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## **Full Length Article**

# Gastrointestinal parasites of sheep in Kafrelsheikh governorate, Egypt: Prevalence, control and public health implications



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#### ABSTRACT

Gastrointestinal (GI) parasitism is a primary cause of losses in sheep production. A crosssectional study was designed to investigate the prevalence, control strategies and public health importance of the GI parasites of sheep from Nile-Delta, Egypt. The prevalence of GI parasites in a total of 224 individual sheep was 50%: Protozoa (29.02%) and helminths (37.05%). The prevalence of helminths infection was by Strongyle-group (19.21%), Paramphistomes (9.38%), Strongyloides papillosus (4.02%), Trichuris spp. (2.68%), Moniezia spp. (0.89%) and Nematodirus spp. (0.45%). No single infection with Fasciola spp. was recorded. The protozoan infections included Eimeria spp. (16.52%), Entamoeba spp. (10.27%), Giardia duodenalis (0.45%), and, for the first time in sheep population in Egypt, Balantidium coli cyst (1.79%). Multivariate logistic regression analysis showed that usage of multiple anti-parasitic drug combinations and "Twice per year treatment" regime were associated with a substantial reduction of parasitic infection among examined sheep. The relative risk (RR) associated with lab technicians' lack of awareness of Fasciola and Balantidium zoonotic parasites was higher by 3 and 9 times than that of Giardia, respectively. In conclusion, GI parasites are endemic at high levels among sheep in the study area. Also, continuous awareness campaigns about zoonotic parasites are essential to reduce the possible public health threats.

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#### 1. Introduction

Sheep production is considered a major sector of meat supply for human consumption in Egypt and contributes to development of the rural areas. In Egypt total sheep population was estimated at 5.5 million heads, most of them allocated in Nile-Delta (Statistics of Live Stocks, 2011). Gastrointestinal (GI) parasitism is considered one of the most serious and underestimated problems, which hinders sheep productivity (Perry and Randolph, 1999). Moreover, studies have shown that some of sheep GI parasites are of public health importance and they were incriminated in zoonotic transmission to human either by direct contact with sheep manure or indirectly through ingestion of contaminated food or water (Byomi et al., 2010; Feng and Xiao, 2011; Ralph et al., 2006). In Egypt, the risk of zoonotic transmission of some diseases via sheep should be carefully considered because majority of sheep flocks are reared as free-range animals with access to various agriculture fields and water sources. Such a rearing system provides many possible routes of environmental contamination by disease pathogens. This study aimed firstly to estimate the prevalence of GI parasites among sheep in Kafrelsheikh governorate of the Nile Delta region, Egypt. Secondly, the efficacy of parasitic infections' treatment regimens was evaluated by shepherd's questionnaires. Finally, public health threats were estimated using lab technician's questionnaires and statistical modeling.

#### 2. Materials and methods

#### 2.1. Study area and design

Kafrelsheikh is one of the five governorates in the Nile Delta and located north to Cairo (31°06′42″N 30°56′45″E). It is an agricultural governorate with a high density of livestock and human population. In Kafrelsheikh governorate, sheep are raised either in small numbers kept in the household by farmers or in village flocks managed by shepherds (Aidaros, 2005). One shepherd would often keep sheep from a number of different owners; as a result, animals from different households are part of the same flock for grazing and breeding during most of the year. Some village flocks are sometimes combined together to make a large flock managed by more than one shepherd. The flocks are reared by the free-range system, where animals graze freely all over the country as there is no regulation of animal movement in Egypt (Aidaros, 2005). Owing to the similarities of sheep husbandry system of management across the Nile Delta, the situation of parasitic diseases in Kafrelsheikh governorate is not likely to differ considerably from neighboring governorates in the Nile Delta region. A cross sectional survey was designed to investigate the prevalence of GI parasites (genera/species) among sheep and risk factors associated with infection. The sampling target population was all sheep in 10 main villages of Kafrelsheikh governorate. Each of these villages was assumed to have similar sheep flock size. Individual sheep was the primary sampling units. The total number of animals was obtained from the census of animal population at Kafrelsheikh governorate 2010. The sample size was estimated using Win episcope 2.0 programme as 270 individual

sheep. This number was divided equally on the 10 villages of Kafrelsheikh governorate. Within each village, 27 sheep were equally divided between the existing flocks. Individual sheep were selected from each herd by simple random sampling.

#### 2.2. Samples and laboratory examination

Between February and July, 2013 a total of 224 (not 270 due to lack of collaboration of some shepherds) fresh fecal samples were collected from the rectum of tested sheep in the study area. Samples were processed for morphological examination by formalin-ethyl ether centrifugation-concentration method according to Garcia (2001). When required wet mounts from sediments were stained with Lugol's iodine 5% and examined under light microscope at high magnification (x400). Identification was done according to Soulsby (1982).

