



Ovine fascioliasis: environmental epidemiology and meta-analysis of the prevalence, Agro-ecological and economic factors in five provinces of the Nile Delta region of Egypt

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ABSTRACT

This study is aimed to determine the prevalence of ovine fascioliasis and to evaluate the impact of putative risk factors in five provinces located in the Nile Delta region of Egypt. A cross-sectional method was conducted from 2014 to 2015 involving the coprological investigation using sedimentation technique and questionnaire survey in Beheira, Kafr-Sheikh, Sharkia, Menofia and Gharbia province. A total of 4920 sheep (n= 80 flocks) was sampled in order to achieve a representative sample of 140 flocks. The dataset was analyzed using comparative meta-analysis, the pooled prevalence was 17.87%, and the higher prevalence was recorded in the Baladi breed 21.86% with 95% confidence interval followed by the Barki 18.12% (95% CI-0.58, 1.42) and the Rahmani 13.58% (95% CI-0.19, 0.25). Sixteen parameters of population and agro-ecological factors were considered for assessing the heterogeneity analysis of age, sex, breeds, body condition scores, flock sizes, and ecological conditions. Low prevalence of fascioliasis was observed in summer, spring, and autumn. Sheep flock sizes ranging from 100-150 and over 150 were associated with a higher prevalence of *Fasciola* than flock size less than 100 sheep. The prevalence was higher in relative humidity ranging from 50 to 60 and over 60, compared to conditions of relative humidity <50. The total weight of infected sheep with fascioliasis was significantly lower (44.17 kg) than healthy sheep (55.29 kg). The monetary value of weight reduction for the infected sheep estimated was 301.55 Egyptian pounds (EGP). Additionally, the average treatment cost/sheep was 46.22 EGP, and the cost of mortality for three-dead sheep was 4800 EGP. The study findings revealed that urgent strategic control measures are required based on attributing risk factors of fascioliasis in the Nile Delta regions of Egypt. Furthermore, epidemiological information about the disease and associated risk factors may aid in developing effective control measures and strategic anthelmintic intervention against fascioliasis and improving the productivity of the sheep industry in Egypt.

Keywords: Ovine; Fascioliasis; Risk factors; Economic impact; Prevalence

1. Introduction

Sheep production and fascioliasis: Fascioliasis is also referred as the common liver fluke or the sheep liver fluke and is one of the neglected tropical (NTD), food-borne, trematode diseases of both ruminants and humans. Sheep fascioliasis can lead to a potentially fatal liver disease of sheep that is often associated with high mortality, diminishing in fleece production, lessening in the growth rate, and dropping in slaughter weight, eventually leading into marked economic losses (Arbabi et al., 2018; El-

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Tahawy et al., 2017a). The total sheep populations in Egypt were estimated at 5.5 million head of breeding sheep and are widely distributed in Nile-Delta regions. Egypt is one of the fascioliasis-endemic areas and has been observed to undergo 30 % of the total annual economic loss inflicted due to the reduced milk and wool production (EL-Tahawy et al., 2018).

The overall prevalence rate of fascioliasis ranged from 0% to 59% in different animal species (sheep, cattle, horses and camels) in different areas of in Egypt. A previous study was extended to the current study evaluated the risk factors associated with the prevalence of fascioliasis affecting cattle population in three provinces of the Nile Delta of Egypt (Kafr El-Sheikh, El-Beheira, and Alexandria) (El-Tahawy et al., 2017a).

Economic impact: The financial loss resulting from fasciolosis is approximately US\$ 2 billion every year worldwide. A previous report by the Egyptian Academy of Scientific Research and Technology demonstrated that the annual financial losses resulting from animal fascioliasis in Egypt are approximately 190 million Egyptian pounds per year (El-Shazly et al., 2006; Lotfy and Hillyer, 2003). Moreover, ovine fascioliasis is major health problem of the sheep industry and produces devastating effects to the farm owners due to high mortality, infertility, and degraded quality of mutton and lamb meat, condemned lamb carcass.

The gastrointestinal tract is developmentally very active in the early period post hatch in poultry species (Uni Z. et al., 2000). The intestinal crypt that form on the day of hatch become defined in the first 48 to 96 hours and continue to grow rapidly during the first 7 days. The intestinal villi increase significantly in diameters during the first 7-10 days after hatch.

The rationale of this study:

Various epidemiological studies in Europe (Beesley et al., 2018), Africa (Jaja et al., 2017), have recognised the key factors of fascioliasis in cattle. Although the global prevalence and economic losses of fascioliasis have been observed, little consideration has been given to the possible association between the prevalence of ovine fascioliasis and investigation of putative risk factors. Fascioliasis is transmitted by contact with infected animals, the existence of contaminated snails, or grazing management which permit animals to access to infected herbage or water containing metacercariae. Accordingly, there is a strong association between the source of ovine fascioliasis and factors associated with agro-ecological and management practices.

