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Epidemiological and ultrasonographic investigation of bovine fascioliasis in smallholder production system in Eastern Nile Delta of Egypt

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ABSTRACT

Regular updating of our knowledge on the epidemiological determinants of bovine fascioliasis is necessary to increase the awareness of the disease's significance and subsequently, improve the control measures. The objectives of this study were (1) to estimate the prevalence of bovine fascioliasis, and identify the association of epidemiological characteristics under traditional householders' production systems, (2) to describe the association between the clinical picture, *Fasciola* spp. egg count and hepatobiliary ultrasonography findings. In total, 270 faecal samples were examined microscopically for the presence or absence of *Fasciola* spp. egg, using the sedimentation-flotation method. Copro-positive animals were subjected to ultrasonographic examination. Overall prevalence of copro-positive animals was 27.4% (22.4–33.0%, 95% CI). The final multivariate analysis showed that there was a significant association between fascioliasis and animal species ($P < 0.03$), and administration of anthelmintic ($P < 0.0001$). Cattle have a less chance of being positive to *Fasciola* spp. by 0.55 (95% CI: 0.30 – 0.99) compared to water buffaloes. Administration of anthelmintic to animals on a regular basis decreased the risk of copro-positivity to *Fasciola* spp by 0.17 (95% CI: 0.07 – 0.36) compared to animals received anthelmintic on an irregular basis. Infected animals having different *Fasciola* spp. egg burden revealed different clinical symptoms associated with hepatobiliary changes on ultrasonographic examination ranged from normal hepatic parenchyma and bile system in low faecal egg load to hyperechogenic hepatic parenchyma, hyperechogenic with distal shadowing bile duct, and distended gallbladder in high faecal egg load of *Fasciola* spp.

In conclusion, the prevalence of bovine fascioliasis is high under the traditional household's production system. Regular administration of anthelmintic significantly reduces the animal's chance of being copro-positive to *Fasciola* spp. Ultrasound poses a valuable prognostic technique for assessment of bovine fascioliasis.

1. Introduction

Bovine fascioliasis is a zoonotic trematodiasis of veterinary and public health importance affecting ruminant animals (Haseeb et al., 2002; Periago et al., 2008). Several millions of dollars have been lost in African countries due to fascioliasis (Dawa et al., 2013; Mucheka et al., 2015). In Egypt, several reasons make the situation of fascioliasis particularly interesting (Hussein and Khalifa, 2010). Specifically, (a) liver fluke fragments were discovered in Egyptian tombs (Esteban et al., 2003) and in an Egyptian mummy (David, 1997), (b) the prevalence of human fascioliasis has significantly increased since 1990 (Soliman, 2008), and (c) transmission is continuous throughout the year, with a peak at the end of spring in June. Both, *F. gigantica* and *F. hepatica* are reported from Egypt (Esteban et al., 1998; Amer et al., 2016). *F. gigantica* has been present since the times of the pharaohs, while *F. hepatica* was imported from Europe at the beginning of the 1900s (WHO, 2007; Soliman, 2008). Moreover, the intermediate hybrid forms were also reported (Periago et al., 2008), suggesting an expected continuous increase in the prevalence of fascioliasis in both human and animals. The annual overall costs due to fascioliasis in Egypt is 221 USD per cow due to reduction in body weight, reduction in milk production, and treatment costs (El-Tahawy et al., 2017). Furthermore, the number of human cases of fascioliasis has dramatically increased in the Lower (Nile Delta) Egypt (Soliman, 2008) and Upper Egypt (Mekky et al., 2015). The prevalence of human cases was increased from 3% in 1990 (WHO, 2007) to 8% in 2013 (Mekky et al., 2015). Due to that critical

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situation, Egypt was one of the first countries, which implemented control activities against human fascioliasis since 1996 (WHO, 2007).

Occurrence of fascioliasis is strongly linked to the freshwater mollusk of the genus *Lymnaea* spp., which acts as an intermediate host of the liver fluke (de Kock et al., 2003; Sharma et al., 2011). In Egypt, the agricultural activities depend exclusively on the water of the Nile River, which is running across the middle of Egypt starting from the south at the Nasser Lake to the north at the Mediterranean Sea. Previous studies have shown that human fascioliasis in Egypt was associated with the presence of ruminants in households, and the watering, and bathing of the animals in the Nile river and its channels (Curtale et al., 2003). Smallholder production system by traditional householders is a common livestock production system in developing countries. This traditional husbandry system is generally extensive, with small herds or small numbers of animals kept for subsistence or to generate an additional income to the household.

A greater insight into the epidemiological characteristics of liver fluke infections is important for improving the control program and, subsequently; minimizing the risk of zoonotic transmission to humans. Therefore, gathering information on the prevalence of the *Fasciola* spp. and potential risk factors associated with the spread of fascioliasis among the animals is necessary in order to understand the disease nature and transmission and subsequently, proposing the appropriate effective control strategies for their treatment and control (Takeuchi-Storm et al., 2017). Risk factors associated with bovine fascioliasis have been reported from large-scale and commercial production systems (Olsen et al., 2015; Takeuchi-Storm et al., 2017). To the best of the authors' knowledge, there is no available literature that documented the prevalence and risk factors among the smallholder production system particularly, in the endemic area, which may have variable infection levels. Furthermore, variations in micro-environment and management practices could influence the disease epidemiology and persistence (Knubben-Schweizer et al., 2010; Knubben-Schweizer and Torgerson, 2015).

