino peritoneal cells were not as effective at mediating killing of juvenile *F. gigantica* parasites. Our final criterion suggested this effector mechanism of ITT sheep should be ineffective in mediating killing of immature *F. hepatica* parasites. This was indeed the case and we demonstrated *F. hepatica* immature parasites were not susceptible to superoxide-dependent cytotoxicity. In support of this observation, a key defence enzyme (SOD) against superoxide radicals was significantly higher in juvenile *F. hepatica* when compared to *F. gigantica* parasites and may explain why juvenile fluke of *F. gigantica* are more effectively killed by peritoneal cells of ITT sheep than those of *F. hepatica*.

In summary, our in vitro studies have demonstrated the effector cells mediating killing of *F. gigantica* immature parasites include ITT macrophages and eosinophils and the molecular mechanism of killing involves superoxide radicals. We then investigated whether our in vivo studies to date would support our hypothesis of this resistance mechanism identified in vitro.

In vivo studies

1. Differential eosinophil and neutrophil counts in ITT and merino sheep after a primary infection with *Fasciola gigantica*

The proportions of blood eosinophils and neutrophils were determined in ITT and merino sheep at different times post infection with *F. gigantica*. Experiments indicated that the proportion of eosinophils in blood of merino sheep started to increase at day eight post infection, reaching a plateau by day 36 post infection. ITT sheep showed a significantly higher percentage of eosinophils than the merino sheep at days 8, 14 and 25 post infection (Figure 8.12a). The relative level of blood neutrophils decreased from day 8 to day 14 in both breeds of sheep (Figure 8.12b). At day 36 post infection, the percentage of neutrophils in blood started to

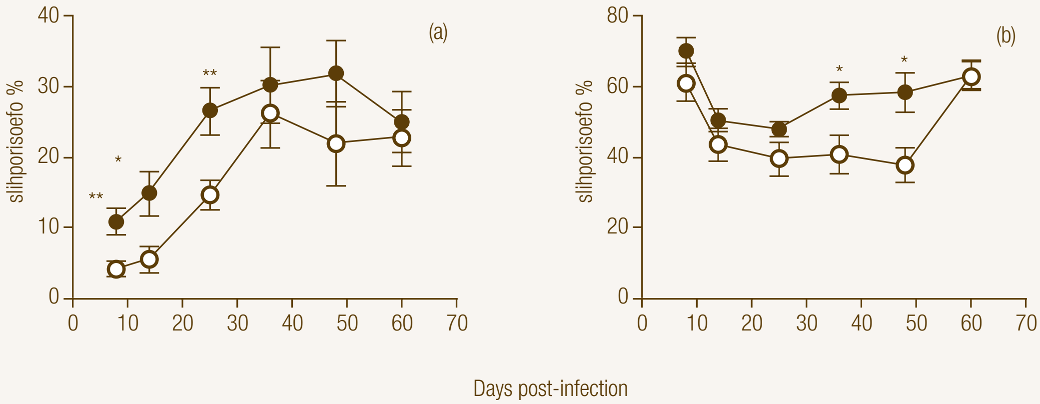


Figure 8.12 Percentage of eosinophils (A) and neutrophils (B) in blood of Medan ITT (●) and merino (**O**) sheep infected with *F. gigantica.* Blood samples were collected at different times post infection and the differential cell counts were performed on Giemsa-stained blood smears. Each point represents the mean percentage + S.E of 12 (ITT) and 10 (merino) samples tested. * $P < 0.023$, ** $P < 0.013$ between the two groups.

increase in ITT sheep, becoming significantly higher than observed in merino sheep at days 36 and 48 (Figure 8.12b).

2. ITT sheep exhibit differences to merino sheep in antibody levels against *Fasciola gigantica*

The Ig subclass antibody responses were measured by ELISA in sera from Medan ITT and merino sheep infected with *F. gigantica*. The levels of specific IgM and IgG_1 were nearly three-fold higher in ITT than in merino sheep (Figure 8.13a–b). IgE antibodies showed a biphasic response, characterised by a first peak of antibodies 14 days post infection, followed by a second peak of a greater magnitude between days 30 to 65 (Figure 8.13d). The first IgE response was higher in Medan ITT than in merino sheep and the second one was of the same level in the two breeds of sheep although it appeared earlier in merinos (Figure 8.13d). merino sheep showed a high IgG_2 antibody response, which starts to develop soon after infection and reaches a peak by day 30 (Figure 8.13c). This IgG_2 response was absent in Medan ITT sheep.

3. Measurement of cytokine production in ITT and merino sheep infected with *Fasciola gigantica*

To study the cytokine changes in gene expression levels during infection we first had to design primers to amplify the cytokine genes as these were not available when we did these studies. Fifteen cytokines in ITT RNA were identified by reverse transcriptase PCR (RT PCR, Table 8.3). Having established the cytokine primers to use we then looked at the expression levels of IL-2, IL-12, TNFα, IFNγ (as indicators of a Th1 responses) and IL-4 and IL-5 (as indicators of Th2 responses) in the hepatic lymph nodes of ITT and merino sheep at 0, 3, 6 and 10 weeks post infection.

Expression levels of each target gene were quantified using the relative quantification method and expressed relative to the tissue-specific calibrator cDNA. All samples from the same tissue origin were amplified in the same experimental run for each target gene and converted to a comparative quantification (calibrator) experiment type (Stratagene) for analysis. Tissue-specific calibrator cDNA was run in quadruplicate on each plate and used to normalise for the different annealing temperatures optimised for each oligonucleotide pair, PCR efficiencies and experimental variation.

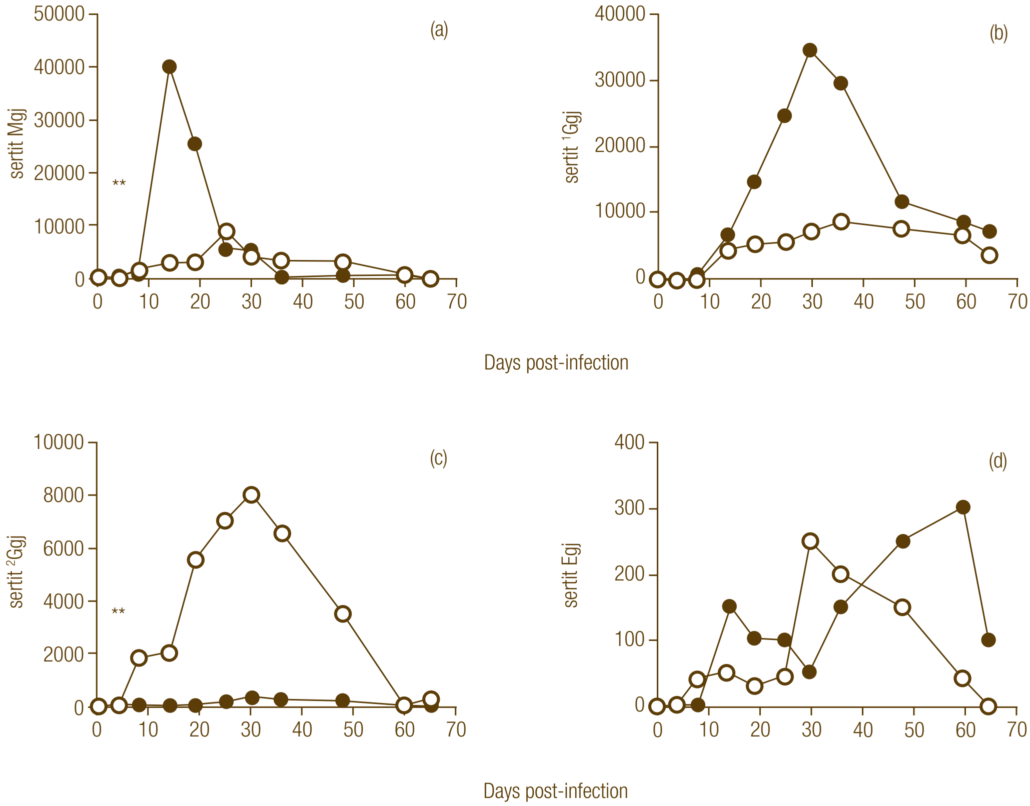


Figure 8.13 The isotype of the antibody responses in sera from Sumatra ITT (●) and merino (○) sheep infected with *Fasciola gigantica.* IgM (A), IgG1 (B), IgG2 (C) and IgE (D) titres were determined by ELISA at different time points post infection. Each point represents the antibody titre of a pool of 12 sera for ITT and of 10 sera for merino sheep.

