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Field Trials to Evaluate Five Fasciolicides against Natural Liver Fluke Infection in Cattle and Sheep in Egypt

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ABSTRACT

Fasciola hepatica, a parasitic trematode, affects cattle and many mammals, including humans. The present study was carried out in Assuit governate, Egypt, over one year from 2018 to 2019, to assess the prevalence of fascioliasis in cattle and sheep. We clinically examined 835 animals (303 cattle and 532 sheep) from different private farms. We performed the fecal examination through a direct smear and did a sedimentation technique. The results demonstrated that fascioliasis was present in 20.8% of cattle and 17.1% of sheep, and the overall prevalence was 18.4%. After assessing associated risk factors, there was a significant association only between sex and infection rate ($P<0.05$). Other assessed risk factors (species, water, and feeding source) did not affect the infection level ($P>0.05$). By calculating the odds ratio, the sex was considered as a risk factor as odds ratio (OR) =5.879, 95% confidence interval (CI) 3.699-9.449. Categorized the animals into six groups. We treated each group with either albendazole, triclabendazole, superzole, clorsulon, or rafoxanide. NC group received no treatment. Animals were subjected to clinical and laboratory examination after the second dose's third and sixth week. The recovery percentage in animals treated with albendazole, triclabendazole, and Superzole was 84%, while 84% and 96% in animals treated with clorsulon and rafoxanide, respectively. All the drugs were effective ($P<0.05$); nonetheless, rafoxanide demonstrated the best recovery percentage (area under curve =0.605 in cattle and =0.615 in sheep).

Key words: Fascioliasis, Cattle, Sheep, Prevalence, Sex, Fasciolicides.

INTRODUCTION

Fasciolosis is a prevalent and severe infectious parasitic disease infecting all domestic ruminants and humans. It has risen to the top of all zoonotic helminths worldwide (Haridy et al. 2002; Piri et al. 2018; Umur et al. 2018; George et al. 2019; Aghayan et al. 2019; Villa-Mancera and Reynoso-Palomar 2019. Alsulami and Mohamed, 2021; Kipyegen et al. 2022). Bovine Fascioliasis always lacks clinical signs, and the infection is usually chronic, manifesting as growth reduction, decreased milk production, low meat quality and quantity, and impaired reproduction (Martínez-Valladares et al. 2010). In addition, liver cirrhosis was reported (Marcos et al. 2007). Human Fascioliasis was becoming a significant public health problem in Egypt, especially in the Nile Delta region (Curtalei et al. 2000). Infected animals are considered the primary source of the infection. *Fasciola hepatica* and *F. gigantica*, are the main causative agents for the disease. Freshwater snails transmit them (lymnaeid) and a variety of other domestic animals acting as reservoir hosts (WHO 2004). *Fasciola gigantica* is the main species in Africa. Most fascioliasis infections emerge at the end of the wet seasons, causing high prevalence (up to 25%) at the beginning of the dry season. Many risk factors were associated with Fasciola infection in cattle and sheep (Theodoropoulos et al. 2010). Evidence on risk factors, especially cattle, is still limited in Egypt. The spread of Fascioliasis in animals is significantly related to different climatic and environmental conditions, such as the presence of wet pasture and water sources. These conditions provide an excellent environment for the growth and spread of free-living fluke stages (Charlier et al. 2011; Relf et al. 2011).

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Other factors can affect the infection level, such as sex, breed, stocking herd level, water, and food source (Kuerpick et al. 2013; Petros et al. 2013). Detecting eggs in feces or adult worms in the liver and bile ducts post-mortem has traditionally been used to diagnose Fascioliasis.