Bovine fascioliasis manifests clinically by weight loss, anemia, weakness, diarrhea, and subcutaneous edema, particularly in submandibular region (Yadav et al., 1999; Sharma et al., 2011). In progressive chronic cases, the liver is extensively damaged and develops hepatic fibrosis (Serra-Freire et al., 1995; Marcos et al., 2007). Diagnosis of fascioliasis relies on clinical signs and detection of the *Fasciola* spp. egg using faecal examination for the initiation of anthelmintic therapy (Van Metre et al., 2007). Ultrasound imaging has been implemented as a diagnostic and prognostic tool for evaluation of abdominal and thoracic disorders in ruminants (Braun, 2009; Mohamed and Oikawa, 2007). Tharwat (2012) described the ultrasonographic findings of bovine fascioliasis, however; the author showed the ultrasonographic picture only in chronic cases of fascioliasis based on a small sample size (16 animals) at different points of time (2003–2007). Additionally, the author used different diagnostic techniques (faecal examination and ELISA) for selection of the positive cases. Previous studies reported a marked variation between the sensitivity of faecal examination and ELISA for diagnosis of fascioliasis (Charlier et al., 2008), especially in old infections and in case of low parasitic burden (Dorchies, 2007). The objectives of this study were (1) to estimate the prevalence of bovine fascioliasis, and identify the association of epidemiological characteristics under traditional householders' production systems at Eastern Nile Delta of Egypt, and (2) to describe the association between the clinical picture, *Fasciola* spp. egg count and hepatobiliary ultrasonography findings associated with bovine fascioliasis.

2. Material and methods

2.1. Study population and animal selection

This study was carried out from September 2016 to the end of August 2017 on cattle and buffaloes from several villages in Sharkia province located in Eastern Nile Delta of Egypt. Villages were selected for inclusion in this study based on the distance (less than 50 km) from Zagazig city, where our laboratory facilities are located at the Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University. The farmers were selected based on the criteria of being household breeders of large ruminants (small-scale production system < 10 animals), and their willingness and voluntary participation after giving them a brief description about the study and its objectives. Animals were selected randomly based on species (cattle and buffaloes), being belonging to household breeders, and having a recent history of decreased milk production, and/or emaciation. Exclusion criteria were applied when the villages are far away from Zagazig city, farmers having large production system (≥ 10 animals) or raising different animal species such as small ruminants and/or donkeys with cattle and buffaloes, and animals undergo anthelmintic treatment at time of sampling and/or have been received anthelmintic treatment from one month before sample collection. In total, 47 household breeders were visited in the villages of three major cities including Zagazig (115 animals from 20 breeders), Bilbeis (85 animals from 15 breeders), and Abo-Hammad (70 animals from 12 breeders). Description of the study population and potential risk factors are listed in Table 1.

2.2. Sample collection and metadata

Fecal samples were collected from the rectum of the selected animal using rectal gloves. Each collected sample was placed in sterile conical tube and labeled with the household identifier number. They were stored in a cold box and transported to the laboratory at the Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Egypt. The samples were stored overnight in the cold box for processing the following morning. Each animal underwent a clinical examination after compiling the case history and data regarding the sampled animal (biodata) including species, sex, age, feeding system type, and history of anthelmintic drenching, was recorded. Information on the hygiene of the place, where the animals are kept and the season, where the animal was sampled were merged with the collected biodata and result of fecal analysis (presence/absence of eggs of *Fasciola* spp.).

Table 1
Description of 270 cattle and buffaloes from smallholder production system in Eastern Nile Delta of Egypt examined for *Fasciola* spp. infection during the period from September 2016 to the end of August 2017.

Item	Level	Animal species (%)		Total (%)
		Cattle	Buffaloes	
Sex	Female	55 (39)	87 (61)	142 (53)
	Male	65 (51)	63 (49)	128 (47)
Locality	Zagazig	47 (41)	68 (59)	115 (42)
	Belbeis	31 (37)	54 (64)	85 (32)
	Abo-Hammad	42 (60)	28 (40)	70 (26)
Age	Old (> 3y)	75 (45)	91 (55)	166 (62)
	Young (≤3y)	45 (43)	59 (57)	104 (39)
Feeding type	Ration	50 (42)	68 (58)	118 (44)
	Free grazing	70 (46)	82 (54)	152 (56)
Frequency of anthelmintic administration	Irregular	80 (44)	100 (56)	180 (67)
	Regular	40 (44)	50 (56)	90 (33)
Hygiene	1 = good	40 (44)	50 (56)	90 (33)
	2 = moderate	33 (41)	48 (59)	81 (30)
	3 = bad	47 (48)	52 (53)	99 (37)
Season	Autumn	23 (46)	27 (54)	50 (19)
	Spring	45 (41)	65 (59)	110 (41)
	Summer	17 (47)	19 (53)	36 (13)
	Winter	35 (47)	39 (53)	74 (27)
Total		120 (44)	150 (56)	270 (100)