

**Comparison of host-parasite relationships of *Fasciola gigantica* infection
in cattle (*Bos indicus*) and swamp buffaloes (*Bubalus bubalis*)**

Thesis submitted by

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in the School of Tropical Veterinary
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STATEMENT ON THE CONTRIBUTION OF OTHERS

My studies were generously funded by the John Allwright Fellowship of the Australian Centre for International Agricultural Research (ACIAR). The financial support for this project was provided by ACIAR project AS1/96/160. Data for the abattoir studies were collected together with my colleagues and some students who were involved with the ACIAR project on fasciolosis at the University of Southern Mindanao, Kabacan, Cotabato. These people have been cited as co-authors in the papers accompanying this thesis.

The experimental animals for the experimental infection were kept at the Philippine Carabao Center at the University of Southern Mindanao (USM), Kabacan, Cotabato, Philippines. Some of the laboratory analyses undertaken for this work were conducted at the Veterinary Hospital of the College of Veterinary Medicine at USM.

This project has the approval of the Animal Ethics Subcommittee (Permit Number A778_02).

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ABSTRACT

The host-parasite interactions in *Fasciola gigantica* infection in cattle and swamp buffaloes have not been extensively investigated. Designing of future approaches for the control of tropical fasciolosis requires an understanding of the host-parasite relationships. This study was therefore undertaken to look at and compare the host-parasite interactions of *F. gigantica* infection between cattle (*Bos indicus*) and swamp buffaloes (*Bubalus bubalis*). This study compared the prevalence of infection, clinico-pathological and parasitological manifestations, sequential production of antibody isotypes and of Th1 and Th2 cytokines, local immune responses, and the histopathology of the infection between cattle and swamp buffaloes during infection with *F. gigantica*.

The study shows that cattle and buffaloes are both susceptible to infection with *F. gigantica* in the Philippines with the infection affecting young and old animals. However, there are indications that these animals differ in their responses to infection.

The red blood cell (RBC) count was significantly higher in infected than in non-infected swamp buffaloes ($P < 0.05$) while there was no significant difference in packed cell volume (PCV) and haemoglobin values between infected and non-infected buffaloes ($P > 0.05$). Red blood cell count was significantly higher in buffaloes with high fluke burdens (> 70 flukes) than those with no flukes or with medium fluke burden (21-70 flukes) ($P < 0.05$). Significantly higher PCV value was also observed in buffaloes with high fluke burdens compared with those with low or medium worm loads ($P < 0.05$). Haemoglobin values did not differ significantly between buffaloes with low, medium, high or no fluke burdens ($P > 0.05$). On the other hand, infected cattle showed significantly lower RBC counts than non-infected cattle ($P < 0.05$) and these counts were negatively related to fluke burden. Packed cell volume was also significantly lower in cattle with high fluke burden than those with fewer flukes ($P < 0.05$). These findings showed that swamp buffaloes were not as severely affected by *F. gigantica* compared with cattle suggesting that they can cope with infection much better than cattle. From these observations, it was concluded that swamp buffaloes are more resilient to *F. gigantica* infection than cattle.

There was a trend of a lower fluke burden and faecal egg counts in naturally infected swamp buffaloes than in cattle. Fluke burdens were also lower in buffaloes than cattle at 3, 7, 12 and 16 weeks post- experimental infection with 1000 metacercariae. Sixteen weeks after the experimental infection, eggs were already seen in cattle but none in buffaloes and only immature flukes were present in buffaloes at this time, indicating that the prepatent period of *F. gigantica* in cattle is shorter than in swamp buffaloes. These findings support a conclusion that swamp buffaloes are more resistant than cattle to *F. gigantica*.

An indirect ELISA was done to assess the sequential production of antibody isotypes IgG1, IgG2 and IgE reacting to *F. gigantica*. Infected cattle and buffaloes showed increased levels of these isotypes relative to the controls. No marked increase in IgG1 and IgG2 occurred in cattle except during the later part of infection. In buffaloes, the elevations of these two isotypes showed a pattern of increasing trend. IgG1 and IgG2 values in buffaloes were higher than in cattle. It is proposed that IgG2 may be associated with resistance against *F. gigantica* in these species, higher IgG2 in buffaloes being related to the higher resistance observed in these animals compared with that in cattle.

The levels of IFN- γ , IL-6 and IL-8 in serum of cattle and buffaloes were assessed by a sandwich ELISA. IFN- γ was not present in detectable levels in the serum of these animals suggesting that this cytokine may not be important in the immune response against *F. gigantica* in cattle and swamp buffaloes. Serum IL-6 levels were higher in infected than in non-infected animals from one to 16 weeks post-infection and higher in cattle than in buffaloes. This suggests that IL-6 is not important in resistance against *F. gigantica* in these animals. Higher serum IL-8 levels were observed in infected buffaloes than in cattle suggesting that this cytokine is associated with the higher degree of resistance against *F. gigantica* in swamp buffaloes than in cattle.

The local immune response in the liver of infected animals was assessed by immunohistochemistry and histology. T and B lymphocytes, plasma cells, eosinophils and mast cells were present in hepatic lesions. A progressive increase in T cell numbers occurred after infection in buffaloes whereas these continuously declined in

cattle after a sharp rise at three weeks post-infection. The numbers of B lymphocytes and plasma cells increased from 3-16 weeks post-infection in both species.

