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Epidemiological and Ultrasonographic Investigation of Bovine Fascioliasis in Smallholder Production System in Eastern Nile Delta of Egypt

Hend M. El Damaty [a], Yasser S. Mahmmod [a, b, c*], Shaimaa Gouda [d], Nader M. Sobhy [a]

[a] Infectious Diseases, Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, 44511-Zagazig, Sharkia Province, Egypt

[b] Centre de Recerca en Sanitat Animal (CReSA, IRTA), Campus de la Universitat Autònoma de Barcelona, 08193-Bellaterra, Cerdanyola del Vallès, Barcelona, Spain (present)

[c] Universitat Autònoma de Barcelona, 08193-Bellaterra, Cerdanyola del Vallès, Barcelona, Spain (present)

[d] Internal Medicine, Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, 44511-Zagazig, Sharkia Province, Egypt

[*] **Corresponding author**

Yasser S. Mahmmod

Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, 08193-Bellaterra, Cerdanyola del Vallès, Barcelona, Spain (present)

Email: yasser.mahmmod@irta.cat; yasser@sund.ku.dk

Mobile: 0034-612567320

ORCID: 0000-0002-1975-3454

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 egg burden.

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.

Keywords: cattle; *Fasciola* spp.; risk factors; ultrasonography; water buffaloes

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 in 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 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 to 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 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), Bilbis (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 tubes 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.).

2.3. Parasitological examination

Fecal samples were brought to the laboratory and were subjected to the parasitological examination. The presence of *Fasciola* spp. eggs in faecal samples was evaluated by a sedimentation-flotation technique (Charlier *et al.*, 2008). Approximately 10 gm of faeces were placed into a paper cup and thoroughly mixed with 200 ml water and filtered 3 times through a metallic tea sieve into a 250-ml beaker to separate large particles. The filtrate was allowed to stand for 20 min after which the sediment was collected in a test tube and centrifuged at 700 x g for 5 min. After centrifugation, the supernatant was discarded and the sediment was suspended in zinc chloride and centrifuged at 180 x g for 3 min. The obtained sediment material was transferred to a microscope slide, covered with a coverslip, and investigated at ×100 magnification. *Fasciola* spp. eggs were identified on the basis of morphological characteristics using light microscopy according to the keys and the guideline given by Bowman *et al.* (2003). The results were expressed as the presence or absence of eggs of *Fasciola* spp.

To investigate the association between the ultrasonographic picture of the liver of infected animals and the severity of *Fasciola* spp. infection and subsequently, predicting its prognostic value, the faecal egg count was estimated per gram (EPG) was determined using McMaster egg counting technique according to Conceição *et al.* (2002). Briefly, 30 gm of faeces were mixed with 60 ml of a 5% water detergent solution in a large test tube and stirred well with a stirring rod. The sample was strained through a large mesh sieve to a conical 1000 ml flask, and filled up with tap water. The sample was allowed to stand for 10 min and then the supernatant discarded and the sediment was re-suspend in tap water. The sedimentation process was done four times and after the last sedimentation, the test tube was filled up to 50 ml volume with tap water. Both chambers of a McMaster slide were filled with a Pasteur pipette and all the *Fasciola* spp. eggs present on the bottom of the chambers were counted. EPG was determined according to the following equation:

$$\text{EPG} = \frac{\text{total number of observed eggs}}{\text{number of chambers}} \times \frac{50\ \text{ml}/30\ \text{g}}{0.15\ \text{ml}}$$

The McMaster technique was applied only to the faecal samples from those animals that were egg-positive on sedimentation-flotation technique. According to the EPG, level of *Fasciola* spp. infection was classified as low (< 30 EPG), moderate (≥ 30 to < 100 EPG) and high (≥ 100 EPG), reflecting the degree of infection severity according to Radfar *et al.* (2015) and Kajugu *et al.* (2015) with modifications.