Ct values were expressed as a value relative to the mean Ct value obtained for the calibrator (given an arbitrary value of 100). Relative values were obtained for all target genes and two housekeeping genes, β-actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). GAPDH and β-actin were both considered to be appropriate internal controls for this study as their Ct values for all samples analysed were within five cycles and analysis of the target genes normalised to either GAPDH or β-actin produced similar results. Results of this study represent target gene expressions normalised to β-actin. In addition, a ratio of IL-4 / IFN-γ mRNA expression relative to the calibrator was calculated using a similar method.

β-actin and GAPDH are genes constitutively expressed in ovine cells. These genes were used as positive controls for the RT-PCR reactions. Having established the cytokine primers to use we then looked at the expression levels of IL-2, IL-12, TNFα, IFNγ (as indicators of a Th1 responses) and IL-4 and IL-5 (as indicators of Th2 responses) in the hepatic lymph nodes of ITT and merino sheep at 0, 3, 6 and 10 weeks post infection. High IL-10 expression usually signifies down-regulated Th1 responses. Although the cytokine gene expression responses did vary over the course of the infection there were no clear differences between the *F. gigantica* infected ITT and merino sheep suggesting a mixed Th1 and Th2 response was elicited (Figure 8.14).

Discussion and conclusions from in vivo studies

Two critical observations from our in vivo studies were made. First, eosinophils were significantly elevated at the time of *F. gigantica* parasite killing in the ITT host relative to the merino host and, second,

Table 8.3 Summary of ovine PCR products for different cytokines detected from various RNA sources in the ITT sheep. ND, not determined; LN, lymphocytes isolated from lymph nodes; Macro, macrophage cultures.

Primer (ovine/ bovine)	Ovine LN	Ovine Macro	40°C		50°C		60°C	
			Lymph	Macro	Lymph	Macro	Lymph	Macro
β-actin	Yes	Yes	–	–	++	++	+++	+++
GAPDH	Yes	Yes	ND	ND	+++	+++	ND	ND
IL1α	Yes	Yes	ND	ND	++	+++	ND	ND
IL1β	Yes	Yes	ND	ND	++	+++	ND	ND
IL2	Yes	–	ND	ND	+++	+	ND	ND
IL3	No	No	–	–	–	–	ND	ND
IL4	Yes	–	ND	ND	+++	–	–	–
IL5	Yes	–	–	–	–	++	ND	ND
IL6	Yes	Yes	–	++	–	++	++	–
IL7	Yes	Yes	–	–	–	–	+	+
IL8	Yes	Yes	++	+++	++	–	–	–
IL10	–	Yes	ND	ND	+	+++	ND	ND
IFNγ	–	Yes	+	–	–	+++	–	–
TNFα	Yes	Yes	–	+++	–	+++	+++	+++
GMCSF	–	Yes	ND	ND	++	+++	ND	ND
GCSF	Possibly	–	–	–	–	–	+	–
IL12P35	–	Possibly	–	+	–	–	–	–
IL12P40	Possibly	–	+	–	–	–	+	–

– denotes no band
\+ denotes band of weak intensity
++ denotes band of moderate intensity
+++ denotes band of strong intensity

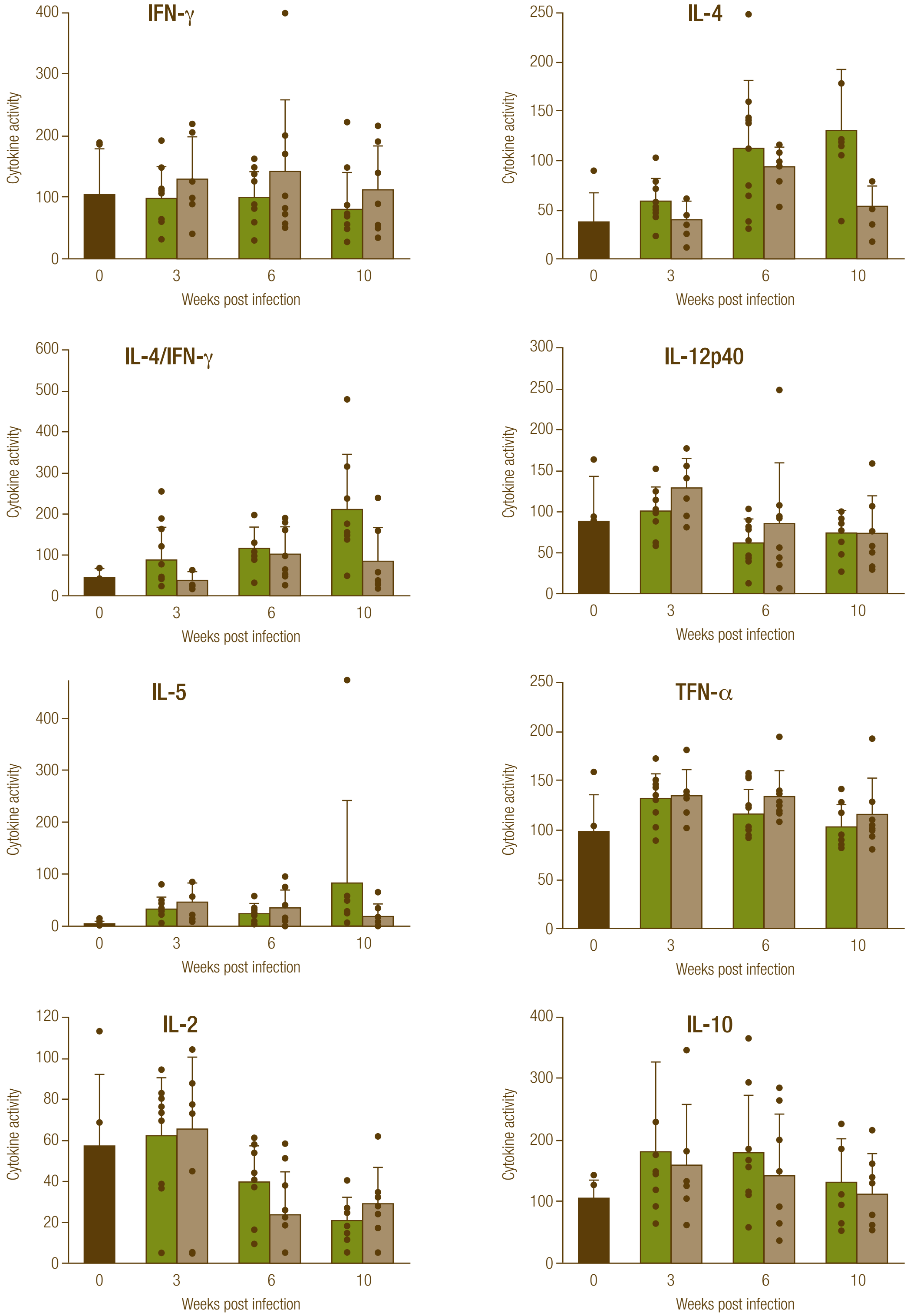


Figure 8.14 Mean cytokine responses to *Fasciola gigantica* infection in the hepatic lymph nodes of ITT (pale brown) and merino (green) sheep. Individual assay results are indicated by the six dots associated with each column. Nb: cytokine activity is relative to the housekeeping gene β-actin.

