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Original papers

Prevalence and histopathological study on cystic hydatidosis in heart and spleen of goat slaughtered at Makkah, Saudi Arabia

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ABSTRACT. Hydatidosis or echinococcosis is considered to be one of the most common zoonotic diseases of the animals. Infection occurs when intermediate hosts such as camel, cattle, sheep, and goats ingested food or water contaminated with eggs from the definitive host (dog). This is a cross-sectional study which was carried out in one of the biggest abattoirs in Makkah in the west of Saudi Arabia. A total number of 38302 goats were examined and recorded at Makkah abattoirs. The examination had been performed to all slaughtered animals on two organs (spleen and heart) for detection of any hydatid cysts during the period from July 2018 until December 2018. The study included also histopathological tissue evaluation. The total infections number of hydatidosis in goats is 0.23%. The infected hearts were 40.35% whereas the infected spleen was 48.48% subsequently in local animals. The imported animals were 2124, the infected animals in heart were 59.64%, whereas the infected animal involving spleen were 51.51%. Meanwhile, results of histopathological examination had shown that most of the hydatid cysts in goats caused progressive focal pressure and degenerative changes in the surrounding tissue.

Keywords: hydatid cyst, goats, heart, spleen, histopathology, Saudi Arabia

Introduction

Hydatidosis is an economic problem facing the Kingdom of Saudi Arabia (KSA) because of many condemnations for infected organs. In addition, it results in a public hazard effect because of the possible transmission to the human beings [1]. This disease is a worldwide lethal zoonosis caused by adult or larval stages of tapeworms of the genus *Echinococcus* [2]. Although, 12 species have been recognized, only four are of public health concern and produce human pathology: *Echinococcus granulosus* (cystic echinococcosis), *E. multilocularis* (alveolar echinococcosis), *E. vogeli*, and *E. oligarthrus* (both causing polycystic echinococcosis). The first two species are etiological agents of life-threatening diseases, having high fatality rate and poor prognosis if careful clinical management is not given [3]. Definitive hosts such as dogs and other carnivores' animals shed eggs in the feces. The intermediate hosts got infection when ingested food or water contaminated with eggs [4].

Humans act as accidental intermediate hosts. In intermediate hosts, the disease is usually detected at *post mortem* inspection. The hydatid cysts grow slowly and take several years to cause symptoms. Liver and lungs are the most common sites of the cysts but could be found in other organs such as spleen, heart, and kidneys [5].

Several studies indicated that hydatid disease is an endemic zoonotic disease in KSA affecting both human and their domestic animals. The prevalence of hydatidosis in sheep was 69.6% in Jeddah [6], 12.61% in Al-Baha [7,8], 6.8% in Najran [9], 13.5% in Al-Taif [10] and 2.83 % in Dammam [1].

Hydatid cysts are mainly located in liver or lungs and may cause pathological damages in these tissues [1]. Extrahepatic abdominal involvement may be primary or secondary. Hydatid infection of the heart is rare, and the clinical presentation is usually insidious but there the lethal hazard of cyst is perforation [11]. Early diagnosis and treatment are critical. On the other hand, hydatid spleen is the most common site after liver in abdomen. Mainly 1.5–3.5% of all cases of abdominal hydatidosis have been reported due to splenic hydatid cyst [12,13].

Some studies have been conducted on the prevalence of cystic hydatidosis in slaughtered animal in different areas of Saudi Arabia, but few or rare studies have addressed the histological appearance. Therefore, the current study was conducted to evaluate the histopathological investigations on hydatidosis among slaughtered goats at Makkah area, Saudi Arabia.

Materials and Methods

Study area. This is a cross-sectional study which was carried out in one of the biggest abattoirs in Makkah province in west of Saudi Arabia. Examination of the slaughtered goats to detect hydatid cysts in heart and spleen was done periodically in the abattoir.

Sample size. A total number of 38302 goats were inspected and recorded at Makkah abattoirs for detection of any hydatid cysts during the period from July 2018 until December 2018.

Slaughtered animals' inspection. Post-mortem examination of the slaughtered animals was carried out by veterinarians through visual inspection of the offal, palpation and incision of visceral organs including particularly the spleen and heart according to the procedure recommended by FAO/UNEP/WHO [14].