#### 2.3. Questionnaires design and target populations

Two structured questionnaires were built in this study. The first one was for estimation of the public health relative risks associated with lack of awareness, presence and prevalence of some parasitic zoonoses among human population at Kafrelsheikh governorate. Only Fasciola species, Giardia spp. and Balantidium spp. were the only parasites included in this questionnaire owing to their characteristic (egg/cyst) morphology based on the light microscope examination routinely used for identification of parasitic infection in human stool samples in the private labs in Egypt. This questionnaire was designed and distributed to some technicians (n = 14) in private human laboratories in the study area. To estimate the lack of awareness of zoonotic parasites among technicians, the questionnaire was supplied with photos of Fasciola egg and Giardia cyst obtained from CDC (2013), while the Balantidium coli cyst photo was obtained from the sheep feces samples in this study. Only the respondents who correctly recognized the parasite egg or cyst where asked for the presence and prevalence of these parasites according to their own lab records. The other questionnaire was designed and distributed to the shepherds (n = 26) of the examined sheep flocks looking for types of anthelmintics used for parasitic control, and the regime of application of these medications. The shepherds in this study always used drug combination instead of single medication per flock. According to the drug combinations used by the shepherds, medications were divided into three groups: [A: Curafluke (5% oral suspension of Fenbendazole and Rafoxanide) + Dovenix (Nitroxinil 2.5%, subcutaneous injection solution) + Ivomec (1% ivermectin, subcutaneous injection solution), B: Dovenix + Ivomec, and C: Curafluke + Dovenix]. Regime of drugs application was estimated as "Twice per year" vs. ">Twice per year". To study the effect of geographical region, three regions were allocated in the study area; each of them represents a set of closely located villages.

#### 2.4. Epidemiological analysis

#### 2.4.1. Prevalence calculation

The prevalence of different parasites among examined sheep was estimated as percentage by dividing the number of infected Table 1 – Results of descriptive statistics and a logistic regression model with the status of GI parasites infection of sheep within the examined small ruminant flocks at Kafrelsheikh governorate, Nile Delta, Egypt, as the response variable.

Variable	Categories	Parasitic infection				Multivariate analysis				
		Yes	No	Total	OR	S.E.	P <	95% CI		
Drug groups	А	39	34	73	-	-	-	-		
	В	29	23	52	3.20	0.52	0.13	0.72-14.33		
	С	26	17	43	1.28	0.52	0.59	00.33-4.49		
Treatment regimes	>Twice/year	44	30	74	-	-	-	-		
	Twice/year	43	42	85	0.28	0.31	0.04	0.08-0.96		
Region	1	33	24	57	-	-	-	-		
	2	22	14	36	3.35	0.55	0.15	0.65–17.33		
	3	39	36	75	1.07	0.42	0.39	0.35-3.30		

animals by the number of tested animals and multiplying the product by 100.

#### 2.4.2. Multivariate logistic regression

Shepherd's responses from questionnaires were analyzed using a multivariate logistic regression model to identify and quantify the strength of association between anthelmintics types, regimes of anthelmintics application and the region of the flock with GI parasites infection among individual sheep. The same model was repeated many times with either of Protozoal infection or Nematodes infection or Trematodes as the response variable.

#### 2.4.3. Relative risk

Lab technicians' responses were considered as relative risks (RR) in the context of public health, as follows:

A. The relative risk of lack of awareness among technicians on different parasites infecting humans was calculated using the lack of awareness of *Giardia* as the baseline group, by the following equation according to Hegazy et al. (2009):

#### (D1/N1)\*(N2/D2)

where D1 is the number of technicians with lack of awareness of a particular parasite. N1 is the total number of asked technicians about this parasite. D2 is the number of technicians with lack of awareness of *Giardia*. N2 is the total number of asked technicians about *Giardia*.

B. The relative risk of presence and prevalence of different parasites infecting humans obtained from the answers of technicians was calculated with *Fasciola* presence and prevalence as the baseline group, using the same equation before but D1 is the number of technicians who confirmed the presence and the prevalence (either high or low) of a particular parasite. N1 is the total number of asked technicians about this parasite. D2 is the number of technicians who confirmed the presence and the prevalence (either high or low) of *Fasciola*. N2 is the total number of asked lab workers about *Fasciola*.

n.b.: When either of D1 or D2 were equal to zero and caused problems with computation of the relative risk, 0.5 was added to all of D1, D2, N1 and N2.<sup>1</sup>

#### 2.5. Statistical analysis

The statistical analysis was conducted using the statistical package EXCEL on MICROSFT OFFICE, Win episcope 2.0 and SAS 9.2 (SAS Institute Inc. 2008) software.

