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Prevalence of *Echinococcus granulosus* (Cestoda: Taeniidae) Infection in Stray Dogs and Local Herbivores in Al-Kharj, Saudi Arabia

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ABSTRACT

Background: *Echinococcus granulosus* is a cyclophyllid cestode parasite that lives as an adult in the intestine of canids and is the most common cause of hydatidosis in ruminants (intermediate hosts). It also offers a public health risk because of the possibility of human infection. The incorrect disposal of dead animals, access of farm dogs to the internal organs of sheep and grazing in areas with wayward dogs are the most common production methods that might increase the risk of sheep being exposed to hydatidosis. The purpose of this study was to investigate the natural spread of *E. granulosus* infection in wayward dogs, as well as the sterility and fertility rates of hydatid cysts in herbivores of Al-Kharj (Saudi Arabia).

Methods: Fecal samples from 150 dogs were collected from several locations in Al-Kharj (Saudi Arabia) where livestock rearing takes place and the existence of wayward and semi-feral canines from August 2021 to February 2022. Parasitic eggs were recovered from fecal samples using the flotation method, followed by centrifugation at 1200 × g. Examination of slaughtered animals was done by comprehensive visual examination, palpation and incision of the internal organs, including lungs, liver, muscles, kidneys and heart. All hydatid cysts detected in the organs were collected for cyst count, cyst fertility testing and protoscolices viability testing. Examination of cysts and viability of protoscolices. A 0.1% aqueous solution of eosin stain was used to examine the survival of protoscolices. According to Smyth and Barret (1980), deceased protoscolices became stained (purplish-red color) five minutes after exposure to staining, whereas living protoscolices remained colorless (recorded as alive) under the microscope.

Result: 1698 sheep, 450 goats and 240 camels (slaughtered in Al-Kharj Abattoir) were examined as to their livers, lungs, muscles, kidneys and hearts were checked for hydatidosis. In a study of 150 dogs, 21 (14%) were shown to be naturally infected with *E. granulosus* eggs. Hydatid cysts were also identified in 156 (9.18%) sheep, 36 (7.5%) goats and 24 (10%) camels. In comparison to other tissues, the liver and lungs had the highest percentage of infestation, with values of 50.6% and 25.6% in sheep, 58.3% and 16.6% in goats and 66.6% and 16% in camels, respectively. The older age groups had higher infection rates than the younger ones. The highest percentage was seen in sheep over the age of 12 months (10.4%) and camels over the age of 6 years (12%). Hydatidosis is a serious economic and zoonotic problem. Therefore, we should take preventative measures in Al-Kharj city to avoid environmental pollution and infection of both humans and animals.

Key words: Dogs, *Echinococcus granulosus*, Herbivores, Zoonosis.

INTRODUCTION

Hydatidosis is an important economic and zoonotic disease caused by the adult *Echinococcus* metacestode. Adult tapeworms in dogs and a range of domestic pet intermediate hosts, such as cattle, sheep, goats and camels, keep the parasite's domestic life cycle going (Sher *et al.,* 2006). Infected dogs can shed enormous numbers of parasite eggs in their feces, polluting various types of soil and spreading disease due to the high biological potential of *E. granulosus* (Gemmell, 1990).

Because of the numerous occurrences of damaged organs, hydatidosis has become an economic burden in many regions of the world. It also offers a public health risk because of the possibility of human infection (Daryani *et al.,* 2007). Hydatidosis in slaughtered animals is detected by postmortem testing. Hydatid cysts are most commonly found in livestock's liver, lungs and spleen (Almalki *et al*., 2017). The incorrect disposal of dead animals, enabling access to farm dogs with the internal organs of sheep, the disinterest of farmers in treating farm dogs for worms and grazing in areas with wayward dogs are the most common production methods that might increase the risk of sheep being exposed to hydatidosis (Christodoulopoulos *et al.,* 2008).

Echinococcus granulosus is common in Iran, Turkey, Iraq, Morocco, Oman, Tunisia and Libya (Sadjjadi, 2006).

