



RESEARCH ARTICLE

An Abattoir-based Survey of Helminthic Liver Infections and Associated Pathological Lesions in Sheep in the Sultanate of Oman

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ABSTRACT

Importation of apparently clinically healthy animals from endemic areas to naïve countries is a major source of infection to the establishment of new pathogens outside borders. A survey investigation between 2018 and 2021 was conducted at the Central Muscat Municipality Slaughterhouse to examine parasitic infections in condemned liver from 948 sheep samples during routine meat inspection. Pathological and parasitological examinations were carried out to identify parasites and associated hepatic lesions. An overall prevalence of 6.01% (57/984) was observed in sheep condemned liver by single infection of *Dicrocoelium dendriticum*, *Cysticercus tenuicollis*, *Stilesia hepatica*, *Fasciola hepatica*, and hydatid cysts. Hepatic lesions varied widely from severe hemorrhagic wavy tracts in case of acute cysticercosis (0.4 %) to indurated dark brown with numerous white scars in livers infected with *D. dendriticum* (4.2 %). Presence of cysts with clear fluid and protoscolices were seen in livers infected with *C. tenuicollis* (0.63%) and hydatidosis (0.1%), detected as larger cysts with white thicker walls embedded in livers. Examination of the bile ducts revealed *S. hepatica* at 0.4% and *F. hepatica* at 0.2%, with thickened bile ducts. Morphometric measurements confirmed the diagnosis of *D. dendriticum* in imported sheep from Romania. To the best of our knowledge, this is the first report of helminthic infections among sheep livers, with reference to dicrocoeliasis in imported sheep in Oman. Expanding of livestock based on animals' importation warrants further quarantine regulations to control emerging parasites and to reduce economic losses due to liver condemnation in the country.

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INTRODUCTION

Parasitic infections represent huge economic losses for livestock production. Direct costs from offal and carcass condemnations or medical expenses can be high. Most lesions are not observed during ante-mortem inspection and are only detected after slaughter. Economic losses result from rejection of affected parts or whole organs (Cadmus and Adesokan, 2009). Liver flukes are frequently reported with significant economic impact due to liver condemnation. Fascioliasis, cysticercosis, echinococcosis and dicrocoeliasis are the most commonly detected parasites in the condemned livers globally (Maraqa *et al.*, 2005). The severity of animal fascioliasis is dependent upon the number of ingested metacercariae and the clinical

signs can vary from sudden death in acute fascioliasis, to extensive hepatic damage along with anemia in the subacute disease. Submandibular edema and decreased milk production are usually seen in chronic fascioliasis (Taylor *et al.*, 2016).

Dicrocoelium dendriticum, known as the lancet fluke, is a small liver fluke that resides in bile ducts and gall bladders of diverse animal species such as cattle, sheep, goats, buffaloes and camels that serve as definitive hosts. It causes a disease known as dicrocoeliasis. Snails and ants act as the first and second intermediate hosts, respectively (Taylor *et al.*, 2016). Moreover, the lancet fluke can occasionally infect horses, dogs, pigs and rabbits and is considered as a rare zoonosis (Otranto and Traversa, 2003; Cengiz *et al.*, 2010). The parasite is most prevalent

throughout Europe, Australia, North America, Japan and some Southeast Asian countries. The trematode can survive and remain inside the definitive host for several years. Thus, in the acute and chronic stages of the infection, pathologic lesions in the liver like cirrhosis and symptoms such as weight loss and anemia are seen. It has a severe economic impact in many countries due to liver failure, weight loss and liver condemnation during meat inspection at slaughterhouse (Otranto and Traversa, 2003; Jahed Khaniki *et al.*, 2013).

