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Parasitic Characterization and Histopathological Alterations in the Local Naemi Sheep, *Ovis aries awassi*, Naturally Infected with *Dicrocoelium dendriticum* in Riyadh, Saudi Arabia

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ABSTRACT

Background: The Naemi sheep, *Ovis aries awassi*, is an important local meat source in Saudi Arabia. The current study investigated the impact of natural infection with *Dicrocoelium dendriticum* flukes on local sheep. Parasitic burden, egg output and gross external and internal appearances of the liver were investigated.

Methods: Fifteen naturally infected sheep were targeted in this study based on positive *D. dendriticum* hepatic infection and fecal egg counts, in addition to five healthy (control) ones for comparison. Adult flukes were also characterized in terms of morphometric and diagnostic morphological characters.

Result: Data showed significant differences in parasite burden between low, median and highly infected sheep as well as many morphological parameters within worms. Livers were compared based on the level of injury, histological alterations and the number of *D. dendriticum* adult worms. Macroscopically, livers were enlarged and darkened in color, with thicker bile ducts and fibrosis. Furthermore, leucocyte infiltration, bile duct fibrosis and hyperplasia were investigated. Hepatic lesions were scored at different degrees of severity and numbers. In infected levels, a positive correlation was observed between the number of adult flukes, external macroscopic lesions, bile duct hyperplasia and fibrosis. On the other hand, a negative correlation between these symptoms and the level of leukocyte infiltration was observed.

Key words: *Dicrocoelium dendriticum*, Fibrosis, Hepatic lesions, Leucocyte infiltration, Naemi sheep, Parasite burden.

INTRODUCTION

Dicrocoeliosis is a zoonotic disease caused by the lancet fluke, *Dicrocoelium dendriticum*, which can be found in the bile ducts and gallbladders of definitive hosts, as domestic and wild ruminants are the most common hosts. In addition, horses, rabbits, dogs, pigs and humans can also be infected (Otranto and Traversa 2003; Chamuah and Raina 2020).

Numerous species of land snails and ants (as the first and second intermediate hosts) are involved in the parasite's complex life cycle (Otranto and Traversa 2002). In the definitive host, *D. dendriticum* spreads directly within the liver and biliary ducts of mammals without affecting other liver structures after animal ingestion of ants carrying metacercariae (Garduño *et al*. 2019).

This parasitic disease causes significant economic losses in the livestock industry by impairing female fertility and slowing animal development, weight loss, growth delay, anemia, digestive disorders, edema, decreased milk production and costs associated with anthelminthic treatment in ruminants (Manga-González *et al*. 2005; Otranto and Traversa 2003; Ferreras-Estrada *et al*. 2007). Ingestion of raw or undercooked infected liver may result in spurious infections (Cabeza-Barrera *et al*. 2011). Fields with dry, chalky, or alkaline soils are ideal for Dicrocoelium intermediate hosts (Manga-González *et al*. 2001; Meenakshisundaram *et al*. 2017). The clinical manifestations that these parasites cause in animals are primarily related to liver and gastric issues. Acute abdominal pain, fever and eosinophilia are the common symptoms of an infection in its early stages (Zeynep Tas *et al*. 2010). Later on, the patient develops diarrhea, anorexia, a high fever and abdominal pain. In chronic cases, the disease can cause jaundice, liver and biliary duct cirrhosis, ascites and cachexia. Perhaps the infected animal dies following severe hepatic complications (Molyneux 2006). *Dendriticum* has low parasitic specificity in definitive and intermediate hosts, according to epidemiological studies (Shamsi *et al*., 2020). A routine *in vivo* diagnosis of Dicrocoeliosis at carpological examination is usually carried out *via* the microscopic

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detection of eggs in the host feces. On the other hand, postmortem/slaughtering examination allows evaluation for histological alteration and detection of adult flukes in the liver and bile ducts (Manga-Gonzalez *et al*. 1991; Rojo-Vázquez *et al*. 2012). Further, these symptoms are directly proportional to parasitic burden and chronic inflammation of the bile duct (Jithendran and Bhat 1996; Beck *et al*. 2015). Macroscopically, the most common liver injuries are bile duct inflammation, enlarged liver and fibrosis (Jithendran and Bhat, 1996). Histopathological changes in experimentally infected lambs were characterized by a wide range of injuries, including periductal fibrosis, ductal reaction and leukocyte infiltration (Ferreras-Estrada *et al*., 2007). Several studies examined the phenotypic expression of inflammatory cells in animals experimentally infected with the liver fluke, *Fasciola hepatica* (Dezfuli *et al*. 2015). The current study provides fundamental information for the investigation of liver fluke infections in Saudi Arabia. Additionally, data suggests that some level of quarantine or border checks may be beneficial in preventing the introduction of *D. dendriticum* or other pathogens into Saudi Arabia until they are accurately screened and diagnosed.

