PSM Veterinary Research 2019; 4(3): 74-88

Research Article 2019 | Volume 4 | Issue 3 | 74-88

An Investigation on the Existence and Health Hazards of Fascioliasis among Buffaloes in Sohag Governorate, Southern Egypt

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Article Info

Open Access

Citation: Mohamed, A.E.A., Ali, O.A., Mosleh, A.M., Ali, M.F., Hassan, D.F., Fereig, R.M., 2019. An Investigation on the Existence and Health Hazards of Fascioliasis among Buffaloes in Sohag Governorate, Southern Egypt. PSM Vet. Res., 4(3): 74-88.

Running title: Fascioliasis in Buffaloes in Egypt

Received: July 24, 2019

Accepted: October 27, 2019

Online first: November 20, 2019

Published: November 30, 2019

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Abstract:
Fascioliasis is a zoonotic disease caused by *Fasciola hepatica* and *Fasciola gigantica*, the common parasites of grazing animals in different regions of the globe including Egypt. This study was conducted on buffaloes for investigating the prevalence and health hazards of fascioliasis in Sohag, Egypt. An overall prevalence rate 36/151 (23.84%) was observed among all tested buffaloes. Among the aforementioned animals, fascioliasis was confirmed in living animals via detecting the operculated golden brown egg in the fecal samples in 33/117 (28.21%), whilst adult fluke was detected in liver samples from slaughtered buffaloes of another group 3/34 (8.82%). No difference was observed in the prevalence in housed buffaloes (24.47%) or in farmed ones (22.81%), (*P*=0.82, Odds ratio=0.5-2.38). Evaluation of health hazards in infected animals (n=33) was employed against a number of non-infected control animals (n=20). Hematological variables in infected buffaloes revealed significant decrease in RBCs count, hemoglobin content, PCV, an increase in WBCs count, neutrophils and eosinophils percents, and decrease in lymphocytes percent, indicating anemia and immunopathology. In addition, hepatic dysfunction was reported as evidenced by a significant decrease in serum albumin and a significant increase in ALT, AST, globulin, and serum total protein in diseased animals in comparison to control ones. Also, there were a significant increase in catalase and malonaldehyde and decrease in superoxide dismutase in diseased animals suggesting altering the redox potential. Postmortem examination revealed the presence of adult worm, necrosis, cirrhosis, mononuclear cell infiltration in hepatocytes and portal canal. These results emphasize the endemicity of fascioliasis among buffaloes in Egypt and the expected significant hazards on animal health and economic aspects.
Keywords: Fascioliasis; *Fasciola gigantica*; *Fasciola hepatica;* Buffalo.

INTRODUCTION

Fascioliasis is a parasitic disease, caused by *Fasciola* hepatica (*F. hepatica*) and *Fasciola gigantica* (*F. gigantica*), causing substantial economic losses. Fascioliasis is an emerging or re-emerging disease in several regions of the world. The infection occurred via oral route after ingestion of encysted metacercariae in case of grazing animals or raw liver in the case of human (Mas-Coma, 2005). More than 700 million of domestic animal populations are at risk of infection, with estimated financial losses in ruminants exceeding US$ 2 billion per year to rural agricultural communities and commercial producers (Spithill and Dalton, 1998). Moreover, it is recorded that 2.4–7 million of humans are suffering from fascioliasis, and 90 million people under the risk of infection (Keiser and Utzinger, 2005). Fascioliasis has been recorded in animal and human populations in several areas in Asia, Africa, Europe, the Americas and Oceania, as well as other temperate countries and regions, (Mas-Coma *et al*., 2005, Mehmood *et al*., 2017).