Histopathological and histochemical examination. Specimens were grossly examined and then investigated under the microscope to evaluate the histopathological morphology of the hydatid cyst and any other tissue alteration should the situation required.

Section for the wall of the cyst with the neighbouring heart and spleen tissue were taken and fixed in 10% neutral buffer formalin solution. After complete fixation has been assured, gradual dehydration of samples was done using different grades of alcohol (ascending grades). Then, the samples were transferred into xylene for clearance and embedded in melted paraffin wax. Sections of 5-micron thickness were prepared and stained with Haematoxylin and Eosin (H&E) and for histochemical analysis were stained using Periodic Acid Schiff stain (PAS) and Masson's trichrome stain [15].

Immunohistochemical study. Sections stained using markers for CD3 and counter stained with Hx according to the Alborg pathology lab Saudi Arabia protocol. CD3 expression was nuclear immuno-positive reaction [16].

Statistical analysis. Effect of goat origin, type of tissue, and sex on hydatid infection and the prevalence for hydatidosis were analysed by the Proc Frequency procedure (SAS, Institute, Inc, 2004). The Pearson's $\chi 2$ (Chi square) statistics were calculated according to Steel and Torrie, [17]. The corresponding histograms were graphed using Microsoft Office Excel program (2007).

Results

Prevalence of cystic echinococcosis in slaughtered animals

This study was conducted on specimens removed from heart and spleen of the studied animal. In the current study, the results recorded in (Tables 1,2 and Fig. 1,2) revealed that the overall prevalence of infected goat at Makkah abattoirs was

Table 1. The overall prevalence of hydatidosis among goat

Normal animals		Infected animals	
No	[%]	No	[%]
38.212/38.302	99.76	90/38.302	0.23

Table 2. Prevalence of hydatidosis in goat origin with regard to their sex

	Sex	Normal		Infected	
		No	[%]	No	[%]
Local	Male	9500	55.97	12	30.76
	Female	7472	44.02	27	69.23
	Total	16972	44.41	39	43.33
Imported	Male	12720	59.89	36	70.59
	Female	8520	40.11	15	29.41
	Total	21240	55.58	51	56.67
Total		38212	99.76	90	0.23

Chi Squares for the effect of sex on hydatid prevalence (Value = 14.079) is high significant (P = 0.000).

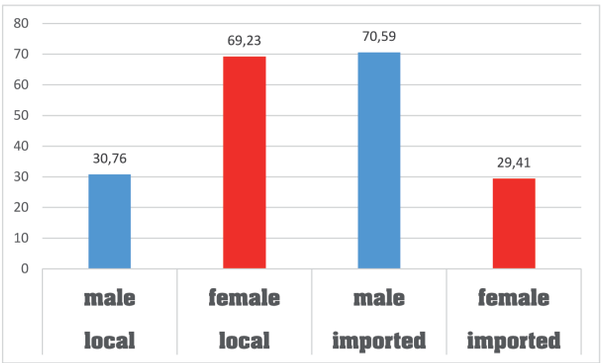


Fig. 1. Effect of goat sex on the prevalence of hydatidosis (%)

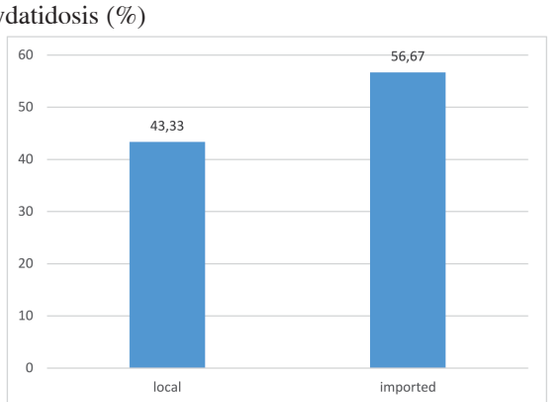


Fig. 2. Effect of goat origin on the prevalence of hydatidosis (%)

0.23% regardless of sex or goat origin. The prevalence was 43.33% in local goat, while in the imported one was 56.67%. The prevalence of hydatidosis in male local goat was 30.76%, but in male imported goat was 70.59%, while the prevalence in female local goat was 69.23and in female imported goat was 29.41% (Table 2; Fig. 1,2). Female goat had higher infection rate than males within the local area. Increased significant difference was observed for the effect of sex on the prevalence of hydatidosis in local and imported goat at ($P<0.05$). The results recorded in (Table 3; Fig. 3,4) revealed the prevalence of hydatid cysts in relation to the organ infected (heart or spleen) in local sheep where the percentages were 40.35% (heart) and 48.48% (spleen), while 59.64% (heart) and 56.67% (spleen) for imported, while the overall prevalence of hydatidosis in heart in local goat was 63.33%, meanwhile the percentage was 36.67% (spleen) for imported one. In the current study, the prevalence of the hydatid cyst was observed in the heart more than in the spleen. Also, we observed that the sex had effect on the prevalence of the hydatidosis among the goats.