Eosinophils were also present in hepatic lesions, which may be partly a consequence of the degranulation of mast cells in hepatic lesions as a result of antigenic stimulation from the flukes. It is concluded that both cellular and humoral responses are induced in the liver of cattle and swamp buffaloes infected with *F. gigantica*. The T cell response in cattle was apparently suppressed after week 3 of infection which may be due partly to the rapid migration of flukes or to a suppression of the local immune response in the liver of cattle by *F. gigantica*. The increasing responsiveness in buffaloes represented by the gradually increasing numbers of T lymphocytes may have contributed to the suppression of development of flukes or delayed their migration in these animals. This difference in the expression of the hepatic T cell response between cattle and swamp buffaloes may be related to the observed differences in their level of resistance against *F. gigantica*.

The percentage of eosinophils in the blood increased in infected animals. The eosinophilia observed may have resulted from the generalized inflammation following liver fluke infection and may not be protective as migrating flukes or dead flukes with surrounding eosinophils were not seen in the liver. Eosinophilia also indicates a stimulation of a Th2-type of immune response in these animals during infection with *F. gigantica*. The kinetics of eosinophilia differed between hosts. A rapid eosinophilia was observed within 1-3 weeks post-infection in cattle whereas this was considerably delayed in buffaloes to weeks 6-11. The slower eosinophil response in buffaloes may be associated with the increased resistance to *F. gigantica* in this host, i.e eosinophils are not an effector cell involved in killing immature *F. gigantica* during the first five weeks of infection.

Histopathology of liver and hepatic lymph nodes revealed some differences in the extent of lesions between cattle and swamp buffaloes at different periods of infection. At three weeks post-infection, focal necrosis was present in cattle but not in buffaloes. The hepatic lymph node (HLN) of cattle showed stronger follicular and parafollicular hyperplasia compared with buffaloes. Lymphocytic infiltration in portal areas was more marked in cattle than buffaloes at seven weeks post-infection and more plasma

cells were present in the medullary cords of HLN of cattle than buffaloes. Marked portal reaction, bile duct hyperplasia and severe cirrhosis were seen in cattle at 12 weeks post-infection. Only moderate cirrhosis was observed in buffaloes at the same time post-infection. At 16 weeks post-infection in cattle necrosis of bile ducts was seen with mostly eosinophils in the inflammatory infiltrate. In buffaloes, most of the inflammatory cells were lymphocytes. These results imply that there was milder damage and inflammatory response in the liver and milder stimulation of the HLN at some stages of infection in buffaloes compared with cattle which could be due to their lower fluke burden or to the delayed migration or suppressed development of flukes in buffaloes.

Results of this study showed that there were similarities and differences in the immune responses of cattle and buffaloes during infection with *F. gigantica*. These varying responses to *F. gigantica* infection represent differences in host-parasite relationships of *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.

List of Published Papers

1. 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 (E. C. Molina, E. A. Gonzaga and L. A. Lumbao, 2005, Tropical Animal Health and Production, 37, 215-221)
2. Cellular and humoral responses in liver of cattle and buffaloes infected with a single dose of *Fasciola gigantica* (Elizabeth C. Molina and Lee F. Skerratt, 2005, Veterinary Parasitology, 131, 157-163)
3. Serum interferon-gamma and interleukins-6 and -8 during infection with *Fasciola gigantica* in cattle and buffaloes (Elizabeth C. Molina, 2005, Journal of Veterinary Science, 6, 135-139)
4. Clinico-pathological indications of resilience of swamp buffaloes (*Bubalus bubalis*) to infection with *Fasciola gigantica* (E. C. Molina, E. O. Sinolinding and Peralta A., 2005, Tropical Animal Health and Production, 37 (6), 451-455)
5. Differences in Susceptibility Between Cattle and Swamp Buffaloes to Infection with *Fasciola gigantica* (E. C. Molina, E. A. Gonzaga, E. O. Sinolinding, L. A. Lumbao, A. Peralta and A. Barraca, 2005, Tropical Animal Health and Production, 37 (8), 611-616)

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LIST OF ABBREVIATIONS

ABTS	2,2-Azino-di-[3-ethylbenthizolin sulfonat (6)]
ADCC	Antibody-dependent cell-mediated cytotoxicity
DAB	Diaminobenzidine
ELISA	Enzyme-linked immunosorbent assay
ES	Excretory-secretory
FABP	Fatty-acid binding protein
FEC	Faecal egg count
GGT	Gamma-glutamyl transferase
GLDH	Glutamate dehydrogenase
GST	Glutathione <i>S</i> -transferase
HRP	Horseradish peroxidase
HLN	Hepatic lymph node
IFN- γ	Interferon-gamma
IgG	Immunoglobulin G
IL	Interleukin
ITT	Indonesian thin-tail
mAb	Monoclonal antibody
MCH	Mean corpuscular haemoglobin
NEJ	Newly excysted juvenile
OD	Optical density
pAb	Polyclonal antibody
PBL	Peripheral blood lymphocyte
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PCV	Packed cell volume
PMN	Polymorphonuclear
TBS	Tris buffered saline
Th	Helper T
TMB	Tetramethylbenzidine
Tris-EDTA	Tris-ethylenediamine tetra-acetic acid