The egg detection method is less sensitive as 30% of animals shed a small number of eggs (Happich and Boray 1969). Control of fasciolosis in ruminants is based primarily on anthelmintic using flukicides, followed by control of snails as intermediate hosts (Pfukenyi et al. 2006). Triclabendazole (TCBZ) is one of the most widely used drugs against Fasciola spp. Due to its high efficacy against both immature and adult flukes, triclabendazole (TCBZ) is one of the most commonly used drugs against Fasciola spp. (Boray et al. 1983). However, many studies have recently reported the development of resistance against the benzimidazole group, including (Albendazole and Triclabendazole) (Moll et al. 2000; Coles 2005; Alvarez-Sanchez et al. 2006), due to the year-round use of these drugs with no monitoring of drug efficacy. Other flukicides available on the market include clorsulon (CLS) and Rafoxanide. The present study aims to determine the prevalence of Fascioliasis in cattle and sheep in the Assuit Governorate, Egypt, to estimate the associated risk factors with Fasciola infection in cattle and sheep there and achieve the appropriate prevalence outcome control measures by selecting the best anthelminthic drug against Fascioliasis.

MATERIALS AND METHODS

Ethical Approval

The study was approved in accordance with the guidelines of institutional animal care. The negative group received the treatment after the study before being admitted to the farms.

Animals

This study was conducted from July 2018 to August 2019 in Assuit Governorate, Egypt. A total number of 815 animals (303 cattle and 532 sheep) were clinically examined from different private farms in some villages in Assuit Governorate, which were visited twice. Examined animals were marked by ear tags with animal ID (identification number). The animals were of different sexes (405 male, 430 female) and most of them (815 animals) were raised on purchased clover, whereas the other animals (20) were raised on cultivated clover, which grows by groundwater. The water source is canal water for 751 animals and groundwater for 84 animals. Clinical examination was done through body temperature, bwt measurement and examination of conjunctiva mucous membrane, edematous swelling in the intermandibular space, and hair—diarrhea afflicted all of the animals.

Fecal Examination

Direct Smear

Fecal samples were collected directly from the rectum or immediately after defecation in a numbered plastic cup labelled with animal ID and collection date. A direct smear was used for qualitative analysis; a small portion of each sample was transferred to a glass slide, mixed with a drop of normal saline and examined under a light microscope with 10 and 40 magnification lenses to detect Fasciola spp. eggs (Kaufmann 1996).

Sedimentation Technique

A mixture of 3 grams of faeces and 30ml of water was left to sediment in a graduated cylinder for 3 minutes and then the supernatant was removed. The sedimentation was repeated twice and then a drop of the sediment was transferred to a microscopic slide, methylene blue (1%) was added (Sirois 2016). A 40X magnification lens was used to detect Fasciola spp. eggs. Sedimentation was repeated after the treatment on the third and sixth weeks. The number of eggs per gram of feces (epg) was quantified by dividing the fecal egg count by a factor of 3.

Flukicides Treatment Schemes

Positive animals were allocated to five treatment groups, and the sixth group is negative control (NC) (Table 1); the animals inside these five groups were divided into three categories based on the intensity of the infection: low infection 1-2 epg, medium infection 2-4 epg and high infection more than 4 epg.

Statistical Analysis

All the obtained data was entered in Microsoft Excel® (Microsoft® office 2013) spreadsheet and then exported into SPSS (version 22, IBM Corp., Armonk, NY) for statistical analysis. Pearson Chi-square test was used to measure the association between risk factors, and the percentage was done by within raw and Fascioliasis prevalence. The Monte Carlo test measured the association between the treatment groups in each species. Odds ratio (OR) were assessed. The receiver operating characteristic (ROC) curve and the area under the curve (AUC) were done to assess the best treatment group. The Marginal Homogeneity test measured the significance between before and after treatment intervention. $P<0.05$ was considered statistically significant.

RESULTS

Parasite Prevalence

In this study, a total number of 835 animals (303 cattle and 532 sheep) were examined clinically and their feces in the laboratory to detect liver fluke infection. Microscopic examination revealed the prevalence rate of infection in cattle was 20.8% and in sheep was 17.1%, with an overall prevalence of 18.4% (Table 2). The clinical signs such as decreased bwt, loss of hair or wool, depression, pale or icteric mucous membranes, sometimes diarrhea, and edematous swelling in the intermandibular space (bottle jaw), primarily determined the outcome. Second, fecal examination was performed, with detection of characteristic Fasciola spps. eggs in the feces of examined animals. The eggs were identified depending on their main morphological features: large, yellowish, operculated, with thin walls and containing one-cell stage embryo.