Cysts appeared of variable sizes and round shapes. Some of the cysts are seen bulging from the surface or deeply seated in the heart or the spleen tissue. The cyst contained cavities had a smooth membrane (Fig. 5).

Histopathological examination had been done not only to evaluate the histological structure of the hydatid cyst but also to evaluate the tissue changes that related to hydatid cyst infection.

H&E examination

The H&E stained sections showed the detailed structure of hydatidosis as it is shown in Figures 6–8. The cyst wall was appeared consisted of three layers. The outer layer, known as fibrous capsule, which represents the host response to the parasite. The middle layer is acellular eosinophilic laminated membrane (laminated layer). The inner germinal layer is thin and with protoscolices. The cyst contained active germinal layers and broad capsules with scolices. The cysts that we have investigated in our study had more than one scolex and with characteristic birefringent hooks. The fibrous layer appeared with heavy infiltration of inflammatory cells and extravasated RBCs. Notice, area of depletion of lymphocytes.

In the current study, H&E examination of the stained heart sections (Fig. 9) showed irregular arranged widely separated cardiac muscle fibers with perinuclear vacuolization and areas of pale

Table 3. The prevalence of hydatidosis in both local and imported goat organs and as regard the sex

	Sex	Heart		Spleen	
		No	[%]	No	[%]
Local	Male	7	30.34	5	31.25
	Female	16	69.56	11	68.75
	Total	23	40.35	16	48.48
Imported	Male	22	64.70	14	70.59
	Female	12	40.11	3	29.41
	Total	34	59.64	17	56.67
Total		57	63.33	33	36.67

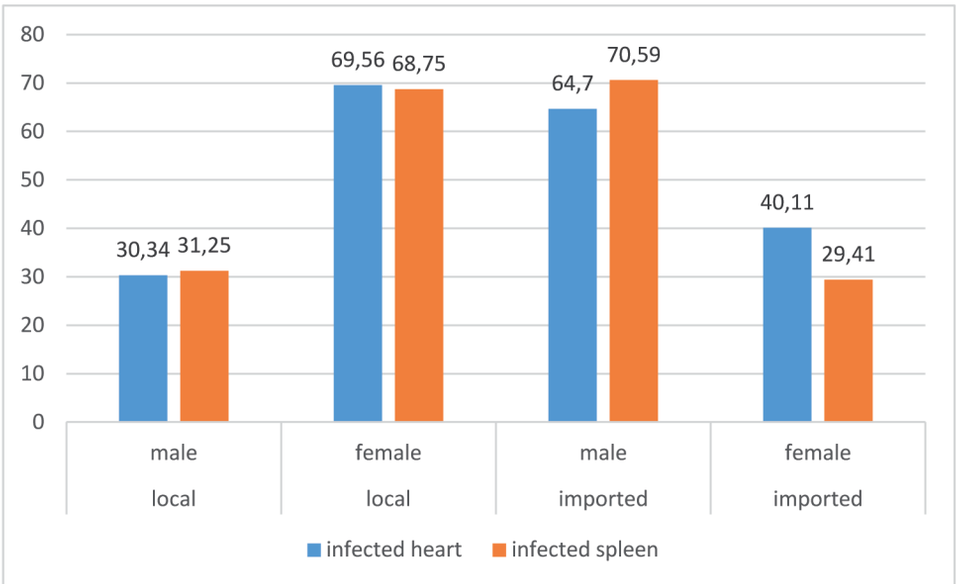


Fig. 3. The incidence of hydatidosis in the goat organs (heart and spleen) as regard sex and goat origin (%)