In Egypt, fascioliasis seems to be a hyperendemic disease because of the demonstration of liver fluke fragments in an Egyptian mummy (David, 1997). Even in the recent history, the number of reported fascioliasis cases was 830.000 individuals (Haseeb *et al*., 2002). In cattle, the prevalence of fascioliasis in the Nile Delta region based on microscopical examination of fecal egg was 9.77%. The age, gender, breed and farming systems were found to be as potential risk of infection (El-Tahawy *et al*., 2017). In Qena governorate in southern Egypt, the overall prevalence was 30.3%, consisting 28.6% in cows, 33.7% in buffaloes, and 17.2% in sheep using coprological examination of the egg via microscopy (Hussein and Khalifa, 2010). Fascioliasis was surveyed by Haridy *et al*. (1999) by macro- and microscopical examination of the liver, and ElKhtam and Khalafalla, (2016), who reported that the prevalence of fascioliasis was 1.58 % among buffaloes and 5.8% among goats in Minoufiya governorate, respectively using liver samples from slaughter houses. In a similar approach, Bazh et al. (2012) reported the prevalence among the infected cattle in El-Beheira via detecting flukes in liver reaches 50%. Dyab et al. (2019) reported 30% prevalence of *Fasciola* spp., in Cattle and Buffaloes Slaughtered in El-Minia Governorate Abattoirs, Egypt.

Fascioliasis is incriminated to induce severe economic losses because of adverse effect on livestock industry (Kaplan, 2001, Assefa, 2005). Acute fascioliasis in the buffaloes is characterized by blood loss anemia and sometimes sudden death may occur, while chronic fascioliasis is characterized by hemolytic anemia, decrease of the dairy production and decrease of the common daily profit and edemas (Salimi-Bejestani *et al*., 2005). In addition to reduction in meat and milk production, fascioliasis has also resulted in reduction of growth rate, fertility, feed intake and resistance of animals to different diseases. Infection with liver flukes is remarkably downgrading the carcass value and therefore reducing the price. Carcasses with liver fluke have lower cold weight, lower conformation scores, and lower levels of fat (Sanchez-Vazquez and Lewis, 2013).

This study was undertaken to solve the status of underestimation of fascioliasis in Sohag governorate, southern Egypt. Fascioliasis seemingly constitutes a serious problem in both veterinary field and public health sector. Although numerous studies investigated the epidemiology of fascioliasis in the Delta region (northern part), the situation is poorly estimated in southern Egypt. Thus, this study aimed to survey fascioliasis and evaluates potential health hazards among buffaloes in Sohag governorate, in southern Egypt. Detection of fascioliasis was relied on demonstration of *Fasciola* egg in fecal matter, clinical signs, and postmortem examination of adult flukes in the liver tissues. In addition, the effect of *Fasciola* infection was estimated by analyses of changes in clinical findings, hematological parameters, biochemical markers, and histopathological picture.

MATERIALS AND METHODS

Ethical approval

All samples of the current study were collected after getting approval from animal owners. The study was approved by the Local Governmental Authority for Veterinary Services in Sohag and Qena governorates, southern Egypt and the Ethics and Welfare Committees of both faculties of Veterinary Medicine in Sohag University and South Valley University, Egypt, which is in accordance with the Institutional Animal Care. All fecal and blood sample collections were performed by skilled persons or veterinarians with non- or minimal invasive methods. The collected samples were used specifically and solely for the purposes of this study.

Animals and geographical locations

A total number of 137 live buffaloes of both genders (male=63; female=54) and from 6 months to 5 years of age were investigated in this study. These animals were selected from different villages at Sohag governorate, southern Egypt (Figure 1). Samples were collected from animals kept in private farms (mass breeding system) or in house (individual breeding system). For analyses of health hazards under the field condition, a number of specially selected animals (n=20) were considered as a control non-infected group (male and female of 6 months to 5 years old). These animals were apparently healthy, exhibiting good body condition, and free from any internal or external parasites by parasitological investigations as illustrated below. Moreover, they were confirmed as *Fasciola*-free after injection with 2 doses of Dovix 25% (nitroxynil) anti fascioliasis drug (Arabco. Med. company, Cairo, Egypt), according to the manufacturer's instructions. Other animals (n=117) were suffering from a variable degree of emaciation, pale mucous membrane, various degrees of diarrhea, dullness and depression. Some of these animals also had submandibular edema and decrease in production and reproduction of most animals. In a total 33 of these animals, fascioliasis was confirmed by finding *Fasciola* eggs in fecal samples, while other cases (n=84) were shown the clinical signs of fascioliasis but the fecal examination revealed absence of *Fasciola* eggs (Table 1). For the purpose of hepatic changes assessment at the cellular level, liver sections from 34 buffaloes admitted to local slaughter houses in Sohag governorate were examined grossly. Among the aforementioned number, 3 positive samples were detected, and were subjected to further histopathological examination.