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Hydatid cysts are often seen in sheep, cattle, goats and camels throughout the Middle East and North Africa, according to many surveys (Sadjjadi, 2006). The majority of slaughterhouse research and surgical examinations in Saudi Arabia have been conducted on hydatidosis in both livestock and humans (Almasri *et al*., 2019). The disease is more frequent in rural populations in endemic regions and it is mainly contracted through contact with sheep and dogs (El-Metenawy, 1999).

Due to a lack of knowledge on *E. granulosus* infection in Al-Kharj city (Saudi Arabia), this study was conducted to investigate its natural incidence in stray dogs and some herbivores animals.

MATERIALS AND METHODS

Study and sampling of dog feces

Fecal samples from 150 dogs were collected from several locations in Al-Kharj (Saudi Arabia) where livestock rearing takes place with the existence of wayward and semi-feral canines. About 14 samples were taken from the ratio of feces seen over a 150-300 m^2 soil surface at each location, placed in a specimen bed and kept at 4°C until analyzed. The sampling was done to demonstrate the *Echinococcus granulosus* eggs that were available in the region, not to count the number of solitary dogs. From August 2021 to February 2022, many locations were visited once a month and data was gathered. According to Teuscher (1965) and Dryden (2005), parasite eggs were recovered from fecal samples using the flotation method, followed by centrifugation at 1200 × g. Because the sample did not harm dogs captured overland, no specific notification to an ethics commission was necessary.

Examination of slaughtered animals

The research involved an energetic slaughterhouse survey, which included sheep, goats and camels transported to the Al-Kharj abattoir for slaughter from diverse locations. After a comprehensive visual examination, palpation and incision of the internal organs, including the lungs, liver, muscles, kidneys and heart, a total of 2418 animals (1698 sheep, 480 goats and 240 camels) were investigated postmortem for the presence of hydatid cysts. A veterinarian oversaw the procedure. The research was carried out following FAO/UNEP/WHO (1994). All hydatid cysts detected in the organs were collected and sent to the laboratory of Parasitology (Department of Zoology, College of Science, King Saud University) for cyst count, cyst fertility testing and protoscolices viability testing.

Examination of cysts and viability of protoscolices

The existence of deterioration and calcification in cysts was checked first. For fertility studies, certain cysts were chosen at random. A needle was inserted into the cyst's wall and opened using a scalpel and scissors to release pressure on the cyst. Microscopically, the contents were examined for the existence of protoscolices. The germinal layer was also examined for the emergence of protoscolices. Severe turbulence or calcified cysts that do not contain protoscolices are considered sterile. A 0.1% aqueous solution of eosin stain was used to examine the survival of protoscolices. According to Smyth and Barret (1980), deceased protoscolices became stained (purplish-red color) five minutes after exposure to staining, whereas living protoscolices remained colorless (recorded as alive) under the microscope.

Histopathological examination

Tissue samples from the affected liver were quickly fixed for at least 24 hours in % neutral buffered formalin. The fixed sample was trimmed, washed, dehydrated with rising ethyl alcohol, then clarified with xylene and embedded in paraffin wax. According to Bancroft *et al.* (2013), sections of 5 µm were cut, stained with hematoxylin and eosin and then examined microscopically.

RESULTS AND DISCUSSION

Natural prevalence of *E. granulosus* eggs in stray dogs

The prevalence rates among 150 fecal samples of stray dogs from different localities of Al-Kharj, 21 (14%) were infected with *E. granulosus* eggs (Table 1). The prevalence of *E. granulosus* in dogs, as well as its metacestode in herbivores, varies greatly over the world (Matossian *et al*., 1977). Studies from several parts of Saudi Arabia have established that *E. granulosus* and Hydatid worms are prevalent there (El-Metenawy, 1999; Hayajneh *et al*., 2014; Almalki, 2021). Almalki (2021) indicated that the frequency of *E. granulosus* in the feces of stray dogs from the city of the Taif region was 15.5%. This might be owing to wild dogs having unrestricted access to potentially harmful materials, as well as the fact that dogs are extensively parasitized animals due to a lack of anthelmintic therapy. Humans in Al-Kharji are in increasing danger of contracting a range of gastrointestinal parasites, some of which are zoonotic diseases carried readily by stray dogs.