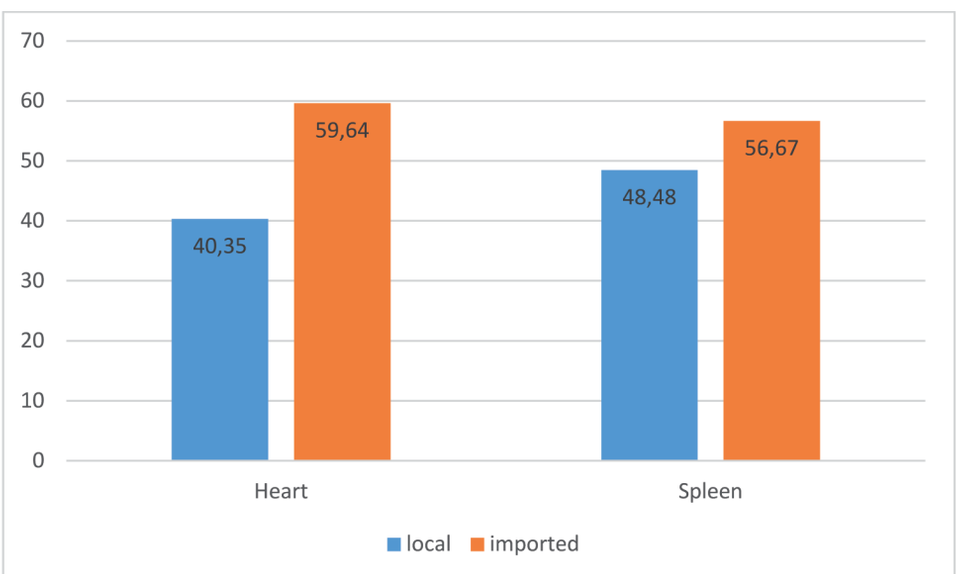


Fig. 4. The incidence of hydatidosis in the goat organs (%)

Fig. 5. Hydatid cyst separated of infected goats

acidophilic sarcoplasm (Fig. 10).

H&E stained sections revealed that the histological structure of the hydatid cyst in the spleen was principally similar to that seen in the heart. The cyst appeared with three layers.

Thick capsule of the spleen with area of depletion of the lymphoid cells in the white pulp was observed (Fig. 11A). Sections of the spleen around the hydatid cyst revealed Disrupted parenchymal architecture with loss of white pulp in some areas and other with irregular arrangement of white pulp scattered irregularly in a background of red pulp. Thick connective tissue septa are detected. Red pulp

