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The Infection Rate of *Fasciola* and *Anaplasma* in Cattle and Buffaloes in Qena, Egypt

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ABSTRACT

Fascioliasis is caused by two species of trematodes that mainly affect the liver. It belongs to the group of foodborne disease infections. It is a zoonotic disease, while anaplasmosis is a tick-borne disease. Both diseases occur worldwide, particularly in regions with intensive animal production. Therefore, the current study aimed to investigate the incidence rate of *Fasciola* and *Anaplasma* infections among cattle and buffalo in a part of southern Egypt, “Qena province,” in different seasons among male and female animals with special reference to the harmful effects of Fasciola in the liver of infected animals. The study was carried out from January 2020 to January 2021. A total of 437 cattle and 188 buffaloes of mixed breeds with an age range from 1 to 3 years were selected randomly for the study. Fecal samples were obtained and analyzed for the presence of fluke eggs using direct smear, flotation, and sedimentation techniques. Samples of liver fluke were collected from the liver of slaughtered animals for histopathology. The results revealed that fascioliasis incidence among cattle and buffalo in Qena was 3.43 and 4.26%, respectively. The microscopic examination of blood smears from cattle and buffaloes revealed that 7.5% had an *Anaplasma* infection; in addition, the infection rate concerning gender indicated that it was higher in females 7.57% than males 6.67%. The findings of fascioliasis and anaplasmosis in farm animals indicate that the danger of both diseases in farm animals, which affects the economic production of animals, increases the risk of infection with zoonotic agents for farmworkers.

Key words: *Anaplasma*, buffalo, cattle, *Fasciola* and Qena.

INTRODUCTION

Fascioliasis is a ruminant disease caused by two main parasitic trematodes, *Fasciola hepatica* and *Fasciola gigantica*. It is a zoonotic disease as it affects animals and humans. In humans, it is called human fascioliasis and was considered a significant illness over the last two decades. Infection in humans occurred by the ingestion of metacercaria on eaten leaves, therefore it is generally known as a food-borne trematode parasite (Mas-Coma et al. 2014).

In animals, it causes enlargement of the liver, in addition to other harmful effects, leading to severe economic losses reached to thousands of dollars (Festus et al. 2017). Liver damage caused by premature *Fasciola gigantica* could also possibly lead the animals to Black disease caused by *Clostridium novyi* and hence increase the fatality rate (Phiri 2006).

It was reported that the severe economic losses do not result from damage of the liver of infected animals only but also include losses from the reduction of milk and wool production, decreased weight gain, metabolic disorders, and decreased fertility and lastly medication expenditures on anthelmintic drugs (Simwanza et al. 2012; Festus et al. 2017). Fascioliasis is related to ovarian inactivity and other infertility disorders in bovine species (Ahmed 2007), it has been observed that 58.4% of repeat breeders in cows are related to *Fasciola hepatica* infection (Simsek et al. 2007).

On the relation between *Fasciola* infection and animal reproduction, *Fasciola* was identified in bovine species suffering from ovarian inactivity and other problems of infertility (Ahmed 2007). Fascioliasis affects the reproductive efficiency of domestic animals by degrading the growth rate of young animals, slowing puberty in breeding animals, and increasing estrous intervals in mature animals (Ahmed 2007). In addition, Simsek et al. (2007) stated that cows inspecting suffered from repeat breeder found that 58.4% of the cases examined were *Fasciola hepatica* seropositive. Fasciolosis also has a

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deleterious impact on steroid hormones, and this is confirmed by Lopez-Diaz et al. (1998) who reported that the serum concentration of estradiol-17 beta has increased in cases of *Fasciola* and resulting in abnormally low concentrations of progesterone. Chronic fasciolosis in cattle was also observed to be associated with decreased fertility due to different factors, including changes in blood metabolites, the decline in growth rates and puberty in growing animals, and sustained anoestrus in mature animals following the loss of body condition (Barakat et al. 2001).

