Coprological study of trematode infections and associated host risk factors in cattle during the dry season in and around Bahir Dar, northwest Ethiopia

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ABSTRACT

This study was conducted to estimate the prevalence and identify risk factors associated with trematode infections in cattle in and around Bahir Dar, northwest Ethiopia. Fecal samples collected from randomly selected 369 cattle were examined using simple sedimentation technique for differential trematode eggs count. The animals were found shedding eggs of three groups of trematodes, namely Fasciola spp., paramphistomes and Schistosoma spp. The overall prevalence of trematodes was 61.0%, and specific prevalence for Fasciola, paramphistomes and Schistosoma was 20.1%, 48.5% and 16.5%, respectively. A substantial overlap was observed in the occurrence of Fasciola and paramphistomes. The prevalence of all the three trematodes identified in this study was significantly \( P < 0.05 \) associated with body condition and breed, while the prevalence of Fasciola and paramphistomes was also associated with age. The mean \( \pm SE \) fecal egg count per gram of feces (EPG) for Fasciola, paramphistomes and Schistosoma was 4.3 \( \pm 0.55 \), 25.7 \( \pm 2.11 \) and 3.1 \( \pm 0.42 \), respectively. EPG of Fasciola was significantly correlated with EPG of paramphistomes \( (P < 0.001) \). The EPG for all the three trematodes was associated with body condition and breed of animals \( (P < 0.05) \), while EPG for paramphistomes was also affected by age of the animals \( (P < 0.05) \). The prevalence of all the three major trematodes of animal health importance with high rate of mixed infection along with poor body condition, suggests substantial economic loss incurred due to reduced productivity in cattle in the study area.

1. Introduction

Trematode infections, especially fasciolosis, are some of the most economically important helminth diseases hampering the productivity of domestic ruminants worldwide (Dargie, 1987; Mage, Bourgne, Toullieu, Rondelaud, & Dreyfuss, 2002; Njau, Kasali, Scholtens, & Toullieu, Rondelaud, & Dreyfuss, 2002; Njau, Kasali, Scholtens, & Toullieu, Rondelaud, & Dreyfuss, 2002). In addition to its effect on productivity, fasciolosis is a cause of significant economic losses through liver condemnation at slaughter (Abbe et al., 2010; Abunna, Asfaw, Megersa, & Regassa, 2010; Berhe, Berhane, & Tadesse, 2009; Phiri, Phiri, Sikasunge, & Monrad, 2005).

There are several genera of paramphistomes: Paramphistomum, Cotylophoron, Calicophoron, Bothriothoron, Orthocoelum and Gigantocotyle, of which Paramphistomum is the most common and widespread in ruminants (Taylor, Coop, & Wall, 2016). Paramphistomes (amphistomes) are traditionally regarded as having no

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clinical significance (Iglesias-Piñeiro et al., 2016). However, heavy infec-
tion with immature flukes, which attach to the lining of the upper part of small
intestine, may cause severe disease which may even result in
death (Lloyd, Boray, & Love, 2007; Rolfe, Boray, Nichols, & Collins,
1991). Moderate infections with the immature fluke may cause reduced
weight gains or milk production, or illness. Most livestock, however,
have only light stomach fluke infections with either adult fluke or small
numbers of immature fluke that they show no signs of disease
(Lloyd et al., 2007).

Schistosomosis in cattle in Africa can be caused by Schistosoma bovis,
S. mattheei and S. leiperi. Schistosomosis is generally considered to be of
low importance in large ruminants, and even where a high prevalence
of the parasite is detected in slaughtered cattle, clinical signs of the
disease are seen only rarely (Urquhart et al., 1996). However, infections
sometimes may result in severe clinical signs (Hansen & Perry, 1994).

