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# Concurrent infections of *Fasciola*, *Schistosoma* and *Amphistomum* spp. in cattle from Kafue and Zambezi river basins of Zambia

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**Abstract**

This study investigated interactions among *Fasciola gigantica*, *Schistosoma* spp. and *Amphistomum* spp. concurrent natural infections in Zambian cattle, based on egg and worm counts. In the abattoir 315 cattle were screened for worms of *F. gigantica* in the liver, *Schistosoma* spp. in mesenteric veins and/or *Amphistomum* spp. in the rumen. One hundred and thirty-three (42.2%) of the abattoir-examined cattle harboured one, two or all three trematodes. Of 133 cattle, 50 were randomly selected for worm and egg counts. The mean numbers (± SD) of *Amphistomum, Schistosoma* and *Fasciola* were 622.08 (± 97.87), 33.68 (± 7.44) and 19.46 (± 4.58), respectively. A total of 32% harboured all the three trematodes, 66% had *F. gigantica* and *Amphistomum* spp. infections, 52% had *Schistosoma* spp. and *Amphistomum* spp. infections while 32% had *F. gigantica* and *Schistosoma* infections. A positive correlation ($P = 0.014$) was found between *F. gigantica* and *Amphistomum* worm burdens. There were no correlations between *Amphistomum* and *Schistosoma* worm burdens and between *F. gigantica* and *Schistosoma* worm burdens. It may be concluded that there is no significant cross-protection among these trematodes in cattle in endemic areas.

## Introduction

Trematode infections are economically important helminth diseases hampering the productivity of domesticated ruminants worldwide (Vercruysse & Claerebout, 2001). *Fasciola gigantica*, *Schistosoma mattheei* and *Amphistomum* spp. are the most common trematodes in Zambia (De Bont *et al.*, 1994; Phiri *et al.*, 2006a). Mixed infections with different trematode species are common since they share the same intermediate snail host and/or similar transmission sites (Pfukenyi *et al.*, 2005; Keyyu *et al.*, 2006; Phiri *et al.*, 2006a).

Heterologous resistance between *Schistosoma* and *Fasciola* species in concurrent infections has been observed in several experimental and farm animals (Hillyer, 2005). Previous reports have demonstrated that *F. hepatica* and *Schistosoma mansoni* cross-protect against each other in mice, causing a reduction in worm burden and/or egg-producing capacity (Hillyer, 1984). Likewise, cattle infected with *Schistosoma bovis* were shown to acquire an enhanced resistance to challenge *Fasciola* spp. infection

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and vice versa (Haroun & Hillyer, 1986; Yagi *et al.*, 1986). An enhanced resistance against *F. hepatica* and *F. gigantica* after primary infection with *S. bovis* has been described in cattle (Sirag *et al.*, 1981; Yagi *et al.*, 1986) and sheep (Monrad *et al.*, 1981). Similarly, a primary infection of *F. gigantica* in calves resulted in enhanced resistance to challenge *S. bovis*, with a 94.2% reduction in worm count (Yagi *et al.*, 1986). These findings imply that these two trematodes may be susceptible to the same or similar immune responses (Spithill *et al.*, 1999) and may have common antigens (Rodriguez-Osorio *et al.*, 1993).

Studies on concurrent naturally acquired infections of *Fasciola*, *Schistosoma* and *Amphistomum* in cattle are lacking. Since concurrent trematode infections may influence the epidemiology of individual fluke populations, it was desirable to study the dynamics of these trematode infections and their relationships in cattle in endemic areas. Therefore, objectives of this study were to determine the occurrence and extent of concurrent *F. gigantica*, *Schistosoma* spp. and *Amphistomum* spp. infections in free-ranging cattle in Zambia, and to investigate their interactions.

## Materials and methods

### *Study area*

This study was carried out at Turn Pike abattoir, located 60 km south of Lusaka. The local cattle came from areas where worm control is not routinely practised. A total of 315 cattle presented for slaughter were examined from June 2005 to July 2006 for worms of *F. gigantica* in the liver, *Schistosoma* spp. in mesenteric blood veins, and *Amphistomum* spp. in the rumen. Worm and faecal egg counts were determined from 50 randomly selected trematode-infected cattle.