It is also critical that the impact of *Fasciola* infection on the duration of the postpartum period is not ignored. The findings revealed that the length of the postpartum cycle of the animals infected with *Fasciola* was observed to be substantially elevated relative to the non-infected ones. The same findings were taken from Ahmed (2007) who observed a rise in the anoestrus cycle in the mature animal infected with *Fasciola.* Disruption during this crucial phase could relate to interruption in the involution of the uterus and disruption during the restoration of estrus activity, which could potentially extend the calving interval and reduce the reproductive and economic productivity (El-Wishy 2007) also, there is the reduction in milk yield during infection with *Fasciola* (Mendes et al. 2008).

In many Asian and African regions, the existence of both types of *Fasciola* was recorded, particularly in areas of the Nile water flow, the gigantic mountain ranges of the lakes, and the arms of the rift valley. In such environments, the respective snail vectors prefer alternating altitudes and climatic conditions (Soliman 2008; Mas-Coma et al. 2009; Howell et al. 2012). Due to their distinct distribution, epidemiology, and management interventions, the differential examination of eggs and specific antigens is significantly preferred (Curtale et al. 2007).

Acute and chronic fascioliasis have been found in different areas of Egypt, it is influencing the general immune status of the animal, furthermore, there is no precise strategy for early detection before the time of egg deposition (Maha 2008).

Fascioliasis is a ruminant liver disease worldwide that is caused by the genus *Fasciola* leading to human illness around the world. The miracidium of the parasite invades one of the numerous water snail hosts at Lymnaea. Infection is the result of encysted metacercariae ingestion present in raw watercress (Carrada-Bravo 2003).

Anaplasmosis, often known as gall sickness, is a tick-borne disease caused by intraerythrocytic rickettsial microorganisms from the order Rickettsiales, family Anaplasmataceae. The erythrocytic *Anaplasma* may infect cattle, sheep, goats, buffalo, and several wild ruminants, though cattle are more vulnerable to infection than buffaloes (Rajput et al. 2005). At least 20 tick species transmit *Anaplasma.* Climate change had a profound impact on the interaction between ticks and their hosts in recent decades (Nasreen et al. 2020).

Anaplasma are major animal and human pathogens that are obligate intracellular, Gram-negative, tick-borne rickettsial bacteria. In tropical and sub-tropical areas, anaplasmosis poses a threat to animal health and output. Due to decreased production, mortality, and work efficiency of afflicted animals, it causes significant economic losses (Jonsson et al. 2008). *Anaplasma ovis*, *A. marginale*, *A. centrale*, *A. platys*, *A. bovis*, and *A. phagocytophilum* are the six known *Anaplasma* species (Ybañez et al. 2014).

Production losses and the cost of treating anaplasmosis are significant economic burden on livestock worldwide (Brown 1997). Early diagnosis of anaplasmosis in farms or in animals introduced to farms is an important decision point for producers to influence production losses and consequent costs (Ashley and Thomas 2021). The main objective of our study was to explore the infection rates of Fascioliasis and Anaplasmosis in cattle and buffalo in the southern part of Egypt in the province of Qena, which is characterized by a hot and dry climate.

MATERIALS AND METHODS

The study was approved by Research Code of Ethics at the South Valley University (RCOE-SVU).

Clinical Examination

The clinical animal evaluation was performed scientifically for age, sex, body mass index, body temperature, heart and respiratory rate and visible mucous membranes.

Study Area

This research was performed in Qena province, which contains several farms for milk production and meat processing. Qena province is in the southern part of Egypt (26° 10′12″N 32° 43′38″E/26.17000 °N 32.72722 °E) and characterized by a temperature range (30 to 45˚C) during the four seasons. The research was carried out/done/performed during the period from January 2020 to January 2021. A total of 437 cattle and 188 buffaloes of mixed breeds with an age range from 1 to 3 years were selected randomly for this study (Fig. 1B).