There are several reports of coproscopic and abattoir surveys indi-
cating the widespread prevalence of liver and stomach flukes in cattle
in Ethiopia (Abebe et al., 2010; Abunna et al., 2010; Amen, Erko, &
Bogale, 2001; Fromsa, Meharenet, & Mekibib, 2011; Yenen, Kebede,
Fentahun, & Chanie, 2012). Prevalence of Schistosoma spp. in cattle in
some parts of the country with permanent water bodies, seasonal
floodings and high rainfall has also been recorded (Ameni et al., 2001;
Chanie, Dejen, & Fentahun, 2012; Fromsa et al., 2011; Habtamu &
Wolde Mariam, 2011; Habtamu, Negash, Sirak, & Chanie, 2013; Yenen
et al., 2012). Some studies demonstrated the occurrence of mixed
trematode infections in cattle in Ethiopia (Ameni et al., 2001;
Fromsa et al., 2011). Several studies in cattle in Ethiopia demonstrated
considerable financial loss due to liver condemnation at slaughter due to Fasciola
infection (Abebe et al., 2010; Abunna et al., 2010; Berhe et al.,
2009).

For a rational and sustainable helminth control programme, a
comprehensive knowledge of the epidemiology of parasites and their
interaction with the host in a specific climate and management system is
a prerequisite (Barger, 1999). Therefore this study was conducted with the
objectives to estimate the prevalence and identify risk factors
associated with infection of cattle with trematodes in and around Bahir
Dar.

2. Materials and methods

2.1. Study area and animals

The study was conducted in Bahir Dar town and adjoining
Sebatamit village (about 4 km east of Bahir Dar), northwest Ethiopia.
Bahir Dar, the capital of Amhara Region, is located on the southern
shore of Lake Tana, the largest lake in Ethiopia and the sources of the
Abay (Blue Nile) river. The town is located approximately 578 km
northwest of Addis Ababa, at a latitude and longitude of 11°36′N and
37°23′E and an elevation of 1,800 meters above sea level. The
climate conforms to the Ethiopian woyanadega (mid altitude area) with average
annual rainfall of 1500 mm, humidity of 57.88 % and annual
minimum and maximum temperatures of 10 and 30 °C, respectively.
The main rainy season extends from late June to late September. The
area has poor drainage and there is annual over flooding during the
rainy seasons leaving pockets of water bodies for long period during the
dry season (Aregay, Bekele, Ferede, & Hailemelekot, 2013). Both the
traditional extensive and semi-intensive farming are practiced in the
study area. Sebatamit is a suburban village about 4 km from Bahir Dar
located on the bank of the River Abay.

The study involved cattle above 6 months of age, both sexes and
local and crossbred (Friesian–Zebu) animals managed under the tradi-
tional smallholder husbandry system where cattle are often kept out-
doors and grazed on communal pastures all day. The animals graze in
the vicinities of Lake Tana, the Blue Nile River and its tributaries
(Andasa and Tikurit) (Habtamu & Wolde Mariam, 2011). The study was
conducted from November 2011 to April 2012.

2.2. Study design and sampling technique

The study was a cross-sectional study involving 369 animals se-
lected using simple random sampling method. Sample size was calcu-
lated according to Thrusfield (2005) with 40% estimated prevalence of
the parasites (Habtamu & Wolde Mariam, 2011; Solomon & Wossene,
2007) and desired 95% confidence interval and 5% precision.

2.3. Fecal sample and data collection

Feces were collected directly from the rectum of the study animals
with gloved hands and were placed in clean universal bottles. The lo-
cality, breed, age, sex and body condition of each study animal were
recorded at the time of sample collection. Age was estimated using
dentition according to Torell, Bruce, Kvasnicka, and Conley (2003)
which involves noting the time of appearance and the degree of wear on
the temporary and permanent teeth. Cattle estimated to be less than 4
years old were considered as young cattle while those 4 years and above
as adults. Body condition was categorized into 3 broad categories (lean,
medium and fat) using recommendations by Nicholson and
Butterworth (1986).