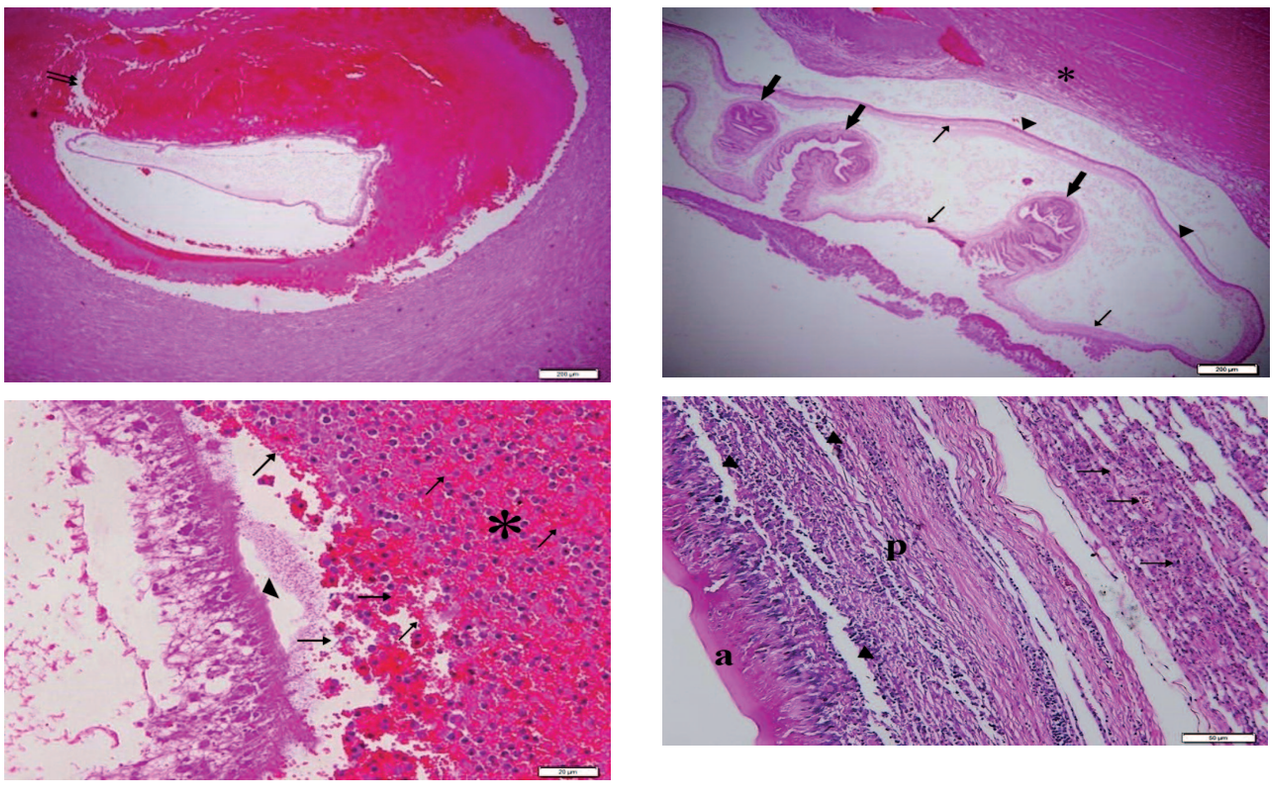


Fig. 6. H&E stained sections from heart of goat showing: A: Section of the hydatid cyst with laminated wall and areas of depletion of lymphocytes (↑); B: Three protoscolices inside the hydatid cyst with characteristic birefringent hooks (thick arrow). Laminated wall of the hydatid cyst appeared outermost fibrous layer (*), middle hyaline acellular laminated layer (▲) and inner germinal layer with protoscolices (↑); C: Eosinophilic acellular cuticular membrane (▲) and thick fibrous layer heavily infiltrated with inflammatory cell lymphocytes and eosinophils (*). Notice, extravasated RBCs between the inflammatory cells (↑);D: Eosinophilic acellular laminated layer (a) and thick fibrous layer (p) with heavy infiltrated inflammatory cells and extravasated RBCs (↑). Notice, area of depletion of lymphocytes (▲).

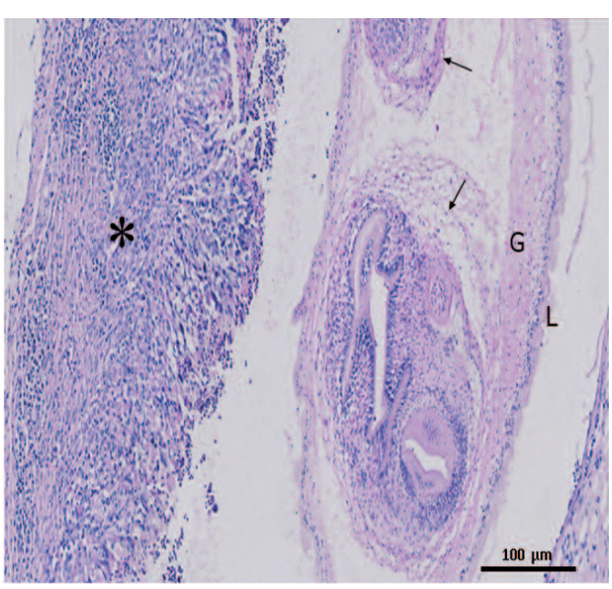


Fig. 7. H&E stained sections from heart of goat showing three protoscolices inside the hydatid cyst. Laminated wall of the hydatid cyst appeared outermost pericyst layer (*), middle hyaline acellular layer (L) and inner germinative layer with protoscolices (G).