MATERIALS AND METHODS

Venue and design of the experiment

The study was carried out during July 2021 in the Riyadh region, Saudi Arabia using naturally *D. dendriticum* infected sacrifices of Naemi sheep, *Ovis aries awassi*. The feces and liver were collected from slaughtered animals in the automatic Riyadh slaughterhouse according to Saudi slaughter standards, with the approval of the slaughterhouse official, to obtain samples and transport them to the parasitological laboratory at the College of Science, King Saud University for experimental purposes.

Animal liver and feces samples were divided into four groups (15 samples with varying degrees of injury + 5 control samples) of similar weights and ages ranging from 2-3 years. According to Ciringoli *et al*. (2010), the affected liver was visually examined and cut to confirm infection. The affected livers were divided into 3 grades based on the degree of liver lesions (LLS), bile duct hyperplasia (BDH) and the degree of fibrosis (severe, moderate and low-injury).

Eggs count

Counts of feces eggs

After confirming *D. dendriticum* infection,15 g feces were taken of each infected sacrifice were taken directly from their rectums and placed in plastic bags. Feces were stored at +4°C until they were used for the egg counts (FECs) using the floatation method according to Urquhart *et al*. (1996). The egg phases and various morphological traits were identified according to Yamaguti (1959) and Soulsby (1982).

Count of gallbladder eggs

Gallbladders were removed from five individual infected livers (n = 5) from each of the highly, moderately and lowly infected groups and stored at +4°C until used for egg counting. Bile samples (10-25 ml each) were individually transferred into conical tubes, centrifuged at 1500 g for 10 min. Supernatants were decanted without disturbance of the resulting biliary pellets. One ml of distilled water was added to each of the biliary pellets and mixed well. Then, then, 20 µl were taken with a micropipette from each mixture and individually observed under a stereomicroscope at 20× and 40× and the counts *D. dendriticum* eggs.

Liver fluke's examination

Parasite burden

Five individual samples (1 g each) from five different individual infected livers (n=5) of each of the highly, moderately and low infected groups were cut into slices and manually squeezed in bowls containing tap water for evaluating the parasitic burden, resulting squeezed liver samples were washed with a stream of running tap water on sieves (0.18 mm diameter each). Flukes in the resulting bowls' contents were fixed by adding a solution of 70% ethyl alcohol. Recovered flukes of each group were counted under a stereomicroscope according to the method Perry *et al*. (2016). Five adult flukes were taken from each liver in order to confirm species identification based on the morphological and morphometric characteristics based on the relevant morphological keys (Taira *et al*. 2006, Otranto *et al*. 2007).

Flukes morphometric characteristics

Ten clear samples of fixed adult *D. dendriticum* flukes (n = 10) were randomly selected for examination under an optical stereomicroscope at 40× magnification according to (Soulsby 1982). Indices of body length and width, the diameter of the oral and ventral suckers, the width and length of the ovary, pharynx, testicles, vitellaria and uterus were measured (Taira *et al*. 2006).

Liver examinations

Anatomopathological examinations

The anatomopathological examinations for the infected livers were performed according to Scala *et al*., (2019). Briefly, seven hepatic areas were targeted: the right lobe and left lobe of both the diaphragmatic face and visceral face, quadrate lobe, caudate lobe and the cut surface. Severity for each area were scored as follows: (0) absence; (1) rare; (2)≤5 cm^2; (3) 5-7.5 cm^2; (4) 7.5-10 cm^2.