#### 2.6. Ethical consideration

Ethical approval was obtained from a committee of Research, Publication and Ethics of the Faculty of Veterinary Medicine, Kafrelsheikh University. All procedures were explained to flock owners and owners' approval were obtained.

#### 3. Results

Fifty percent of 224 sheep fecal samples examined were infected with one or more GI parasites. The prevalence of infection with different GI helminths was (37.05%); nematode (26.79%), trematode (9.38%) and cestode (0.89%). Paramphistomes eggs were detected in 9.38% of examined samples. No other type of trematode eggs was observed. Moniezia spp. eggs were the only cestodes found in 0.89% of examined samples. The most prevalent eggs of GI nematodes found in fecal examination was Strongyle-type (19.21%), followed by Strongyloides papillosus (4.02%), Trichuris spp. (2.68%) and Nematodirus spp. (0.45%), while the enteric protozoa prevalence (26.79%) were Eimeria spp. oocysts (16.52%), Entamoeba spp. cyst (10.27%), B. coli cyst (1.79%) and lastly Giardia duodenalis cyst in one sample only (0.45%). Multivariate logistic regression analysis showed no significance between A, B and C groups of anthelmintics medications used in this study. Yet the likelihood of GI parasites infection in sheep which received anthelmintics in both groups B and C was higher than sheep in group A (Table 1). Group C (OR = 1.28; 95% CI: 0.33-4.49) was slightly better than group B (OR = 3.20; 95% CI: 0.72-14.33). Treatment regime as "twice per year" dosing of anthelmintics was significantly better (P < 0.04) than ">twice per year" (OR = 0.28; 95% CI: 0.08-0.96) (Table 1). Another multivariate analysis model showed that the likelihood of protozoan infection in sheep groups, which received anthelmintics containing Ivermectin compound was significantly lower than the sheep in the other group (Table 2). The region showed no effect on efficiency of anti-parasitic drugs used in this study (Tables 1

<sup>&</sup>lt;sup>1</sup> (http://www.medcalc.org/calc/relative\_risk.php online).

Table 2 – Results of descriptive statistics and a logistic regression model with the status of GI protozoan infection of sheep within the examined small ruminant flocks Kafrelsheikh governorate, Nile Delta, Egypt, as the response variable.

Variable	Categories	Pr	Protozoal infection			Multivariate analysis			
		Yes	No	Total	OR	S.E.	P <	95% CI	
Drug groups	А	20	53	73	-	-	-	-	
	В	8	44	52	0.38	0.34	0.01	0.10-1.35	
	С	19	24	43	2.05	0.33	0.02	00.56-7.16	
Region	1	16	41	57	-	-	-	-	
	2	12	24	36	0.56	0.32	0.41	0.20-1.72	
	3	56	19	75	0.76	0.38	0.98	0.21-2.77	
OR: Odd Ratio: S.E.: Standard Error: CI: Confidence Interval.									

Table 3 – The relative risk of lack of awareness of different parasites infecting humans among lab workers at Kafrelsheikh governorate, Nile Delta, Egypt.

Parasite	Awareness of	of parasite	Total	RR			
	Yes	No					
Giardia	14 (100%)	0 (0%)	14	1.0			
Fasciola	13 (92.2%)	1 (7.7%)	14	3.0			
Balantidium	10 (71.40%)	4 (28.6%)	14	9.0			
RR: Relative Risk.							

and 2). The Relative risk of lack of awareness of some zoonotic parasites potentially transmitted by sheep manure among technicians is shown in Table 3. Giardia cyst was recognized by all questioned labs (100%), while for Fasciola eggs and B. coli cyst the RR of lack of awareness was 3 and 9 times higher than that of Giardia, respectively. Some of the technicians (46%) confirmed the presence of Fasciola in human stool samples (Table 4). The RR of presence of B. coli cyst and Giardia cyst was 1.08 and 2.16 times higher than Fasciola respectively. On the other hand, majority of technicians (83%) described Fasciola prevalence as low (Table 4) and the RR of prevalence of other parasites in human stool was estimated at 0.47 and 4 times for B. coli cyst and Giardia cyst than Fasciola, respectively.