The larval stage of *Taenia hydatigena*, *Cysticercus tenuicollis*, is a tapeworm of dogs that migrates from the small intestine of intermediate hosts (small ruminants) and then through the liver to develop mature cysticerci in the omentum, mesentery, and peritoneum (Corda *et al.*, 2020). The migration of a vast number of larvae can cause severe acute infections, traumatic hepatitis and peritonitis with mortality rates of up to 19% in lambs (Scala *et al.*, 2016). Hydatidosis caused by larval stage of dog's tapeworm (*Echinococcus granulosus*) is a cosmopolitan cyclozoonosis with devastating economic impacts on humans and livestock. The disease is transmitted through the ingestion of infective eggs of *Echinococcus granulosus* (Dakkak, 2010). Dogs are the definitive hosts and livestock are the intermediate hosts while humans are considered accidental intermediate hosts. Hydatidosis is endemic in Oman and the infection rate of hydatid cysts was reported as 0.07% in the slaughtered sheep by Al Kitani *et al.* (2015). *Stilesia hepatica* is a nonpathogenic tapeworm of the bile ducts that is commonly found in small ruminants during meat inspection where livers are condemned for esthetic reasons rather than food safety concerns (Nginyi *et al.*, 1993).

This manuscript reports the prevalence of helminths recovered from condemned sheep livers in the Central Muscat Municipality Slaughterhouse in Oman with emphasis on the pathology and morphometry of the recovered parasites.

MATERIALS AND METHODS

Study design and samples collection: A total of 948 livers from imported adult sheep breeds (Somali, South African, Sudanese and Romani) were inspected between 2018 and 2021 during the routine meat inspection in the Central Muscat Municipality Slaughterhouse, Muscat, Oman. Based on the gross lesions, 57 suspected condemned livers were transferred in polyethylene bags in an icebox to the Veterinary Pathology Laboratory at the College of Agricultural and Marine Sciences, Sultan Qaboos University for further laboratory investigations.

Parasitological examination: Parasites and cysts were collected from the incised condemned livers and identified either macroscopically or by stereomicroscope. Morphometric measurements were conducted for smaller lancet flukes following the procedure of Taira *et al.* (2006) with minor modifications. Briefly, the flukes were soaked for 24 hours in tap water in petri dishes to flatten and ten flukes were laid on a glass slide without mounting or coverslip and measured under light microscope (Olympus BX51 microscope attached to Olympus DP70 camera, Olympus Co., Tokyo, Japan). Measurements included total body length, body width, external oral sucker diameter,

external ventral sucker diameter, testes length, testes width, total length of vitelline glands and anterior to maximal body width.

Pathological examination: Livers were examined grossly, incised, and samples were collected and fixed in 10% neutral buffered formalin. Tissues were dehydrated, cleared in xylene, embedded in paraffin using the standard technique and 4µm thickness sections were cut by a rotary microtome and stained by hematoxylin and eosin. Consecutive sections were stained by trichrome stain (Abcam: ab150686, Cambridge, UK) to assess fibrosis (Bancroft and Gamble, 2007). Slides were examined under an Olympus BX51 microscope attached to an Olympus DP70 camera.

RESULTS

Parasitological findings: The detected parasites and their percentages are summarized in Table 1. The examined condemned livers were infected with *D. dendriticum* (4.2%, 40/984), which were imported with Romani sheep. The bodies of the flukes were flat, elongated and translucent revealing all internal organs (Fig. 1). The common characteristic features of the examined *D. dendriticum*; rounded external oral and ventral suckers, two globular tandem orientation of the testes, a round ovary located posterior to the testis. In addition to two vitelline glands in the periphery of the middle segment of the fluke, and ellipsoidal golden-brown eggs occupying the convoluted uterus in the posterior two-third of the fluke that were only visible when stained (Fig. 2). The morphological measurements of the identified *D. dendriticum* are shown in Table (2). Only two livers (0.2%, 2/984) were infected with *F. hepatica* (Fig. 1) where the affected animals were imported from South Africa. Infections by tapeworms included hydatid cyst (0.1%, 1/984), acute cysticercosis (0.4%, 4/984), chronic cysticercosis (0.6%, 6/984), and *S. hepatica* (0.4%, 4/984) as shown in Fig. 1.