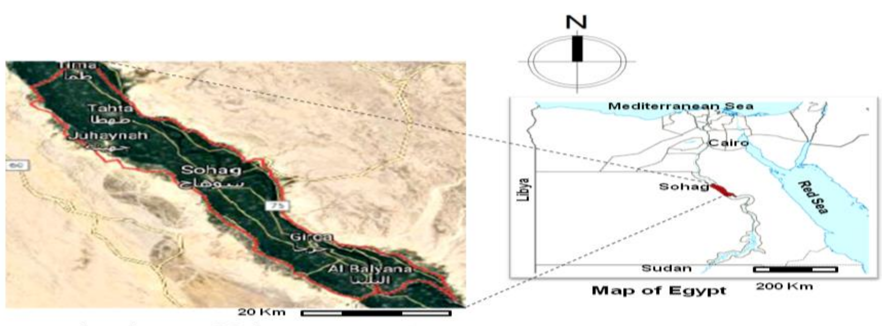

Fig. 1. Map of Egypt. The dark colored-area in the graphical map is showing the location of Sohag governorate in southern Egypt. Landscape map is illustrating the agricultural parts of Sohag surrounded by semi-desert and desert areas.

Survey, case history questionnaire and clinical investigations

A specified questionnaire was designed to record the information and complaints of the animal owner, exhibited clinical signs, and management practices. The collected data were organized with our recorded signs and observations on each animal and breeding site. Careful routine clinical examination of these studied cases was carried out as the methods described previously (Radostits *et al*., 2007). A summary for investigated clinical findings is summarized in Table 2.

Fecal samples and parasitological investigations

Fecal samples were collected directly from the rectum of diseased and control animals in a labeled clean, dry plastic bag for examination of specific eggs by using direct smear and concentration sedimentation technique (Coles, 1986).

Collection of blood samples and analyses of biomarkers

The blood samples were collected from the jugular vein from each animal after clipping and disinfecting of the area of the vein. Two types of blood samples were collected. For hematological picture, about 2.5 ml whole blood was taken from the jugular vein of each animal in a test tube with EDTA, this sample was used for detection of (RBCs, Hb., PCV, MCV, MCH, MCHC, WBCs, DLC) as described formerly (Coles, 1986).

While, serum was harvested by collecting about 7.5 ml of blood in a centrifuge tube without anticoagulant, blood samples were allowed to stand for clotting. The clot was detached from the wall of the centrifuge tube by glass rod, and then samples were centrifuged at 3000 rpm for 15 min to force the clot to the bottom of the tubes. Serum was stored at -20˚ until testing (Coles, 1986). The separated sera were used for detection of some liver enzymes aspartate transaminase (AST), alanine transaminase (ALT) (IU/L) (Breuer, 1996). Also, serum was used for detection of total proteins, albumin (gm/dl) (Tietz *et al*., 1990), oxidants include malondialdehyde (MDA) (µmol/L) (Ohkawa *et al*., 1979), and antioxidants include catalase (CAT) (IU/L) (Aebi, 1984) and superoxide dismutase (SOD) (IU/L) (Nishikimi *et al*., 1972).

Gross and microscopical examination of liver

Firstly, gross examination of sectioned bile duct was conducted to detect the adult worm. Then, the specimens were fixed in neutral buffer formalin dehydrated in the alcohol ascending grader of ethyl, cleared and embedded in paraffin block sections of 5-7 m were taken from each block and stained with H&E stain (Bancroff *et al*., 1990).

Statistical analysis

Differences in the incidence of *Fasciola* infection in buffaloes were detected using a chi-square test. Statistical analyses between infected and non-infected groups were measured by Student *t* test with GraphPad Prism version 5 (GraphPad Software Inc., La Jolla, CA, USA). A *P* value of < 0.05 was considered as a statistically significant value. The 95% confidence intervals of a proportion including continuity correction and odds ratios were calculated using www.vassarstats.net.

