

Concurrent Infection of Fascioliasis and Trypanosomosis and Associated Risk Factors in Local Zebu Breed Cattle of Western Ethiopia

This article was published in the following Dove Press journal:
Veterinary Medicine: Research and Reports

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Background: A cross-sectional study was conducted from late October 2016 to June 2017, with the primary objective of estimating and analyzing the concurrent occurrence of both fascioliasis and trypanosomosis infections and associated risk factors along the tsetse-infested Didessa river basin.

Methods: The methodology applied was based on stratified sampling for the parasitological study, with entomological and malacological surveys, including fly dissection.

Results: The result of variance-ratio testing between trypanosomosis and fascioliasis infections (mean prevalence 0.117 ± 0.322 and 0.283 ± 0.451 , respectively), was statistically significant ($P[F > f] = 0$), with higher observed fascioliasis infection ($n=147$, 28.27%). Severe anemia was observed in trypanosomosis infection, with mean packed cell volume of 19.57 (OR=0.71, $P > |z| = 0.000$), and vast fascioliasis infections identified among cattle with medium and poor body condition in terms of weight ($n=91$ [32.73%] and $n=38$ [21.47%]). On entomological study, 578 (62.62%) and 345 (37.38%) female and male *Glossina tachinoides* fly species were cached, respectively, with overall mean flies/trap/day of 5.19 ($n=923$). Despite the prevalence of trypanosomosis in infected cattle, of 130 *G. tachinoides* flies dissected, only three were found to be positive for an infection rate of 2.31%. Malacological study identified three snail species known to maintain fascioliasis: *Lymnea truncatula* ($n=28$, 45.16%), *Lymnea natalensis* ($n=23$, 37.10%), and *Biomphalaria* ($n=11$, 17.74%). Concurrent infection with fascioliasis and trypanosomosis was mainly associated with the co-occurrence of their intermediate host snails and *Glossina* flies, respectively, with 4.42% ($n=23$) prevalence.

Conclusion: This study clearly demonstrated that the former parasite was highly associated with emaciation, whereas the second was responsible for anemia. In future, researchers should focus solely on estimating meat and milk production of local cattle to assess the economic impact of the study parasites.

Keywords: concurrent infection, fascioliasis, trypanosomosis, associated risk factors, cattle

Introduction

Ethiopia has a huge and diverse livestock population that plays an important role in the economy and livelihoods of farmers and pastoralists. Despite the excess animal population, production and productivity is scanty and even below the average for most countries in sub-Saharan Africa. This may be due to poor nutrition, reproduction insufficiency, management constraints, and prevailing animal diseases.¹ Among the diseases, fascioliasis and trypanosomosis are the major impediments to livestock

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development and agricultural production, specifically for pastoralists living along the major river basins. In Ethiopia, about 180,000–200,000 km² of agriculturally suitable land in the west, southwest, and northwestern lowlands and associated river basins of the country is infested by tsetse flies, trypanosomes, snails, and helminthic parasites.^{2–4}

Among the genera in the Fasciolidae trematode family, *Fasciola* is a helminthic parasite affecting mainly ruminant livestock.⁵ The commonly known species of *F. hepatica* and *F. gigantica* are the usual causes of fascioliasis in livestock, while rare in humans. However, sometimes they can affect humans through drinking contaminated water or consumption of aquatic plants containing encysted metacercaria.⁶ The parasite lives part of its life in aquatic snails that act as intermediate hosts and are found in and around wet areas, such as irrigation, water holes, and waterlogged areas.⁷

African animal trypanosomosis (AAT) is a parasitic infection caused by an extracellular flagellate. Trypanosomes are unicellular protozoan parasites of the phylum Sarcomastigophora, order Kinetoplastida, family Trypanosomatidae, and genus *Trypanosoma*.^{8,9} Trypanosomosis in Africa is mainly restricted to areas in which the vector, the tsetse fly (*Glossina* spp.) can survive. However, it may exist outside the tsetse-belt areas and transmitted mechanically by biting flies of the genera *Tabanus*, *Haematopota*, *Chrysops*, and *Stomoxys*. This type of transmission has caused the acyclic (mechanical) spread of trypanosomosis outside tsetse-infested areas.⁹ The most known pathogenic trypanosomes are *T. congolense* and *T. vivax*, which are responsible for the most important form of AAT and prevalent species in domestic animals.¹⁰