Blood Samples

A total of 200 blood samples were taken from cattle and buffaloes with tick infestations, characterized clinically by fevers, jaundice, and pale mucous membranes. Blood was drawn from a jugular vein and thin blood smears were made immediately. Blood smears were labeled, air-dried, fixed with methanol, stained with Giemsa stain, and inspected microscopically for the presence of *Anaplasma* using a compound microscope at 100 magnifications, scanning at least 25 fields per slide. The parasites were recognized based on their morphology (Quinn et al. 2011) (Fig. 1A).

Fecal Samples

Fecal samples were obtained directly from each animal's rectum in a plastic jar according to Charles (1998), and preserved at 5°C. The obtained samples were checked within 36 hours. Fecal samples were analyzed for the presence of fluke eggs using direct smear, flotation, and sedimentation techniques (Urquhart et al. 1996).

Postmortem Sample Collection from Liver

Samples of liver fluke were collected from the liver of freshly slaughtered selected animals (buffaloes and cattle), in the slaughterhouses of Qena city.

Histopathological Examination

Specimens from different parts of the liver of the slaughtered cattle were taken for studying histopathological changes. These materials were fixed immediately in 10% formal saline, dehydrated, cleared, embedded in paraffin, sectioned at 4µm and stained with Hematoxylin and Eosin.

RESULTS

The incidence rate of *Fasciola* infection among 437 cattle and 188 buffaloes was 3.43and 4.26% respectively (Table 1). In addition, the infection rate concerning gender indicated that it was higher in females (3.82%) than males (2.67%) (Table 2). Investigation of the incidence in the different seasons of the year revealed that it was the highest in spring at 5.14% (Table 3).

Microscopic Examination

Analysis of blood smears by microscopic examination revealed that 15 (7.5%) blood samples out of 200 samples of cattle and buffaloes had *Anaplasma marginale* infection depending on the microscopic examination (Table 4, Fig. 1). In addition, the infection rate concerning gender indicated that it was higher in females (7.57%) than males (6.67%) (Table 5). Investigation of the incidence in the different seasons of the year revealed that it was the highest in spring 17.78% (Table 6).

Histopathology

Fasciola hepatica infection-induced hyperplasia in the wall of the bile duct with epithelium sloughing in the lumen. Also, biliary cirrhosis showed extensive proliferation of fibrous connective tissue around the intrahepatic bile ducts associated with mononuclear inflammatory infiltration (Fig. 2A). Additionally, the infection with the mature *Fasciola hepatica* also induced hyperplastic proliferation in the biliary epithelium forming papillomatous projections toward the lumen which destroyed the whole bile duct (Fig. 2B). Moreover, other bile ducts exhibited hyperplasia of the epithelial lining which formed a gland-like pattern (adenomatous hyperplasia) (Fig. 2C). The hyperplastic proliferation of biliary epithelium was noticed with the formation of newly formed bile ductulus. This was associated with heavy inflammatory cell infiltration in the fibrous connective tissue between newly formed bile ductulus with yellowish bile pigment secretions in the dilated bile ducts (Fig. 2D).

DISCUSSION

Sheep and cows are the most definitive hosts of *Fasciola hepatica*; goats, bison, ponies, camels, pigs, and deer can likewise be infected. The infection might be transmitted by freshwater plants, including water lettuce, mint, horse feed, and parsley. Animals become infected with *Fasciola* when they were ingested vegetation contaminated with metacercariae and the disease can present in acute, subacute, or chronic forms, but the chronic form being the most prevalent in cattle. (Bista et al. 2018; Alemneh et al. 2019). Human beings could get the infection by drinking defiled water containing reasonable metacercariae (Chan and Lam 1987). The frequency of animals and human disease rises during wet seasons because of an expanded number of snails and longer endurance of encysted cercariae (Arjona et al. 1995). In this study, the rate of *Fasciola* infection was (3.68%), this result is lower than previously reported in Qena Provence in cows 28.6 and buffaloes 33.7% (Abdel-Nasser and Khalifa 2010) and lower than other results recorded by Ekwunife and Eneanya (2006), Daryani et al. (2007). Concerning species-specific, buffaloes were more infected with *Fasciola* (4.26 %) than cattle (3.43%) in the current study, and this can be attributed to the more exposure of the buffaloes to water and pasture than cattle and sheep in Qena. These results almost agree with the results of the previous study indicated that hepatic fascioliasis due to *Fasciola gigantica* was 4.8, 3.3, 0.72 and 0.13% in buffalo, cattle, sheep and goats, respectively in the abattoirs of Basrah in southern Iraq (Mahdi and Al-Baldawi 1987).