### *Parasitological examinations*

*Fasciola* and *Amphistomum* eggs were detected and quantified by the sieving and sedimentation technique as described by Taira *et al.* (1983). *Schistosoma* faecal egg counts were determined using a modification of the concentration technique by Lawrence (1970). Each *Schistosoma* egg counted represented 10 eggs per gram (De Bont *et al.*, 1995).

### *Worm counts*

The intestine was isolated and mesenteries were spread out for thorough examination of mesenteric veins for schistosomes. The rumen was opened and washed lightly with tap water to expose amphistomes. Schistosomes were counted *in situ* in the abattoir, while amphistomes were picked from the mucosa of the whole rumen using tissue forceps, and counted. Sharp incisions were made on the liver surface, through major bile ducts and into the parenchyma to expose liver flukes. To determine liver fluke burden, liver dissections were done as described by Phiri *et al.* (2006b).

### *Statistical analysis*

Data of faecal egg counts (EPG) and worm counts from sampled animals were log transformed (count +1) to stabilize variances. Trematode frequencies were calculated using SPSS version 11.0 for Windows (SPSS Institute, Chicago, Illinois, USA). Chi-square was used to determine differences in worm burdens and faecal egg counts among the three trematodes. Pearson's correlation coefficient and linear regression were used to determine and describe relationships among the three trematodes. A significant relationship was denoted by a $P$ value of less than 0.05.

## Results

Of 315 cattle presented for slaughter, 133 were positive for at least one trematode infection. From these, 50 animals were randomly selected for further studies.

### *Coproscopy (n = 50)*

On coproscopic examination, 58% were positive for fascioliasis, 76% for amphistomiasis, 22% for schistosomiasis and 20% were positive for *Schistosoma* miracidia. Faecal egg counts varied throughout the study period, but ranged from 0 to 95 with a mean (± SEM) of 8.02 ± 2.23 for *F. gigantica*, 0–90 with a mean (± SEM) of 7.0 ± 2.42 for *Schistosoma* species and 0–183 with a mean (± SEM) of 17.7 ± 4.49 for *Amphistomum* species. A positive correlation ($r = 0.560$, $P < 0.001$) was obtained between *F. gigantica* and *Amphistomum* EPG.

### *Worm burdens*

At meat inspection, 68% of the 50 randomly selected trematode-infected cattle were positive for fascioliasis, 56% for schistosomiasis and 92% were positive for amphistomiasis. The range of individual worm burdens was wide and there were variations in worm burdens among the three trematodes (table 1). Heavy worm burdens characterized *Amphistomum* infections, of which 48% of the *Amphistomum* infected cattle ($n = 46$) harboured more than 500 amphistomes. Low worm burdens characterized *Schistosoma* infections, of which 68% of the *Schistosoma* infected cattle ($n = 28$) harboured fewer than 100 worms in the mesenteric blood vessels.

Mixed trematode infections were common, of which 32% of the 50 randomly selected trematode-infected cattle harboured all three trematodes. *Fasciola gigantica* and *Amphistomum* dual infections were the highest (34%) while *F. gigantica* and *Schistosoma* dual infections were the lowest (0%). *Schistosoma* and *Amphistomum* dual

Table 1. *Fasciola gigantica, Schistosoma* spp. and *Amphistomum* spp. worm burdens in 50 randomly selected trematode-infected cattle from the Kafue and Zambezi river basins of Zambia.

| Trematode species | Worm burden, range | Mean ± SEM |
|---|---|---|
| *Fasciola gigantica* | 1–176 | 19.46 ± 4.58 |
| *Schistosoma* spp. | 1–186 | 33.68 ± 7.44 |
| *Amphistomum* spp. | 2–2974 | 622.08 ± 97.87 |

infections were represented by 20%. Only 6% of sampled animals had *Amphistomum*, 2% *F. gigantica* and 4% *Schistosoma* single infections.