2.4. Ultrasonographic examination

Animals that were tested positive for *Fasciola* spp. on faecal examination were subjected to ultrasonography. The ultrasonographic examination was performed by using 3.5 and 5.0MHz convex transducers (Sono scape, A5V, China) while the animals were standing. The ultrasonography of the liver was performed on the right side in the 9th, 10th, or 11th intercostal spaces as described previously (Braun et al., 1995; Mohamed and Oikawa, 2007). The abdomen and thoracic cavity were scanned from both right and left sides. In preparation for ultrasonography, the skin was shaved and swabbed with alcohol 70% to remove excess oil, and coupling gel was finally applied to the intercostal spaces and the entire abdomen were clipped. Ten copro-negative apparently healthy animals (five cattle and five buffaloes) were used as a negative control.

2.5. Statistical analyses

A dichotomous outcome variable was computed as the presence or absence of eggs of *Fasciola* spp. in each animal. The explanatory variables (potential risk factors) of interest included in the model analysis were animal species (cattle/buffaloes), sex (male/female), age, feeding system type, frequency of anthelmintic administration (regular/irregular), housing hygiene, and season (summer, autumn, winter and spring).The animals were categorized into two age groups; animal ≤ 3 years were termed as young while those > 3 years were termed old. (Jaja *et al.*, 2017). Housing hygiene was classified into three groups according to the cleanliness of the bedding and environment of the animal's housing into 1= good hygiene, 2= moderate hygiene and 3= bad hygiene. The feeding was separated into two groups based on the feeding system type into free grazing group where the animals rely mainly on free grazing and ration group, where the animals offered a daily balanced ration of concentrates and green fodder mixture.

At first, a descriptive statistics and distribution of the potential risk factors were carried out. Statistical analyses were carried out using a logistic regression model to investigate the association between infection with fascioliasis (presence or absence of *Fasciola* egg in faeces) and the studied risk factors. The model was constructed using the "glm" function with a binomial distribution in R version 3.3.3 (R Core Team, 2015). A univariable model was carried out to test the unconditional associations between dependent and independent variables and to determine which of the potential factors should be included in the multivariable logistic regression analysis. Statistical significance at this step was determined at *P-value* $\leq$ 0.20 (Dohoo *et al.*, 2009). A full multivariable model analysis including the significant variables from univariable modelling and all 2-way interaction between variables was done. The final model was generated using a backward step-wise elimination by dropping the least significant variable until we got the final model, which included only variables with *P-value* $\leq$ 0.05. Interaction terms which did not significantly contribute to the regression model were removed from the model one-by-one. The P-value, odds ratio (OR) with a 95% confidence interval (95% CI), and regression coefficient (b) were recorded for each variable. In all statistical analyses, the results were considered to be significant at *P-value* $\leq$ 0.05.

3. Results

In Sharkia province, one of the largest agriculture provinces in Egypt, the most common traditional livestock farming system among Egyptian farmers is extensive systems type.

Feeding system depends mainly on free grazing the daylight on the farmer's own grass/crop field, which relies exclusively on the Nile River water for irrigation, and at evening the farmer supplemented the animal with hay, wheat straw with or without some concentrate. Some farmers feeding their animals on total mixed ration with vitamins and mineral supplements. Housing was a tie stall barn, bedding material was mud, straw and in some cases mixed with sawdust or straw, which is not regularly scraped or changed. Animals are commonly received an anthelmintic dosing such as Ivermectin to combat the parasitic infestations. Some farmers drenching their animals with other types anthelmintic such as albendazole in combination with or without ivermectin injection. Some farmers administer the anthelmintic to their animals on a regular basis (once every three months), while others may use it from time to time on an irregular basis.

3.1. Prevalence of fascioliasis and associated risk factors

Cross-tabulation of dependent and independent variables are listed in Table 2. The overall prevalence of *Fasciola* spp. egg-positive animals was 27.4% [95% CI; 22.4 – 33.0], Table 2. A lower prevalence of fascioliasis was observed in cattle (21%, N=25/120) compared with buffaloes (33%, N= 49/150). The prevalence of fascioliasis in animals raised on free grazing feeding system type (38%, N=57/152) was higher than those animals raised on ration feeding system (14%, N=17/118). The prevalence of fascioliasis in animals received anthelmintic on an irregular basis (36%, N=65/180) was higher than those animals received anthelmintic on regular basis (10%, N=9/90).

The result of the univariate analysis is presented in Table 3. The feeding type, frequency of anthelmintic administration, and housing hygiene were significantly associated with copro-positivity to *Fasciola* spp. at *P-value* < 0.0001. Meanwhile, the animal species, sex and season were significantly associated with copro-positivity to *Fasciola* spp. at *P-value* < 0.05, whereas age was not.