Both fascioliasis and trypanosomosis probably lead to high mortality and poor productivity. They also cause considerable economic loss through organ condemnation at meat inspection, reduced productivity (weight gain, milk yield, and traction power), rendering large areas of Ethiopia totally unsuitable for cattle-based agriculture, and lowered immunity (resistance) of cattle to other concurrent disease infections.^{11,12} Despite the concurrent occurrence of fascioliasis and trypanosomosis infection in the region, scientific investigation and information on the topic was very scanty. Therefore, the primary objective of this study was to investigate/estimate and analyze the concurrent occurrence of these infections and associated risk factors in the tsetse-infested Didessa river basin.

Methods

Study Area

Bedele and Jimma Arjo districts are located 480 and 378.4 km west of Addis Ababa, respectively, at an elevation of 1,200–2,200 m above sea level. The climate alternates, with long summer rain (June–September), a short rainy season (March–April), and a dry winter season (October–February). The study area (Figure 1) receives a mean annual rainfall of 1,200–1,800 mm, with optimum temperature of 25°C. Agriculture is the mainstay of peoples' livelihoods, with a mixed farming system. Livestock plays an integral role in agriculture.¹³

Study Design and Sample-Size

Determination

This cross-sectional study was conducted from late October 2016 to June 2017 with the primary objective of estimating and analyzing the concurrent occurrence of fascioliasis and trypanosomosis infections and associated risk factors along the tsetse-infested Didessa river basin. To obtain a representative sample area, stratified sampling was applied, as the target populations were all local zebu-breed cattle of all age-groups and both sexes in each randomly selected study site. The sample size was determined using¹⁵ expected disease prevalence in the area of 50%, since there had been no officially reported prevalence previously. A minimum 384 head of cattle was required, and 520 animals were included, increasing precision of the study:

$$n = \frac{Z^2 pq}{e^2}, \text{ where, } z = 1.96 \text{ CL}(\alpha) \text{ of } 95\%,$$

$$p = \text{prevalence, } e = \text{margin of error, } q = 1 - p$$

The methodology applied was based on stratified sampling for the parasitological study and entomological and malacological surveys, including fly dissection. Blood and fecal sample collection, laboratory presentation, diagnosis, recording of associated risk factors, and analysis of both study parasites were performed simultaneously to assess concurrent occurrence.

Blood-Sample Diagnostic Procedure

Paired blood samples were collected from the auricular vein (marginal ear vein) of each animal using two hematocrit capillary tubes after the animal had been restrained properly. Each tube was filled to 75% of its height and sealed with crystal