Histopathological alterations

Upon being slaughtered and macroscopic evaluation, representative tissue samples from each confirmed infected carcass's liver were taken. Prior to embedding in paraffin wax, liver tissues were fixed in 10% neutral-buffered formalin and then dehydrated in ascending concentrations of alcohols. Hematoxylin-eosin was used to stain sections cut at a thickness of 5 microns (Wu and Danielson 1995, Perry *et al*. 2016). The most representative lesions were identified including: bile duct hyperplasia, fibrosis and degree of leucocyte infiltration. Severity categories of fibrosis was assessed according to the ratio between fibrosis in the examined area into: 0 (absent), 1 (mild; <15%), 2 (moderate; 15-35%), 3 (high; 35-50%) and 4 (severe; >50%), according to Piegari *et al*, (2021). The rate was investigated via observing five fields in ten different individual infected livers (n = 10) at magnification of 40× each. Moreover, the severity grading of bile duct damage (based on the number of sectioned biliary branches in the affected area) was categorized as: 0 (absent), 1 (mild; <10), 2 (moderate; 10-15), 3 (high; 15-20) and 4 (severe severe; >20). These degrees were obtained from 10 different individually observed damaged fields (n = 10) at 40× magnification each. Finally, based on the hepatic enlargement, inflammation was categorized into: 0(absent), 1 (mild, with lymphatic aggregation but without liver enlargement), 2 (moderate, with leucocytic infiltration), 3 (high, with higher leucocytic infiltration) and 4 (severe, with severe leucocytic infiltration). These degrees were obtained from 10 different individually observed damaged fields (n = 10) at 40× magnification each. All histopathological degrees were assessed by analyzing the fields with image processing software (Adobe Photoshop, v: 2020, Adobe, USA).

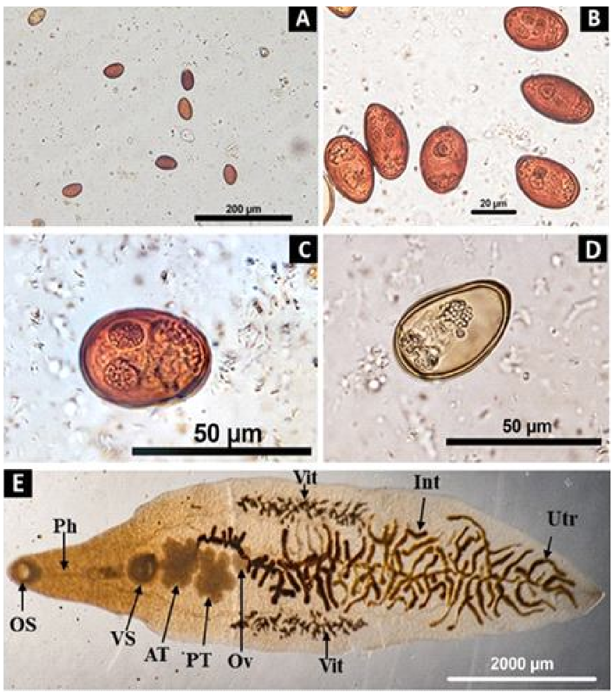

Fig 1(a): A photo plate showing two stages of *D. dendriticum* fluke isolated from naturally infected Naemi sheep's livers. A, B and C: magnifications of the brown-reddish eggs. D: an egg showing a developing meracedium. Eggs were recovered from the infected gallbladder. E: An adult stage recovered from a naturally infected animal shows the oral sucker (OS), pharynx (Ph), ventral sucker (VS), delicately branched anterior and posterior testes (AT and PT respectively) and ovary (Ov), the two branched vitellaria (Vt), intestine (Int) and uterus (Utr). Adult flukes were recovered from infected livers.

Statistical analysis

Statistical analyses of data have been undertaken using MINITAB software (MINITAB, State College, PA, v: 18.1, 2018). Data of flukes' morphometric measurements, eggs counts and parasite burden were tested first for normality (using Anderson-Darling Normality test) and for variances homogeneity, prior to any further analysis. Consequently, based on the type of data, two-sample t-test was used for comparing differences between some relevant data of body measurements. While data of egg counts and parasite burden were not normally distributed and thus, Mann-Whitney U was used for individual comparisons. Values were expressed as means ± standard errors at a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Egg counts

Eggs were counted of both feces and gallbladder of infected sheep. Most eggs were brownish in color (Fig 1A, B and C) and some were containing developing meracedia (Fig 1D). Gallbladders of highly infected animals showed 3.92- and 14.0-folds eggs number of that of moderate and low infected ones (442±67.2, 1579±138, 6190±676) respectively ($P \leq 0.5$; n = 5) (Table 1). In comparison, those numbers of eggs were significantly lower in feces. Whereas, feces of highly infected sheep showed 3.55 and 8.09 folds that of moderate and low infected ones (48.8±3.44, 111.2±10.7, 395±36.1) respectively (Table 1).