IgG_2 responses correlated with susceptibility to infection.

The production of IgG_2 has been shown to be strongly up-regulated by IFN-γ in mice, humans and cattle (quoted in Hansen et al. 1999). It is also likely the elevated IgG_2 levels in the merino sheep associated with *F. gigantica* susceptibility are related to elevated IFN-γ levels. This observation is intriguing given our observation in the merino sheep and others (Bielefeldt Ohmann and Babiuk 1984), that IFN-γ inhibited superoxide production. Thus, the immunological pathways, which would inhibit our observed effector mechanism in the ITT sheep, appear up-regulated in the susceptible merino host. This cytokine, produced mainly by NK and T-helper type 1 (Th1) cells, enhances phagocytosis and tumour-killing activities and inhibits B-cell responses, including IgG and IgE production by counteracting most of the activities mediated by the T-helper type 2 (Th2) cytokine, IL-4. In our experimental conditions, the low production of IgG_2 antibodies in resistant ITT sheep suggests that Th_1-like responses may be down-regulated in this breed during the infection with *F. gigantica*. When we tried to determine this by studying cytokine responses during infection at key time points we were unable to find significant differences in Th1 cytokines (IFNg; IL-12) or Th2 cytokines (IL-4;IL-5) between ITT and merino sheep. This finding suggests more detailed studies are needed to test this hypothesis. However, there was a trend for higher levels of IgM, IgG_1, IgE antibodies, and, significantly, the percentage of eosinophils in blood (a key hallmark of Th2 responses) was higher in ITT sheep than in merino sheep. Th2 clones provide help for the stimulation of all the above antibody subclasses through the production of IL-4, IL-5 and IL-6. IL4 up-regulates production of IgM, IgG_1 and IgE by bovine lymphocytes in vitro (Estes et al. 1995). Moreover, IL-5 has been implicated in eosinophil activation and shown to enhance killing of nematode larvae by sheep eosinophils (Rainbird et al. 1998). Taken together, these observations indicate that the enhanced resistance of ITT sheep to *F. gigantica* infection is concomitant with a Th2-like pattern of immune responses.

The development of an immunodiagnostic test for a phenotypic indicator of parasite burdens was attempted by the combinational analysis of key parameters measured in a group of experimental sheep challenged with *F. gigantica* and *F. hepatica*. In this study we have shown combinations of certain biochemical and immunological parameters give an accurate estimation of infection (up to 80% of the fluke burden variation can be explained by these parameters). In this regard, this study has been unique and very successful. However, the ultimate aim of this study was to develop a non-invasive parameter test for fluke burden prediction in animals of unknown parasite status that could be performed easily and routinely. In this study, the best predictive parameters required a complicated number of samples to be taken and used to predict infection rate. This would obviously be impractical in the field. However, no publication or report to date has been able to test and accurately predict infection rates in any animal model and this study demonstrates the leading-edge scientific studies of this ACIAR project.

References

Bielefeldt-Ohmann H. and Babiuk L.A. 1984. Effect of bovine recombinant alpha-1 interferon on inflammatory responses of bovine phagocytes. Journal of Interferon Research 4, 249–63.

Estes D.M., Closser N.M. and Allen G.K. 1994. IFN-[gamma] Stimulates IgG2 production from bovine B cells co-stimulated with anti-[mu] and mitogen. Cellular Immunology 154, 287–295.

Hansen D.S., Clery D.G., Estuningsih S.E., Widjajanti S., Partoutomo S., and Spithill T.W. 1999. Immune responses in Indonesian thin tail and merino sheep during a primary infection with *Fasciola gigantica*: lack of specific IgG2 antibody response is associated with increased resistance to infection in Indonesian sheep. International Journal for Parasitology 29, 1027–35.

Hughes D.L. 1987. *Fasciola* and fascioloides. Pp. 91–114 in 'Immune responses in parasitic infections: immunology, immunopathology, and immunoprophylaxis. Volume II: Trematodes and cestodes' ed. by E.J.L. Soulsby. CRC Press: Florida.

Piedrafita D., Parsons J.C., Sandeman M., Wood P.S., Estuningsih E., Partoutomo S. and Spithill T.W. 2001. Antibody-dependent cell-mediated cytotoxicity to newly excysted juvenile *Fasciola hepatica* in vitro mediated by reactive nitrogen intermediates. Parasite Immunology 23, 473–82.

Piedrafita D., Spithill T.W., Dalton J.P., Brindley P.J., Sandeman M.R., Wood P.R. and Parsons J.C. 2000. Juvenile *Fasciola hepatica* are resistant to killing in vitro by free radicals compared with larvae of *Schistosoma mansoni*. Parasite Immunology 22, 287–96.

Rainbird M.A., MacMillan D. and Meeusen E.N.T. 1998. Eosinophil-mediated killing of *Haemonchus contortus* larvae:effect of eosinophil activation and role of antibody, complement and interleukin-5. Parasite Immunology 12, 15–32.

Roberts J.A., Widjajanti S., Wiedosari E., Partoutomo S. and Spithill T.W. 1997. Resistance of Indonesian thin tail sheep against *Fasciola gigantica* and *F. hepatica*. Veterinary Parasitology 68, 69–7.

Spithill T.W., Smooker P.M. and Copeman B. 1999. *Fasciola gigantica*: epidemiology, control, immunology and molecular biology. Pp. 465–520 in ‘Fasciolosis’, ed. by J.P. Dalton. CAB International: Wallingford, UK.

Appendix

KINGDOM OF CAMBODIA
NATION RELIGION KING

Ministry of Agriculture, Forestry and Fisheries

Technology Implementation Procedure

Fasciolosis of Cattle and Buffaloes and Its Control Measures

Prepared by :

- The Department of Agricultural Extension in association with
- The Department of Animal Health and Production

Supported by :

- The Australian Centre for International Agricultural Research (ACIAR)
- The Cambodia Australia Agricultural Extension Project

Author: **Dr. SUON SOTHOEUN**
Engineer of Animal Health and Production
Ph. D in Veterinary Sciences
Department of Animal Health and Production
Phone: 012 714 682
Email: Sothoeundahp@online.com.kh

Date: January 2007

Foreword

The Ministry of Agriculture, Forestry and Fisheries (MAFF) receives funds from the Australian Agency for International Development (AusAID) for the Cambodia Australia Agricultural Extension Project II (CAAEP II – 2001 – 2007). CAAEP has the goal of strengthening the national institutional and extension systems in Cambodia through improving agricultural extension staff's capacity and agricultural extension planning in line with the Royal Government of Cambodia Decentralization & Deconcentration policy. Tools to help this process are Commune Agro-ecosystems Analysis and Technology Implementation Procedures (TIP).

TIPs are produced by local Subject Matter Specialists (SMS) and have been reviewed, and endorsed by a committee of stakeholders called the TIP Oversight Committee and by the relevant MAFF technical department. TIP terms of reference call for a manual based on *local best practice* with respect to information, technology, extension methodology, implementing procedure and cost analysis. The manuals are for farm advisers from district agricultural offices (DAO) and other development workers in the provinces using and working directly to train and transfer these technologies to Cambodia's farmers and producers who will experience increased potential in their different geographic locations in accordance with the local needs and socio-economic conditions.