### *Interactions among trematodes*

A positive correlation ($r^2 = 0.12$, $P = 0.014$) was obtained between *F. gigantica* and *Amphistomum* worm counts (fig. 1), and also between *F. gigantica* worm burden and *Amphistomum* faecal egg counts ($r^2 = 0.338$, $P < 0.001$).

There were no correlations ($r^2 = 0.022$, $P = 0.302$) between *Amphistomum* and *Schistosoma* worm burdens and between *F. gigantica* and *Schistosoma* worm burdens ($r^2 = 0.015$, $P = 0.390$).

## Discussion

This study has revealed, for the first time, the presence and extent of fascioliasis, schistosomiasis and amphistomiasis concurrent infections in cattle in Zambia, based on both egg and worm counts. Our observation of concurrent *F. gigantica* and *Amphistomum* species infections is in agreement with earlier reports in Zambia (Phiri *et al.*, 2006a) and elsewhere (Szmidt-Adjide *et al.*, 2000; Pfukenyi *et al.*, 2005; Keyyu *et al.*, 2006). This finding demonstrates that interactive infections between these two trematodes are mutually inclusive. Phiri *et al.* (2006a) recorded a positive correlation ($r^2 = 0.043$) between *F. gigantica* and *Amphistomum* species using faecal egg counts. Our study, however, recorded a stronger correlation ($r^2 = 0.120$) between these two trematodes using worm burdens. Worm burden may therefore be a better parameter for determining the relationship between these two trematodes than EPG, as pathophysiological changes in the host may result in fluctuations in faecal egg outputs (Boray, 1969). Faecal egg counts

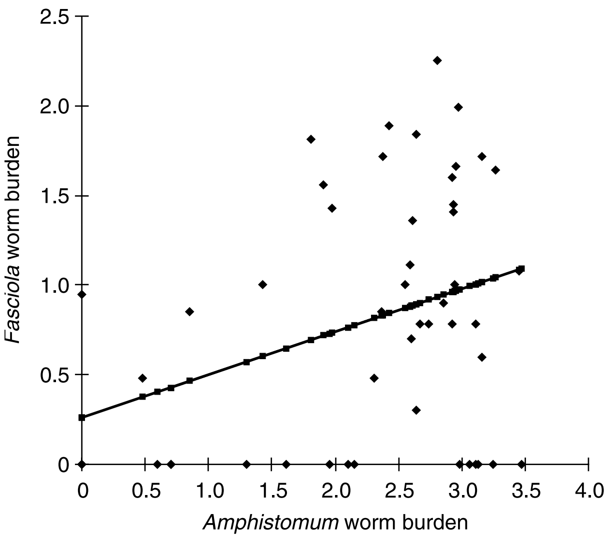


Fig. 1. Positive correlation ($r^2 = 0.12$) between *Fasciola gigantica* and *Amphistomum* worm burdens in cattle from Kafue and Zambezi river basins of Zambia.

may also be influenced by the amount of faeces produced per day, time of day of faecal sampling, low sensitivity of coproscopic techniques and other factors. Moreover, acquisition of immunity by cattle in chronic infections may depress egg output (Bitakaramire, 1973).

Although an increase in *F. gigantica* worm burden was associated with an increase in *Amphistomum* worm burden, differences in their worm burdens could be due to the density and uptake of the specific metacercariae by the host and/or the specific development of metacercariae in the definitive host (Szmidt-Adjide *et al.*, 2000). Despite scarcity of reports on economic effects of amphistomiasis in cattle, the heterologous interaction of *Amphistomum* and *F. gigantica* may compound the economical effects of liver flukes to the livestock industry. These effects could be induced by the combined pathology caused by individual trematode populations, especially by immature flukes in young animals.

Although *Schistosoma* and *Amphistomum* spp. co-joint infections were common, there was no significant relationship between these trematodes. This may be attributed to possible simultaneous exposure of cattle to both trematodes in endemic areas and other unknown factors. Knowles & Jones (1989) observed that concurrent *Calicophoron microbothrium* and *Schistosoma bovis* infection might occur in the snail *Bulinus tropicus*, given that *B. tropicus* is an intermediate host for both trematodes. As a result of the above, it may be difficult to observe the relationship between these trematodes in cattle in endemic areas where both parasites may become established in the definitive host at the same time.