#### 4. Discussion

The high overall prevalence of GI parasites (50%) in this study indicates a very intense transmission within the sheep population. These results may reflect the actual figure of GI parasites

prevalence in the whole Nile Delta region because of the close similarity of husbandry systems. The quite high prevalence of GI parasites put in question the efficacy of different anthelmintics by the shepherd. Low efficacy of the medicines may be attributed to the misuse of these drugs, dose, frequency of application. This is because shepherds do not usually seek the veterinary supervision and they give these medicines as a routine regime. Also, another reason is the freerange husbandry of sheep in the study area, where animals in this rearing system are exposed to many potential sources of parasitic infection via contaminated pastures and water sources as reported by Kijlstra et al. (2009). Helminthiasis was more prevalent in examined sheep (37.05%) than protozoan infection, which is in much agreement with previous studies (Bhat et al., 2012). Paramphistomes "rumen or stomach flukes" was the only trematode helminths found during fecal examination and its prevalence (9.38%) is higher than other parts in the Egyptian Delta (Sultan et al., 2010). The shepherds' response obtained from the questionnaire in this study showed that Niclosamide, the specific anthelmintic for Paramphistomes, is not being used and this may be the reason behind high prevalence of Paramphistomes. Liver flukes of the genus Fasciola did not score any infection in all examined animals. This result disagree with most of the previous reports on ovine fascioliasis in Egypt (Abouzeid et al., 2010; Sultan et al., 2010) and other countries (Negasi et al., 2012). However, a recent study showed a significant decrease in the prevalence of fascioliasis over three years of investigation of sheep in the Mid-Nile Delta of Egypt (Elmonir et al., 2015). These findings could be attributed to successful implementation of control programs for fascioliasis in Egypt (Youssef and Uga, 2014). Moreover, improved public awareness about the disease impact on animal health and productivity has strengthened the usage of fasciolicidal

Table 4 – The relative risk of presence and prevalence of different parasites infecting humans in Kafrelsheikh povernorate, Nile Delta, Egypt,

Parasite	Presence of	Presence of parasite		tal RR Prevale		of parasite	Total	RR
	Yes	No			Low*	High**		
Fasciola	6 (46%)	7 (54%)	13	1.0	5 (83%)	1 (17%)	6	1.0
Balantidium	5 (50%)	5 (50%)	10	1.08	4 (100%)	0 (0%)	4	0.47
Giardia	14 (100%)	0 (0%)	14	2.1	4 (33%)	8 (67%)	12	4

RR: Relative Risk.

\* Low: 1-25%.

\*\* High: >25%.

compounds for prevention or treatment. Consequently, this resulted in the decrease in the prevalence of adult Fasciola among farm animals during the last few years in Egypt. Adult tapeworm detected in the current study was Moniezia spp. with a prevalence of 0.89%. This was much lower than previous records in Egypt-Delta (Sultan et al., 2010) or abroad (Negasi et al., 2012). Frequent ivermectin dosing by shepherds in the study area fights the persistence of intermediate host mites on feed of animals. The GI nematodes were the most prevalent helminths infection in the examined sheep. These results were comparable to other reports in Egypt (Abouzeid et al., 2010; Sultan et al., 2010) and elsewhere (Bhat et al., 2012). The most common type of nematode eggs found in this study was Strongyle eggs. Ovine Strongyle parasitic group contains many species of veterinary importance, e.g. Haemonchus spp. and public health importance, e.g. Trichostrongylus colubriformis (Perry and Randolph, 1999; Soulsby, 1982). In the current study, enteric protozoa prevalence was quite high. Our results indicated that Eimeria oocysts prevalence was 16.52%. This was higher than a previous report in Egypt (6.7%) (Abouzeid et al., 2010). Uninucleated Entamoeba species have been found before in sheep feces during investigations on GI parasites (Toulah, 2007), but not in Egypt (Byomi et al., 2010). Balantidium coli cyst was observed in the current survey with low prevalence (1.79%). All samples containing B. coli cyst came from one herd only; this herd consisted of mixed animal species (cattle, buffaloes, sheep, goat and donkeys). Pigs are considered the main reservoir of B. coli (Schuster and Ramirez-Avila, 2008), yet it was also recorded before in cattle, buffaloes (Bilal et al., 2009), sheep (Jamil et al., 2014) and even in donkeys (Khan et al., 2013). All of these available research studies did not record this infection before in sheep of Egypt. Sheep in the study area may be infected with B. coli through cross infection with infected animals (i.e. cattle, buffaloes, donkeys) reared in the same herd. Sheep can also catch the infection from contaminated pastures and water sources by human or animal excreta especially with the increased chances of exposure to infection from these sources in the free-range rearing system (Kijlstra et al., 2009). Further studies should be conducted in the future to elucidate the mechanisms of transmission and species involved in such infection in Egypt. Giardia duodenalis is an enteric protozoan with a wide host range including humans. Our study showed that G. duodenalis prevalence was the lowest (0.45%) among sheep enteric protozoa in the examined samples. Previously, G. duodenalis was recorded in sheep located in Nile-Delta of Egypt with a high prevalence (25.2%) (Byomi et al., 2010).