Effect of Risk Factors on Fascioliasis Prevalence

Table 3 summarizes the association between fascioliasis prevalence and the possible risk factors. Statistically, chi-square results revealed that there was only a significant association between sex and fascioliasis

Table 1: Treatment groups against Fascioliasis

Groups	Treatment			Animals examined		
	Drug	Dose (mg/kg)	Route	Total	Cattle	Sheep
1st	Albendazole	12	per os	25	10	15
2nd	Triclabendazole	12	Per os	25	10	15
3rd	Superzole	12	Per os	25	10	15
4th	Clorsulon	12	subcutaneously	25	10	15
5th	Rafoxanide	10	Subcutaneously	25	10	15
6th (NC)	No treatment			29	11	18

Table 2: Prevalence of liver fluke infection by fecal examination

Animals	No. Of examined animals	No. Of positive animals	Prevalence%
Cattle	303	63	20.8
Sheep	532	91	17.1
Total	835	154	18.4

Table 3: The association between risk factors and Fasciola prevalence

Risk factors		Total num.	Positive	%	X^2	P value	Odds ratio 95% CL
Animal species	Cattle	303	63	20.8	1.745a	0.187	1.272(0.890-1.819)
	Sheep	532	91	17.1			
Animal sex	Male	405	26	6.4	75.584a	0.000	5.879(3.699-9.345)
	Female	430	128	29.8			
Water source	canal water	751	144	19.2	2.654a	0.103	1.756(0.885-3.482)
	ground water	84	10	11.9			
Feeding source	Farm made	20	3	15	1.000^		0.776 (0.225-2.682)
	Purchased	815	151	18.5			

prevalence (P<0.05), the sex had a significant effect on the infection level, females had a higher infection rate (29.8%) than males (6.4%), OR=5.879, 95% CL (3.699-9.354). Other risk factors of animal species, water source, and feeding source had no significant effect on the prevalence of Fasciola infection (P<0.05).

Effect of the Treatment on the Treatment Groups

As shown in Table 4, there was a significant statistical difference between the pre and post-treatment groups in all animals (P<0.05), indicating all the used drugs were effective against Fascioliasis. The number of recovered animals in the first group was 18 out of 25 after using f Albendazole, Triclabendazole, and Superzole with a recovery percentage of 72% and 21 out of 25 after using Clorsulon (84%) and 24 out of 25 after using Rafoxanide (96%). As displayed in Table 5, there is an association between the treatment groups (P<0.05) in cattle and sheep and demonstrated the recovery percentage in each species. Rafoxanide achieved the best recovery percentage in cattle (90%) and sheep (100%). The Roc curve predicted that Rafoxanide was the best drug compared to the other used drugs in cattle and sheep (AUC>0.5) (Fig. 1 & 2)). The animals' recovery was determined by the absence of the eggs in the faeces and improvement or disappearance of the clinical signs such as stopping diarrhea and improving bwt.

DISCUSSION

In the present study, the total prevalence rate of Fasciola infection in cattle was 20.8% (63 out of 303). These results agrees with the findings of Maha (2008), who found a 27% infection level in cattle and buffalo in the Assuit Governorate and nearly agrees with another study conducted in Egypt that reported a 30.88% prevalence rate in cattle (Elshraway and Mahmoud 2017). Nevertheless, this finding is not consistent with some other studies conducted in Egypt that reported a lower prevalence rate in the Nile delta, such as El-Tahawy et al. (2017) reported a 9.77% prevalence of fascioliasis in cattle. Haridy et al. (2002) reported 5.3%, and ElKhtam and Khalafalla (2016) reported a 5.8% prevalence rate in cattle. In contrast, Bazh et al. (2012) reported a 50% prevalence of fascioliasis in cattle in Egypt. In the present study, 17.1% prevalence rate was found in sheep. Much higher (27 and 40%) prevalence has been reported from Egypt by Maha (2008) and Morsy et al. (2005), respectively.