To fully understand the interaction between the prevalence and risk factors of ovine fascioliasis in the Nile Delta region of Egypt, various epidemiological characteristics or factors are required to be investigated. These factors include age, sex, breeds, body condition scores of sheep, flock size, and different ecological conditions, and age and education level of the sheep owner. The findings from this investigation would be of a benefit for the assessment and management aspect of fascioliasis in sheep control programs (Roberts and Suhardono, 1996). Therefore, the presented study was aimed to determine the prevalence of ovine fascioliasis in different breeds of sheep flocks in the Nile Delta in Egypt

Table 1: Logistic regression analysis of risk factors associated with Fascioliasis in sheep (final model, including breed, season, sex, age, farmer age, type of grazing and duration, type of pasture and environment-related factors)

Risk factor	Groups	OR	95% CI	P-value
Population Parameters				
Breed	Barki	0.91	0.58-1.42	0.68
	Rahmani	0.32	0.19-0.52	<0.0001
	Baladi		Reference	
Province	Beheira	2.9	1.89-3.91	<0.0001
	Kafr el-Sheikh	2.29	1.27-4.14	<0.001
	Menofyia	3.42	2.29-5.87	<0.0001
	Sharkia	4.69	2.55-4.28	<0.0001
	Gharbia		Reference	
Sex	Male	0.26	0.20-0.34	<0.0001
	Female		Reference	
Age	2-Mar	0.08	0.05-0.13	<0.0001
	>3	0.2	0.14-0.30	<0.0001
	<2		Reference	
Sheep flock Parameters				
Flock size	Flock size 100-150	2.27	1.39-3.84	<0.0001
	Flock size >150	1.94	1.44-2.62	<0.0001
	Flock size <100		Reference	
Flock contact	Flock contact -Yes	2.27	1.07-3.05	<0.0001
	Flock contact- No		Reference	
Duration of grazing	6 month	2.15	1.05-4.37	<0.05
	9 month	4.17	2.65-6.56	<0.0001
	12 month	3.11	2.07-4.68	<0.0001
	3 month		Reference	
Type of pasture	Public pasture	2.81	2.13-3.70	<0.0001
	Private pasture		Reference	
Sheep farming management factors				
Water supply	Tap water	0.61	0.44-0.85	<0.001
	Stream water			
Prophylactic treatment	Prophylactic treatment -Yes	0.16	0.12-0.23	<0.0001
	Prophylactic treatment -No		Reference	
Body condition score	Medium	2.82	1.89-4.20	<0.0001
	Poor	2.6	1.88-3.60	<0.0001
	Good		Reference	
Education level of owner	Higher education	0.04	0.03-0.07	<0.0001
	Basic education		Reference	
Education level of owner	Higher education	0.04	0.03-0.07	<0.0001
	Basic education		Reference	
Age of owner	Age 40-50 years	0.54	0.39-0.74	<0.0001
	Age >50 years	0.84	0.60-1.17	0.3
	Age <40 years		Reference	

Agro-ecological parameters

Season	Spring	0.17	0.10-0.30	<0.0001
	Summer	0.24	0.14-0.39	<0.0001
	Autumn	0.42	0.29-0.61	<0.0001
	Winter		Reference	
Temperature	26-31 degree Celsius	1.26	0.95-1.67	0.1
	>31 degree Celsius	1.1	0.80-1.50	0.54
	<26 degree Celsius		Reference	
Relative humidity	50-60	0.33	0.23-0.49	<0.0001
	>60	0.63	0.46-0.86	<0.001
	<50		Reference	

N no of total samples n no of positive samples

Table 2: Economic impact of fascioliasis on sheep productivity

	Criteria	Case	Mean ± Std Error mean
1	Total weight (kg)	Fasciola	55.29 ± 2.06b
		Free-control	47.14± 1.17a
2	Value of weight reduction (EGP)	Fasciola	301.55 ± 4.45
		Free-control	-
	Treatment cost (EGP)	Fasciola	46.22± 0.76
		Free-control	-
3	Mortality value (EGP)	Fasciola	4800
		Free-control	-

Means within the same column carrying different letters are significantly at P<0.05.

Population and Sheep flock parameters - prevalence of Fascioliasis in Nile Delta sheep