Characterization of *E. granulosus* eggs

The eggs are spherical and yellow in color, about 30-40 ìm in diameter and resemble the eggs of other tapeworm species. They contain the embryo of a hexacanth or oncosphere. The eggs are protected by transparent coats that include a sticky covering that adheres to animal hair and other materials that aid in their survival, as well as an inner layer (thick radially striated embryophore) (Fig 1).

Natural prevalence of hydatidosis in herbivores

For hydatidosis testing, 1698 sheep, 480 goats and 240 camels were used. Hydatid cysts were discovered in 156

Table 1: Number of fecal samples of stray dogs infected with *E. granulosus.*

Animal species	Health status		Total
	Infected	Non-infected	
Stray dogs	21 (14%)	129 (86%)	150 (100%)

(9.18%) sheep, 36 (7.5%) goats and 24 (10%) camels. The magnitude of the disease between the species was significant (Table 2). Camels were found to have a higher prevalence (10%) than sheep (9.18%) and goats (6.56%). However, the prevalence rates in camels, sheep and goats were lower in this research than in the study of Ibrahim (2010) in the Al Baha region. The prevalence of *E. granulosus* infection in sheep was 13.5% higher in the prior study in Al Taif than in our study. The higher percentage of parasite infection in dogs recorded in Al Taif (15.5%) compared to Al-Kharj (14%) was the explanation for the disparities between these findings and those of the previous research. Variability in frequency between animal species may be linked to *E. granulosus* strain differences in general (McManus, 2006). Metwally *et al*. (2018) followed by Al-Mutairi *et al*. (2020) reported the presence of *Echinococcus granulosus* (genotypes G1-G3) in sheep and camel.

Table 2: Number of sheep, goats and camels infected with hydatid cyst slaughtered in Al-Kharj abattoir.

Animal species	Health status		Total
	Infected	Non-infected	
Sheep	156 (9.18%)	1542 (90.8%)	1698 (100%)
Goats	36 (7.5%)	444 (92.5%)	480 (100%)
Camels	24 (10%)	216 (90%)	240 (100%)

In sheep, goats and camels, the distribution and number of organs infected with hydatid cysts have been described (Fig 2, Table 3). In sheep, liver, lung, muscles, kidney and heart were all involved in 50.6%, 25.6%, 9.6%, 6.4% and 7.6%, respectively; in goats, 58.3%, 16.6%, 13.8%, 5.5% and 5.5%; and in camels, 66.6%, 16%, 8.3%, 0.0% and 8.3%. Hydatid cysts are primarily detected in the liver, lungs and muscles, according to the findings of this study. The livers and lungs of sheep, goats and camels were shown to be more commonly infected with hydatid cysts than the muscles, kidneys and heart, which was consistent with Kamhawi *et al*. (1995) and Haridy *et al*. (2006). The liver and lungs are the first large capillary sites encountered in the biosphere of migrating *Echinococcus* eggs (hexacanth embryos) via the portal vein pathway and they predominantly mediate the sequence of filtration systems liver and lungs before any other peripheral organs are involved (Kebede *et al*., 2009).

The number of hydatid cysts in animals of various ages was assessed (Table 4). In sheep and goats, the age of animals was divided into two groups: G1<12 months and G2>12 months. In camels, the age of animals was divided into two groups G3<5 years and G4>5 years. Sheep and goats were the oldest animals reported at 36 months, while

Table 3: Organs infected with hydatid cysts in sheep, goats and camels.

Animal species	Organs					Total
	Liver	Lung	Muscles	Kidney	Heart	
Sheep	79 (50.6%)	40 (25.6%)	15 (9.6%)	10 (6.4%)	12 (7.6%)	156 (100%)
Goats	21 (58.3%)	6 (16.6%)	5 (13.8%)	2 (5.5%)	2 (5.5%)	36 (100%)
Camels	16 (66.6%)	4 (16%)	2 (8.3%)	0 (0.00%)	2 (8.3%)	24 (100%)

Table 4: The relationship between age and health status in sheep, goats and camels.