Regarding the effect of the risk factors on the infection level, sex was the only risk factor that affected the infection in the present study. This higher prevalence of infection in females can be attributed to several stressful physiological factors such as pregnancy, parturition, and lactation, which suppress the immune system (Sirois 2016). The current results agree with many other previous studies (Karim et al. 2015; Rizwan et al. 2016; Asrat 2018), those reported that fascioliasis prevalence was higher in females than males. On the contrary, other studies reported no significant difference between males and females (Mulatu and Addis 2011; Gebreyohannes et al. 2013; Mathewos et al. 2014). Another study reported a higher prevalence rate of fasciolosis in males (El-Tahawy et al. 2017). With respect to species, there was no association between the animal species and fascioliasis prevalence. This finding is inconsistent with another study that reported that the species was considered a risk factor (95% CI=3.8–4.5%) in sheep, 95% CI=8.0–9.9%) and in cattle (Khademvatan et al. 2019) as well.

In the current study, there was no significant difference between the farms' types (the traditional ones used the canal water and the cultivated clover, and the other farms used the groundwater). These results do not agree with the findings of El-Tahawy et al. (2017), who found that the conventional farms were at higher risk of fascioliasis than organic ones (P<0.005). Yildirim et al. (2007) reported a higher prevalence rate of liver flukes in the traditional farms (76.5%) than the small-scale dairy farms (37.2%).

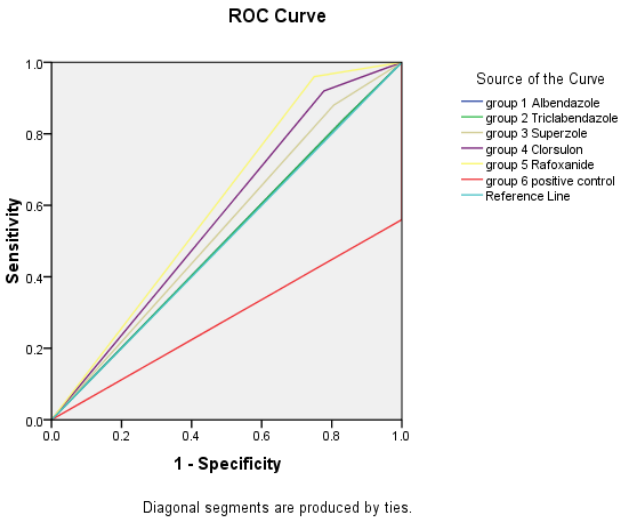

Fig. 1: (ROC) curve of predicting the best treatment group in cattle.

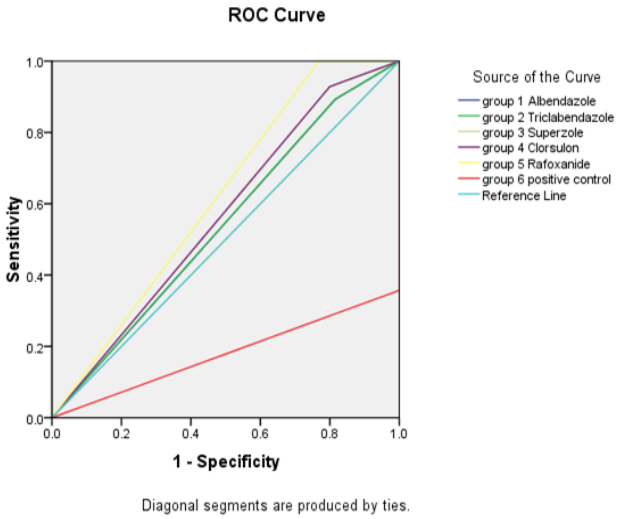

Fig. 2: (ROC) curve of predicting the best treatment group in sheep.