RESULTS

Prevalence of *Fasciola* infection among buffaloes in Sohag, Egypt

In the present study, high prevalence of fascioliasis in buffaloes was recorded, which was confirmed via demonstration of the eggs in feces of tested animals. An overall infection rate 36/151 (23.84%) was observed among all tested buffaloes. in alive emaciated animals, fascioliasis was confirmed via detecting eggs in the fecal samples in 33/117 (28.21%), (95% CI=20.47-37.41). On the other hand, the lower prevalence rate was recorded when finding adult flukes in liver samples was targeted 3/34 (8.82%), (95% CI=2.31-24.81). No difference was observed in prevalence in housed buffaloes (24.47%) or in farmed ones (22.81%), (*P*=0.82, Odds ratio=0.5-2.38) in a totally tested animals or even in alive ones consisting housed buffaloes (33.3%) or in farmed ones (22.81%), (*P*=0.20, Odds ratio=0.75-3.84) (Table 1). These results indicate the high prevalence of fascioliasis in either housed or farmed buffaloes in Sohag governorate.

Effects of *Fasciola* infection on clinical findings

Regarding apparent clinical examination, although some animals also showed clinical signs of fascioliasis, they were not considered as *Fasciola*-positive cases. All infected animals showed pale mucous membranes, dullness, depression, loss of appetite, easily detached hair and various degrees of diarrhea and edema. These clinical signs either solitarily or collectively have not been recorded in the healthy buffaloes (Table 2).

Effects of *Fasciola* infection on hematological picture

There was a marked decrease in the total RBCs count, Hb content and PCV in *Fasciola*-infected animals than control animals, which was consistent in both housed and farmed groups ($P < 0.05$) (Table 3). On the other hand, a significant increase in the total WBCs was reported in the diseased group when compared to the control animals. This increase was also noticed in some other kind of tested immune cells such as in case of neutrophilia, and eosinophilia in infected groups against healthy groups of either housed or farmed animals. On the contrary, lymphopnea and monocytopnea was also recorded among infected buffalo groups (Table 4). These results may suggest the suffering of *Fasciola*-infected buffaloes from anemia and specific inflammatory reactions.

Table 1. Prevalence of *Fasciola* infection among buffaloes in Sohag, Egypt.

Examination tool	Housed		Farmed		Housed vs Farmed		Total		
	Tested	Infected (%)*	Tested	Infected (%)*	OR (95% CI)[a]	P value	Tested	Infected (%)*	95% CI[a]
Fecal finding of egg	60	20 (33.3)	57	13 (22.81)	1.69 (0.75-3.84)	0.20	117	33 (28.21)	20.47-37.41
Adult flukes in liver	34	3 (8.82)	-	-	-	-	34	3 (8.82)	2.31-24.81
Combined test	94	23 (24.47)	57	13 (22.81)	1.1 (0.5-2.38)	0.82	151	36 (23.84)	17.45-31.59

*, refers to the positive animals to fascioliasis, detected by finding eggs in fecal samples and showing one or more of characteristic clinical signs (live animals), and adult flukes in liver sections in slaughtered animals.

OR= odds ratio, CI= confidence interval; calculated according to method described by at http://vassarstats.net/.

Table 2. Effect of *Fasciola* infection on some clinical parameters of buffaloes.

Clinical parameters/ Aspect of inspection	Non-infected animals (n=20)	Infected animals (n=33)
Appetite	Good	Inappetance or mild loss
Body condition	Good to vey good	Poor (emaciated)
Coat	Glossy	Easily detached
Mucous membranes	Rosy red	Pale
Mental satus	Active	Dull/depressed
Edema	None	Bottle jaw
Feces characteristics	Normal	Diarrhea/indigestible food
Productive potential*	High	Reduced
Reproductive status*	High	Reduced

T/L; Tera/litre, g/dl; grams/deciliter, fl; femotolitres/cell, pg; picograms/cells, SD; standard deviation, ns; non-significant. *, statistically significant differences were observed between the healthy and infected group with the Student's t test ($P < 0.05$). Data are normally distributed with equity of variances between the samples.

Table 3. Effect of *Fasciola* infection on hemogram of buffaloes.