2.4. Coprological examination

Coprological examination was made at Bahir Dar Regional Veterinary
Laboratory; fecal samples, when not examined immediately on arrival, were stored in a refrigerator at 4 °C until examined. Simple
sedimentation technique for detection and count of trematode eggs
(DAFWA, 2013; Hansen & Perry, 1994; Svendsen, 1997) was used with
minor modifications. Briefly, 3 g of feces was put in a container and 40-
50 ml tap water was added and mixed thoroughly. The suspension was
filtered through a tea strainer and allowed to stand for 5 min. The
supernatant was discarded carefully and the sediment was re-suspended
in tap water. The sedimentation process was repeated multiple times
until the fecal debris and coloring material was removed and the super-
natant appear clear. After the last sedimentation the supernatant
was removed very carefully and the sediment was recovered into a test
tube and re-suspended in about 5 ml tap water, a drop of methylene
blue was added, and allowed to stand for 5 min. For effective staining
of the debris which leaves the trematode eggs un-stained, all the material
was transferred into a Petri dish and examined under low power ob-
jective. Trematode egg counts were performed by moving the Petri dish
in such a way that every field was examined. Yellow color of Fasciola
spp. eggs was used to differentiate them from those of paramphistomes
(Urquhart et al., 1996). The differential EPG was calculated by dividing the
specific parasite egg count in a sample of 3 g by three.

2.5. Data management and statistical analyses

Data collected during sample collection and results of coprological
examinations were entered and stored for analysis into Microsoft Excel
spread sheet. The effect of age, sex, breed and body condition on in-
fecion with trematodes was analyzed using multiple logistic regression
model/analysis. Univariable logistic analysis was used to assess the
relationship between mixed and single trematode infections with body
condition. The Goodman and Kruskal’s gamma statistics was used as a
measure of correlation of occurrence of the three trematodes. A Venn
diagram (Dohoo, Martin, & Stryhn, 2009) was produced to illustrate the
level of coinfection with trematodes. The egg count data (EPG) were log
transformed [log10(EPG + 1)] and EPG difference between sex, age and
breed was analyzed using two-sample t-test, while one-way analysis of
variance (ANOVA) was used to test the EPG between body conditions.
Pair wise comparison of means was carried using the Bonferroni ad-
justment. An animal positive for at least 1 egg was considered positive
for the respective trematode infection. Linear regression was used to
determine relationships among the EPG of the three trematodes. All
The overall prevalence of trematodes observed in this study was 61.0% (95% CI: 55.9, 65.8). Specific prevalence was 20.1% (95% CI: 16.3, 24.5), 48.5% (95% CI: 43.4, 53.6), and 16.5% (95% CI: 13.1, 20.7) for Fasciola spp., paramphistomes and Schistosoma spp., respectively. Single infection with the respective trematodes (when an animal was infected with only one of the three trematodes) was 3.8% (95% CI: 2.3, 6.3), 28.7% (95% CI: 24.3, 33.6) and 6.0% (95% CI: 3.9, 8.9), respectively. Mixed infections with at least two parasites were recorded in 85 (23.0%, 95% CI: 19.0, 27.6) cattle (Table 1).

There was a substantial overlap in the infection of individual animals with Fasciola, paramphistomes and Schistosoma (Fig. 1). The overlap was significant (gamma = 0.457) between Fasciola and paramphistomes, while it was random for Schistosoma vs. Fasciola (0.104) and Schistosoma vs. paramphistomes (−0.023).

Table 2 shows the results of multivariable logistic regression analysis of association between trematodes prevalence and potential predictors. Prevalences of Fasciola, paramphistomes and Schistosoma were significantly associated with body condition and breed of the study animals (P < 0.01). The prevalence of all the three trematodes was higher in animals with lean body condition than with fat body condition and in local cattle than crosses. Cattle younger than four years were associated with higher prevalence of Fasciola and paramphistomes (P < 0.05). Only Schistosoma prevalence was associated with sex, where females were more affected than their male counterparts (P < 0.05). Fasciola prevalence, however, showed a tendency to be greater in females than in males (P = 0.058).

We have attempted to assess the association between mixed infections of trematodes with body condition. Body condition was significantly associated with concurrent infection with Fasciola and paramphistomes (P < 0.01) and paramphistomes and Schistosoma (P < 0.001). Highest prevalences of co-infections were observed in animals with lean body condition than animals with better body conditions. However, while co-infections involving paramphistomes were significantly associated with body condition, single infection with paramphistomes did not (P > 0.05) (Table 3).

Interestingly, there was no animal with fat body condition score in those with single Fasciola infection. But the number of observation (n = 14) was not sufficient to make statistical comparison. The number of animals found co-infected with Fasciola and Schistosoma (n = 9) and with single Schistosoma (22) were also not sufficient to make statistical comparisons and therefore were omitted from the analysis.