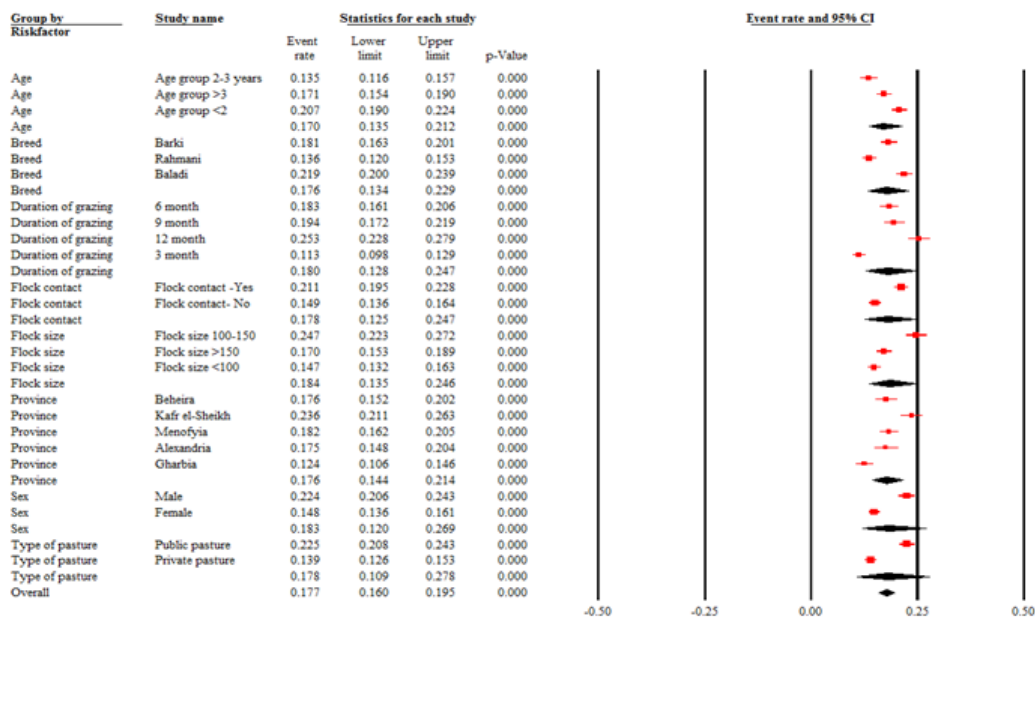


Figure 1: Meta-analysis of the prevalence of Fascioliasis in Nile Delta sheep comparing the putative risk factors of population and sheep flock parameters is indicated in the forest plot. The pooled rate ratio values were calculated by the statistical software called CMA (version 3.3070, USA). The black diamond represents the combined effect estimate of the prevalence of fascioliasis in Nile Delta sheep with multiple population and sheep flock parameters randomly assigned for this evaluation. The red square with the line indicated the effect size of the prevalence fascioliasis of the Nile Delta sheep population with a 95% confidence interval. The ratio>0 suggests no difference in risk of fascioliasis of the Nile Delta sheep, whereas a risk ratio < 0 suggests an increased risk of fascioliasis.

Sheep farming management and Agro-ecological parameters - prevalence of Fascioliasis in Nile Delta sheep

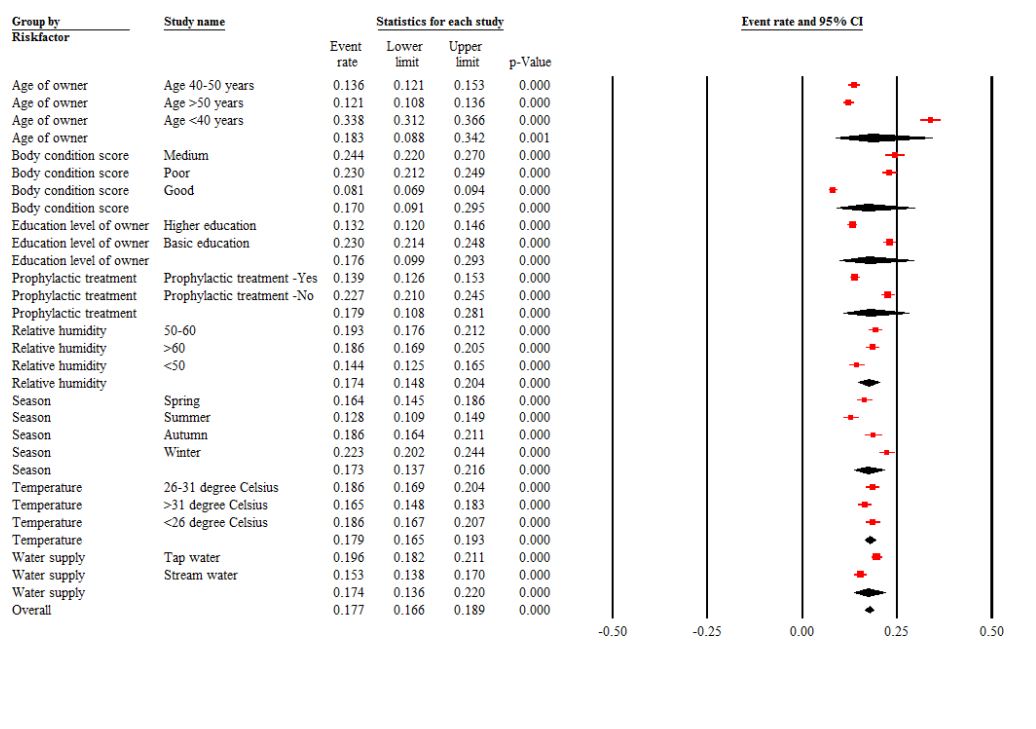


Figure 2: Meta-analysis of the prevalence of Fascioliasis in Nile Delta sheep comparing the putative risk factors of sheep farming management and agro-ecological parameters is indicated in the forest plot. The pooled rate ratio values were calculated by the statistical software called CMA (version 3.3070, USA). The black diamond represents the combined effect estimate of the prevalence of fascioliasis in Nile Delta sheep with multiple population and sheep flock parameters randomly assigned for this evaluation. The red square with the line indicated the effect size of the prevalence fascioliasis of the Nile Delta sheep population with a 95% confidence interval. The ratio>0 suggests no difference in risk of fascioliasis of the Nile Delta sheep whereas a risk ratio < 0 suggests an increased risk of fascioliasis.