TIPs are very important manuals to help provincial and district agricultural staff overcome many problems facing Cambodian farmers who are struggling to deal with a rapidly changing agricultural environment of production and diversification while at the same time ensuring food security, improving family income and reducing poverty, in line with the rectangular strategic of the Royal Government of Cambodia.

The Ministry of Agriculture, Forestry and Fisheries fully supports this series of manuals. They are the main resources for provincial and district agricultural staff, NGOs, and other development agencies and stakeholders who will spread their contents widely among Cambodia's farmers who will intern obtain the fruitful outcomes they expect.

Signed and sealed by

H.E Dr. Chan Sarun, Minister

Ministry of Agriculture, Forestry and Fisheries

Unofficial translation

Preface

The term TIP stands for 'Technology Implementation Procedure'. TIPs explain how to implement improved agricultural technologies that help to solve important problems faced by farmers. They comprise the entire package of information, procedures and materials necessary for an extension worker to pass on the technology to farmers.

The TIP concept has been developed to facilitate extension across communes, districts and provinces throughout Cambodia. TIPs are intended for use by extension workers, NGOs and others working in agricultural development at the field level. As such, TIPs must be :

- Flexible – so that they can be replicated anywhere in Cambodia
- Simple – so that they can be used by anyone working at the field level
- Comprehensive – so that they provide all necessary information

TIP development is prioritized based on key farmer problems identified from a variety of sources, most importantly the Department of Agricultural Extension's (DAE) Farming Systems Management Information System (FSMIS) database which contains information on major farmer problems identified by Commune Agro-ecosystems Analysis (CAEA) implemented across Cambodia. TIP topics are obtained from a variety of sources including:

- National research institutes
- Existing extension programs
- Innovative farmers
- Donor projects
- NGOs
- Private sector
- International research agencies

TIPs are commissioned by MAFF's DAE, and are prepared by experienced subject matter specialists who are leaders in their respective fields in Cambodia. TIPs thus represent 'current best practice' for implementing any improved technology. Draft TIPs undergo a peer review before they are endorsed by MAFF and approved for field use.

Fasciolosis of Cattle and Buffaloes and Its Control Measures

Introduction

In Cambodia, cattle and buffalo play a very important role because they provide draught power for ploughing and harrowing, produce manure for fertilizer, and serve as an animal bank for conversion to cash when money is required urgently. Cattle and buffalo production is constrained by low productivity, morbidity and mortality rates due to diseases, especially fasciolosis. Even though fasciolosis causes huge losses among cattle and buffalo in Cambodia, study of the disease began as recently as 1990. From then until 2002, scientists have collected epidemiological data and have accumulated much information about fasciolosis, its seasonal nature and spread and its impact on cattle and buffalo economics. Thus fasciolosis control measures have been devised for the Cambodian situation.

The recommendations on control measures against cattle and buffalo fasciolosis are not yet adequate. Experts and specialists on fasciolosis have introduced recommendations for control in high risk areas. Farmers, animal owners and stakeholders have been exposed to the effects of the disease, the control measures, and the costs incurred and benefits obtained from undertaking control measures. Information obtained by researchers on cost effectiveness and efficacy of control of fasciolosis confirms that the TIP on fasciolosis is technically sound and can be applied in Cambodia.

The fasciolosis control measures in this TIP have been tested successfully. The TIP was reviewed by the TIP oversight committee (TIPOC) comprising specialists and experienced and qualified staff from concerned institutions at national and provincial levels. This TIP may be used with confidence for education, training and extension for the purpose of control and eradication of fasciolosis which is spreading in parts of the region and in Cambodia.

It is hoped that this TIP will reduce cattle and buffalo mortality and morbidity rates. It is expected that productivity will increase and as a result farm incomes will improve.

1. Description

Summary of the TIP on fasciolosis and its control measures

a. Issues addressed by the TIP:

Farmers & animal owners in some parts of the country have observed that their cattle are in poor condition, have diarrhoea, that their coats are ragged and dull, that they demonstrate low fertility and have long inter-calving intervals, have insufficient strength for draught and in some cases, their animals have died. Examination of livers at the slaughter house reveals that some are damaged and swollen. Cutting bile duct canals is like cutting sand and cirrhosis is seen in the livers. Bile ducts are enlarged, have a bad smell and sticky, dark yellowish coloured fluid within is accompanied by the presence of flat leaf-shaped worms with a length of between 3.5 and 7.5 cm. In general, the price of live cattle infected with Fasciola is less than for uninfected animals.

b. Goals:

According to studies conducted by the Department of Animal Health and Production with participation of the Department of Agricultural Extension, the Office of Animal Health and Production, Kandal province, and the Australian Centre for International Agricultural Research (ACIAR), the problems can be resolved. Technology in this TIP on fasciolosis is based on these studies. By using the TIP for fasciolosis spread of the disease will be halted, animals will regain health, fertility will be restored and animals will be sold for a better price.

c. Methods:

Education, training and extension programs on fasciolosis such as "school-on-air" have been used for farmers, animal owners and stakeholders. Programs covering epidemiology of the disease and mode of spread, impacts, costs and benefits for control and methods for fasciolosis control and its eradication have been developed and tested. The effective measures for fasciolosis control include biological measures, grazing management and drugs.

d. Benefits for farmers, animal owners:

The TIP on fasciolosis will assist farmers & animal owners to understand the impact of fasciolosis in terms of economic losses and the benefits obtained from control. In the regions where prevalence of fasciolosis is higher than 30 percent, cattle are affected thus,

- Weight: 41 kg lighter than non-infected cattle;
- Reproduction: 10 percent lower pregnancy rate than in non-infected females;
- Liver damage: 2.50 kg of damage to the liver;
- Draught: not enough strength for draught; and
- Product quality: lower in infected than in non-infected cattle.

Analysis of the cumulative cost of the three (3) major impacts of fasciolosis is estimated at 109.00USD/head for castrated cattle and 80.00 USD/head for female. If fasciolosis control measures are implemented, farmers may expect a benefit which will amount to 76.00 USD/head.

Technical Suitability Criteria

Conditions required to exist in order that the TIP on fasciolosis will provide the outcomes expected:

- **Gender:** Women are eligible for involvement in implementation of the TIP on fasciolosis, and have a special role in implementation of biological control. Women collect cattle & buffalo dung and store it in a trench. This dung should be kept for a specific time before using it as fertilizer in the field. In addition, women as money controllers could make a decision to spend money preferentially for treatment of animals against fasciolosis.
- **Climate/water:** Fasciolosis occurs in wet areas where water lies continuously through rainy and dry seasons. Hot and dry weather could minimize the spread of the disease due to the fact that the intermediate host of Fasciola, the mollusc Lymnea, could not spread very far. Most of the molluscs and the Fasciola larvae (metacercariae) may die from desiccation.
- **Geography:** Implementation of the TIP on fasciolosis could be conducted in all parts of Cambodia where fasciolosis is present.
- **Labour requirement:** Men, women and boys aged from 16 year old can be involved in the implementation of the TIP.
- **Farmer/animal owner resources requirement:** Farmers/animal owners require funds to construct dung trench roof and fence. Trench size for dung storage is 7-9 m^2 for three cattle. Locally available construction materials should be used. In addition, around 50 000 riels is required for treatment of fasciolosis, 50 000 riels being the maximum cost of drugs for treatment of the disease.
- **Number of cattle per family:** The relevance of the TIP does not depend on the number of cattle in the family. The TIP can be implemented whatever the number.