Age group	Animal species	Health status		Total
		Infected	Non-infected	
1	Sheep	40 (6.74%)	551 (6.74%)	591 (100%)
	Goats	27 (8.85%)	278 (91.1%)	305 (100%)
2	Sheep	116 (10.4%)	991 (89.5%)	1107 (100%)
	Goats	9 (5.1%)	166 (94.8%)	175 (100%)
3	Camels	18 (9.4%)	172 (90.5%)	190 (100%)
4	Camels	6 (12%)	44 (88%)	50 (100%)

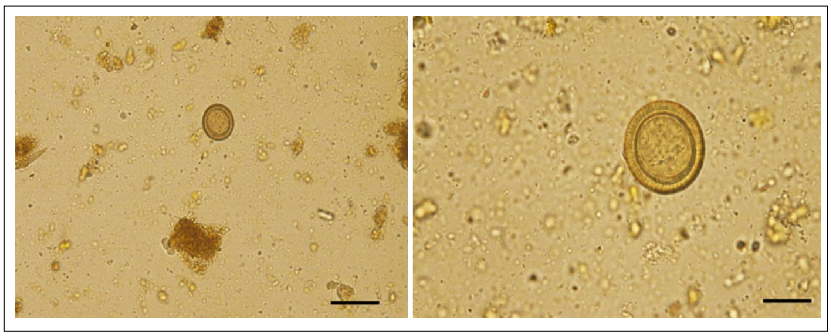

Fig 1: *Echinococcus granulosus* egg in stray dogs feces observed under an optical microscope 20× and 100×. Scale bar= 20 µm.

Table 5: Type of hydatid cyst (sterile, fertile and calcified) in different organs of sheep, goats and camels slaughtered in Al-Kharj abattoir.

Animal species	Cyst			Total
	Fertile	Sterile	Calcified	
Sheep	89 (57%)	44 (28.2%)	23 (14.7%)	156 (100%)
Goats	15 (41.6%)	16 (44.4%)	5 (13.8%)	36 (100%)
Camels	8 (33.3%)	13 (54.1%)	3 (12.5%)	24(100%)

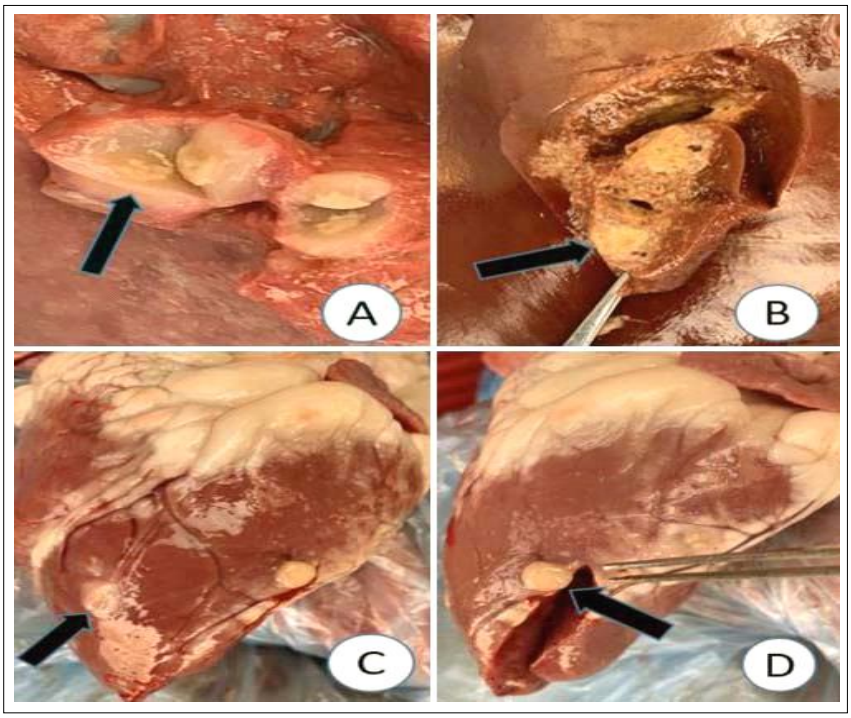

Fig 2: Hydatid cysts in sheep; caseous cyst with a cavity filled with a thick matrix of cheesy consistency in the lung (A), calcified cyst in the liver (B) multiple hydatid cysts in the heart (C and D).