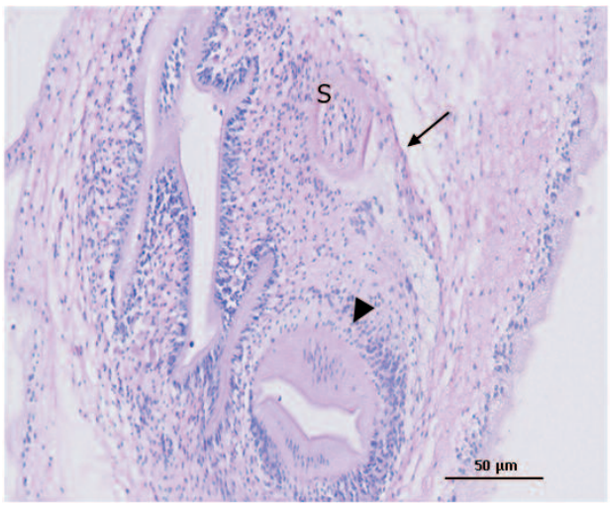
of spleen with megakaryocytes (↑↑) and dilated splenic sinusoids were also detected in some specimens (Fig. 11B,C).

Histochemical study

Using Masson trichrome stain, the collagen fibers in the hydatid cyst capsules, perivascular region, in between the cardiac muscles, and spleen capsules were seen. The collagen fibers content and the acellular laminated membranes took the green color (Fig. 12,13). Moreover, stained sections with PAS showed the laminated membranes, germinal layers, and protoscolices with positive PAS magenta color (Fig. 14).

Immunohistochemical findings

Slides stained with markers for CD3 showed positive reaction for CD3.The reaction was obviously seen in the cyst wall cells and infiltrating cells in tissue in close proximity to the cyst wall. The reactions manifested by scattered brown stained cells in sections obtained from the slaughtered



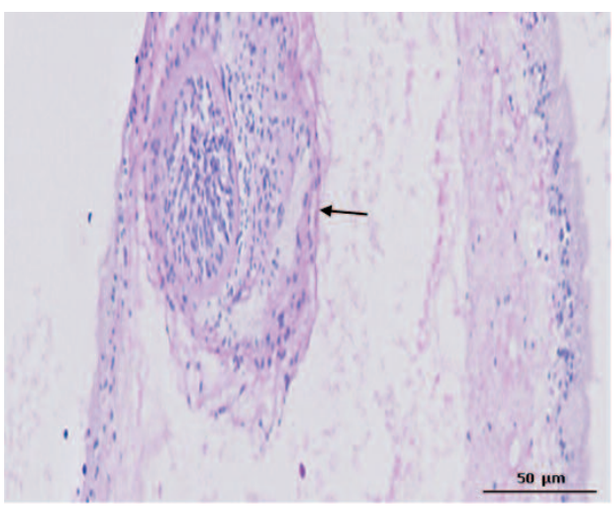


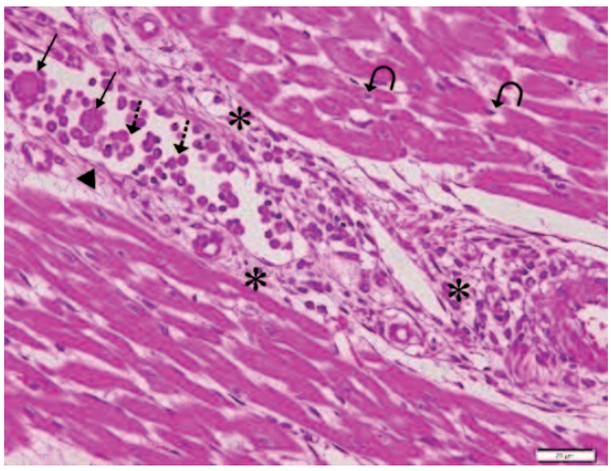
Fig. 8. Higher magnification shows the structure of protoscolex

animals (Fig.15,16).

Discussion

Hydatidosis is an economic problem impact in livestock because of many condemnations for infected organs. In addition, it results in a public hazard effect because of the possible transmission to the human beings. Therefore, it is reasonable to find reliable data for monitoring epidemiologic aspects of disease and prepare a concrete data for future comparison. Although abattoir surveys have restrictions and are not optimal sources, they are an economical way of collecting information on livestock disease [1].