The result of final multivariable logistic regressions is listed in Table 4. The resulting final model consisted of the significant variables, animal species and frequency of anthelmintic administration. Administration of anthelmintic to animals on a regular basis decreased the risk of copro-positivity to *Fasciola* spp. to 0.17 (95% CI: 0.07 – 0.36) compared with animals received anthelmintic on an irregular basis. Cattle have a less chance of being positive to *Fasciola* spp. to 0.55 (95% CI: 0.30 – 0.99) compared with buffaloes.

3.2. Ultrasonography findings

Results of ultrasonographic and clinical findings associated with the different levels of *Fasciola* spp. infections according to egg count (EPG) are presented in Table 5. On ultrasonography examination, the first group of copro-positive animals (n= 38; 14 cattle and 24 buffaloes) having a low level of egg count on faecal examination showed a normal hepatic parenchyma and bile system. The predominant clinical finding in this group was non-specific symptoms such as decrease in milk production and/or loss of body weight. The second group of (n= 20; 6 cattle and 14 buffaloes) having a moderate level egg count showed on hyperechogenic hepatic parenchyma with multiple echogenic foci and normal bile system (Figure 1). The predominant clinical finding in this group was paleness in mucous membranes. The third final group (n= 16; 5 cattle and 11 buffaloes) having a high level of egg load revealed hyperechogenic hepatic parenchyma, hyperechogenic with distal shadowing bile duct, and distended gallbladder, Figure (2 a, b). Additionally, anechoic fluid was imaged in the abdominal and thoracic cavities in one buffalo in the third group, Figure (3). Clinically, the predominant findings include severe emaciation, subcutaneous oedema in submandibular space “bottle jaw”, and anemic to icteric mucous membranes.

4. Discussion

4.1. Prevalence of bovine fascioliasis

The overall prevalence of *Fasciola* spp. was found to be 27.4%, revealing a high level of infection that requires an effective control program. The high overall prevalence reported in our study may reflect the favorable conditions for the precipitance of transmission cycle of liver flukes among the cattle and buffaloes raised under the smallholder's production system. This study was conducted in the eastern region of Nile Delta of Egypt, where the majority of the farmers use Nile River's water for irrigation of their land and drinking purpose of their animals. That's why; ruminant animals in such areas are at high risk of liver flukes endemicity (Haseeb *et al.*, 2002; El-Tahawy *et al.*, 2017). Moreover, climatic conditions, use of traditional husbandry methods and improper management may contribute greatly to parasite and its vector growth, development and transmission (El-Tahawy *et al.*, 2017). Lack of knowledge on how to control the animal health problems properly and the low education level of the farmers particularly, managing the smallholder production system, could partially explain the high prevalence of fascioliasis under such environment. That comes in agreement with Seimenis (2012) who concluded that farmers' poor knowledge and access to adequate veterinary services and improper use of anthelminthic may alter the dynamics of *Fasciola* spp. in endemic areas and further promote the prevalence of fascioliasis. Additionally, this high prevalence could argue the remarkably high prevalence of human fascioliasis in Egypt (Haseeb *et al.*, 2002)

4.2. Associated risk factors with bovine fascioliasis

Our findings demonstrated that cattle are likely have a less chance of being positive to *Fasciola* spp. infection by 0.55 (95% CI: 0.30 – 0.99) compared with buffaloes. A plausible explanation for this finding that water buffalo is a swamp animal prefer to swim frequently in the Nile water. Therefore, it is frequently exposed to the intermediate host of *Fasciola* spp. parasite, where snails are commonly inhabited in the water channels, rivers and swamps (de Kock *et al.*, 2003). Subsequently, animals become infected by drinking contaminated drinking water and/or ingesting the vegetation growing on the edges of the water canals, which intensively harbor the metacercaria cysts (infective stages) of *Fasciola* spp. making buffaloes under high risk of infection (Kaplan, 2001). The household Egyptian farmers have a common habit to bring the buffaloes to the river canal for bathing and/or drinking purposes at early morning time when they go to their own crop field and at evening time when they bring the animals back to their house (Curtale *et al.*, 2000, 2003). Our findings are in agreement with Hussein and Khalifa (2010) who found water buffaloes have been possessed a higher prevalence (33.7%) of *Fasciola* spp. infection, compared to cattle (28.6%). Molina *et al.* (2005) reported a difference in host-parasite relationships of *F. gigantica* infection between cattle and swamp buffaloes and may be linked to the observed varying levels of resistance and resilience to infection between these hosts. Furthermore, Genetic variations within the host between cattle and buffaloes may explain the variation in their susceptibility (Molina and Skerratt, 2005). Mehmood *et al.* (2017) added that the difference of fascioliasis prevalence between cattle and buffalo populations might be attributed to the difference in management practices and pattern of animal movements for grazing near the waterlogged area.