In this study, few animals harboured both *F. gigantica* and *Schistosoma* worms. Since both trematodes cause significant liver pathology (Ferreras *et al.*, 2000; Phiri *et al.*, 2006b), when one infection is established, it may exclude the other from establishing. Almeida *et al.* (2003) demonstrated that sheep vaccinated against rSm14 were significantly protected against challenge infections with *F. hepatica* metacercariae, and were completely free of histopathological hepatic damage related to liver fluke infections. Although the correlation coefficient between the two trematodes in our study was negative, the relationship was not significant ($P = 0.390$). In experimental studies, primary infection with *Schistosoma* or *Fasciola* species in ruminants has been reported to result in enhanced resistance to heterologous challenge (Bushara *et al.*, 1978; Sirag *et al.*, 1981; Yagi *et al.*, 1986; Ferreras *et al.*, 2000; Almeida *et al.*, 2003). Our findings however, were not conclusive, since this study was done in naturally infected cattle from endemic areas with no known duration of infections. It was not possible to determine which trematode infected the host first, or the sequence of subsequent infections. Possible simultaneous exposure of cattle to both trematodes in endemic areas could have contributed to our failure to assess the effects of co-joint infections on their worm burdens and egg production.

In conclusion, for control programmes of trematodes to be effective in endemic areas, their design must consider the existence of concurrent infections. It is difficult to conclude on the relationship among and between those trematodes in natural infections. The number of cattle in this study is relatively low, especially considering the high trematode prevalence ratio in the study area. Experimental studies on concurrent *Fasciola* and *Amphistomum*, and *Schistosoma* and *Amphistomum* infections may also give a clear explanation on interactions of these trematodes in cattle.

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## References

**Almeida, M.S., Torloni, H., Lee-Ho, P., Villar, M.M., Taumaturgo, N., Simpson, A.J. & Tendler, M.** (2003) Vaccination against *Fasciola hepatica* infection using a *Schistosoma mansoni* defined recombinant antigen, Sm14. *Parasite Immunology* **25**, 135–137.

**Bitakaramire, P.K.** (1973) Preliminary studies on the immunization of cattle against fasciolosis using gamma-irradiated metacercariae of *Fasciola hepatica*. pp. 465–525 *in* Dalton, J.P. (*Ed.*) *Fasciolosis*. Wallingford, UK, CABI Publishing.

**Boray, J.C.** (1969) Experimental fasciolosis in Australia. *Advances in Parasitology* **7**, 95–209.

**Bushara, H.O., Hussein, M.F., Saad, A.M., Taylor, M.G., Dargie, J.D., Marshall, T.F. de C, & Nelson, G.S.** (1978) Immunization of calves against *Schistosoma bovis* using irradiated cercaria or schistosomula of *S. bovis*. *Parasitology* **77**, 303–311.

**De Bont, J., Vercruysse, J., Southgate, V.R., Rollinson, D. & Kaukas, A.** (1994) Cattle schistosomiasis in Zambia. *Journal of Helminthology* **68**, 295–299.

**De Bont, J., Vercruysse, J., Sabbe, F., Southgate, V.R. & Rollinson, D.** (1995) *Schistosoma mattheei* infections in cattle: changes associated with season and age. *Veterinary Parasitology* **57**, 299–307.

**Ferreras, M.C., Garcia-Iglesias, M.J., Manga-Gonzalez, M.Y., Perez-Martinez, C., Mizinska, Y., Romajo, V., Gonzalez-Lanza, M.C., Escudero, A. & Garcia-Marin, J.F.** (2000) Histopathological and immunohistochemical study of lambs experimentally infected with *Fasciola hepatica* and *Schistosoma bovis*. *Journal of Veterinary Medicine B* **47**, 763–773.

**Haroun, E.M. & Hillyer, G.V.** (1986) Resistance to fasciolosis – a review. *Veterinary Parasitology* **20**, 63–93.

**Hillyer, G.V.** (1984) Immunity to schistosomes using heterologous trematode antigens. *Veterinary Parasitology* **14**, 263–283.