Table 3: Hypothesis testing and Heterogeneity testing of the impact of multiple risk factors on the prevalence of Fascioliasis in Nile Delta sheep

Risk factors	Number of groups	Effect size	Z value	P value	Q value	df (Q)	I squared	Standard Error	Variance	Tau squared
Population factors										
Age	3	0.170 (0.136-0.212)	-40.55	0	25.549	2	92.172	0.06	0.004	0.053
Breed	3	0.176 (0.134-0.229)	-40.34	0	38.952	2	94.865	0.083	0.007	0.078
Sex	2	0.183 (0.120-0.269)	-40.26	0	46.1	1	97.831	0.183	0.033	0.126
Province	5	0.176 (0.144-0.214)	-12.43	0	43.231	4	90.747	0.055	0.003	0.07
Sheep flock factors										
Flock size		0.184 (0.135-0.346)	-40.27	0	43.231	4	90.747	0.055	0.003	0.07
Flock contact	2	0.178 (0.125-0.247)	-7.264	0	31.545	1	96.83	0.125	0.016	0.086
Type of pasture	2	0.178 (0.108-0.278)	-5.23	0	60.261	1	98.341	0.242	0.059	0.168
Duration of grazing	4	0.180 (0.128-0.247)	-7.412	0	88.491	3	96.61	0.137	0.019	0.162
Sheep farming factors										
Water supply	2	0.174 (0.136-0.220)	-10.46	0	14.665	1	93.181	0.063	0.004	0.041
Prophylactic treatment	2	0.179 (0.108-0.281)	-5.113	0	62.794	1	98.408	0.251	0.063	0.175
Body condition score	3	0.170 (0.091-0.295)	-4.359	0	169.97	2	98.823	0.411	0.169	0.393
Education level of owner	2	0.176 (0.099-0.293)	-4.359	0	78.908	1	98.733	0.323	0.104	0.226
Age of the owner	3	0.183 (0.088-0.342)	-3.473	0.001	252.32	2	99.207	0.56	0.314	0.554
Agro-ecological factors										
Season	4	0.173 (0.137-0.216)	-11.11	0	41.321	3	92.74	0.066	0.004	0.073
Temperature	3	0.179 (0.165-0.193)	-31.28	0	3.412	2	41.387	0.007	0	0.003
Relative humidity	3	0.174 (0.148-0.204)	-15.73	0	13.442	2	85.121	0.03	0.001	0.025

and to assess the impact putative risk factors with references to parameters of sheep management and agro-ecology.

2. Material and methods

2.1. Ethical approval

The animal study was carried out by the Egyptian laws and University guidelines for animal care. The study was approved by the Local Governmental Authority for Veterinary Services in Kafr El-Sheikh, El-Beheira, Sharkia, Menofia and Gharbia governorates and the Ethics and Welfare Committees of both faculties of Veterinary Medicine in Kafr El-Sheikh University and Damanhour University, Egypt, which is in accordance with the Institutional Animal Care.

2.2. Study area

A cross-sectional surveillance study was conducted in five provinces in the Nile Delta region of Egypt, namely Kafr Sheikh, Beheira, Sharkia, Menofia and Gharbia (Fig. 1). These provinces constitute 75% of the total production of sheep in Egypt. Kafr El-Sheikh is located at the latitude of 31° 6' 22.752" N, and longitude of 30° 56' 31.11" E; El-Beheira at latitude of 30° 50' 53.1564" N and longitude of 30° 20' 36.7836" E; Sharkia at latitude of 31° 50' " N and longitude of 30° 59" E; Menofia at 30° 35' 50.082" N and 30° 59' 15.4752" E, and Gharbia located at 30° 52' 31.2816" N and 31° 2' 0.636" E.

2.3. Routine data collection and sampling

A set out 4920 sheep (n= 80 flocks) were sampled from 140 flocks in five provinces in the Nile Delta region of Egypt. The age, sex, breed, body condition score, flock size, and different ecological condition were the parameters of assessment. The selected sheep flock was visited between January 2014 and February 2015 and investigated across four seasons (winter, summer, autumn, and spring). A survey questionnaire comprised of ten closed questions related to the potential risk factors was collected from the sheep owners including age, breed, sex, body condition score, the season of sampling, prophylactic treatment, flock management, and farmer status.

2.4. Coproscopic examination

Fecal samples were collected in sterile polyethylene bags. Samples were preserved in 10% formalin and labelled. Coproscopy was performed by the sedimentation technique depicted by (Sargison et al., 2016). Briefly, three gram of fecal samples was suspended with 20ml of 0.9 % saline, sieved through a grid (size 250 µm) and then to centrifuge tube. The solution was decanted and rewashed with saline, and this process was repeated twice. The blend was centrifuged for three minutes at 1500 rpm, and by discarding the supernatant carefully. Sediment were stained with a drop of methylene blue and mixed thoroughly. A drop of sediment was taken into a microscope slide, covered droplet with a coverslip and then examined under a microscope at 100 X magnification. The Fasciola fluke eggs were clearly differentiated based on the fact of its brownish color due to bile staining and more regularly shaped than paramphistome eggs.

2.5. Agro-ecological risk factors

Season: seasonal fascioliasis is classified based on the animals infected with fascioliasis within the following time intervals; autumn-fascioliasis (21 September to 20 November), winter-fascioliasis (21 December to 20 February), spring-fascioliasis (21 March to 20 May), and summer-fascioliasis (21 June to 20 August) (Kantzoura et al., 2011; Tum et al., 2004).

Body condition: sheep body condition scores were estimated according to the methods described by Nicholson and Butterworth (1986). On a scale of 1-5, a score of 1 is extremely thin, whereas a score of 5 is extremely fat. Sheep body condition scores of 2 and three are considered as medium and sound body condition respectively whereas 1 or 5 scores were considered of poor body condition.