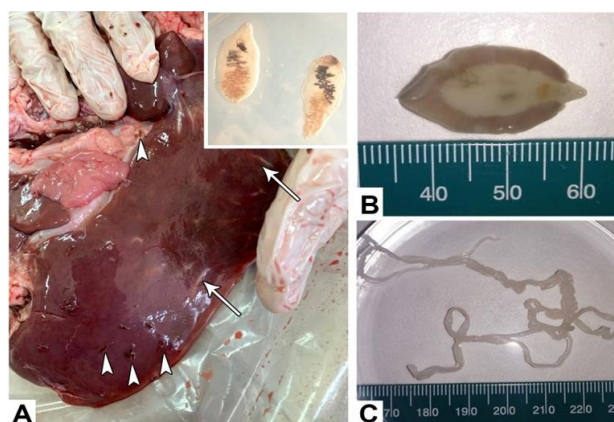


Fig. 1: A) Condemned liver from an imported Romani sheep showing numerous white striated scars (arrows) and multiple live adult *Dicrocoelium dendriticum* (arrowheads) oozing from the bile duct and contaminating the liver surface (Arrowheads). (Inset: Squash form of the adult *Dicrocoelium dendriticum* under stereomicroscope); B) Mature *Fasciola hepatica* trematode isolated from an imported South African sheep; C) Mature *Stilesia hepatica* cestode isolated from an imported Somali sheep.

Pathological findings: The examined condemned liver samples showed various lesions in accordance with the

identified parasite. Livers infected with *D. dendriticum* were indurated, enlarged, dark brown with numerous white strike scars. Upon incision, myriads of small flukes mixed with bile appeared from the thickened bile ducts (Fig. 1). Histopathological examination revealed the presence of longitudinal and cross sections of *D. dendriticum* resulted in severe thickening of the bile ducts with inflammatory cells particularly lymphocytes, macrophages, plasma cells, and fibrous connective tissues (Fig. 2). Thickened bile duct walls due to periductal fibrosis was mainly in the portal areas as demonstrated using the Masson's trichrome stain (Fig. 2). Pressure atrophy of the surrounding hepatic cells was prominent. The hepatic portal areas showed marked infiltration with lymphocytes, plasma cells, macrophages and few eosinophils. Papillary proliferation of the bile duct

mucosa with extensive inflammatory cells infiltrations was seen (Fig. 2). Condemned *F. hepatica* infected livers were normal in both size and color. Few numbers of parasites were detected upon incision of the bile ducts without any noticeable gross lesions. Histologically, few eosinophils were seen in the portal areas. Condemned livers that were affected by acute cysticercosis showed multiple tortuous hemorrhagic tracts and whitish larvae (Fig. 3), the livers were enlarged with rounded ends. Microscopically, injured hepatic parenchyma due to multiple recent and old migratory tracts were found filled with erythrocytes along with cross sections of cestode larvae, and margined with inflammatory cells and fibrin (Fig. 3). Livers affected with chronic cysticercosis exhibited either single or multiple thin walled parasitic *C. tenuicollis* cysts attached to the hepatic capsules. The cysts contained clear fluid with an apparent whitish lump containing the protoscolex (Fig. 3).