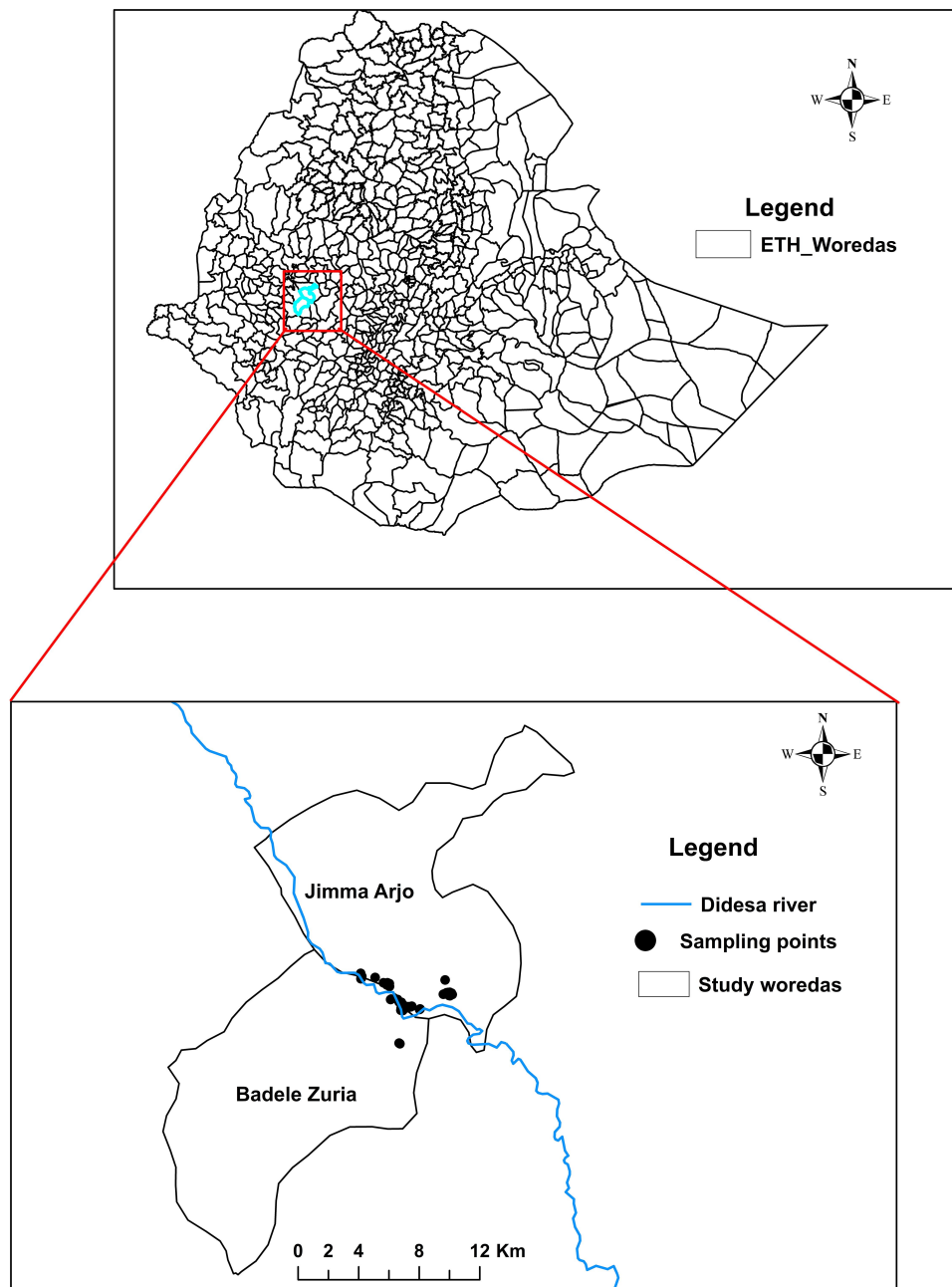


Figure 1 Map of the study area.¹⁴

sealant. The first tube was used to measure packed cell volume (PCV) values for determination of anemia.¹⁶ According to Murray et al, only cattle with $PCV \leq 24$ are considered anemic. Then, the second tube was cut 1 mm below the buffy coat to include the top layer of red blood cells. The content of the tube was expressed onto a clean microscopic slide, mixed, and covered with a coverslip. Finally, prepared slides were examined for the presence of trypanosomes based on the type of movement in the microscopic field. Trypanosome species were confirmed after Giemsa staining and examination under

oil-immersion microscopy (100× magnification) by morphological characteristics of the parasites. During sample collection, the body condition of each animal was categorized as poor, medium, and good,¹⁷ and all associated risk factors, eg, age, sex, and altitude, were recorded.

Entomological Study and Fly Dissection

Monopyramidal traps (n =89) were deployed in the Didesa river basin of Bedele and Jimma Arjo districts. All traps were baited uniformly with octenol (1-octen-

3-ol), acetone, and 3-week-old cow urine. All bait was placed on the ground about 30 cm upwind of the trap in separate bottles. The poles of traps were greased to prevent predation, mainly by ants. The traps were positioned for 2 consecutive days with a mean gap between traps of 250 m, in areas most likely for finding tsetse, based on the presence of gallery forests and the location of rivers and streams after clearing up to a 2–3 m radius of the trap site to enhance the visibility of the traps and prevent possible fire damage.¹⁸ Then, after 48 hours, the catch in each trap was sorted by fly species and counted, identified, and analyzed. The apparent density (arithmetic mean catches per trap per day) of flies was calculated by dividing the total number of tsetse flies captured (ΣF) by the product of the number of functioning traps used to catch them (T) and the number of days for which the traps were operational (D): $FTD = \Sigma F / T \times D$. The sex of all collected flies was identified by observing the posterior end of the ventral aspect of the abdomen by hand lens. Male flies were identified by the enlarged hypopygium in the posterior ventral part of the abdomen, which is absent in female flies.¹⁹ The coordinates of each trap position were recorded using GPS, and a map of the study area was drawn.