Application of prophylactic treatment: the most commonly used prophylactic treatment choices against fascioliasis in sheep farms of Egypt are albendazole and rafoxanide, and triclabendazole (EL-Tahawy et al., 2018). Sheep have received a dose of one, or a mix of these medications during the time between twenty-one days and thirty days in the farming environment. Animals that received a dose of anthelmintic drugs at least once during the study period were considered to be in a prophylactic state, which was subsequently recorded in our survey.

Type of farm and water supply: the age of the sheep farmers and their education levels were recorded at the time of the survey. The duration of grazing among sheep flocks was grouped into three months, six months, nine months, and 12 months. The type of pasture during grazing (private or public) and the source of water supply (tap water or stream) were also recorded.

The climate conditions: temperature and humidity in Egypt were extracted from weather forecasts websites (<http://www.wunderground.com> and www.freemeteo.com). Then, the average monthly data of the five provinces of Egypt were calculated.

Economic impact

The economic losses due to fascioliasis can be calculated through estimation of the treatment cost, reduction in body weight, and mortality of the affected animals according to the method described by El-Tahawy et al. (2017a). Body weight of affected animals was compared with the healthy control animals based on the price of one-kg of live body weight equal to 37 Egyptian Pound (EGP). Also, the cost of Fasciola treatment was estimated based on the cost of the chosen drug and the course of the treatment in addition to the cost of mortality.

2.6. Statistical analysis

The association between the potential risk factors and the prevalence of fascioliasis was performed by multiple logistic regression and univariate analysis. The Chi-square test was used to test the independent categorical variables with the response Fasciola prevalence. The variables which are significant at $p < 0.05$ were analyzed analysed for multicollinearity and multivariate logistic regression model. The overall fit of the logistic regression models was assessed using the Hosmer-Lemeshow goodness-of-fit statistics. Data are adjusted as odds ratios (OR) with 95% confidence intervals (95% CI). Missing observations were excluded from the analysis. All the analysis was performed using Epi info™ statistical package. The economic data were analyzed analysed by T-independent sample test between Fasciola and healthy sheep.

Meta-analysis of the prevalence of fascioliasis:

The objective of this meta-analysis was to summarise collected and available information on the prevalence of fascioliasis, and the impact of risk factors. Several multilevel random-effect meta-analysis models were fitted to estimate the mean prevalence rate of fascioliasis and to compare them among the breed, season, sex, age, farmer age and education, type of grazing and duration, type of pasture and environment-related factors. Comprehensive Meta-Analysis software (Hanna et al., 2019) was used to perform the meta-analysis to generate forest plots using rate ratio and associated 95% Confidence Interval of the prevalence of fascioliasis obtained from the selected risk factors. The results of the pooled estimated effect size of the prevalence of fascioliasis and its impact on affecting sheep flocks in relation to about the putative risk factors. The meta-analysis utilized utilised the random effect model to study heterogeneity, which is based on the Higgins I² statistic and Cochran's Q-test. The forest plots generated were analysed to elucidate the outcome effects and effect estimates of different risk factors in determining the prevalence of fascioliasis in five provinces of the Nile Delta of Egypt.

3. Results

A total of 4920 sheep were investigated for risk of fascioliasis in five major significant provinces, Beheira, Kafr-Sheikh, Sharkia, Menofia and Gharbia in Egypt (Figure 1). The overall prevalence of ovine fascioliasis was 17.87% in the Nile Delta region of Egypt. A total of 80 sheep owners were interviewed. The 95% CI of the prevalence was performed along with a measure of heterogeneities between farm and agro-ecological parameters for ovine fascioliasis (Table 1). The predictors were fitted in a meta-regression model to explain the proportion between parameters. Findings from the meta-analysis of the different risk factors were shown in Figure (2-5). Heterogeneity analysis was occurred to indicate that differences in parameters were calculated. The heterogeneity-chi square was expressed by the ratio of cochrans value (Q) and degree of freedom (df) and was ignored if the df is less than one.

3.1. Characteristics of risk factors included in the meta-analysis

Population parameters

Breed: Three major breeds were investigated in this study; namely Barki, Rahmani and Baladi. The prevalence of fascioliasis in Barki and Rahmani breed was 18.12% (280/1545) and 13.58% (228/1678) respectively. It was noted as 21.86% (371/1697) in Baladi breed. The odds ratio (OR) for Barki sheep risk factor interaction with fascioliasis was 0.91 (95% CI 0.58-1.42, $P=0.68$) and for Rahmani sheep was 0.32 (95% CI 0.19-0.52; $P<0.0001$) (Table 1). In the fixed effect model, the calculated Cochran value (Q) was 38.95 (df= 2 and $p<0.0001$) (Figure 2 and 3).

Nile Delta provinces: The provinces, Beheira (OR = 2.90 (95% CI =1.89-3.91; $P<0.0001$)), Kafr El-sheikh (OR = 2.29 (95% CI =1.27-4.14; $P<0.001$)), Menofia (OR = 3.42 (95% CI =2.29-5.87; $P<0.0001$)) and Sharkia (OR = 4.69 (95% CI =2.55-4.28; $P<0.0001$)) have shown statistically significant higher risk factor interaction with prevalence of fascioliasis compared to Gharbia governorate (Table 1). Fixed meta-regression showed Q-value 43.23 with df= 4 and $p<0.0001$ (Figure 2 and 3).