Parasitic burden

In the twenty animals examined (five control and fifteen infected), the number of *D. dendriticum* adult worms (Fig 1E)

Table 1: Egg counts and parasite burden in Naemi sheep naturally infected with *D. dendriticum*. Values are presented in means±SE (n = 5).

Parameter	Counts		
	Low infection	Moderate infection	High infection
Gallbladder (eggs)/20 µl	442±67.2	1579±138	6190±676
Feces (eggs)/20 µl	48.8±3.44*	111.2±10.7**	395±36.1***
Parasite burden (in liver)/g liver	644±61.83	2145±262.95	5072.6±863.17
Replicates (n)	5		

***Means significantly higher comparing to moderate and low infection; **Means significantly higher comparing to low infection; *Means significantly lower comparing to moderate and highly infection.

was ranged from 0 (in control) to 5099±952/g in infected livers. Parasitic burden was recorded as ranging from 450-845 (mean: 690±69; n = 5) of low-infected group, 1127-3714 (mean: 2145±441; n = 5) of moderately-infected group and 3071- 8420 (mean: 5099±952; n = 5) of highly-infected group (Table 1 and Fig 2B, C, D and E). Tow-Sample t-Test showed that the mean parasitic burden in highly infected livers was significantly higher than both moderate and low infected ones (5099±952 v 2145±441 and 690±69; p<0.05; n = 5) respectively (Table 1). Moreover, the mean parasitic burden in moderately infected livers was significantly higher than the low infected ones (2145±441 v 690±69; p<0.05; n = 5) (Table 1). These results show clearly that parasite burden in the highly infected livers was 2.37 and 7.38 folds of that in moderate and low infected livers respectively. The negative control animals (healthy group) did not show any pathological or parasitic signs in their livers or feces.

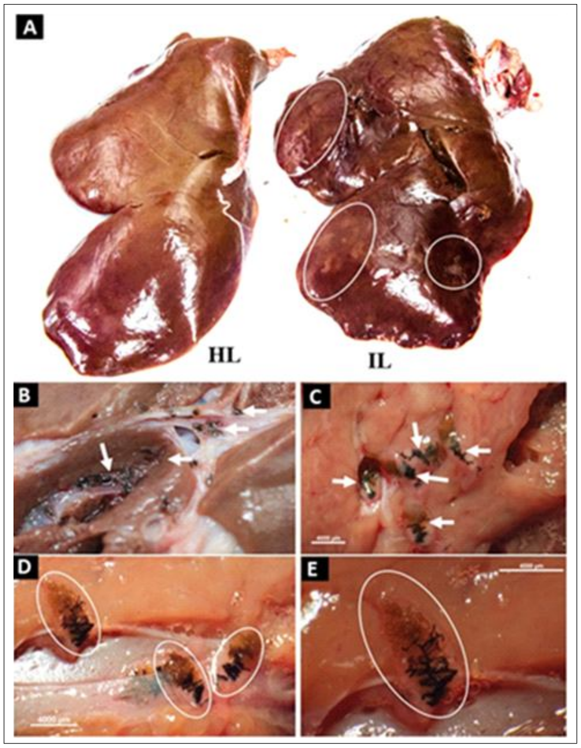

Fig 2(b): A photo plate showing cut surface signs of sheep livers infected with *D. dendriticum* worms. A healthy liver (HL) as a control, with a bright brown-reddish color and an infected liver (IL), with a dark brown color and whitish surface fibrotic lesions (inside whit circles). B: A cut surface of an infected liver shows clumps of worms inside the gallbladder duct (white arrows). C, D and E: cut surface of infected liver showing mature flukes at various magnifications.