Parameter	Housed Healthy No.= 12 Mean ± SD	Infected No.= 20 Mean ± SD	Significance	Farmed Healthy No.= 8 Mean ± SD	Infected No.= 13 Mean ± SD	Significance
RBCs, T/L	5.8±0.8	4.4±0.67	*	6.4±0.93	4.8±0.62	*
Hb, g/dl	11±0.87	6.9±0.86	**	11.2±1.4	7.1±0.90	**
PCV, %	31.7±5.7	22.2±2.4	**	33.5±4.1	21±2.6	**
MCV, fl	46.5±9	49.9±3.4	ns	51.2±3.1	48.1±3.6	ns
MCH, pg	16.6±1.7	15.7±2.5	ns	15.3±1.3	16.2±1.2	ns
MCHC, g/dl	34±0.17	31.2±4	ns	32±1	33.6±0.07	ns

T/L; Tera/litre, g/dl; grams/deciliter, fl; femotolitres/cell, pg; picograms/cells, SD; standard deviation, ns; non-significant.*, statistically significant differences were observed between the healthy and infected group with the Student's t test ($P < 0.05$). Data are normally distributed with equity of variances between the samples.

Table 4. Effect of *Fasciola* infection on leucogram of buffaloes.

Parameter	Housed Healthy No.= 12 Mean ± SD	Infected No.= 20 Mean ± SD	Significance	Farmed Healthy No.= 8 Mean ± SD	Infected No.= 13 Mean ± SD	Significance
WBCs, G/L#	9.6±2	16.5±2.1	**	9.3±1	16.2±2.4	**
Neutrophils, %	42.7±7.6	52.6±4.5	**	35.2±5.5	46.8±6.8	**
Lymphocytes, %	44.2±9	40.6±5.4	*	56.8±7.9	42.2±6.6	**
Monocytes, %	8.7±9	4.7±0.98	**	6.7±1.4	4.3±2.1	*
Eosinophils, %	1.14±0.24	3.2±1.08	**	1.8±0.48	3.6±1.2	**

G/L; giga/litre, SD; standard deviation. *, statistically significant differences were observed between the healthy and infected group with the Student's t test ($P < 0.05$). Data are normally distributed with equity of variances between the samples.

Blood biochemistry and *Fasciola* infection in buffaloes

The total protein and albumin concentrations were found to have significantly decreased in the *Fasciola*-infected buffaloes when compared to non-infected animals ($P < 0.05$). On the contrary, globulin level has been markedly increased in infected than healthy groups. As a result, the albumin/globulin ratio was also remarkably reduced in infected animals against those of healthy ones (Table 5). In addition, there was an increase in AST and ALT levels in *Fasciola*-infected buffaloes group when compared with control one in both housed and farmed animals (Table 6). Consistently, there was a significant increase in MDA and CAT and marked decrease in SOD in *Fasciola*-infected buffaloes when compared with control ones (Table 7). These results are coinciding with expected damage of liver in the case of fascioliasis characterized by disturbance in protein synthesis, exogenous release of enzymes and creating a status of redox imbalance.

Table 5. Effect of *Fasciola* infection on protein and protein fractions in serum of buffaloes.

Parameter	Housed Healthy No.= 12 Mean ± SD	Infected No.= 20 Mean ± SD	Significance	Farmed Healthy No.= 8 Mean ± SD	Infected No.= 13 Mean ± SD	Significance
Total protein, g/dl	7.2±0.58	5.9±0.31	*	7.2±0.1	6.1±0.1	*
Albumin, g/dl	4.6±0.34	2.2±0.3	*	4.6±0.24	2.5±0.37	*
Globulins, g/dl	2.6±0.37	3.7±0.19	*	2.6±0.27	3.6±0.2	*
A/G ratio	1.8±0.37	0.62±0.09	*	1.7±0.24	0.68±0.12	*

g/dl; grams/deciliter, SD; standard deviation.

*, statistically significant differences were observed between the healthy and infected group with the Student's t test (P < 0.05). Data are normally distributed with equity of variances between the samples.

Table 6. Effect of *Fasciola* infection on some liver enzymes in serum of buffaloes.

Parameter	Housed Healthy No.= 12 Mean ± SD	Infected No.= 20 Mean ± SD	Significance	Farmed Healthy No.= 8 Mean ± SD	Infected No.= 13 Mean ± SD	Significance
ALT, IU/L	35.3±4.2	94.5±16.5	**	35.6±2.5	95±3.8	**
AST, IU/L	36.1±6	76.7±5.1	**	37.2±1.4	76.4±3.1	**

IU/L, international unit per litre, SD; standard deviation.

*, statistically significant differences were observed between the healthy and infected group with the Student's t test (P < 0.05). Data are normally distributed with equity of variances between the samples.