Other recommendations

The use of combined methods for control of fasciolosis is more effective than the selection any single methods. Moreover, prompt implementation of control of fasciolosis by farmers & animal owners who live in the same region or village could reduce the spread of the disease and later, achieve eradication of fasciolosis effectively and sustainably.

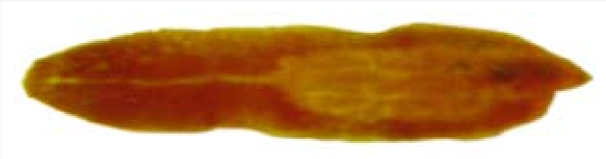
2. Technical guidelines

Fasciolosis of Cattle and Buffaloes and its Control Measures

Fasciolosis of cattle and buffaloes is caused by a trematode, family-Fasciolidea, genus-Fasciola. In Cambodia, the species *Fasciola gigantica* is found. Prevalence of fasciolosis in cattle and buffaloes is high in some parts of the provinces of Kandal, Prey Veng, Svay Rieng, Kampong Cham and Kampong Thom. Adult Fasciola live in bile ducts and gall bladders and young Fasciola locate in liver tissues. Effects of fasciolosis are seen in reduced weight gain, mortality, low and slow reproduction, liver damage, and lack of physical strength expressed as draught power.

2.1 Morphology

Fasciola gigantica-is leaf shaped, grayish-brown in color, and is 3.5-7.5 cm in length. The shoulder and tail are parallel and are 0.65-1.2 cm in width.



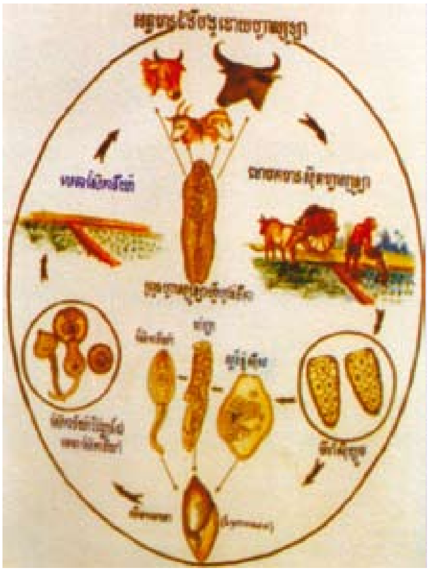
Fasciola gigantica

2.2 Life-cycle

Adult Fasciola lives in the bile ducts and gall bladder of the liver and young fluke lives in the liver tissues. The eggs of Fasciola enter the duodenum with the bile and leave the host in the faeces. The rate of development and the hatching of F. gigantica eggs depends on the surrounding environment's temperature, oxygen levels and humidity. At a temperature of 20-26 ^{0}C eggs hatch in about 10-12 days producing the first larval stage, the *miracidium*. At temperatures over 40 ^{0}C, eggs will die and in darkness, eggs develop well, though miracidia are not hatched out. A miracidium is about 0.15 mm in length, its head is covered with gland tissues for penetrating into the intermediate host snail and with cilia surrounding it is equipped for movement. In the outside environment, miracidia survive for 2-3 hours. In the event that miracidia do not penetrate into the intermediate host snail Lymnea spp, they die. Following penetration, it casts off its ciliate covering and develops into the sporocysts, then rediae and cercariae. Development from miracidium to cercaria is 4-7 weeks. Cercariae leave the snail and within a few minutes to two hours the cercariae settle on blades of grass, water plants, rice stalks etc. just below water-level. Later, after casting off their tails, they secrete a covering from the cystogenous glands forming cysts at the surface of the water which sink to the bottom. The encysted cercaria is called a metacercaria which is now infective. Cattle and buffaloes became infected by ingesting grass, water plant, rice stalk, rice straw and etc. with metacercaria or swallow them in drinking water. In some cases, infection can occur from mother to the offspring via the placenta.

Following ingestion of the metacecariae into the intestine they become Fasciola larvae which migrate to liver through two routes:

- Larvae of Fasciola may migrate through the abdominal cavity and penetrate the liver capsule. They are later found moving through the liver parenchyma. Within three weeks, Fasciola larvae will reach the bile ducts;
- Excystation of larvae occurs in the duodenum through the bloodstream across the intestinal vein. They then proceed to the bile ducts of the liver. The development of larval Fasciola to adult Fasciola will take for 2,5-4 months. Fasciola may live for 3-5 years in an animal.



Life-cycle of Fasciola

Lymnea- intermediate host of Fasciola

Lymnea acuminata

The snail intermediate host of Fasciola is called Lymnea. Dr. Suon Sothoeun conducted a research study on the snail intermediate in Cambodia, collecting snail samples and sending them to the Institute of Zoology in the Ukraine for morphological study and taxonomy. Results of the study indicated that the mollusc which is the intermediate host of Fasciola is genus Lymnea, species acuminata. This snail is commonly found in clear, slow flowing water, with a pH range from 5.8-9.0. The snail size is 2.5-3.0 cm. The mollusc Lymnea likes to float on the water surface. At four (4) months the snail starts to lay eggs. Each laying can produce 12-96 eggs which are hatched after two (2) weeks. The hatching percentage is up to 100 percent. During the dry season, Lymnea will bury itself in the mud and survive for some months. Lymnea is common in all kinds of ponds be they natural ponds, man-made ponds or canals, drainage ditches, rice field paddy and in fallow fields.

2.3 Symptoms

Clinical signs are not distinct and are similar to other diseases. In severe cases, the most characteristic signs are:

- Diarrhoea, characterised by dark brown faeces spotting the rump of the animal. The faeces have an offensive odour;
- Dry, unkempt and dull coat;
- Reduced weight gain, wasted physical condition;
- Oedema of mucous membranes and accompanying pale colour;
- Oedema may be seen in the jaw and chest areas;
- Panting & breathlessness; animal foundering and in some cases, death.

2.4 Pathology

Lymphatic nodes of the liver are swollen, bile ducts are enlarged containing a foul smelling, sticky, dark yellowish coloured fluid in the presence of the fluke, Fasciola. Cutting bile duct canals gives an impression of cutting through sand and cirrhosis is evident in the liver. In cases where few flukes are present, there are few changes in the liver. However, with severe and chronic infection, it is noted that the liver is swollen and haemorrhaging. Autopsy of such livers reveals immature flukes, the small, white, round and leaf-shaped larvae of Fasciola, sized between 0.3 and 1.1 cm. At the same time, adult flukes may be found.

2.5 Diagnosis

Diagnosis is obtained from clinical symptoms, disease epidemiology, faecal examination for Fasciola eggs and from examination of the liver. Diagnosis could be made by serological tests however these serum tests are not available in Cambodia. Liver examination is conducted to detect Fasciola and to record changes in the liver.

a. Sample collection

The hand should be wrapped in a thin, soft plastic bag (preferably lubricated with paraffin) to take about 100g faeces directly from the anus. If a plastic glove is used, after taking the faecal sample from an animal the plastic glove must be washed or rinsed with water to wash away any eggs of Fasciola that might stick to the glove. Cleaning the glove is to prevent contamination of samples and passing of infection from one animal to another. Use of a plastic bag is easy, cheap and comfortable. After taking the faecal sample the plastic bag is sealed and marked for identification. If faeces cannot be collected from the anus, the upper part of a dung pad could be sampled, again 100g, if it was passed by the animal not more than 24 hours prior to sampling.

b. Faecal sample preservation

For faeces preservation for submission to the laboratory, faeces have to be stored at not more than 10 ^{0}C in an ice box.

c. Sending faecal sample

Faecal samples should be sent to the laboratory as soon as possible. The sample should be accompanied by a form containing the following information:

- Name of the animal owner
- Address (village, commune, district and province)
- Date collection of sample
- Animal species
- Age of animal
- Animal identification.