Dissection was carried out as described by Meharenet and Alemu,²⁰ starting by removing the wings and legs after wing-fray and ovary analysis had determined the age of male and female flies, respectively. Then, freshly killed tsetse flies were dissected under microscopy using 0.9% normal saline. Trypanosome infections in dissected body parts of tsetse flies (ie, midgut, salivary gland, and mouthpart or proboscis) were observed using a compound microscope (magnification 40 \times), and consequently parasite positive samples were again stained with Giemsa and examined under compound oil-immersion microscopy for trypanosome species identification based on morphology.^{21,22}

Coprosopic (Fecal Sample) Examination

Fecal samples were collected directly from the rectum using sterile gloves after blood samples had been collected. The fecal samples were placed in a clean universal bottle preserved with 10% formalin and closed with a screw top in airtight condition. The bottles were labeled with unique identification numbers that matched the detailed data recorded using the standard format. The samples were transported to Bedele Regional Laboratory for gross and microscopic examination. For qualitative

study,²³ a routinely used sedimentation-bead technique was used for identification of *Fasciola* eggs.

Malacological Study

Snails were collected by hand (gloved) or scooping from marshy grass lands and waterlogged areas around the Didessa river basin. In sum, 62 snails were collected, placed in plastic bags with fresh algae, aquatic vegetation, and aerated water, and transported to Bedele Regional Laboratory for identification. Snail identification was conducted at the laboratory based on morphology.

Data Analysis

Data processing was carried out using both qualitative and quantitative data analysis. For qualitative testing, tables, frequency distribution, and means \pm SD analysis were some of the methods employed. For quantitative testing, prevalence rates and differences in mean PCV based on disease prevalence were analyzed using independent *t*-tests, and Chi-square² tests were used to estimate significance with adjusted ORs. Test statistics were used to assess overall disease prevalence with precision of 5% and 95% CIs. We used StataCorp for analysis.

Results

Of a total of 520 bovine blood specimens examined for trypanosomosis infection, 61 were diagnosed positive, for prevalence of 11.73%. These were composed of 39 (7.50%), 18 (3.46%), and 4 (0.77%) *T. congolense*, *T. vivax*, and mixed infections (*T. congolense* and *T. vivax*), respectively. Concurrently, 520 bovine fecal samples were examined for fascioliasis infection, with 147 diagnosed positive for prevalence of 28.27%. Variance-ratio tests for trypanosomosis and fascioliasis infections resulted in means of 0.117 \pm 0.322 and 0.283 \pm 0.451, respectively, and were statistically significant ($P [F > f] = 0$) for higher fascioliasis infections. Among the total animals tested, 23 (4.42%) had coinfection of trypanosomosis and fascioliasis (Tables 1 and 2).

Among the associated risk factors considered, body condition and anemia were found to be statistically significant (AOR 1.70, 95% CI 1.10–2.68, $P=0.01$, and AOR 0.13, 95% CI 0.06–0.27, respectively $P=0$) for fascioliasis and trypanosomosis infections. As shown in Table 2, animals with good body condition had less chance of being infected by fascioliasis, while nonanemic animals had lower risk of having trypanosomosis infection than animals with poor body condition and anemia, respectively. Higher prevalence of *Fasciola*

Table 1 Concurrent Infection and Variance Ratios of the Study Parasites

	Infections (n)	Prevalence (%)	Mean Prevalence	SE	SD	CI	P(F>f)
<i>T. congolense</i>	39	7.50	0.15	0.023	0.527	0.105,0.195	0*
<i>T. vivax</i>	18	3.46	0.04	0.008	0.183	0.189,0.050	
Mixed infection	4	0.77	0.02	0.012	0.262	0.001,0.046	
Trypanosomosis	61	11.73	0.12	0.014	0.322	0.089, 0.145	0*
Fascioliasis	147	28.27	0.28	0.012	0.451	0.244, 0.322	
Concurrent infection (both parasites)	23	4.42	0.44	0.009	0.206	0.027, 0.062	

Note: *Statistically significant.