Table 7. Effect of *Fasciola* infection on oxidants and anti-oxidants compounds in serum of buffaloes.

Parameter	Housed Healthy No.= 12 Mean ± SD	Infected No.= 20 Mean ± SD	Significance	Farmed Healthy No.= 8 Mean ± SD	Infected No.= 13 Mean ± SD	Significance
MDA, µmol/L	0.21±0.03	0.60±0.111	*	0.18±0.05	0.55±0.03	*
CAT, IU/L	97.2±10	301.5±9.5	**	115±8.3	306.2±14	**
SOD, IU/L	351±14.4	207±13.5	**	349.4±10.8	206.7±10.5	**

µmol/l; micro mole per litre, IU/L, international unit per litre, SD; standard deviation.

*, statistically significant differences were observed between the healthy and infected group with the Student's t test ($P < 0.05$). Data are normally distributed with equity of variances between the samples.

Gross and histopathological findings

Regarding *Fasciola*-induced pathology in liver, gross lesion in the liver of infected buffalo with *Fasciola* revealed distinct necrosis, cirrhosis, adult *Fasciola* and dilated bile duct (Figure 2A). Microscopical examination showed morphological and architectural changes in the hepatic tissues, including connective tissue formation and mononuclear cell infiltration in hepatocytes and portal area (Figure 2B). These lesions corroborate the expected health hazards as a result of induced chronic hepatitis and hepatic damage ends by hepatic failure and death.

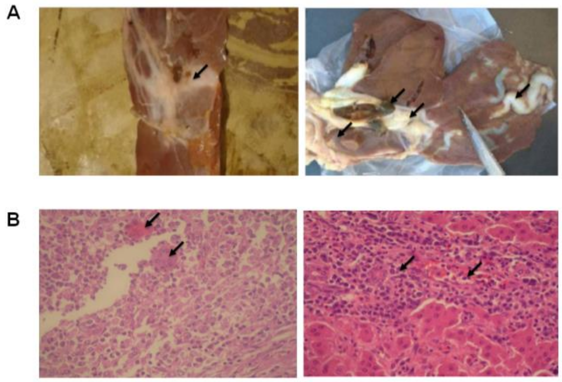

Fig. 2. Gross and microscopical lesions of *Fasciola*-infected liver tissues. (A) Gross lesion in liver of infected buffalo with *Fasciola;* left panel shows fibrosis and right panel indicates necrosis, cirrhosis, adult *Fasciola* and dilated bile duct. (B) Histopathological changes in hepatic tissues showing connective tissue capsule of granuloma consists of multinucleated giant cells and mononuclear cells, and portal area infiltrated with mononuclear chronic inflammatory cells with slight hemorrhages between hepatocytes (right panel). Magnifying lens x40 was used for microscopical examinations.

DISCUSSION

Buffaloes represent an essential part of the animal production sector in Egypt. The world buffalo population averaged 170 million heads (FAO, 2003). Buffalo industry is increasingly important in different parts of the world because of cheap and high quality source of meat (Dixon *et al*., 2001). On the contrary, cattle industry recently has become limited due to the high susceptibility of cattle to numerous parasitic diseases, including fascioliasis (Keyyu *et al*., 2005), and this will adversely affect the food security of the human. Parasitic infestation is of economic and public health importance (Iqbal *et al.,* 2014; Iqbal and Ashraf, 2017; Muhammad *et al.,* 2015) and an important factor contributing to low productivity. In the present study, high prevalence of fascioliasis (23.84%) was recorded with no significant difference to the breeding system in totally tested buffaloes. However, in the tested alive animals, slightly higher rate of infection was noticed in housed than farmed buffaloes. The latest findings may be attributed to owner habits of bathing their animals on the shores of River Nile and its originating ditches and streams which are considered main source of fascioliasis because of containing infective metacercariae (Mas-Coma *et al*., 2005). Our recorded prevalence rate was markedly higher than those estimated among cattle in Nile Delta region based on microscopical examination of fecal egg was 9.77% (El-Tahawy *et al*., 2017), and 1.58% in buffaloes (Haridy *et al*., 1999). However, it was lower than those recorded by Hussein and Khalifa (2010) among buffaloes (33.7%) in Qena governorate in southern Egypt, and than those reported by Bazh *et al*. (2012) in cattle (50%) in El- Beheira via detecting flukes in liver. These variations are commonly recorded in similar epidemiological studies attributing to sample collection site and timing and used tests.