The frequency of anthelmintic administration was significantly associated with the liver fluke infection. Animals receiving anthelmintic on a regular basis have a less risk of being copro-positive to *Fasciola* spp. to 0.17 (95% CI: 0.07 – 0.36) compared with animals received anthelmintic on an irregular basis. This is consistent with previous studies, which reported that anthelmintic administration to reduce parasitism of liver flukes in the host and therefore, the egg elimination and pasture contamination (Torgerson and Claxton, 1999; Knubben-Schweizer *et al.*, 2010). Due to the lack of records of anthelmintic administration, it was hard to follow the type and dose of administrated anthelmintic because that was carried out basically by the veterinarians who did not usually keep a record to the treated cases. However, we got an overview about the type of anthelmintic commonly used in animals in that geographical area. The most commonly used anthelmintic is ivermectin alone or in combination with clorsulon (e.g., ivomec plus), which are effective against wide range of external and internal parasites. Previous studies concluded that use of ivermectin in combination with clorsulon is an effective flukicide (Rehbein and Visser, 1999; Elitok *et al.*, 2006; Hutchinson *et al.*, 2009). Some farmers drenching their animals with other types anthelmintic such as albendazole or triclabendazole. The misuse of drug in general and anthelmintic in particular is a common habit in rural areas due to lack of knowledge and absence of a national database recording system for registering the use of veterinary drugs in Egypt. Due to the irregular use of anthelmintic and/or suboptimum anthelmintic dosing, we think resistance to anthelmintic has been emerged in the study area. This comes in agreement with Shokier *et al.* (2013) who concluded that albendazole and rafoxanide groups yielded lower efficacy levels against *fasciola*, which could be an indicator for albendazole and rafoxanide resistant *Fasciola* in cattle. The same conclusion has been reported by other studies (Martínez-Valladares *et al.*, 2014: Novobilský *et al.*, 2016).

Moreover, Chaudhri et al. (1993) demonstrated that control of liver fluke infection in livestock could be achieved with any effective flukicide administered regularly at an interval of not more than three months. Similarly, Keyyu *et al.* (2009a) concluded that strategic community-based worm control program (drenching albendazole 10% at 10 mg/kg four times a year) significantly reduced the proportion of animals passing *Fasciola* eggs by 82.5% compared to the village without applying of such program ($P < 0.05$). In another study (Keyyu *et al.*, 2009b), the authors concluded that a programme of four regular strategic anthelmintic treatments per year was effective in controlling gastrointestinal nematodes and *F. gigantica* and improved weight gain. The clue behind the regular administration of anthelmintic was explained by Gupta and Singh (2002) who concluded that chemotherapy of fascioliasis in cattle and buffaloes require an extensive post-treatment follow up. That is because the infected animals usually harbor different stages of the flukes, from immature to mature level and the drug to be chosen should have an effect on a wide range of parasitic stages or else, a repeated deworming schedule should be regarded.

Frequency of anthelmintic administration may not be the only factor that reduces the prevalence of fascioliasis but also, the type and efficiency of the administered anthelmintic could have an important role (Richards *et al.*, 1990), which may vary the according to the stage of liver fluke (mature or immature) and its species (Hutchinson *et al.*, 2009). Additionally, the host characteristics such as age, species, and health status cannot be separated from the anthelmintic impact (Kuchai *et al.*, 2011). Keyyu et al. (2003) concluded that the control practices for parasitic infection in various cattle management systems are entirely based on the routine use of anthelmintic, which in turn, depending mainly on the availability of money to buy drugs. Similarly, lack of out-of-pocket resources creates a heavy burden on the farmers in developing countries for combating the animal infectious diseases and neglected zoonotic diseases (Seimenis, 2012). That could be a possible explanation for the irregular administration of anthelmintic followed by householder's farmers under this study.