Table 2 Infections of Study Parasites in Cattle Grouped Under Epidemiologically Associated Risk Factors

	Observation (n)	Fascioliasis			Trypanosomosis		
		Positive (%)	AOR (CI)	P	Positive (%)	AOR (CI)	P
Age							
Young	229	59 (25.76)	1 (1)	0.26	28 (12.23)	1 (1)	0.76
Adult	291	88 (30.24)	1.25 (0.83–1.88)	0.26	33 (11.34)	0.92 (0.52–1.63)	0.76
Sex							
Male	291	91 (31.27)	1 (1)	0.09	35 (12.03)	1 (1)	0.81
Female	229	56 (24.45)	1.41 (0.94–2.12)	0.09	26 (11.35)	1.07 (0.60–1.91)	0.81
Body condition							
Poor	177	38 (21.47)	1 (1)	0.01*	23 (12.99)	1 (1)	0.52
Medium	278	91 (32.73)	1.70 (1.10–2.68)	0.01*	31 (11.15)	0.83 (0.47–1.52)	0.52
Good	65	18 (27.69)	0.41 (0.09–0.63)	0.01*	7 (10.77)	0.16 (–0.52 to 0.53)	0.52
Anemia status							
Anemic	224	67 (29.91)	1 (1)	0.47	50 (22.32)	1 (1)	0*
Nonanemic	296	80 (27.03)	— (0.58, 1.30)	0.47	11 (3.72)	–13 (0.06, 0.27)	0*

Note: *Statistically significant.

infection occurred among cattle with medium and poor body condition (n=91, 32.73%; n=38, 21.47%), whereas comparatively higher trypanosomosis infection was observed (n=50, 22.32%) among anemic cattle with PCV <24. Milder anemia

was observed in fascioliasis-infected cattle (mean PCV23.37 ±4.71, OR 0.93; $P>|z|=0$), and severe anemia was characterized by trypanosomosis infection (mean PCV=19.57±4.73 OR 0.74; $P>|z|=0$; Table 3).

Table 3 Association of Anemia with Fascioliasis and Trypanosomosis Infections

	Observation (n)	Mean PCV	SD	OR	SE	z-value	P> z
Fascioliasis-negative	374	24.61	3.70	2.36 (0.76–7.33)	0.14	1.48	0.139
Fascioliasis-positive	147	23.37	4.71	0.93 (0.89–0.97)	0.02	–3.10	0*
Trypanosomosis-negative	459	24.88	3.51	128.90 (24.56–676.49)	248.97	6.20	0*
Trypanosomosis-positive	61	19.57	4.73	0.74 (0.69–0.79)	0.03	–8.03	0*
Concurrent infection (both parasites)	23	22.87	0.21	0.96 (0.01–72.13)	0.05	–1.68	0.094

Note: *Statistically significant.

Table 4 Entomological Study and *Glossina tachinoides* Fly Dissection

Sex	<i>Glossina tachinoides</i> Infection	Infection Rate	Dissected Flies, n	Flies Collected, n	FTD	Traps Deployed, n
Male	1	1.75	57	345	1.94	89
Female	2	2.74	73	578	3.25	
Total	3	2.31	130	923	5.19	

From the entomological study, 578 (62.62%) and 345 (37.38%) female and male *G. tachinoides* flies were collected for an overall FTD of 5.19 (n=923). Despite the prevalence of trypanosomosis in infected cattle (n=130), *G. tachinoides* flies were dissected, and three found positive (two were harboring *T. congolense* and the remaining *T. vivax*) for an infection rate of 2.31%. All were identified at different sampling points (Table 4).