### 3.2. Fecal egg count

The mean (± SE) count of Fasciola, paramphistomes and Schistosoma eggs was 4.3 (± 0.55), 25.7 (± 2.11) and 3.1 (± 0.42) per gram of feces (Table 4), with a range of 0 to 57, 0 to 371 and 0 to 43, respectively. Mean fecal egg count per gram of feces (EPG) for all the three trematodes identified in this study was significantly associated with body condition score of the study animals (P < 0.001). EPG was highest in cattle with lean body condition. EPG was also significantly different among breeds (P < 0.05); indigenous animals shed higher number of trematode eggs (for all the 3 species) compared to crossbred animals. Fecal egg count of only paramphistomes was, however, affected by age (P < 0.001) whereby EPG was higher in younger cattle of < 4 years compared to cattle ≥ 4 years. Sex did not significantly affect trematode egg counts (P > 0.05) (Table 4).

A positive correlation (r² = 0.0443, P = 0.000) was obtained between Fasciola and paramphistomes EPG. However, there were no correlations (r² = 0.0028, P = 0.313) between Fasciola and Schistosoma EPG and between paramphistomes and Schistosoma EPG (r² = 0.0001, P = 0.815).

### 4. Discussion

In this study, the highest prevalence and EPG was recorded for paramphistomes (48.5%, 25.7) followed by Fasciola (20.1%, 4.3) and Schistosoma (16.5%, 3.1). Similar pattern of occurrence, where paramphistomes top the prevalence followed by Fasciola and Schistosoma, respectively has been reported for the three trematodes from different parts of Ethiopia (Ameni et al., 2001; Yenehe et al., 2012) and elsewhere in Africa (Nzalawze, Kassuku, Stothard, Coles, & Eisler, 2014). Keyyu, Monrad, Kyvsgaard, and Kassuku (2005) reported consistently higher prevalence of paramphistomes than Fasciola gigantica in cattle managed under different conditions in Tanzania.

The higher prevalence and EPG of paramphistomes may partly be explained by the fact that the adult parasite is considered non pathogenic and subsequently is not targeted by anthelmintic treatment. It might also be related to the biology of the parasite and the intermediate hosts. Adult paramphistomes may survive in the host for years (Hansen & Perry, 1994) and are very prolific expelling many eggs (Dorchies, 2006) while multiplication of the parasite in infected snails is massive (Hansen & Perry, 1994). The intermediate hosts of paramphistomes are also extremely adaptable and prolific breeders (Hansen & Perry, 1994). Limitation of availability of effective drugs against paramphistomes (Dorchies, 2006) might have also contributed to the relative high prevalence of the parasite. The common anthelmintics used for routine de-worming against important nematodes and liver

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### Table 1
Prevalence of trematodes in cattle in and around Bahir Dar (n = 369).

| Parasite species | Number positive | Prevalence (95% CI) |
|------------------|-----------------|--------------------|
| Fasciola         | 74              | 20.1% (16.3, 24.5)  |
| Paramphistomes   | 179             | 48.5% (43.4, 53.6)  |
| Schistosoma      | 61              | 16.5% (13.1, 20.7)  |
| Fasciola + Paramphistomes | 45 | 12.2% (9.2, 16.0)  |
| Fasciola + Schistosoma | 9 | 2.4% (1.3, 4.6) |
| Paramphistomes + Schistosoma | 27 | 7.3% (5.1, 10.5) |
| Fasciola + Paramphistomes + Schistosoma | 4 | 11.1% (0.4, 2.9) |
| Mixed            | 85              | 23.0% (19.0, 27.6)  |
| Trematodes       | 225             | 61.0% (55.9, 65.8)  |

Fig. 1. A Venn diagram showing overlap in coprological prevalences of Fasciola, paramphistomes and Schistosoma in cattle.
fluke in Ethiopia such as albendazole, ivermectin and triclabendazole have little or no effect on paramphistomes (Rolfe & Borray, 1988, 1993). Higher and increasing prevalence of paramphistomes compared to liver fluke has been documented in France partly due to the lack of an effective treatment against cattle paramphistomosis (Mage et al., 2002).