Incidence of *Fascioliasis* in female animals was more than males (Ramesh et al. 2018), there is a relationship between host sex and the intensity of helminths infection (Rahman and Collins, 1992), this might be also due to the stress during pregnancy and parturition (Spithill et al. 1999). The effect of seasonal variations on the rate of incidence was investigated in this study. The higher rate (5.14%) was observed during the spring, autumn and winter respectively, while the lowest rate was observed

Table 1: The rate of *Fasciola* infection in cattle and buffaloes

Species	Cattle	Buffalo	Total
Number	437	188	625
Positive	15	8	23
Percent	3.43	4.26	3.68

Table 2: The rate of *Fasciola* infection in cattle and buffaloes regarding to sex

Sex	Male	Female	Total
Total number	75	550	625
Positive	2	21	23
Percent	2.67	3.82	3.68

Table 3: Seasonal difference for *Fasciola* infection in cattle and buffaloes

Season	Winter	Spring	Summer	Autumn	Total
Number	155	214	112	144	625
Positive	5	11	2	5	23
Percent	3.23	5.14	1.79	3.47	3.68

Table 4: The *Anaplasma* infection rate in cattle and buffaloes in Qena

Species	Cattle	Buffalo	Total
Number	141	59	200
Positive	14	1	15
Percent	9.92	1.69	7.50

Table 5: The rate of *Anaplasma* infection in cattle and buffaloes regarding to sex

Sex	Male	Female	Total
Total number	15	185	200
Positive	1	14	15
Percent	6.67	7.57	7.50

Table 6: Seasonal difference for *Anaplasma* infection in cattle and buffaloes

Season	Winter	Spring	Summer	Autumn	Total
Number	55	45	45	55	200
Positive	2	8	1	4	15
Percent	3.64	17.78	2.22	7.27	7.50

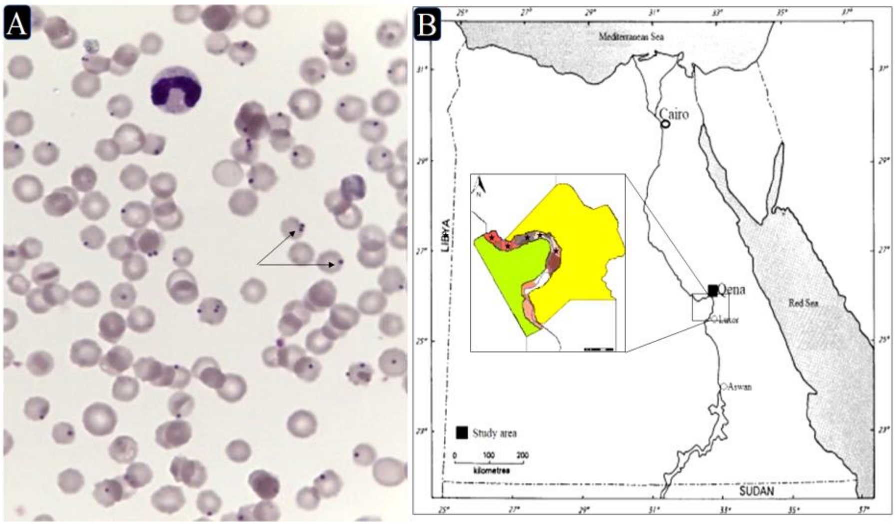

Fig. 1: Blood smear showing *Anaplasma* in the margin of the erythrocytes (A). The map of Egypt showing the area of study and sites of samples collection (B).