2.6 Control measures and eradication

The three (3) measures provided below could control and eradicate fasciolosis successfully and sustainably. These measures which have been implemented in Cambodia, are drawn from the results of the research study on epidemiology of fasciolosis; on animal husbandry management practices; their appropriateness to the situations of Cambodian farmers and the experiences of farmers. It is true that for the control and eradication of fasciolosis there are other methods which could be used. However, it is contended that they could not be used effectively and sustainably in Cambodia in comparison to these selected methods. The control measures and eradication recommendations are as follows:

a. Biological control: collection and storage of cattle and buffalo faeces in a trench;
b. Cattle/buffalo grazing management;
c. Treatment/control: use of drugs and schedule for drug use.

a. Biological control

Collection and storage of cattle and buffalo faeces in a trench reduces the spread of fasciolosis. Fasciola eggs leave the infected host or sick animal in the faeces. After storage for two months the Fasciola eggs will have succumbed to high temperatures prevailing in the trench. The trench should be located away from run-off water, above the water table and away from animal access.

Size of trench

- Length: 3.50 m
- Width: 2.50 m
- Depth: 1.50 m

Roof and fence for the trench:

The trench requires proper roof and fence to protect dung from rain and sun light which will cause it to lose value as a fertilizer. In addition, the trench should be fenced for human and animal safety and to prevent loss of dung. The roof is made of local materials.

Trench for dung storage

Dung shelter

Duration of dung storage

Dung is stored in the trench for two months before using it as a fertilizer. During this period, Fasciola eggs in the faeces will die at temperatures of around 60 ^{0}C in the dung mass.

B. Cattle & buffalo grazing management and feeding

Grazing management

- Dry season rice area: cattle and buffalo may become infected in the dry season rice field by ingestion of metacercariae with water, grasses, water plants or rice stalks in drainage ditches or in rice fields. Infection occurs from January to April. Infection can be circumvented by grazing animals away from these areas in the January to April period.
- Orchards: cattle and buffalo may similarly be infected in orchards by ingestion of metacercariae in water and from grasses, water plants, man-made ponds or drainage ditches. Infection occurs from September to December. For the prevention of infection, cattle & buffalo should graze in other places during this period.
- Household area: cattle & buffalo could be infected in the household area again by ingestion of metacercariae with water, grasses, water plants, man-made ponds or drainage ditches. Infection occurs from September to April. For the prevention of the infection, cattle & buffalo should graze in other places during this period.

Watering

Underground water from wells or water from a river should be used for cattle or buffalo to avoid the possible fasciolosis infection.

Feeding

Feeding cattle & buffalo should be avoided from places where infection is likely and as described above under *grazing management*. Grass should be cut about 5 cm above water level because metacercariae encyst on these plants close to the water surface.

c. Treatment/control: use of drugs and schedule for drug use

The drugs listed below are for the treatment and control of fasciolosis in cattle and buffalo to reduce prevalence, mortality and morbidity rates, improve animal health, improve strength and enhance reproductivity.

- Triclabendazole/Fasinex. Australian.
- Genesis Ultra Pour On. Australian.
- Albendasole/Farmbazan. French.
- Dovenix-25%; Nitroxinile. French.

Fasinex

Farmbazan

Genesis Ultra Pour On

Dovenix

Schedule for drug use

These four drugs are highly effective for the treatment and control of cattle and buffalo fasciolosis. While they are effective, the timing their use is equally as important as their efficacy. The best time for treatment for control of fasciolosis is when it is assessed that animals might be free from infection as the occurrence of fasciolosis is seasonal. Thus the recommended schedule for drug use is as follows:

- Triclabendazole/Fasinex: use once/year in May;
- Genesis Ultra Pour On: use once/year in May;
- Albendasole/Farmbazan: use twice/year:
  - First time: May
  - Second time: July
- Dovenix-25%; Nitroxinile: use twice/year:
  - First time: May
  - Second time: July

2.7 Economic benefits

2.7.1 Impact

Fasciolosis has considerable impact on national economy as young animals can die, suffer reduced weight gain, become physically weak and have reduced reproductive rate, have low productivity and low quality of product demonstrated by reduction of protein, fatty acids and mineral content in meat and liver. In addition, there is discharge from the liver due to liver damage..

In places where the prevalence of fasciolosis is higher than 30 percent, specific effects of the disease are:

- Reduced weight gain: female cow-21.3 kg/head and male-41.0 kg/head
- Reproduction: 10 percent lower pregnancy rate than in non-infected females
- Liver damage: 2.5 kg of damaged organ in each liver
- Weakness: insufficient strength for draught. The strength is reduced by about 40 percent
- Quality of product: reduced nutritional value in meat and liver and the water content of these products is high
- Some animal die
- Sale price of live infected animals is low.

2.7.2 Costs and benefits for control

Research work on fasciolosis in Cambodia has been conducted by an expert and specialists from the Department of Animal Health and Production and has involved staff of the Department of Agricultural Extension, officers of the Offices of Animal Health and Production of Kampong Cham and Kandal together with staff of the Saang District Agricultural Office. The fasciolosis study measures the costs and benefits of the control program. Based on this pioneering work the technology implementation procedure (TIP) on fasciolosis could be used as a model for the fasciolosis control program in other places where it is a threat. Below are the costs and benefits of the fasciolosis control program.

a. Costs of control by farmers

- Trench with roof for dung storage-60 000 riels (20 000-60 000 riels). The trench could be used at least for two years. In this case the cost is 30 000 riels (a range of 10 000-30 000 riels).
- Drug/control-20 000 riels (a range of 10 000-20 000 riels)/head/year.
- Total costs: 50 000 riels (a range of 20 000 riels-35 000 riels)/year/head

Costs and benefits for fasciolosis control (animal/year)

Effect	Gain per cow/Y	Minimum cost	Maximum cost	Average cost
Female				
Weight	21.3 Kg/head	170 400 riels	213 000 riels	191 700 riels
Pregnancy	10%	60 000 riels	80 000 riels	70 000 riels
Liver for sale	2.5 Kg	15 000 riels	25 000 riels	20 000 riels
	Total	**245 400 riels**	**318 000 riels**	**281 700 riels**
Male				
Weight	41.0 Kg/head	328 000 riels	410 000 riels	369 000 riels
Liver for sale	2.5 Kg	15 000 riels	25 000 riels	20 000 riels
Strength	40%	–	–	–
	Total	**343 000 riels**	**435 000 riels**	**389 000 riels**
Control costs				
Dung shelter		10 000 riels	30 000 riels	20 000 riels
Drug		10 000 riels	20 000 riels	15 000 riels
	Total	**20 000 riels**	**50 000 riels**	**35 000 riels**
Benefits				
Female		225 400 riels	268 000 riels	246 700 riels
Male		323 000 riels	385 000 riels	354 000 riels
	Average	**274 200 riels**	**326 500 riels**	**300 350 riels**

Note:
- Average price of calf sold after two years divided by number of years of investment.
  - Male of two years old: 1 200 000 riels; one year old: 600 000 riels
  - Female of two years old: 1 600 000 riels; one year old:800 000 riels
  - Price of live cow per Kg: 8 000 riels-10 000 riels; average: 9 000 riels
  - Liver per Kg: 6 000 riels-10 000 riels; average: 8 000 riels
  - Liver damage per animal: 2.5 Kg/liver

b. Benefits

Female

- Average benefits: 246 700 riels/head/year (225 400-268 000 riels)

Male

- Average benefits: 354 000 riels/head/year (323 000-385 000 riels)

Average benefits from fasciolosis control

- Average benefits: 300 350 riels/head/year (274 200 riels-326 500 riels)

2.7.3 Environmental effects

Technology introduced for the implementation in this program "fasciolosis in cattle and buffalo and its control measures", especially the introduction of the biological control measure improves hygiene at household level. The health of the farm family is protected by placing a control on a source of possible infection. An added benefit is that farmers will have manure in greater quantity and it will be of better quality. Farm incomes are accordingly raised and living standards positively impacted.