4.3. Ultrasonography of hepatic fascioliasis

The ultrasound examination revealed a good correlation between the hepatobiliary changes associated with fascioliasis, the degree of disease severity based on faecal egg load and the clinical manifestations appeared on the infected animal. This is concur with Yadav *et al.* (1999) who found that egg counts > 300/g indicate an acute fasciolosis, while its subsequent fall to 100–200/g was symbolic chronic phase of the disease based on experimental approach. The ultrasonographic finding showed normal hepatic parenchyma and normal bile system in infected animals with low fluke egg load and mild clinical manifestation. This finding indicate that liver fluke infection did not cause marked/ substantial changes in hepatic tissue or bile duct. Possible explanations (1) infected animals are in the early stages of infection which was initiated with few number of parasites, and (2) the animal is in the chronic phase of infection but the number of liver fluke colonizing the liver tissue are few in number. This comes in agreement with previous studies (Braun *et al.*, 1995; Sharma *et al.*, 2011). Animals with moderate egg load and clinical manifestation showed involvement of hepatic parenchyma. This result indicates degenerative hyperplasia of hepatic parenchyma due to the presence of adult flukes in the liver (Braun et al., 1999).

Animals showed marked clinical manifestation at high egg count showed severe damage in hepatic tissue represented by shadowing distal to hyperechogenic bile duct indicating bile duct calcification (obstructive cholestasis) resulting from chronic fascioliasis due to the presence of adult worms in the bile duct (Braun, 2009; Mohamed, 2012). The dilatation in the gallbladder is due to lack of stimulation of the reflex emptying process (Braun, 2009). In chronic fascioliasis, calcified bile ducts result in discrete sonographic changes in the liver parenchyma, represented by intensely hyperechogenic and an acoustic shadow distally (Braun, 2009). In liver fluke infection, cholangitis is developed by the irritation of the spines on the tegument of the parasites and their released toxic metabolites (Javaregowda and Rani, 2017). In the present study one buffalo showed an anechoic fluid in the abdominal and thoracic cavities, which is an indicator of ascites and hydropericardium. The possible explanation could be hypoalbuminemia and decrease in intravascular osmotic pressure, which allowed fluids accumulation within the abdominal and thoracic cavities (Braun, 2009; Mohamed, 2012). That concurs with Edith *et al.* (2012) who demonstrated that hypoalbuminemia is a marked syndrome in the chronic phase of liver fluke infection.

It may worth to mention that the changes and degree of pathological changes in the hepatic tissue and bile system as well as the accompanied clinical manifestations are dependent on many factors related to the parasite, host and animal management. Factors related to the parasite such as species of liver fluke, number of infective dose, and development of flukes attaining maturity and becoming adults in hepatic major bile ducts and the gallbladder (Yadav *et al*., 1999). Factors related to the host such as age, sex, species, pregnancy, history of previous exposure and vaccination, frequency, type of anthelmintic administration, immune system status and general health status. Other factors may participate in the pathway of liver fluke infection such as feeding system (grazing or ration), type of production system (small or large), raising mixed animal species together, and application of the hygienic and prophylactic measure. The coprological analysis is still commonly employed to diagnose bovine fascioliasis despite the fact that eggs cannot be detected until after the latent period of infection 8–15 weeks when much of the liver damage has already occurred (Hillyer, 1999; Sanchez-Andrade *et al.*, 2002). Furthermore, detection of *Fasciola* spp. eggs can be unreliable even during the patent period because the eggs are expelled intermittently, depending on the evacuation of the gallbladder (Briskey, 1998). Future studies are necessary to study if ultrasound can ultimately predict the infection of the liver with *Fasciola* spp. before detection of eggs in the faeces. In regard to the study generalizability, we emphasized that the findings of this study are limited by the pre-specified selection of the study population from small-holders production systems and inclusion criteria of animals with health issues such as decreased in milk production and emaciation in the current study.