The malacological study collected a total of 62 snails from different biotopes (watering and grazing lands) and identified as three snail species known to maintain fascioliasis: *Lymnea truncatula* (n=28, 45.16%), *L. natalensis* (n=23, 37.10%), and *Biomphalaria* (n=11, 17.74%).

Discussion

Bovine fascioliasis is one of the most important parasitic diseases in cattle, causing mortality and production losses in various parts of Ethiopia. It is a priority disease in both highland and lowland areas.²⁴ This fluke's distribution is worldwide in areas where cattle, sheep, and goats are raised and there is a niche for the *Lymnaeidae* snail as a maintenance or intermediate host.²⁵ Similarly, AAT and its primary vector, the tsetse, are among the biggest constraints to sustainable livestock production in Africa, specifically Ethiopia.²⁶ Although extensive trypanosomosis and tsetse (T&T)-control operations have been running since the beginning of the twentieth century, tsetse infestation in sub-Saharan Africa and Ethiopia has hardly receded.²⁷ In the context of AAT or T&T control, achieving eradication of the disease has been highly debated and many disease experts believe that sustained reduction in disease incidence to a locally acceptable level ("control") is a more realistic target.²⁸ However, geographic variation in T&T-species distribution and ecoepidemiology of the disease and concurrent occurrence with other gastrointestinal parasites, mainly fascioliasis, make it highly harmful to livestock production.²⁹

Mathewos et al²⁹ suggested that concurrent infection of *Trypanosoma* and *Fasciola* was highly pathogenic, resulting in anemia and even death in cattle if not treated early. Contrary

to this, the current study showed that animals with concurrent infections had better PCV than those that had trypanosomosis infection only. Inconsistently with the present findings, Dwinger et al and Kaufmann et al^{30,31} confirmed an increase in pathogenicity when helminthosis is aggravated by trypanosome infections. Furthermore, Mathewos et al²⁹ confirmed an improvement in body-weight gain and PCV levels when infected cattle were treated with a combination of trichlabendazole and trypanidum/samorin, which was consistent with this finding, suggesting that each parasite is highly responsible for anemia and loss of body weight (condition).

The conducted entomological survey confirmed the presence of *G. tachinoides* with trypanosomosis infection. When *Glossina* flies are infected with trypanosomes, they gate infection throughout their lifetime, which makes them cyclic transmitters of the disease.³² The trypanosomosis prevalence found was due to the high *Glossina* fly density at the study area and confirmed trypanosome infection in densely distributed flies.

One of the most important factors in the occurrence and prevalence of *Fasciola* infection in the study area was the availability of suitable snail habitats.³³ This study demonstrated that *L. truncatula*, *L. natalensis*, and *Biomphalaria* species are highly dominant snail species, and they are known intermediate hosts for fascioliasis. Other studies were also in agreement with this finding, which confirms that the distribution and occurrence of *Lymnaeidae* snails results in high fascioliasis prevalence.^{33–36}

Conclusion

Concurrent infection of fascioliasis and trypanosomosis was mainly associated with the cooccurrence of their intermediate host snails and *Glossina* flies, respectively. This study clearly demonstrated that the former parasite was highly associated with emaciation, whereas the second was responsible for anemia. The major findings of the present study strongly suggest that concurrent infection with *Trypanosoma* and *Fasciola* is the most harmful form of parasitosis in animals within the study area. In future, it is recommended that researchers focus solely on estimating meat and milk

production of the local cattle to access the economic impact of the study parasites.

Data-Sharing Statement

The data sets supporting the conclusions of this research article are available upon request to the corresponding author.

Ethics Approval and Consent to Participate

The National Institute for the Control and Eradication of Tsetse Fly and Trypanosomiasis and Ministry of Agriculture, Ethiopia authorized the fieldwork. The purpose of the study was clearly explained to the cattle owners and veterinary officers, and consent was obtained verbally from the institute's technical team and cattle owners. Participants' involvement in the study was on a voluntary basis. Those who were unwilling to participate or wished to quit were informed that they may do so without any restriction. This study did not involve any human/animal trials or experiments.

Funding

The authors received no specific funding for this work.

Disclosure

The authors report no conflicts of interest for this work.

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