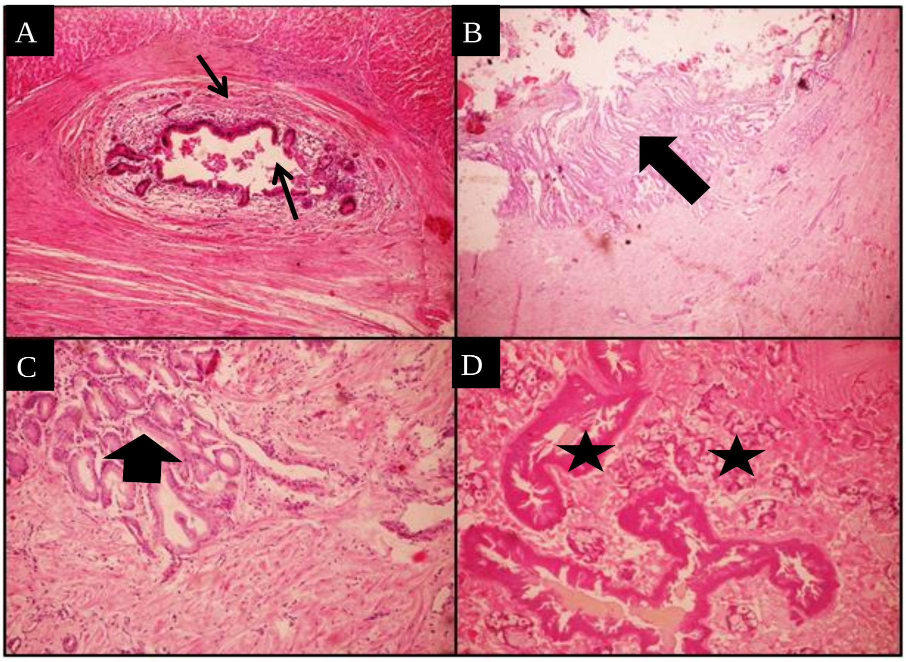

Fig. 2: Biliary cirrhosis with bile duct hyperplasia and inflammatory cells infiltration (thin arrow) (A). The proliferation of the epithelial lining of the bile ducts forming papillary projection in the ductal lumen (thick arrow) (B). Bile duct hyperplasia showing proliferation of the epithelial lining of the bile ducts which formed gland-like pattern (Arrowhead)(C). The hyperplastic proliferation of the biliary epithelium with newly formed bile ductulus (stars)(D).

during the summer season (1.79 %). These results are in the same line with Abu-Elwafa and Al-Araby (2008), who found that spring had the highest incidence than winter, summer, and autumn. On contrary, Maqbool et al. (2002) recorded that the highest incidence of *Fasciola* occurring during the autumn followed by spring and winter while the lowest rate was recorded during the summer also, the highest fascioliasis was recorded during autumn and winter by (Nagwa and Wafaa 2017). This difference may be related to the difference in the geographic area of Qena province and its reflection on the climatic conditions and daily temperature which affected *Fasciola* infestation.

The histopathological examination of the liver shows biliary cirrhosis with bile duct hyperplasia and inflammatory cells infiltration and proliferation of the epithelial lining of the bile ducts forming papillary projection in the ductal lumen and the bile duct hyperplasia showing proliferation of the epithelial lining of the bile ducts which formed gland-like pattern, these findings are consistent with the fact that bile duct thickening is attributed to the persistent presence of *Fasciola* infection by previous studies (Okaiyeto et al. 2012).