Flukes characterization

Adult *D. dendriticum* flukes were recovered from ten infected livers (n=10) and their characteristic and distinguishing the parts of the parasite were investigated. Flukes were gravid (Fig 1E) and active. Averages minor morphometric as well as morphological variations in terms of comparative characteristics (measurements in µm) have been given in Table (2). The translucent, dorsoventrally flattened body measures long (L) (7405.0±269.0 µm, wide (W) 2292.2±68. 4 µm). The oral sucker is sub-terminal and measures, (L 573.6±47.8, W 423.1± 23.9 µm); the slightly large ventral sucker (537.1±21.5, W 484.1±26.9 µm) is in the anterior quarter of body.

The small round pharynx (1060.0±97.7 µm) lies next to the oral sucker; it is followed by the esophagus that extends between the pharynx and the ventral sucker (L: 960.0±89.2 µm) and two long, slender intestinal caeca in the last quarter of the body. The slender excretory bladder reaches anterior to the ovary (L: 405.7±23.4, W: 322.5±12.4 µm). Anterior testes (L: 662.7±34.5, W: 520.4±19.2 µm). Posterior testes (L: 760.0±51.9, W: 629.6±21.4 µm). Lift vitellaria (L: 450.1±16.4, W: 2047.1±46.7 µm). Right vitellaria (L: 2018.4±40.5, W: 501.6±1.69 µm). Uterus (L: 538.6±17.8, W: 502.1±14.8 µm). Two-sample t-test showed similarity between the sizes of oral and ventral suckers, anterior and posterior tests and right and lift vitellaria (P>0.05; n = 10). Finally, lengths of vitellaria, uterus and pharynx were measuring 27.6, 7.2 and 14.3% of body length respectively.

Table 2: Morphometric indices of adult *D. dendriticum* collected from naturally infected Naemi sheep's livers.

Parameter	Dimension	Mean measurement ±SE (µm)	Parameter	Dimension	Mean measurement ±SE (µm)
Body	Width	2292.2±68.4	Anterior testes	Width	520.4±19.2
	Length	7405.0±269.0		Length	662.7±34.5
Oral sucker	Width	423.1±23.9	Posterior testes	Width	629.6±21.4
	Length	573.6±47.8		Length	760.0±51.9
Ventral sucker	Width	484.1±26.9	Lift vitellaria	Width	450.1±16.4
	Length	537.1±21.5		Length	2047.1±46.7
Pharynx	Length	1060.0±97.7	Right vitellaria	Width	501.6±1.69
Esophagus	Length	960.0±89.2		Length	2018.4±40.5
Ovary	Width	322.5±12.4	Uterus	Width	502.1±14.8
	Length	405.7±23.4		Length	538.6±17.8

Values are presented in means±SE (n = 10).

Liver investigations

Hepatic pathological analysis

Healthy livers (HL) of control group showed no external macroscopic abnormalities (Fig 2A). However, *D. dendriticum* -infected livers (IL) showed darker appearance and moderate to high fibrosis associated with white patches on the surface (Fig 2A). In some infected livers, it was noticeable that the parasites were full and having catarrhal exudate within liver lumen (Fig 2B). Worm burden was recorded as low, moderate and high (Table 1) based on the infection intensity. The cut surface was moderately congested with a low burden of adult worm (Fig 2C, D and E).

Hepatic histological alteration

Livers of the control group showed no histological changes. In comparison, the main pathological lesions in the infected livers were showing various degrees of fibrosis, hyperplasia and dilation of biliary duct and signs of inflammation (Fig 3). Highly infected group (recorded 5099 ± 952 flukes/g) (Table 1) showed enlargement in both biliary duct and portal areas, associated with severe fibrosis. Besides, leukocyte infiltration was abundant within the biliary ducts those also showed severe hyperplasia (Table 3 and Fig 3). The moderately infected livers (recorded 2145 ± 441 flukes/g) (Table 1) showed low to moderate fibrosis as well as hyperplasia and severe leukocytic infiltration and aggregation in and around the biliary duct (Table 3 and Fig 3). Finally, livers in low infected group (690 ± 69 flukes/g) (Table 1) showed low ductal fibrosis and severe leukocytic infiltration and lymphoid aggregates within the bile ducts (Fig 3).