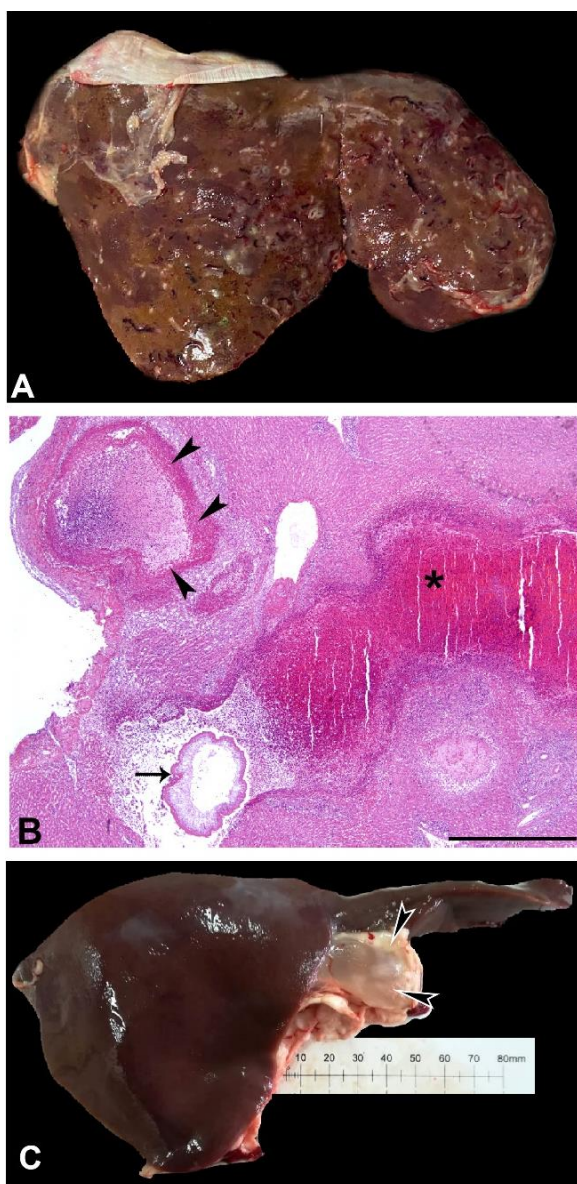


Fig. 3: A) Condemned liver from an imported sheep showing multiple hemorrhagic tracts and whitish *Cysticercus tenuicollis* larvae; B) Photomicrograph of the same liver in A) showing a cross section of cestode larva (arrow) damaging the hepatic parenchyma and creating a recent migratory tract this filled with erythrocytes (asterisk) and margined with inflammatory cells, nearby, another older migratory tract can be seen surrounded by fibrin (arrowheads) (Stain: H&E; Bar= 1 mm); C) A single thin walled parasitic *Cysticercus tenuicollis* cyst attached to the hepatic capsule and containing clear fluid (arrowheads).