Gender and age of the sheep: Both the gender and age of the sheep was significantly associated with fascioliasis. Results revealed that male sheep showed a statistically significant higher risk factor interaction with fascioliasis than female sheep (OR = 0.26 (95% CI =0.20-0.34; $P<0.0001$) (Table 1). The prevalence was relatively higher in adult sheep (age of sheep ranged 2 to 3 years old (13.49 %) and age more than three years (17.11 %) than young age group with less than two years old (20.66%). The

heterogeneity analysis showed that Q value was 25.54 and 46.10 with df 2 and 1 for age and sex of sheep, respectively (Figure. 2 and 3).

Sheep flock size: Sheep are best known for their strong flocking so, the flock contact with others has a great significant role in the spreading of Fasciola infection. The odds ratio of flock size interaction with fascioliasis ranged 100 to 150 and those over 150 sheep (Table 1) were significantly higher than those flock size less than 100 ($P < 0.0001$). Meta-regression showed high heterogeneity with Q-value 50.24 (df- 2) (Figure 2 and 3).

Sheep flock contact: The odds ratio of sheep flock contact (yes) interaction with fascioliasis was 2.27 ($P < 0.0001$) when compared with sheep flock contact (no). Also, sheep flock contact (yes) showed higher prevalence heterogeneity (495/2347) than sheep flock contact (no) (Table 1, Figure 3).

Sheep grazing factors

Effective grazing management is an important part of the overall sheep ranch (or farm), and sheep often graze pastures where Fasciola is endemic. The outcome of Fasciola infection in grazing sheep is severe anemia, liver failure and death in 8–10 weeks. The heterogeneity analysis of different parameters was presented in a forest plot (Figure 2 and 3).

Duration of grazing: The sheep flocks grazed six months (18.27%), nine months (19.44%), and twelve months (25.26%) were associated with a higher prevalence of fascioliasis than those grazed three months (Table 1). As we expected, the sheep flock grazed in the pasture for 12 months showed the highest interaction with fascioliasis (OR of 3.11; 95% CI 2.07 - 4.68).

Type of the pasture: While considering the type of the pasture, the public pasture showed a higher prevalence (22.49) than the private pasture (13.91%).

Sheep farming management characteristics

Water source: water source is considered as an infection source for Fasciola. While comparing the risk factor impact of fascioliasis, the tap water showed a lower prevalence than stream water (Table 1). The odd ratio for the water source interaction was 0.61 (95% CI 0.44-0.85; $P < 0.001$). Fixed heterogeneity analysis showed that the calculated Cochran value was 14.66 (df- 1 and $p < 0.0001$) (Figure 4 and 5).

Prophylactic treatment: As in Table (1) and forest plot (Figure 4 and 5) among the 2697 treated sheep, 375 (13.90%) were infected with Fasciola whereas 504 sheep (22.67%) had fascioliasis among 2223 untreated sheep.

Body condition scores: This score is a crucial factor for the prevalence of Fasciola among sheep flocks. Medium and poor body condition scores (24.40% and 23.01%) have associated with a higher prevalence of fascioliasis than body condition (Table 1 and Figure 4 and 5).

Age and education of the sheep farmers: Both age and education level of the sheep farmer was explored in this study. The higher level of farmer education was statistically associated with low prevalence than lower basic level (13.20% and 23.02%). In addition, also, the age of sheep owner ranged 40 to 50 and over 50 were associated with lower prevalence than those below 40 years old (Table 1 and Figure 4 and 5).

Agro-ecological parameters

Seasonal variation: The seasonal variation of ovine fascioliasis was presented in Table (1). Spring, summer, and winter seasons have associated with a lower prevalence of fascioliasis than an autumn season. The odd ratio of seasonal interaction for fascioliasis in spring (0.17; $P < 0.0001$), summer (0.24; $P < 0.0001$), and winter (0.42; $P < 0.0001$). Strong heterogeneity was expressed in fixed analysis with Q value= 41.32 (df- 3 and $p < 0.0001$) (Figure 4 and 5).

Temperature: Temperatures ranging from 26 to 31 degree Celsius, and over 31 degree Celsius were associated with a higher prevalence of fascioliasis as compared to temperatures less than 26 degree Celsius (Table 1). Calculated Q value for heterogeneity was 3.4 (df- 2 and $p < 0.1$) (Figure 4 and 5).

Relative humidity: The relative humidity ranged 50 to 60, and over 60 showed a higher prevalence of Fasciola than the humidity less than 50 (Table 1 and Figure 4 and 5).

Economic impact

Table (2) presents the different factors involved in the economic effects of fascioliasis on the productivity of sheep flocks. Sheep-infected Fasciola showed a lower total body weight (55.29 Kg) when compared with the total body weight of healthy sheep (47.14 Kg). The monetary losses due to the weight reduction were estimated as 301.55 EGP. The cost of the treatment for sheep with fascioliasis approximately estimated 46.22 EGP/sheep. Furthermore, the value of mortality due to the death of sheep was 4800 EGP/three sheep.