This result revealed that the infestation of *Fasciola* in domestic ruminants is already present and represents one of the major problems facing buffaloes, cattle, and sheep farms in Qena. It is also obvious that the infection depends on variant parameters such as sex, season, and breed. This information on the prevalence of fascioliasis; is very important for controlling the infection in Qena and to minimize the economic losses which require doing an accurate public measure. Seasonally, anthelminthic treatment should be given to get the maximum benefits and, to improve the control methods of fascioliasis in animals. It is necessary to increase public awareness about the mode of transmission, the life cycle of *Fasciola*, and good washing of fresh watercress for a few minutes before use. On the other hand, procedures must be done to lower infection rates and to protect animal and human life such as the application of modern methods for decreasing the snails, meat examination ought to be strengthened in all abattoirs to enhance the health of animals and humans.

The anaplasmosis is endemic in cattle in Mexico, Central and South America, and the Caribbean countries (Kocan and de la Fuente 2003). In the acute stage of the infection, between 10 to 90% of the erythrocytes may be parasitized, depending on the *Anaplasma* strain and the host sensitivity, and about 15% of the erythrocytes were parasitized before any clinical signs of disease appear (Radostits et al. 2007). The extensive phagocytosis of infected erythrocytes by the bovine reticuloendothelial system causes clinical manifestations of anaplasmosis such as anemia and icterus without hemoglobinemia and hemoglobinuria (de la Fuente et al. 2001; Potgieter and Stoltsz, 2004). Fever, weight loss, abortion, lethargy, and mortality are some of the other signs of anaplasmosis (Kocan et al. 2003). Regardless of an animal age at the time of infection, cattle infected with *A. marginale* remain infected carriers throughout the rest of their lives, whether they develop clinical disease or not (Richey 1991).

In this study, the percent of *Anaplasma* infection in Qena was 9.92 and 1.69% in cattle and buffalo, these results were lower than the detection of *Anaplasma marginale* by a recent study in Middle and Upper Egypt by blood smear analysis 16.2% of cattle and 2.4% of buffaloes (Amira et al. 2020). The Incidence of anaplasmosis in female animals was 7.57% which was more than males 6.67%. Females have been found to have a greater incidence of anaplasmosis than males in previous research, which might explain that females are the preferred host for ticks and hence more vulnerable to diseases (Seo et al. 2018). The effect of seasonal variations in the rate of incidence was investigated in this study which revealed that the higher rate (17.78%) was observed during the spring, while the lowest rate (2.22%) was observed during the summer season, these results do not agree with the result reported by (Khan et al. 2019) which indicated that summer has a higher incidence of *Anaplasma* in cattle than the spring. Our findings were lower than the average *A. ovis* infection rate in goats in China (25.2%) (from 0% in summer to 51.6%) (Wang et al. 2021), Tunisia (65.3%) (Ben Said et al. 2015) and Italy (31.7%). (Torina et al. 2012). Infection with *Anaplasma* decreased the haemoglobin and packed cell volume in cattle (Gannguly et al. 2017) and the neonatal elk calf can death from vertical transmission of *A. ovis* (Hendrix et al. 2019). The variations in reported infection rates of *Anaplasma* species for different areas may be due to several factors, including sample test methods and times used, differences in animal feeding and husbandry, the presence and frequency of ticks and other vectors, climatic and environmental factors at the sampling point and differences in host resistance (Torina et al. 2008).

Conclusion

Future studies should be included from different geographical regions in the other southern parts of Egypt especially those near Qena province to detect the rate of spreading of the infection, methods of spreading, and the level of harm resulted from this spread. These studies will provide a clear picture and better understanding of fascioliasis and anaplasmosis in animals that could lead to the development of more effective control measures.

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Author Contributions

Hassan Y. A. H. Mahmoud, Abdel Alim Ahmed Ali, Ataf M. Khalil, Yahia A. Amin, and Alsagher O. Ali designed the research and performed laboratory work, analyzed the data, and wrote the manuscript, performed laboratory work, and reviewed the manuscript.

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