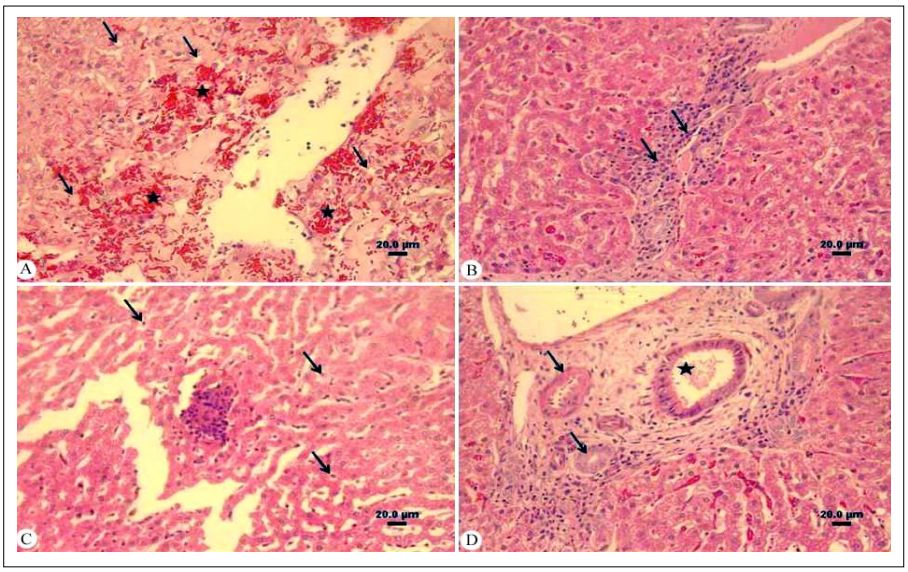

Fig 3: Liver of sheep with hydatidosis infection. (A) Focal steatosis (arrows) and multi-spot of hemorrhage (asterisk). (B) Chronic inflammation with diffuse infiltration of lymphocytes (arrows). (C) Periportal fibrosis (arrows). (D) Increase of thickness wall of the vein (arrows), Congestion of vein (asterisk). Scale bar= 20 µm.

7 years old for camels. The infection rates and intensity were higher in the older age groups. Varcasia *et al*. (2007), Hayajneh *et al*. (2014) and Almalki (2021) reported a positive correlation between infection intensity and host-age group and the age-dependent increase in infection rate among investigated animals.

The cysts were examined for fertility, sterility, calcification and viability (Table 5). The sheep had a higher fertility rate (57%) than goats (41.6%) and camels (33.3%). The difference in fertility and rates of viable protoscolices from fertile cysts may be connected to the variation in per-host immune responses, according to the data (57% fertile, 28.2% sterile and 14.7% calcified cysts in sheep). Furthermore, genotype may influence the fertility of hydrated cysts in the intermediate host (McManus, 2006). Unfortunately, no studies on per-host hydrated cyst genotype have been conducted in this region.

Histopathological examination

The livers of sheep infected with hydatidosis revealed localized steatosis and a multi-spot hemorrhage. Chronic inflammation with lymphocyte infiltration, periportal fibrosis with increased wall thickness and venous congestion (Fig 3). The parasite's protoscolices were observed in liver sections of hydatid-infected animals during the histopathologic investigation. Mononuclear cells such as lymphocytes and neutrophils made up the majority of cellular infiltrations. Hypersensitivity responses in cells associated with hydatid cysts were delayed. Mnati *et al*. (2020) described lesions associated with hydatid cysts, which were similar to our findings.

CONCLUSION

It may be concluded that the current study demonstrated significant findings of intestinal helminths *Echinococcus granulosus* in the stray dogs which were examined since these serve as hosts for a variety of enteric parasites. As a result, preventative measures must be taken in the Al-Kharj region to avoid environmental pollution and infection of both humans and animals.

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Conflict of interest: None.

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