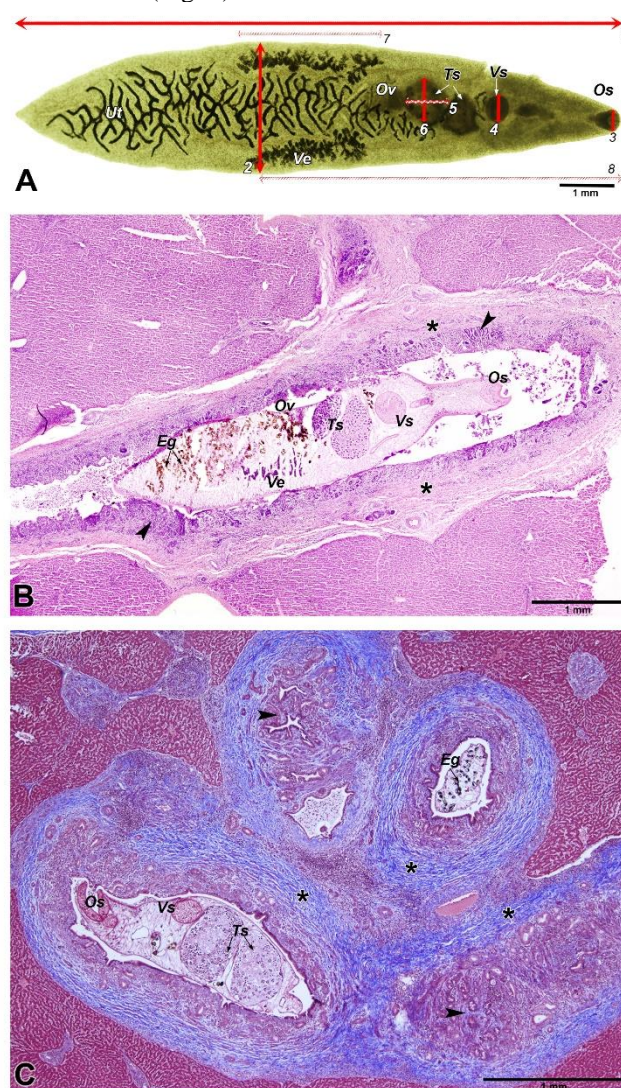


Fig. 2: A) Unstained adult *Dicrocoelium dendriticum* elongated form with locations for morphometrical measurements labeled 1-8; Os= Oral sucker; Vs= Ventral sucker; Ts= testis; Ov= Ovary; Ve= Vetteline glands; Ut= Uterus; B) Photomicrograph of a condemned liver sample showing a longitudinal section of an adult fluke inside the hyperplastic and fibrosed bile duct (Stain: H&E); C) Photomicrograph of a condemned liver sample showing hyperplastic bile ducts with sections of adult flukes and blue stained prominent periductal fibrosis (Stain: Masson's trichrome); Arrowheads; Papillary proliferation of the bile duct mucosa with extensive inflammatory cells infiltrations; Asterisks: Severe thickening of the bile duct walls in the form of periductal fibrosis; Bar= 1mm.

Table 1: Prevalence of liver helminths detected in the Central Muscat Municipality Slaughterhouse during the period 2018-2021

Number of examined livers	<i>Hydatid cyst</i>	<i>Acute cysticercosis</i>	<i>Chronic cysticercosis</i>	<i>Dicrocoelium dendriticum</i>	<i>Fasciola hepatica</i>	<i>Stilesia hepatica</i>	Overall prevalence
984	1 (0.1%)	4 (0.4%)	6 (0.6%)	40 (4.2%)	2 (0.2%)	4 (0.4%)	57 (6%)

Table 2: Morphometric measurements of *Dicrocoelium dendriticum* elongated form recovered from condemned sheep livers

Parameter	<i>Dicrocoelium dendriticum</i> (n =10) Min-max (mean±SD)
Body	
1) Length	4.44-11.21 mm (7.00±2.24)
2) Width	1.24-2.41 mm (1.83±0.32)
3) External Oral sucker diameter	0.14-0.34 mm (0.25± 0.06)
4) External Ventral sucker diameter	0.32-0.55 mm (0.41±0.08)
Tests	
5) Length	0.43-0.76 mm (0.52±0.11)
6) Width	0.35-0.61 mm (0.43 ±0.09)
7) Length of Vitelline glands	1.22-2.63 mm (1.84 ±0.51)
8) Anterior to maximal body width	2.23-6.78 mm (3.95±1.65)

Livers infected with *S. hepatica* were apparently normal and the adult cestode were incidentally detected during the routine inspection of the bile ducts.

DISCUSSION

The Sultanate of Oman imported 1,054,038 live sheep and goats from various countries during the first half of 2020 to meet the high demand of the Omani markets. In this research we hypothesized that imported sheep could be a source of infections to either Omani livestock or humans in the country. We examined 948 livers from slaughtered sheep in the Central Muscat Municipality Slaughterhouse, Muscat, Oman, where different helminths and associated hepatic lesions in the condemned livers were identified. Examined sheep livers that were infected with *D. dendriticum* showed prominent whitish bile ductules due to cholangitis and scarring and marked fibrosis was confined to portal areas surrounding the bile ducts. The lack of migratory tracks in our examined specimens supports the observations of Massoud (1981) who mentioned that the lancet fluke enter the common bile duct through the intestinal opening rather than migration through the liver parenchyma. There were major lesions in the bile ducts, including extensive glandular hyperplasia, papilliform projections of the lining epithelium, and thick connective tissue capsules. Portal areas showed infiltration with lymphocytes, plasma cells, macrophages and few eosinophils as previously stated by Massoud (1981). The testicles are tandem in *D. dendriticum*, but bilateral in *D. chinensis* (Taira *et al.*, 2006; Taylor *et al.*, 2016). The examined flukes in our study had a tandem pattern, confirming the diagnosis of *D. dendriticum* induced dicrocoeliasis and consistent with other published data of Taira *et al.* (2006) and Otranto *et al.* (2007). In this study, all the condemned livers that were infected with *D. dendriticum* were from one shipment of 3000 live sheep imported from Romania. In similar study, Maraqa *et al.* (2005) reported a prevalence of 57.5% of *D. dendriticum* in sheep imported from Romania to Jordan. Another study conducted in Iran found that 1% of sheep and cattle, and 0.8% of goat livers were condemned in the abattoir due to *D. dendriticum* particularly in the autumn for sheep and cattle, and in winter for goats (Shahbazi *et al.*, 2016). In 2021, Manuchar *et al.* reported that 29.4% of sheep livers were rejected due to *D. dendriticum* in local and imported sheep in a slaughterhouse in Northern Iraq over a period of