5. Conclusions

We reported a high prevalence of bovine fascioliasis among cattle and buffaloes raised under the traditional household (smallholders) suggesting the need for more efficient control measures under this type of livestock production system. We identified the potential risk factors associated with bovine fascioliasis may be helpful to construct the appropriate preventive measures. Regular administration of anthelmintic significantly reduces the animal's chance of being copro-positive to *Fasciola* spp. Higher egg count of *Fasciola* spp. in faecal sample is reflected on the animal with severe clinical symptoms and excrescent hepatobiliary damages. Ultrasound is a valuable diagnostic and prognostic technique for assessment of bovine fascioliasis.

Conflict of interest

The authors declare that they have no financial or personal relationships, which may have inappropriately influenced them in writing this article.

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Figure captions:

Figure 1. Hyperechogenic hepatic parenchyma with multiple echogenic foci in a cow with hepatic fascioliasis (Imaged from 10^{th} right intercoastal space).

Figure 2. **A:** Hepatic parenchyma from right 10^{th} inter coastal space showed hyperechogenic bile duct with distal shadowing (arrows). **B:** Distended gallbladder (GB) of a buffalo with hepatic fascioliasis imaged from 9^{th} right intercoastal space.

Figure 3. Anechoic fluid in abdominal cavity (ascites) in a buffalo with severe hepatic lesions

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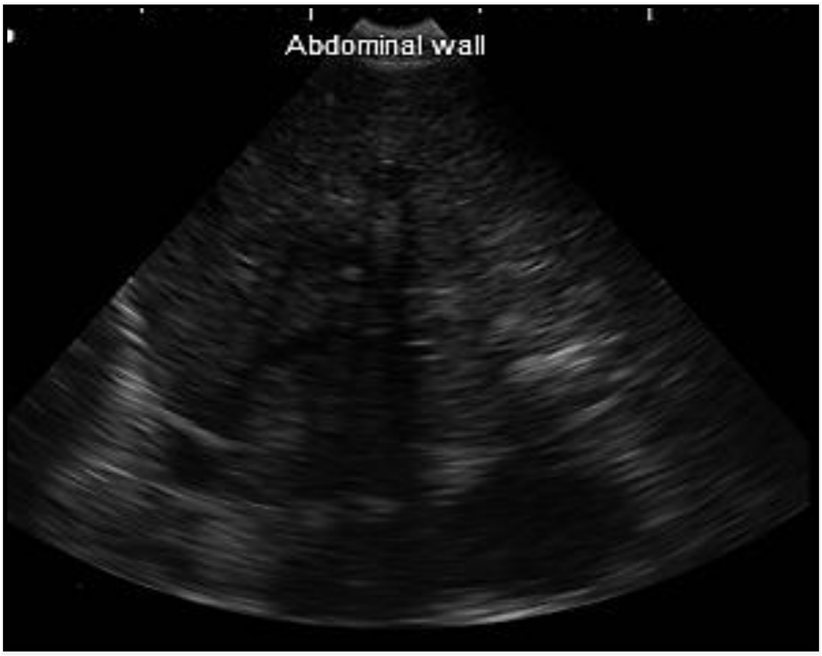

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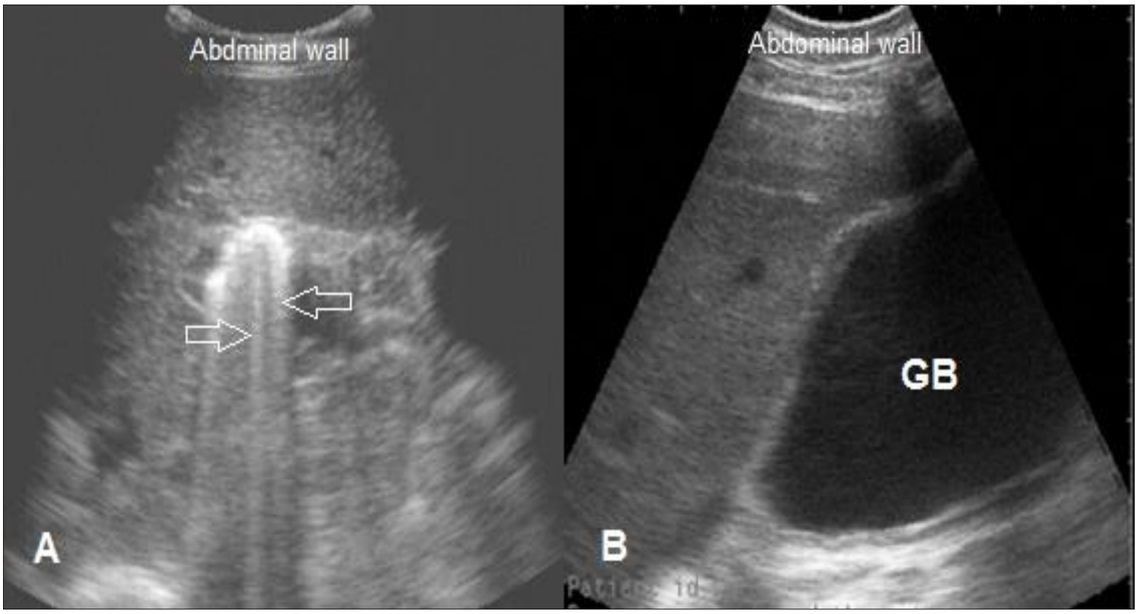