Multivariate analysis of a questionnaire distributed to shepherds showed no statistical difference between different anthelmintic combinations on the parasitic overall prevalence. Yet parasitic infection rates in sheep flocks were inversely proportional to the number of anthelmintic types used, i.e. higher number of anthelmintic types associated with lower parasitic prevalence. This may be due to the fact that the use of multiple drug types increases the anthelmintics spectrum and reduces the chances of drug resistance by certain parasites to a single drug. For the treatment regime "Twice per year" was significantly better than " > Twice per year" (P < 0.04). Although these finding may seem illogical, it could be explained by the numbers of drugs administrated per each application during the year for the same flock. For instance, using multiple

drugs per each application for two times per year may be better than using a single drug for each application several times per year for the same flock. Results reported by Belizario et al. (2003) supported this theory as they showed that anthelmintics used in combinations were significantly better than single drug administration for treatment of nematodes.

Ivermectin compound significantly affect the protozoal infection in our study, which does not agree with previous studies (Campbell et al., 1983). A more recent study showed that ivermectin therapy was effective against protozoal infection, (i.e. giardiasis and cryptosporidiosis) in rat models (Youssef et al., 1996), which agrees with our finding and suggests its possible activity on protozoa by other novel mechanisms.

Fasciola was absent in the examined sheep flocks in this study, yet 46% of the technicians reported the presence of Fasciola eggs in human population. Majority of the questioned labs (83%) recorded low prevalence of Fasciola in human population of Kafrelsheikh governorate. These results agreed with previous studies in other governorates of the Nile Delta of Egypt (El-Shazly et al., 2006). The ongoing Fasciola infection among humans in Kafrelsheikh governorate and its absence in sheep flocks in the same region may indicate the negligible role of sheep in the epidemiological cycle of Fascioliasis in the study region and strongly suggests other possible sources of infection. The ovine Strongyle parasitic group, the most common type of nematode eggs in our study, contains the genus Trichostrongylus, which is a zoonotic nematode parasite, ubiquitous among herbivorous animals worldwide (Ralph et al., 2006). Only molecular approach or larval culture technique could distinguish Trichostrongylus eggs from human hookworm eggs (i.e. Ancylostoma spp.) as reported by Ralph et al. (2006). Both techniques were not used in the routine examination of human stool for parasitic infection in the private labs of Kafrelsheikh governorate, which depends basically on the morphological identification of the parasitic eggs or cysts by light microscope. Therefore, the presence and the prevalence of Trichostrongylus couldn't be estimated based on the lab technicians' response to questionnaires in this study. This highlighted the potential misdiagnosis and underestimation of Trichostrongylus infection among human population in Kafrelsheikh governorate. Unlike other parasitic zoonoses in this study, Giardia was well recognized by all questioned technicians (100%). Moreover, 67% of the labs recorded high prevalence among human population in Kafrelsheikh governorate, which was in agreement with other reports in Egypt (Abaza et al., 1998; Byomi et al., 2010). Even with what seems to be a small role in the Giardia epidemiological cycle, sheep should not be underestimated as a potential reservoir especially in areas with bad sanitation and where there is closecontact between animals and humans in Egypt (Byomi et al., 2010). Balantidium coli is the only ciliate known to infect animals and human. Fifty percent of the questioned lab technicians reported the existence of B. coli among human population of Kafrelsheikh governorate in a low prevalence. This low prevalence in human population is in agreement with previous studies in Egypt (2.38%) (Abaza et al., 1998). The main public health problem with Balantidiasis in this study was the lack of awareness of B. coli cyst by technicians. This problem will indeed affect the estimation of actual human prevalence in Kafrelsheikh governorate. According to our knowledge, this is

the first report that detects B. coli cysts in the stool of sheep in Egypt and it raises the question about the actual role of sheep in the epidemiological cycle of Balantidiasis.

In conclusion, this study provides the baseline information about the status of GI parasites of sheep in Kafrelsheikh governorate, Egypt. This study also highlighted the importance of awareness of the zoonotic parasites especially among laboratory workers for accurate estimation of these zoonoses' prevalence and impact among human population. Efficient veterinary care, routine epidemiological surveillance and enhanced educational campaigns about sheep parasitic zoonoses are mandatory measures to enhance the productivity and reduce the public health risks associated with sheep rearing in Egypt.

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