In our conducted comparative study to evaluate the health hazards, systemic clinical examinations were applied and revealed distinct clinical symptoms among infected and non-infected animal groups. The pale mucous membrane in fascioliasis as observed in our study might be related to anemia, which attributed to acute blood loss by the migratory stages in the liver and the fibrosis that occurred in the chronic stage of infection (Radostits *et al*., 2007, Wiedosari *et al*., 2006). The bottle jaw; the characteristic sign of chronic fascioliasis was observed in some infected animals (Khan *et al*., 2009, Kuchai *et al*., 2011).

Moreover, regarding hemogram and leucogram, there was a marked decrease in the total RBCs count, Hb content and PCV in *Fasciola*-infected animals than control animals. The reasons of RBCs deficiency might be attributed to the bleeding and insufficiency of bone marrow responding to new RBCs formation because of the infection, in addition to the sucking of 0.2 ml of blood daily by worms, and bleeding of 0.5 ml of blood daily in muscles, also a flow of immature RBCs to the circulatory system (Kramer, 2000; Lotfy *et al*., 2003). Whilst, a significant increase in the total WBCs was reported in the diseased group comparing to the control group might be attributed to the cellular immune response for combating the parasite by killing or phagocytosis. The changes in the differential leukocytic counts, including the increase in neutrophils and eosinophils, decrease in monocytes and lymphocytes. These distinct responses may be related to the body's defense against *Fasciola* obstructive effects or due to the toxin mediated lesion of the bone marrow or due to inflammation and infection resulting from the activity of the flukes in the bile ducts (Radostits *et al*., 2007). Eosinophilia in infected buffaloes in comparison with non-infected animals resulting from the ability of eosinophils to destroy the parasite larval stages by their attachment on parasite wall and secretion of granules that induce external parasite wall damage (Strandmark *et al*., 2016). Monocytopenia might be due to increased chemotaxis to the inflammatory process in the bile ducts and increasing of neutrophils number in infected animals may attributed to secondary bacterial infection occurred in diseased animals during migration of flukes through the liver parenchyma (Coles, 1986). These results were in agreement with that reported by Egpu *et al*. (2013) and not agree with that reported by Singh et al. (2011).

Moreover, the marked decrease in total protein concentration in the *Fasciola*-infected buffaloes than non-infected animals might be assumed to blood loss (Angulo-Cubillan *et al*., 2007). This result was in agreement with that reported by Gupta et al. (2012), and different with that reported by Singh et al. (2011). Albumin in this study was less in *Fasciola*-infected animals than control one, and this was due to inhibition of its synthesis, rapid breakdown and losses (Coles, 1986), or may be due to increased leakage into the gut. This result coincided with that reported by Gupta et al. (2012), and conflicted with that reported by Singh et al. (2011). Globulin in this study increased in *Fasciola*-infected animals than control ones. It was attributed to biliary obstruction, and the immune response against

Fasciola invasion to the bile duct. This result was in agreement with that reported by Coppo et al. (2011), and Singh et al. (2011) and not agreed with that reported by Gupta et al. (2012). The albumin/globulin ratio decreased in *Fasciola*-infected animals than control ones (Table 5). This result was in agreement with that reported by Coppo et al. (2011) and not agreed with that reported by Gupta et al. (2012).

We also recorded an increase in AST and ALT levels in infected than non-infected buffaloes. High AST and ALT values in *Fasciola*-infected buffaloes is attributed to hepatocyte degeneration and fibrosis beside to blockage of the bile ducts by migration of juvenile flukes during the first stage of infection. Differences in activities of liver enzymes like AST and ALT in serum generally indicate pathological changes of tissue and organ (Tanritanir *et al*., 2009). Mert *et al*. (2006) reported that the cause of elevation of liver enzymes was attributed to the liver injuries which lead to the liver enzymes to spill into the blood, causing elevations of serum liver enzymes (Mert *et al.,* 2006). These results coincide with that reported by Gupta et al. (2012), Kitilia and Megersa (2014), and contradicted with that reported by Hammam et al. (2012), and Okaiyeto et al. (2012).