The prevalence of Fasciola spp. observed in our study is comparable to the 24% prevalence reported by Yeneneh et al. (2012). It was however lower compared to other recent reports from areas adjoining Lake Tana (Gebrie, Gebreyohannes, & Tesfaye, 2015; Tsegaye, Abebaw, & Girma, 2012) and reports from other parts of the country demonstrated co-infection of cattle with Fasciola and paramphistomes; ParaSch, paramphistomes and Schistosoma; BC, body condition

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The prevalence of paramphistomes recorded in the present study (48.5%) was comparable to an earlier report (45.8%) from an area not far from our study area (Yeneneh et al., 2012) and western Ethiopia (44.2%) (Fromsa et al., 2011) which is characterized by a humid tropical climate of heavy annual rainfall (Vanleeuwen, Tolosa, Sirak, & Aregay, 2013; Berhe et al., 2009; Fromsa et al., 2011; Yilma & Mesfin, 2000).

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Nemera, & Belaine, 2014). Studies conducted near a large marshy area traversed by a river (Ameni et al., 2001) in northeastern Ethiopia (75%) and around a small lake (Kifleyohannes, Kebede, Hagos, Weldu, & Michael, 2015) in northern Ethiopia (65.3%) demonstrated higher prevalence of paramphistomes. Many other studies in Ethiopia, however, reported lower prevalence of the parasite compared to our finding (G. Mariam, Mohamed, Ibrahim, & Baye, 2014; Kemal & Terefe, 2013; Telila et al., 2014; Yohannes, Birasa, Damena, Tasew, & Degefu, 2013). The higher prevalence of paramphistomes, whose intermediate hosts are aquatic snails, observed in the present study could be explained by the fact that our study was conducted near permanent water bodies as opposed to some of the other studies which were conducted in drier areas with no major permanent water bodies.

Prevalence of Schistosoma observed in our study (16.5%) was comparable to the findings of Chanie et al. (2012) (13.7%) in an area adjacent to Lake Tana and Fromsa et al. (2011) (13.5%) from western Ethiopia. The prevalence was, however, low compared to earlier reports from Lake Tana basin (Habtamu & Wolde Mariam, 2011; Habtamu et al., 2013; Yeneneh et al., 2012) and a report from northeastern Ethiopia (28%) (Ameni et al., 2001). The variations observed among studies in trematode prevalence in general may be attributed to differences in climato-ecological conditions among study areas, difference in rainfall between study years, differences in study seasons and difference in animal management practices.

Prevalence as well as EPG of all the three trematodes considered in this study were highest in lean animals compared to animals with medium and fat body condition. Heavy infection with Fasciola in cattle, especially in young stock, may result in severe disease characterized by anemia, hypoalbuminemia (edema), ill thrift and weight loss (Boray, 1969; Love, 2017; Urquhart et al., 1996). Similarly, heavy infection with immature stomach flukes may cause decreased appetite, listlessness and weight loss (Rolle et al., 1991).

Even moderate infections with Fasciola (Hope-Cawdery et al., 1977; Ross, 1970; Wamme, Hammond, Harrison, & Ouyang-Abuje, 1999) and immature paramphistomes (Lloyd et al., 2007) may affect weight gain. Loss of appetite, which might contribute to the poor body condition, is also one of the clinical signs of chronic fasciolosis (Love, 2017).

However, it should be noted that it is difficult to separate the effects of the different genera of trematodes on body condition as they tend to occur together. Our finding supports previous reports which associated fasciolosis (Fromsa et al., 2011; Mesheha & Tesfaye, 2017; Phiri et al., 2006) and paramphistomosis (Fromsa et al., 2011; G. Mariam et al., 2014; Melaku & Addis, 2012) and schistosomiasis (Fromsa et al., 2011; Lulie & Guadu, 2014) with poor body condition.