4. Discussion

Egypt is considered as one of the fascioliasis-endemic areas in the world (Amer et al., 2016; Dietrich et al., 2015), and the disease burden is high in the Nile Delta region. Both species, Fasciola hepatica and Fasciola gigantica, are commonly seen present in Egyptian sheep, and the occurrence of the hybrid form has been reported (Amer et al., 2011). This disease is severely affecting the sheep farming industry and causing severe

economic loss, especially in the reduction of wool production, meat, and total body weight (Amer et al., 2016). There is a scarcity of studies available on the association and interaction between the prevalence and environmental risk factors of ovine fascioliasis in the Nile Delta region of Egypt. Therefore, this present study was conducted to determine the causal association between prevalence of ovine fascioliasis in different breeds of sheep flocks in the Nile Delta in Egypt and epidemiological characteristics/agro-ecological risk factors.

The prevalence of fascioliasis in Baladi breed is markedly higher compared to in Barki and Rahmani breed. The Nile Delta Province, Alexandria showed a higher risk factor interaction with the prevalence of fascioliasis. A similar trend was observed in male sheep and sheep groups aged below two years old that showed a high prevalence. The sheep flock size and contact were positively correlated with the fascioliasis. Grazing duration of sheep flocks, stream water, prophylactic treatment, age and education of the sheep farmer, and public pasture had a positive impact on the common sheep fluke infection. Seasonal variation, temperature and relative humidity of sheep farming environment were playing a significant role in the prevalence of fascioliasis.

The previous study revealed that there was an extensive wide variety of seroprevalence for ovine fascioliasis (Moghaddam et al., 2004). These differences in the prevalence of fascioliasis are most likely caused by agro-biological risk factors and climatic differences between the regions of the study, in addition to the variation in the management frameworks (Abunna et al., 2010).

The present study showed a wide variation of the prevalence of Fasciola among different governorates, demonstrating a higher prevalence rate in the tested provinces than the reference one. Our findings were consistent with the previous studies on the Nile Delta region. Ezzat (1949) stated that *F. gigantica* flourished in the northern parts of the Nile Delta, causing severe economic losses in the sheep flocks. Furthermore, the author of this particular study mentioned that the disease was widespread among sheep and calves in Dakhla and Kharga Oases about 90%. Haridy et al. (1999) reported that the overall prevalence rate of fascioliasis was 2.02% for slaughtered sheep. The highest prevalence was reported in Behera Governorate, while the lowest was observed in Kafr El Sheikh (El-Bahy, 1997). (El-Bahy, 1997).

Furthermore, Soliman (1998) observed the prevalence of fascioliasis as 52.8, 47.5 and 20 % in cattle, buffaloes and sheep, respectively in Beheira Governorate, Sharkia Governorate had the highest prevalence, as shown in our findings that were consistent with Haggag report (Haggag, 2008). The authors of this previous study showed the overall infection rates with fascioliasis in Beheira and Sharkia Governorates were 17.77% and 31.85% by using fecal examination and indirect hemagglutination assay (IHA), respectively.

Fasciola infection between the different sheep breeds in the present study was consistent with previous finding (Khallaayoune et al., 1991). The evidence of this particular impact due to breed could be associated with the farming system (Sanchez-Andrade et al., 2002). Munguia-Xochihua and colleagues (Munguia-Xochihua et al., 2007) highlighted that there were no differences in the infection rate between different breeds of sheep. On the other hand, Mir et al. (2008) showed that the prevalence of fascioliasis was higher in the exotic breed more than the native sheep breed. Furthermore, Eguale and colleagues (2009) confirmed that there was a difference in the vulnerability of the examined sheep breeds to Fasciola infections.

Age variation in sheep flocks in the current study revealed that sheep group aged below two years old had a higher prevalence than those with over three years old and those with 2 to 3 years old. The likely explanation for the lower prevalence in higher age groups could be because of the self-cure phenomenon and high gained resistance, which increments with age. It has been accounted for that host may recuperate from parasitic disease with increasing age and thus are safe from infection. Our findings are in agreement with the studies of Mungui-Xochihua et al. (2007), who recorded that the higher prevalence of fascioliasis was reported in sheep between 2-4 years. Moreover, Ghazani et al. (2008) mentioned that there was a statistical difference between the age of more than one year and less than one year (more in less than 1 year). Besides, Mir et al. (2008) estimated the prevalence of fascioliasis in sheep and found it was higher in sheep more than four years old (42.8%), followed by age group 2-4 years old (37.7%) and age group 0-2 years old (18.79%).

The seasonal variation of the prevalence for ovine fascioliasis revealed that the autumn season was associated with a higher rate than other seasons. Other studies were also indicated the similar findings where Taylor (Taylor, 2007) stated that the favourable weather condition for Fasciola egg to hatch and for snail multiplication was a rainy season with temperature below 10°C. The seasonality pattern in the fascioliasis has also been observed by Ahmed and colleagues (Ahmed et al., 2007) who uncovered that atmosphere condition especially precipitation, that is regularly connected

as well as acting against the prevalence of *Fasciola* infection because this weather pattern is reasonable for snails to reproduce and to survive longer under clammy conditions. Furthermore, Hossain et al. (Hossain et al., 2011) documented that the frequency of fascioliasis was observed to be profoundly higher amid the rainy season than that of a dry one.