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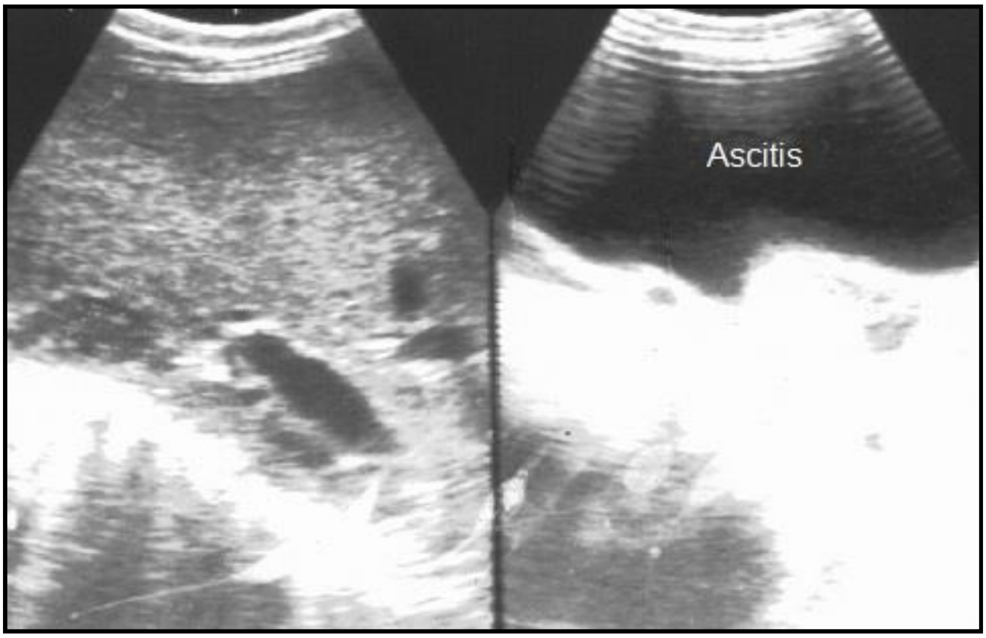

Table 1. Description of 270 cattle and buffaloes from smallholder production system in Eastern Nile Delta of Egypt examined for *Fasciola* spp. infection during the period from September 2016 to the end of August 2017.

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)
	Belbis	31 (37)	54 (64)	85 (32)
	Abo-Hammad	42 (60)	28 (40)	70 (26)
Age	Adult (>3)	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	40 (44)	50 (56)	90 (33)
	2	33 (41)	48 (59)	81 (30)
	3	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)

Table 2. Distribution and description of potential risk factors associated with bovine fascioliasis in 270 animals from smallholder production system in Eastern Nile Delta of Egypt based on faecal examination.