3. Extension guidelines

3.1 Objectives

Extension on the control and eradication of cattle and buffalo fasciolosis has the following objectives:

- To improve understanding and knowledge of fasciolosis (epidemiology, mode of spread) and its control measures among staff of the Department of Animal Health and Production and staff of the Offices of Animal Health and Production. They must have the capacity and capability to teach other stakeholders, especially farmers about control and eradication of fasciolosis;
- To improve knowledge and understanding of fasciolosis (epidemiology, mode of spread) and its control measures among extension workers and village livestock agents so that they have the capacity to conduct extension work for stakeholders, and farmers as well as deepening their own knowledge of treatment and control of the disease;
- To put in place among animal owners & farmers and stakeholders a good working knowledge and understanding of fasciolosis (epidemiology, mode of spread) and its control measures;
- To establish among staff of the Department of Animal Health and Production, staff of the Offices of Animal Health and Production, extension workers, village livestock agents, animal owners-farmers and stakeholders a good working knowledge of costs and benefits associated with fasciolosis control.

3.2 Extension methodology

Extension methods:

- Awareness of fasciolosis is obtained by the program "school-on-air" to stakeholders, animal owners and farmers. The extension & training activity is in three modules, each module lasting for two days. Each module starts three months after the previous one. It includes theory and practice, game play and responding to questions.
- Meetings with farmers and stakeholders. Farmers and stakeholders' are interviewed on fasciolosis and its control measures, and their attitude for the fasciolosis control program is assessed.
- Pilot demonstration and display of posters and written extension material.
- Distribution of extension materials.
- Extension via television and radio.

3.3 Site selection

The site(s) being selected for the implementation of TIP on fasciolosis are those areas where prevalence of fasciolosis is higher than 30 percent.

3.4 Farmers/stakeholders selection

Participants to be selected as recipients of training, education and extension should meet the following criteria:

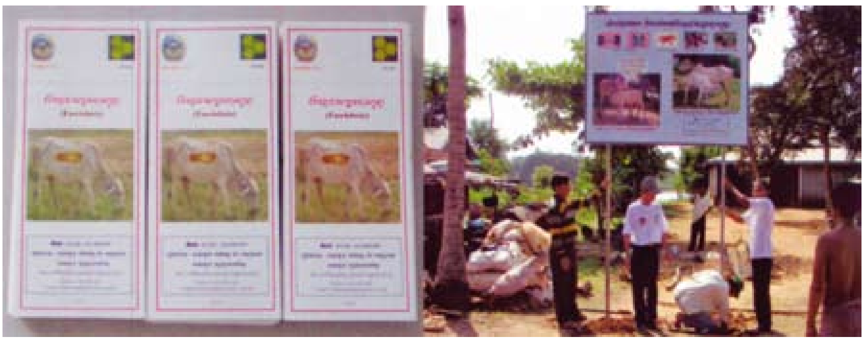
- Those who have cattle & buffalo in an area where fasciolosis is a risk.
- Aged from 16 to 55 years.
- Has a duty to take care of cattle & buffalo and be responsible for their feeding.

Local authorities such as commune and village leaders are invited to participate in the education and extension training courses.

The recommended size of the group of farmers and stakeholders for training is 30. The group is divided into six groups of five for discussion, to play the games and do the presentations which will test that they have fully understood the theory provided during the course. Practical work, games and presentations during the course are specific to each course.

3.5 Equipment and extension materials

Equipment and extension materials for training, education and extension activities comprise leaflets on fasciolosis, posters, banners, radio spots and teaching materials.. Teaching materials are drawings, pictures, fresh, and dried and preserved specimens of the snail identified as the mollusc Lymnea, the intermediate host of Fasciola. They include different kinds of drugs for treatment of fasciolosis, antibiotics and vitamins, a chart life-cycle of *Fasciola gigantica* and pictures of domesticated animals to demonstrate aspects of the training. Teaching materials also include flip charts and kits for group presentations from plenary sessions and discussions.



Leaflet and signboard on fasciolosis

Banner

Mollusk Lymnea

Triclabendazole/Fasinex

Genesis Ultra

Dovenix-25%/Nitroxinile

Farmbazan

Genta-Tylosin

Septotryl 24%

Drugs for participants' selection

Pictures display for participants' playing games

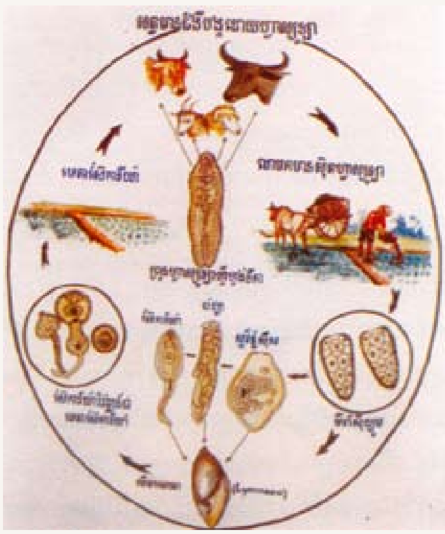


Chart life-cycle for fasciolosis

3.6 Education, Training and extension

The training, education and extension program for the farmers and stakeholders on fasciolosis and its control measures consists of three modules:

Module I

This module, participants are introduced to facts about fasciolosis:

a. Theory

- Morphology of Fasciola
- Life-cycle of fasciolosis, the relationship between susceptible animals and intermediate host of Fasciola mollusk Lymnea, how the disease is spread and infection occurs,
- Symptoms,
- Fasciolosis effects.
- Treatment and prevention.

b. Practices and playing games

Game 1: What are the susceptible animals for fasciolosis? 45 minutes.

- Individual articipants select animal pictures which s/he thinks is of an animal susceptible to fasciolosis (different animal species are displayed for selection).
- Participants report to the groups explaining why s/he came to the conclusion that the animal is susceptible.
- The trainer provides an explanation of the correctness or otherwise of the answers.

Game 2: Which snail/mollusc is the intermediate host of Fasciola? What is the name of the snail/mollusk? Where is its habitat? 45 minutes.

- Individual participants select the snail species s/he thinks is the intermediate host of Fasciola. Different snail/mollusk species collected from the field are displayed for participants' selection. The exhibits could be live or dried & preserved snails or sometimes pictures of snails. The participant inspects the exhibits and draws his/her conclusions.
- Participants report their conclusions to the groups and explain how he or she reached that conclusion. Participants report to the group about the characteristics of the snail's habitat.
- The trainer confirms participant responses and provides further information.

Game 3: Matching the pictures from stages of the life-cycle of Fasciola. 45 minutes.

- Participants are asked to match pictures of the life-cycle of *Fasciola gigantica*.
- Pictures are cut separately: provided are pictures of cattle & buffalo; dung; miracidium, sporocysts, rediae, cercariae, metacercariae, snail/mollusk Lymnea etc.
- The trainers provide comment to the group on participant responses and provide additional explanations.