5 months. Interestingly, Jithendran and Bhat (1996) reported an overall prevalence of 24.1% and 12.3% of dicrocoeliasis in sheep and goats slaughtered at an abattoir in Palampur, India, where many of which were exported to Oman. Spurious human dicrocoeliasis is observed more frequently following the ingesting of an infected raw animal liver with adult worms (Moure *et al.*, 2017). True infection is very rare and occurs following the accidental ingestion of infected ants and can lead to chronic cholangitis with adenomatous proliferation of the bile ducts (Cabeza-Barrera *et al.*, 2011). Thus, an importation of sheep from endemic areas without an accurate diagnosis at the entry point of the country could tremendously contribute to the spreading and establishing of new pathogens beyond the original borders country.

Although mature *C. tenuicollis* is a common finding in sheep and goats during meat inspection, the cysts are usually detected in the peritoneum, mesentery and omentum and to a lesser extent attached to the hepatic capsule. Previously, an abattoir study in Oman reported a prevalence of 1.9% *C. tenuicollis* cysts in livers of slaughtered caprine species (Johnson *et al.*, 1999). The authors did not report any case of acute cysticercosis in contrast to our findings where we detected acute cases of cysticercosis at 0.4% and chronic cysticercosis at 0.6% of the examined sheep livers. In another study, *C. tenuicollis* cysts were detected at 0.5% of the examined slaughtered sheep without determining the cysts locations (Al Kitani *et al.*, 2015). According to our study, we found one case (0.1%) of hydatid cysts in the examined livers, which is in line with the findings of Al Kitani *et al.*, (2015) who reported an infection rate of 0.07% in slaughtered sheep from four slaughterhouses in Oman. This low prevalence rate is probably attributed to the younger age of the slaughtered sheep which is usually less than one year old (Brik *et al.*, 2018). The prevalence of *S. hepatica* in this study was 0.4% which lead to the condemnation of the livers despite being nonpathogenic to the animals as well as to humans (Mungube *et al.*, 2006).

Conclusions: To the best of our knowledge, this is the first report of dicrocoeliasis in imported sheep in Oman. Several species of metazoan parasites were detected in this study, where some of them can affect the health condition of livestock and humans. Further studies are required to assess the economic losses due to helminthic-induced liver condemnations encountered in this study and active survey

to determine the situation of gastro-intestinal parasites in the living animals. Moreover, current regulations for importing live animals from the suspected countries need to be reassessed. The Omani authorities should take with caution the findings of this research to enhance their control program to prevent the establishment of exotic parasites in the country, as these parasites induce huge economical losses and health impairment in both animals and humans.

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Authors contribution: HA, EHJ and EIE designed the study and secured the research fund. HA, EIE, AA, ME, MNA executed the experiment. HA and EIE analyzed the data and interpreted the results. HA drafted the manuscript, EIE and MNA critically revised the manuscript. All authors have read and approved the manuscript.

Conflict of interest statement: The authors declare that they have no conflict of interest.

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