Flock size has a significant role in the spreading of the *Fasciola* (El-Tahawy et al., 2017a). Our findings indicated that the larger size ranged 100 to 150 and over 150 had a higher prevalence of *Fasciola* than the lower smaller flock size less than 100. This higher rate was possibly associated with the higher density of the sheep flock that could be considered a key risk factor for more frequent contact with *Fasciola* infection between the sheep (Kantzoura et al., 2011). In respect to the relationship between the sheep sex and prevalence of *Fasciola*, our finding showed a higher in female than the male that was consistent with the findings of Khan et al. (2010). The authors of this previous study showed that sex is a common determinant of parasitism and female sheep are highly prone to parasitism during pregnancy and pre-parturient period because of anxiety and diminished immune status. Apart from the change in physiological condition during lactation and absence of appropriate nutrition, the longtime contact of the animals to disease factors and their grazing in submerged zones may be the reason for more significant prevalence rate in females (Hossain et al., 2011).

Prevalence of *Fasciola* was varied between the sheep either grazed on public or private pasture. The obtained results demonstrated that sheep grazed in public pasture has more risk for *Fasciola* than those grazed in private one. Our findings were in agreement with the studies of Kantzoura et al. (2011), who investigated that the sheep grazed on public pasture have a higher odds ratio of *Fasciola* than those grazed in private. Durr and colleagues (2005) highlighted that sheep flocks that utilize private pastures had a higher risk of *Fasciola* infection than flocks that grazed on public pastures. The authors of this previous study explained that private pasture has a little space area whereas sheep flock grazed on open fields for a lengthy period. Along these lines, there is a consistent shedding of eggs on these fields. Likewise, private pastures are normally flooded, and the watering system has been observed to be a key factor for the existence of fascioliasis.

The results depicted that stream water was associated with a higher prevalence of fascioliasis than tap water. Njau et al. (1989) stated that wet fields associated with mud are considered as a critical risk factor because this sort of environment is ideal for the proliferation (survival) of snails. The occasional swarming of sheep flocks in the banks of infected water gives a critical chance to transmission. Soulsby (1982) reported that the higher infection rate of sheep fascioliasis might be due to exposure to encysted metacercaria during their grazing adjacent to water canals in addition to their higher susceptibility and low immunity.

The duration of grazing has a part in the spread of fascioliasis among different sheep breeds. The results revealed that sheep grazing for nine and twelve months have a higher chance to contract fascioliasis compared to those that grazed for only three months. This might be explained, as when the duration of grazing takes place over a longer more extended period, the rate of contacts or transmission between the infected sheep and healthy sheep is also increased, thereby increasing the rate of infection.

The poor and medium body condition scores have a higher prevalence of fascioliasis. These findings were consistent with the previous reports on the high pervasiveness of *Fasciola* infection among the sheep with poor body condition. Devendra and Marca (Devendra and Marca, 1983) showed that sheep with poor body condition are defenseless defenceless against a parasitic disease. The predominance of fascioliasis in connection to poor body conditions could be further demonstrated by the fact that parasitism of *Fasciola* and migration of immature worms including suck blood and tissue fluid and damage to the parenchyma of the liver (Marquardt et al., 2000).

The age and education level of the farmer/handler had additionally been examined by Cringoli and colleagues (2004) and were not observed to be significant. In the present study, the age of owners and their poor education level were perceived as critical factors of the prevalence of fascioliasis. The well-established and learned owners often exhibit better stockmanship abilities and improving sheep health.

The ecological conditions in the present study indicated that temperature less than 26°C and humidity less than 50°C are considered as risk factors for the spreading of *Fasciola* among sheep flocks. Khanjari et al. (2014) reported that development of the intermediate host, temperature (> 9.5 °C), rainfall and soil moisture are important factors influencing the development of the parasite from *Fasciola* egg to miracidium. Also, a previous study has demonstrated that the parasite development is arrested below 10 °C or over 30°C. Therefore, the disease transmission of *F. hepatica* is reliant on temperature, which controls the life cycle stages in the mammalian host.

The economic effect of *Fasciola*, our findings were strengthened by the investigations of Ngategize and colleagues (1993) who evaluated that financial losses of fascioliasis as 46.5, 48.8 and 4.7% as brought on by mortality, profitability (weight reduction) and liver judgment, individually. An incidence rate above 90% of *F. hepatica* eggs among sheep and dairy cattle was seen in the condition of Rio Grande do Sul Brazil where economic losses of 12-13% were observed under strict liver/meat quality guidelines (Marques and Scrofrernek, 2003). The financial losses due to the contamination of 250,000 fluke-contaminated livers (cost US\$ (140,000,000)) demonstrating to 15% of the livers examined by the authorities based in the meat pressing plants (El-Tahawy et al., 2017a). Haseeb et al. (2002) stated that the loss in meat and milk in Egypt due to fascioliasis was 30% every year. That is almost equivalent to one billion Egyptian pounds.

Conclusions

The present study highlights the epidemiological examination of putative risk factors associated with the prevalence of fascioliasis in the Egyptian sheep flocks. The study demonstrated that there was a strong association between the prevalence of fascioliasis and key factors such as flock location, age of the animal, sex, breed, type of pasture, water supply, farmer status, and other ecological conditions. Also, our findings revealed that the association of financial/economic impact and the prevalence of fascioliasis that leads to extraordinary losses through a decrease in body weight, treatment expenses, and early mortality. The outcomes of this study may guide proper *Fasciola* control techniques in Egypt.

Competing Interests

The authors have no conflict of interest.

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