In addition, there was an increase in MDA in *Fasciola*-infected buffaloes when compared with control ones. This result attributed to that in fascioliasis, it was noticed that the phagocytic response of the liver cell to the parasite invasion and growth leads to free radical-mediated oxidative stress. This response triggers the initiation and development of lipid peroxidation and increased MDA concentrations in the liver (Maffei Facino *et al*., 1989). Another reason is that upon parasitic infection, the host cells apply various defense mechanisms to eliminate the parasite. These include the production of reactive oxygen species (ROS), reactive nitrogen species (RNS) and digestion by hydrolytic enzymes (Wochna *et al*., 2005). The high chemical reactivity of the reactive oxygen species leads to reactions with almost all constituents of the cell, including proteins, lipids, and DNA. Enhanced lipid peroxidation is an indirect biomarker of free radical generation (Halliwell and Chirico, 1993). Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, which is the most abundant MDA (Romero *et al*., 1998). These results were in agreement with that reported by El-Khadrawy et al. (2008), El-Moghazy, (2011), and Bahrami et al. (2014) and not in agreeing with that reported by Fulya and Sema, (2003). The antioxidant system consists of enzymes and vitamins having cellular protective action against oxidative stress resulting in damage of cell, organ and tissue as a result of parasitic invasion (Dede *et al.,* 2000). In the present study SOD activity in the *Fasciola*-infected animals were found to be significantly lower than control ones. The drop in SOD activity could be explained by the superoxide anion dismutation to hydrogen peroxide caused by the overproduction of the superoxide anion linked to oxidative stress (Santiard *et al*., 1995). This result was in concordance with that mentioned by El-Moghazy et al. (2011) and Atteya et al. (2015). The data of the present study showed that the activity of CAT was significantly increased in the liver tissue of *Fasciola*-infected animals in comparison with control animals. The cause of increase catalase production might be related to the catalase recalling from remote organs and tissues to the liver to support its antioxidant defense under condition of showing production of free radicals as in case of fascioliasis. These results were in agreement with that reported by Kolodziejczk et al. (2005), and different than that reported by Atteya et al. (2015) which reported that fascioliasis in animal cause decrease in CAT antioxidant.

Regarding *Fasciola*-induced pathology in liver, the cause of infiltration of high number of lymphocytes were due to cellular immune response of animals against *Fasciola* invasion (Mendes *et al*., 2010), while the hepatocellular necrosis and abscess formation can be observed in *Fasciola*-infected liver, which happen due to the invasion of the liver by migrating immature liver fluke that damages the tissue and provide anaerobic condition that allowed the germination and proliferation of bacteria and induce necrosis and abscess (Badr and Nasr, 2009, Nabil *et al*., 2013).

CONCLUSION

This study confirms the existence of *Fasciola* in farm animals of Egypt such as buffaloes. This situation raises questions regarding the inappropriateness of previously and currently used control measures against fascioliasis in Egypt. It was further corroborated that the examined farmed or housed can be infected with *Fasciola* parasites, meaning that this parasite is well established in the study area, thereby leading to significant weight loss and decrease in milk or meat production. Although, many antiparasitic drugs are routinely used in the control of the helminthes at farm levels, including fascioliasis, further research is necessary to evaluate the overall situation and revise the currently used control measures. Severe health hazards were recognized on infected animals by different tools of assessments. This study will help with the need for proper management practices including effective regular deworming be employed to improve the health status of buffaloes in the study area which has an implication on sustainable meat and milk production. However, higher number of investigated animals would be of more appropriateness in next studies.

ACKNOWLEDGEMENT

We would like to thank the staff of the Department of Animal Medicine, Faculty of Veterinary Medicine, South Valley University, Egypt for their support and providing all facilities to perform this study. Also, we appreciate the cooperation and help from veterinarians, animal owners and farmers for technical assistance in sample collection and providing us with the required information.

ABBREVIATIONS

ALT, alanine transaminase; AST, aspartate transaminase; CAT, catalase; DLC, differential leucocytic count; Hb, hemohlobin; MCH, mean corpuscular hemoglobin, MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MDA, Malondialdehyde; PCV, packed cell volume; RBCs, red blood cells; SOD, superoxide dismutase; WBCs, white blood cells.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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