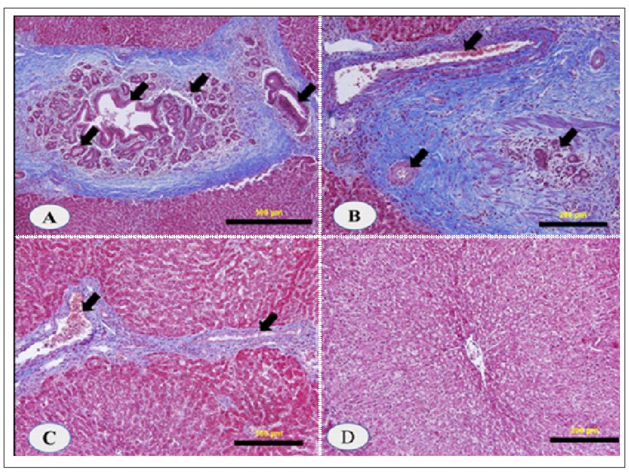

Fig 3(c): *D. dendriticum* infection causes histological changes in sections of experimental livers stained with hematoxylin and eosin. A: Fibrosis and leukocyte infiltration around the biliary ducts in a severely infected liver. B: moderately infected liver with leukocyte infiltration and fibrosis around the biliary ducts. C: a low-infection liver with mild fibrosis and severe leukocyte infiltration. D: A healthy (control) liver showing healthy integrity of hepatic tissue construction.

The recorded liver egg counts, parasite burden, gallbladder hyperplasia and fibrosis all revealed a positive significant correlation ($P<0.01$, n = 5) (Table 3). On the other hand, however, a significant negative correlation between parasite burden, egg numbers, fibrosis, bile duct hyperplasia and leukocyte aggregation and infiltration were clearly observed ($P<0.05$) (Table 3). Furthermore, Mann-Whitney U test revealed that highly infected livers had a statistically higher biliary duct fibrosis and hyperplasia than both the moderately and low infected ones ($P<0.05$, n = 5) (Table 3). In contrast, highly infected livers had less leukocyte infiltration than moderately and low infected ones ($P<0.05$, n=5).

The inflammation (lesion) was graded into: 0 (absent), 1 (mild), 2 (moderate), 3 (high) and 4 (severe). Severity ratios of fibrosis was graded into: 0 (absent), 1 (mild; <10%), 2 (moderate; 10-30%), 3 (high; 30-50%) and 4 (severe>50%). Bile duct hyperplasia was assessed as the number of branches noticed in affected area as: 0 (absent), 1 (mild; <10), 2 (moderate; 10-15) and 3 (severe; >15). The degree of leucocytic infiltration was graded into: 0 (absent), 1 (mild), 2 (moderate), 3 (high) and 4 (severe). Each category of the macroscopic and histological alterations was carried out in 5 different individual livers (n = 5).

The Naemi sheep, *Ovis aries awassi*, is an important local meat source that meets human needs for protein in Saudi Arabia. Infected sheep were checked for the impact of natural infection with *D. dendriticum* flukes on the local Naemi sheep breeds. Morphometric analysis, parasitic encumbrance, egg output and histopathological alterations in the infected liver were investigated. Data revealed a positive link between the parasite burden and egg production. As a result, a large number of *D. dendriticum* flukes retrieved was linked to a higher number of eggs released into the feces. Moreover, it was noticed that worm burden and hepatic pathological features have a substantial positive relationship. In a study undertaken by Campo *et al*. (2000), they found comparable results in lambs experimentally infected with different dosages of *D. dendriticum*

Table 3: Scoring assigned to hepatic macroscopic and histopathological analysis in the local Naemi sheep naturally infected with *D. dendriticum.*

Experimental groups	No. of liver lesions	Fibrosis	Bile duct hyperplasia	Leucocyte infiltration
No. of liver lesio	0	3.8±1	3.2±1	2.4±1
Fibrosis	0	3±1	2.4±1	1±1
Bile duct hyperplasia	0	2.6±1	2.2±1	1±1
Leucocyte infiltration	0	1±1	2±1	3±1

Values are presented in means±SE.