Table 4: Differences between the treatment groups in all animals before and after the treatment

Pre-treatment	Infection Type	Numubers	Post -treatment (Infection)			Recovery		Marginal Homogeneity P value
			High	Medium	Low	Number	%	
Group 1 Albendazole	HI	5	0	0	0	5		0.016
	MI	8	0	1	0	7	72	
	LI	12	5	1	0	6		
Group 2 Triclabendazole	HI	7	0	1	0	6		0.000
	MI	16	0	5	0	11		
	LI	2	0	1	0	1	72	
Group 3 Superzole	HI	7	0	2	0	5		0.000
	MI	10	0	0	0	10		
	LI	8	0	3	0	5	72	
Group 4 Clorsulon	HI	7	0	2	0	5		0.000
	MI	12	0	0	0	12		
	LI	6	0	2	0	4	84	
Group 5 Rafoxanide	HI	8	0	1	0	7		0.000
	MI	11	0	0	0	11	96	
	LI	6	0	0	0	6		

High Infection = HI; Medium Infection = MI; Low Infection = LI.

Table 5: Percentage of recovery after using of different fasciolicides in each species

Fasciolicides	Cattle			Monte Carlo	Sheep			Monte Carlo
	Dis.	Rec.	Recovery%		Dis.	Rec.	Recovery%	
Albendazole	10	6	60		15	12	80	
Triclabendazole			60		15	12	80	
Superzole	10	6	60					
Clorsulon	10	6	80		15	12	80	
Rafoxanide	10	8	90	P= 0.000	15	13	84	P = 0.000
	10	9			15	15	100	
Negative – Control (NC)	11	0	0		18	0	0	

In the present study, five anthelmintics were used against fascioliasis infection, and two of these belonged to the benzimidazole group (albendazole and triclabendazole). According to our findings, both drugs had the recovery rate in cattle (60%) and sheep (80%). These results are consistent with the findings of Moll et al. (2000), who reported low efficacy of triclabendazole against fascioliasis in cattle and sheep in North Holland. However, Elitok et al. (2006) who reported the same lower efficacy of albendazole (66.6%) in Turkey. Keyyu et al. (2008) stated that 14-33% of the treated animals with albendazole were positive for Fasciola eggs two weeks after the treatment.

Moreover, another study in Egypt also reported a lower efficacy (75%) of albendazole (Shokier et al. 2013). Albendazole and triclabendazole are commonly used drugs in Egypt to treat and control gastrointestinal helminths. These were applied randomly by the farmers (sub-doses and incomplete follow-up), which led to the development of resistant strains. The third compound was superzole, that is mainly used to treat tapeworms and sometimes works against liver flukes. It had a decreased recovery rate than in the benzimidazole group in cattle (60%) and sheep (80%). The fourth compound, clorsulon belongs to the sulphonamide group; it is more effective against mature than immature flukes, and it can be applied either orally or subcutaneously. Our results indicated a better recovery rate using clorsulon (80 and 84% in cattle and sheep, respectively). These results agree with another study

conducted in Turkey that recorded a higher efficacy of clorsulon against Fascioliasis in cattle (Elitok et al. 2006). In addition, similar results in other studies confirmed the high efficacy rate of clorsulon in cattle; 100% adult fluke reduction (Wallace et al. 1985) and 91.2% adult fluke reduction (Zimmerman et al. 1986). The last drug is rafoxanide, which belongs to salicylanilides (Yeung et al. 2010), and it also affects mature and developmental stages. Our study demonstrated the best recovery rate in cattle (90%) and sheep (100%), which could be due to the little use of this drug in this area. These findings contradict another study conducted in Turkey in cattle, which demonstrated a lower efficacy of rafoxanide as the percentage of egg reduction was 68.2% (Elitok et al. 2006). These findings may be attributed to the regular use of this drug in this area, leading to resistance development (Shokier et al. 2013).

Conclusion

The present study detected a moderate fascioliasis prevalence in cattle and sheep in the Assuit Governorate, Egypt. Only sex significantly affected Fasciola prevalence in the epidemiological study of risk factors. The findings indicate that Rafoxanide is a highly effective drug for treating Fasciola spp. in cattle and sheep, although resistance to the benzimidazole group has developed in Egypt. This outcome could help improve management strategies in controlling Fasciola infection in Egypt.

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Author's Contribution

All the others contributed to the study's design, materials, analysis, and writing

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