Item	Level	No of examined animals	No of infected animals (%)	95% CI (%)
Locality	Zagazig	115	38 (33)	25 – 42
	Belbis	85	21 (25)	17 – 35
	Abo-Hammad	70	15 (21)	13 – 32
Species	Buffaloes	150	49 (33)	26 – 41
	Cattle	120	25 (21)	15 – 29
Sex	Female	142	46 (32)	25 – 41
	Male	128	28 (22)	16 – 30
Age	Adult (>3)	166	50 (30)	24 – 38
	Young (≤3y)	104	24 (23)	16 – 32
Feeding type	Ration	118	17 (14)	9 – 22
	Free grazing	152	57 (38)	30 – 45
Frequency of anthelmintic administration	Irregular	180	65 (36)	30 – 43
	Regular	90	9 (10)	5 – 18
Hygiene	1	90	9 (10)	5 – 18
	2	81	27 (33)	24 – 44
	3	99	38 (38)	29 – 48
Season	Autumn	50	11 (22)	13 – 35
	Spring	110	39 (36)	27 – 45
	Summer	36	5 (14)	6 – 29
	Winter	74	19 (26)	17 – 37
Total		270	74 (27)	22 – 33

Table 3. Univariable logistic regression for potential risk factors associated with bovine fascioliasis in 270 animals from smallholder production system in Eastern Nile Delta of Egypt (*P-value* ≤ 0.25).

Variable	Level	Estimate	OR [2]	95% CI [3]	P-value
Species	Buffaloes	Ref [1]	Ref	Ref	0.029
	Cattle	-0.61	0.54	0.31 - 0.94	
Sex	Female	Ref	Ref	Ref	0.05
	Male	-0.54	0.58	0.34 - 1.004	
Age	Adult (>3)	Ref	Ref	Ref	0.20
	Young (≤3y)	-0.36	0.70	0.40 - 1.21	
Feeding type	Ration	Ref	Ref	Ref	< 0.0001
	Free grazing	1.27	3.57	1.97 - 6.72	
Frequency of anthelmintic administration	Irregular	Ref	Ref	Ref	< 0.0001
	Regular	-1.63	0.20	0.09 - 0.40	
Hygiene	1	Ref	Ref	Ref	< 0.0001
	2	1.50	4.50	2.03 - 10.83	
	3	1.72	5.61	2.62 - 13.16	
Season	Autumn	Ref	Ref	Ref	0.045
	Spring	0.67	1.95	0.92 - 4.38	
	Summer	-0.56	0.57	0.17 - 1.75	
	Winter	0.20	1.23	0.53 - 2.93	

[1] Ref = reference value; [2] OR= odds ratio; [3] CI= confidence interval

Table 4. Final model of multivariable logistic regression analysis for identification of risk factors associated with bovine fascioliasis in 270 animals from smallholder production system in Eastern Nile Delta of Egypt (*P-value* ≤ 0.05).

Variable	Level	Estimate	OR [2]	95% CI [3]	P-value
Intercept	---	-0.97	0.38	0.22 - 0.64	0.00041
Species	Buffaloes	Ref [1]	Ref	Ref	0.029
	Cattle	-0.61	0.55	0.30 – 0.99	
Frequency of anthelmintic administration	Irregular	Ref	Ref	Ref	< 0.0001
	Regular	-1.77	0.17	0.07 - 0.36	

[1] Ref = reference value; [2] OR= odds ratio; [3] CI= confidence interval

Table 5. Ultrasonographic and clinical findings associated with the different levels of *Fasciola* spp. infections according to egg count (EPG) in infected animals (n=74) from smallholder production system in Eastern Nile Delta of Egypt

Burden of *Fasciola* spp.	No of animals (%)	Clinical picture	Ultrasonographic findings
Low (< 30 EPG)	38 (51.4)	Mild manifestations; - Diarrhea - Decrease milk production - Loss of weight	- Normal hepatic parenchyma - Normal bile system
Moderate (≥ 30 to < 100 EPG)	20 (27)	Moderate manifestations; - Watery diarrhea, - Emaciation, - Anorexia, - Paleness in mucous membranes	- Hyperechogenic hepatic parenchyma with multiple echogenic foci - Normal bile system
High (≥ 100 EPG),	16 (21.6)	Severe manifestations; - Severe emaciation, - Watery diarrhea, - Anorexia - Subcutenous oedema, - Anemic to icteric mucous membranes	- Hyperechogenic hepatic parenchyma, - Hyperechogenic with distal shadowing bile duct, - Distended gall bladder - Anechoic fluid was imaged in abdominal and thoracic cavity
Total	74 (100)	---	---