in the first observed correlation (Campo, Manga-González and González-Lanza 2000). Other studies carried out on lancet liver flukes by Manga-González *et al*., (2007); Beck *et al*., (2014); Korchan, (2015); Samadie *et al*., (2016), revealed a link between egg disposal and parasitic burden in both naturally and experimentally infected animals. All these studies revealed that the presence of infection increases with time. For other parasite diseases, similar results were obtained (Manga-González and González-Lanza 2005, Beck *et al*. 2014, Korchan 2015, Samadie *et al*. 2016). Other research has found a significant link between fecal egg production and worm burden (Radfar, Nourollahi-Fard and Mohammadyari 2015), as well as a significant positive link between fecal egg count and gallbladder and liver burden worm in sheep (Piegari *et al*. 2021). Further, Jithendran and Bhat found comparable results in naturally infected sheep with *D. dendriticum* based on the parasite burden and hepatic macroscopic lesions (Jithendran and Bhat 1996).

The *hepatic* pathological features and morphological characteristics monitored in the infected sheep of the current study were found to be different. These features and characteristics were assessed in terms of leucocyte infiltration, gallbladder hyperplasia and fibrosis. In these particular features, the number of fecal eggs produced and parasite burden were positively correlated with gallbladder hyperplasia and fibrosis, but on the other hand, they were negatively correlated with leukocyte infiltration. Because *D. dendriticum* completes its life cycle in the biliary ducts, the link between gallbladder hyperplasia and parasite burden could be interpreted as an irritation caused by the adult flukes' suckers (Samadieh *et al*. 2017). In this context, previous investigations on infected livers revealed cholangiocyte self-proliferation after bile duct ligation, implying a link between bile duct injury and hyperplasia (Piegari *et al*., 2021). Evidence for this has been provided by recent studies as they proved that hepatocytes can trans-differentiate into cholangiocytes as a result of severe bile duct injury, resulting in ductal hyperplasia (Sato *et al*. 2019). Moreover, other studies found comparable results in the association between fecal egg number, parasite burden, biliary duct fibrosis and hyperplasia (Nelwan 2019). In spontaneously infected cattle with *F. hepatica*, we showed a significant link between the ductal response and liver fibrosis. On the other hand, it is thought that macrophages release pro-fibrotic and anti-inflammatory mediators that cause fibrogenesis and concurrent inhibition of cholangiocyte proliferation, which could be the reason behind the observed reduction in fibroblastic proliferation and fibrosis in the liver (Sato *et al*. 2019).

Based on the numerous morphometric and morphological parameters of adult flukes, such as size, shape, length and width of various internal body organs, the parasite recorded in the current study coincides with the previously described *D. dendriticum* (Rudolphi 1802). In addition, eggs of this parasite species were also found to be abundant in the local host species' feces. Moreover, our findings on the area of the body of this parasite are consistent with the findings of other similar studies (Beck *et al*. 2014). Similarly, a mean body length of *D. dendriticum* obtained from sheep was 7.2 mm, with a mean body width of 2.5 mm (Kuchai, Chishti and Dar 2011). This is similar to the finding of the current study as it showed a mean body length for *D. dendriticum* flukes obtained from the local sheep of 7.405.0 ±2.690 mm and a mean body width of 2.292±0.68 mm. However, other studies indicated significantly smaller metric parameters as they recorded a body length of no longer than 4.6 mm and 1.2 width (Kruchynenko *et al*., 2020). Evidence of a statistically high coefficient of direct correlation between body and vitellaria lengths in trematodes has been previously provided (Masand Bargues 1997; Sundnes 2003). In the current study, this correlation was extended to the uterus too. The parasitic encumbrance, hyperplasia of the biliary duct, as well as fibrosis, were negatively correlated with the intensity of leucocyte infiltration. These findings provide a potential explanation for the mechanisms related to the interaction between the parasite, fibrosis and histological alterations. However, further studies are strongly recommended to confirm this hypothesis.

CONCLUSION

The findings of this study suggest that *D. dendriticum*, a parasite that naturally infects the local Naemi sheep, *Ovis aries awassi*, in Saudi Arabia, may have an impact on the country's most important meat source. As a result, additional research in this area will allow us to learn more about the host-parasite interaction mechanisms as well as the complex response to parasitic infections, laying the groundwork for future research into effective treatment strategies and therapies in support of encouraging the local in production this important local meat resource.

Data availabilty

Data supporting this research are available from corresponding author on reasonable request.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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