**Hillyer, G.V.** (2005) *Fasciola* antigens as vaccines against fasciolosis and schistosomiasis. *Journal of Helminthology* **79**, 241–247.

**Keyyu, J.D., Kassuku, A.A., Msaliwa, L.P., Monrad, J. & Kyvsgaard, N.C.** (2006) Cross-sectional prevalence of helminth infections in cattle of traditional, small scale and large scale dairy farms in Iringa district, Tanzania. *Veterinary Research Communications* **30**, 45–55.

**Knowles, R.J. & Jones, A.** (1989) The influence of *Calicophoron microbothrium* on the susceptibility of *B. tropicus* to *Schistosoma bovis*. *Parasitological Research* **75**, 381–391.

**Lawrence, J.A.** (1970) Examination of ruminal faeces for schistosome eggs. *Rhodesia Veterinary Journal* **1**, 49–52.

**Monrad, J., Christensen, N.O., Nansen, P. & Frandsen, F.** (1981) Resistance to *Fasciola hepatica* in sheep harbouring primary *Schistosoma bovis* infections. *Journal of Helminthology* **55**, 261–271.

**Pfukenyi, D.M., Mukaratirwa, S., Willingham, A.L. & Monrad, J.** (2005) Epidemiological studies of amphistome infections in cattle in the highveld and lowveld communal grazing areas of Zimbabwe. *Onderstepoort Journal of Veterinary Research* **72**, 67–86.

**Phiri, A.M., Phiri, I.K. & Monrad, J.** (2006*a*) Prevalence of amphistomiasis and its association with *Fasciola gigantica* infections in Zambian cattle from communal grazing areas. *Journal of Helminthology* **80**, 65–68.

**Phiri, A.M., Phiri, I.K., Sikasunge, C.S., Chembensofu, M. & Monrad, J.** (2006*b*) Comparative fluke burden and pathology in condemned and non-condemned cattle livers from selected abattoirs in Zambia. *Onderstepoort Journal of Veterinary Research* **73**, 275–281.

**Rodriguez-Osorio, M., Gomez-Garcia, Vi., Rojas-Gonzalez, J., Romajo-Martin, V., Manga-Gonzalez, Y. & Gonzalez-Lanza, C.** (1993) Resistance to *Schistosoma bovis* in sheep induced by an experimental *Fasciola hepatica* infection. *Journal of Parasitology* **72**, 223–225.

**Sirag, S.B., Christensen, N.O., Nansen, P., Monrad, J. & Frandsen, F.** (1981) Resistance of *Fasciola hepatica* in calves harbouring primary patent *Schistosoma bovis* infection. *Journal of Helminthology* **55**, 63–70.

**Spithill, T.W., Smooker, P.M. & Bruce, C.D.** (1999) *Fasciola gigantica*: Epidemiology, control, immunology and molecular biology. pp. 465–525 *in* Dalton, J.P. (*Ed.*) *Fasciolosis*. Wallingford, UK, CABI Publishing.

**Szmidt-Adjide, V., Abrous, M., Adjide, C.C. & Dreyfuss, G.** (2000) Prevalence of *Paramphistomum daubney* in central France. *Veterinary Parasitology* **87**, 133–138.

**Taira, R., Suzuki, K. & Boray, J.C.** (1983) Detection and quantification of *Fasciola* eggs in cattle using the Beads technique. *Japanese Journal of Veterinary Parasitology* **32**, 279–286.

**Vercruysse, J. & Claerebout, E.** (2001) Treatment vs. non-treatment of helminth infections in cattle: defining the thresholds. *Veterinary Parasitology* **98**, 195–214.

**Yagi, A.I., Younis, S.A., Haroun, E.M., Gameel, A.A., Bushara, H.O. & Taylor, M.G.** (1986) Studies on heterologous resistance between *Schistosoma bovis* and *Fasciola gigantica* in Sudanese cattle. *Journal of Helminthology* **60**, 55–59.

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