Module 2
In this module participants are taken through aspects of control of fasciolosis

a. Theory

- Revision module 1
- Information about fasciolosis control measures:
  - Biological control: collection and storage cattle/buffalo dung in a trench
  - Grazing management: safe & unsafe grazing zones, disease occurrence, grazing management
  - Use of anthelmintics

Game 1: To avoid infection with fasciolosis, where should animals be grazed? Draw on the map, zone(s) of fasciolosis risk and indicate periods when infection might occur. 45 minutes.

- Participant draws on the map, marking zone(s) of fasciolosis risk and indicates the time of high infection risk.
- S/he reports to the group
- The trainer confirms accuracy of participant answers and provides additional explanations.

Game 2: By which method could one expect to reduce the spread of Fasciola infection, by storing cattle/buffalo dung in a trench or by leaving it uncollected?

- Participant select picture(s), prepared for the game and
- Report to the group about his or her decision
- The trainer comments to the group on the answers and provides additional explanations.

Game 3: Which drug(s) can be used for treatment & control of fasciolosis? 45 minutes.

- Different kinds of drug as presented in the veterinary pharmacy in manufacturers' containers including drugs against fasciolosis, vitamins, and antibiotics are displayed for participant selection.
- Participants select any drugs they think could be used for treatment of fasciolosis. S/he explains to the group his/ her choice.
- The trainer confirms or denies participant answers and provides additional explanations.

Game 4: Schedules for drug use against fasciolosis. 45 minutes.

- Participants select drugs for treatment against fasciolosis and report to the group the timing of the schedule for treatment.
- The trainer confirms or denies responses from the group and provides additional explanations.

Module 3
In the third module, training on the control measures against fasciolosis and eradication of the disease is reinforced:

a. Theory

- Revision of the effects of fasciolosis

- Control measures against fasciolosis and eradication of the disease:
  - Biological control: collection and storage cattle/buffalo dung in a trench
  - Grazing management: safe and unsafe grazing zones, disease occurrence, grazing management to avoid infection
  - Use of anthelmintics: use of drugs and time/schedule for drugs use.

b. Practices and playing games

Question 1: What are impacts, caused by fasciolosis?

- Participants work in small groups. A representative from each group responds to the question.
- The trainer confirms or rejects participant answers and provides additional explanations.

Question 2: How many methods are there for control and prevention of fasciolosis?

- Participants work in small groups. A representative from each group responds to the question.
- The trainer confirms or rejects participant answers and provides additional explanations.

Question 3: What measures have to be taken to avoid fasciolosis infection?

- Participants work in small groups. A representative from each group responds to the question.
- The trainer confirms or rejects participant answers and provides additional explanations.

3.7 Monitoring and evaluation

Farmers, animal owners and stakeholders are taught about fasciolosis and its control measures. Education and training consists of three modules for implementation over six months. Extension staff have a responsibility to conduct evaluation of results. A recommended procedure for monitoring and evaluation of the extension program is as follows:

- Conduct meetings with farmers, animal owners and stakeholders to explain and inform them of the importance of the fasciolosis program and obtain commitment by stimulating their interest;
- Prior to implementation of the program, conduct a survey of farmers', animal owners' and stakeholders' knowledge and understanding of fasciolosis and its control program, especially concerning changes in animal productivity. Some 10-15 percent of farmers/animal owners/stakeholders from the target area should be selected for the survey and interview. The survey should be conducted both halves of one year so that animal performance in the main seasons can be assessed;
- Implement the planned extension program on fasciolosis
- After the extension activity monitor the target group every three months. Collect information from them on outcomes including any changes in their attitudes to control of fasciolosis;
- Continue to monitor the results of program beyond the first three months. The introduction of control measures takes not fewer than nine months. And the animal production response will take longer.

3.8 Work plan and budget

This TIP on fasciolosis and its control measures could be applied any time of the year without limitation however, considering the training and extension course should be organised to be at the most convenient time for farmers.

The work plan and budget for the TIP on fasciolosis is as follows:

Costs for fasciolosis control/head/year

Activity/Control methods	Cost/Head/Year/Range	Total (average)
Drugs	20 000 riels (10 000–20 000)	20 000 riels
Extension materials	12 000 riels (8 000–12 000)	12 000 riels
Education, training, extension to farmers, animal owners, stakeholders	20 000 riels (10 000–20 000)	20 000 riels
Dung storage house	30 000 riels (10 000–30 000)	30 000 riels
Meetings with farmers, animal owners, stakeholders	16 000 riels (8 000–16 000)	16 000 riels
Training extension staff	12 000 riels (8 000–12 000)	12 000 riels
	110 000 riels (59 000–110 000)	**110 000 riels**

Expenses for the TIP on fasciolosis is 110 000 riels (59 000-110 000 riels) for one animal for one year. The total costs included:

- Farmer, animal owner will spend 50 000 riels (20 000-50 000) for drug against fasciolosis and dung storage shelter;
- The program will spend 60 000 riels (39 000-60 000) for costs of meetings, education, training and extension, survey, monitoring and evaluation.

Work plan and budget for implementation of TIP on fasciolosis and its control measures (12 months)

Activity	Unit	Quantity	Unit price	Total
Extension materials	–	–	–	**720 000 riels**
Education, training, extension to farmers, animal owners, stakeholders	Time	3	400 000 riels	**1 200 000 riels**
Meetings with farmers, animal owners, stakeholders, survey, monitoring and evaluation:				**960 000 riels**
• Meetings with farmers, stakeholders	Time	3	200 000 riels	600 000 riels
• Survey	Time	3	60 000 riels	180 000 riels
• Monitoring and evaluation	Time	3	60 000 riels	180 000 riels
Training extension staff				**720 000 riels**
			Total	**3 600 000 riels**

3.9 Resources materials

A DAHP researcher has conducted a study of fasciolosis and its control since 1990. After 1998, the researcher and others from the Department of Animal Health and Production have continued the fasciolosis research study with project support from the Australian Centre for International Agricultural Research (ACIAR),. The relevant research studies on fasciolosis are as follows:

- Control of Fasciolosis in Cattle and Buffalo in Cambodia, Indonesia and the Philippines; ACIAR, AS1/96/160;
- Development of a Model for the Control of Fasciolosis in Cattle and Buffaloes in the Kingdom of Cambodia; ACIAR/2002/099.

These projects produced fasciolosis reports, research and laboratory protocols, and survey and research study formats. The projects also produced extension materials such as leaflets, posters, banners, radio spots, and TV spots and other education and training materials.

All information on fasciolosis can be found at the Department of Animal Health and Production.

THE CALENDER OF TREATMENT, ANIMAL GRAZING MANAGEMENT AND FEEDING FOR THE CONTROL OF FASCIOLOSIS IN CATTLE IN SAANG DISTRICT KANDAL PROVINCE

The control method	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun	July	Aug.
Infected period												
Infected place 1. Dry season rice field					Dry season rice area							
2. Chamcar	Chamcar area											
3. Household	House area											
4. Treatment: Triclabendasole (12mg./kg. body weight)									*			
Albendasole (15mg./Kg. body weight)									*		*	
Dovenix (10mg./Kg. body weight)									*		*	

Notice :

1. Dry season rice area: Grass, rice stem (after harvest) and water with metacecaria were sources of infection
2. Chamcar area: Grass, water from man made ponds and canals for water stock with metacecaria were main sources of infection
3. Household area: Drinking water from man made ponds and canals and grass with metacecaria were main sources of infection
4. * : Treatment month

For further information please contact:

Department of Agricultural Extension
Ministry of Agriculture Forestry and Fisheries

200, Preah Norodom Boulevard
Phnom Penh, Cambodia
Phone: 012 833 777 or 01282 6617
Fax: 855 23 210 948 or 855 23 213 011
Email: kunso@camnet.com.kh or mak_soeun@camnet.com.kh

www.aciar.gov.au