The significant association of paramphistomes (when an animal was considered paramphistomes positive irrespective of its status to the other two trematodes) with body condition observed in our analysis was not repeated when animals infected only with paramphistomes (single infection) were considered. It is possible that the result was influenced by those animals which were also co-infected with other trematodes (especially Fasciola) in the former, as paramphistome positive animals were more likely to be also positive to Fasciola than their paramphistome negative counterparts. It is also possible that animals found with single paramphistome infection in the study were de-wormed with anthelmintics effective against liver fluke and possibly to nematodes. The result may even suggest additive or synergistic pathogenic effect of co-infection with trematodes. High mortality rate in concurrent infection involving F. gigantica and Schistosoma bovis in dairy cows has been reported from the Sudan (A/Rahman et al., 2007). After observing a positive correlation ($r^2 = 0.12$) between F. gigantica and paramphistomes worm count in naturally infected cattle, Yabe et al. (2008) suggested that the heterologous interaction of these two parasites may compound the economical effects of liver flukes to the livestock industry.

The prevalence of Fasciola and paramphistomes and EPG of paramphistomes were higher in young cattle, less than 4 years old, compared to their adult counterparts. Concurrent with our finding other researchers also recorded higher prevalence of Fasciola in younger cattle (Aregay et al., 2013; Mesheha & Tesfaye, 2017). However, many studies on paramphistomes in Ethiopia and elsewhere didn’t find difference in prevalence among age groups (Fromsa et al., 2011; G. Mariam et al., 2014; Khedri, Radfar, Borji, & Mirzaei, 2015; Kifleyohannes et al., 2015; Phiri et al., 2006). The variation might be partly attributed to differences in classification of age categories among the studies. Development of immunity due to exposure to Fasciola, which limits the lifespan of the primary infection, slows the migration of secondary infection and eventually reduces the number of flukes established (Urquhart et al., 1996), may be responsible for lower prevalence of Fasciola in older cattle. Similarly, development of a good acquired immunity against paramphistomes (Rolle et al., 1991) has been stated.

Contrary to our expectation and some earlier reports from Ethiopia (Gebrie et al., 2015; Habtamu & Wolde Mariam, 2011), local cattle were found to be more affected with the three trematodes and shed higher number of eggs in their feces than crossbred animals. This difference is likely to be due to exposure difference rather than difference in natural resistance, as studies involving F. gigantica suggested that local cattle (Bos indicus) appear to be more resistant than B. taurus to infection with Fasciola (Bitakaramire, 1973; Castelino & Preston, 1979).

It is possible that more attention is paid to valuable (crossbred) animals that their chance of grazing fluke infested areas is limited and/or they are more frequently de-wormed than local animals. In agreement with our observation some studies reported higher prevalence of trematodes in local cattle than crossbred animals (Islam, Begum, Alam, & Mamun, 2011; Mesheha & Tesfaye, 2017; Tsegaye et al., 2012).

We found a high correlation ($\gamma = 0.457$) between Fasciola and paramphistomes occurrence and their EPG ($r^2 = 0.044$). Similarly, Phiri et al. (2006) recorded a positive correlation ($r^2 = 0.043$) between EPGs of F. gigantica and paramphistome species. Yabe et al. (2008) recorded even stronger correlation ($r^2 = 0.120$) between these two trematodes using worm burdens. The correlation may be partially explained by the existence of common factors that contribute to the occurrence of these parasites.

In this study, few animals ($n = 9$) harbored both Fasciola and Schistosoma and neither the occurrence nor the EPG of these trematodes were correlated. Yabe et al. (2008) recorded a similar finding and explained it with the significant liver pathology both trematodes cause which may exclude establishment of the other when one infection is established.

In conclusion, the present study demonstrated that the major trematodes of animal health and welfare importance are relatively highly prevalent in the study area especially in young cattle with high rate of mixed infections. The finding suggests that there is considerable economic loss due to trematode infections through reduced production efficiency of cattle in the study area. The prevalence of Fasciola spp. and Schistosoma spp. may also show the risk these parasites could pose to public health. Introduction of measures which would help minimize exposure of cattle to the parasites, such as keeping cattle away from grazing high risk areas, and strategic use of anthelmintics effective against mixed trematode infections especially in young stock may be considered. Empirical diagnosis and treatment of cattle, especially showing poor growth or weight loss, in the study area should take trematodes into consideration.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This research work was approved by the Research and Publication Committee of the School of Veterinary Medicine of Hawassa University.
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