Infected intermediate hosts are usually asymptomatic. Ultrasonography is done in only few cases, otherwise no accurate method used for the routine diagnosis of the infection in living animals [10,18]. The abattoirs are the best places to survey



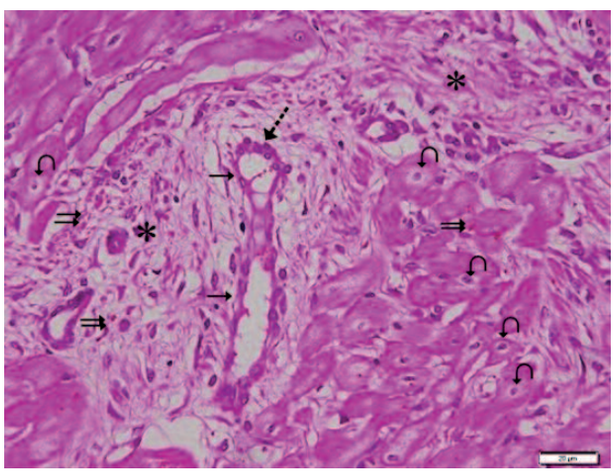


Fig. 9. H&E stained sections from heart of goat showing: A: Fertile hydatid cyst (▲) with active germinal layer. Broad capsule contained scolices (↑). Hydatid sand (—>) appeared inside the hydatid cyst. Outer pericyte layer (*) is heavily infiltrated with inflammatory cells. Irregular arranged widely separated cardiac muscle fibres with perinuclear vacuolization (curved arrow) appear. B: Hydatid cyst (↑) with active germinal layer contained scolices (—>).
Outer fibrous layer (*) is heavily infiltrated with inflammatory cells. Irregular arranged widely separated cardiac muscle fibres with perinuclear vacuolization (curved arrow) appear.
Notice extravasated RBCs (↑↑).

hydatidosis in livestock because diagnosing hydatidosis and determining its prevalence occurred in various species of slaughtered animals. Diagnosis occurred through meat examination and *post-mortem* investigation [9]. In the current study, one of the biggest abattoirs in Makkah area in west of Saudi Arabia has been visited to monitor the goats and to collect specimens from suspected animals. The abattoir was visited to examine for the presence of hydatid cysts in heart and spleen of the goats subjected to this study.

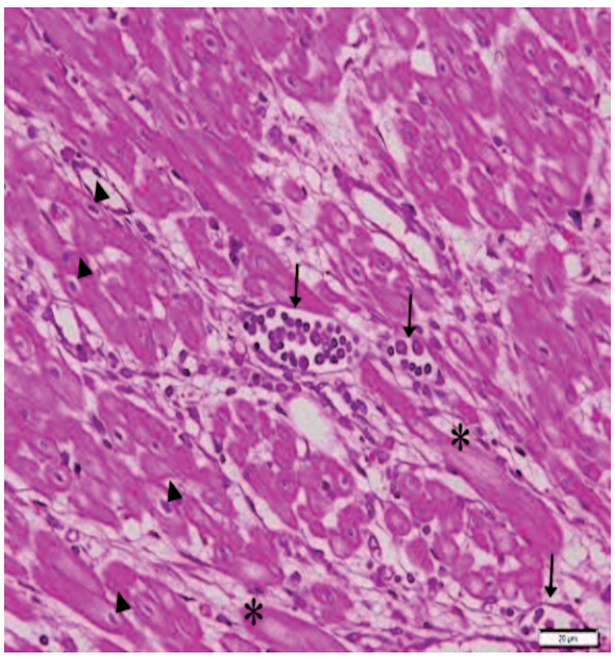


Fig. 10. H&E stained sections from heart of goat showing hydatid cyst (↑) with active germinal layer contained scolices. Irregular arranged widely separated cardiac muscle fiberswith perinuclear vacuolization (▲) appear. Notice, areas of pale acidophilic sarcoplasm (*).

To us, it is well known that most pathogenesis for majority of hydatidosis affected animals is nearly similar. The cysts cause focal lesions at the sites of predilection where it is finally implanted. The most common site of infection in the liver and lungs in goats. Also, they may occur in other locations including the spleen, soft tissue, heart, and spinal extradural space [19]. Because the devastating number of the previous studies have been performed on liver, lungs or other epithelial tissues, we have investigated the heart and spleen of the goats to find out the percentage and the frequency of hydatosis in the slaughtered goats.

This study was conducted on specimens removed from heart and spleen of the slaughtered animals. In the current study, the total infections rate of hydatidosis in goats is 90 (0.23%). This is no doubt is low prevalence and when review the literature and compared with other disposed animals to infections such as cattle, camels, and sheep we found some similarities. Therefore, our findings were in accordance with Shahbazi et al. [20]. who reported that a significant difference in the prevalence of hydatidosis among studied animals with higher prevalence in cattle than sheep, with the

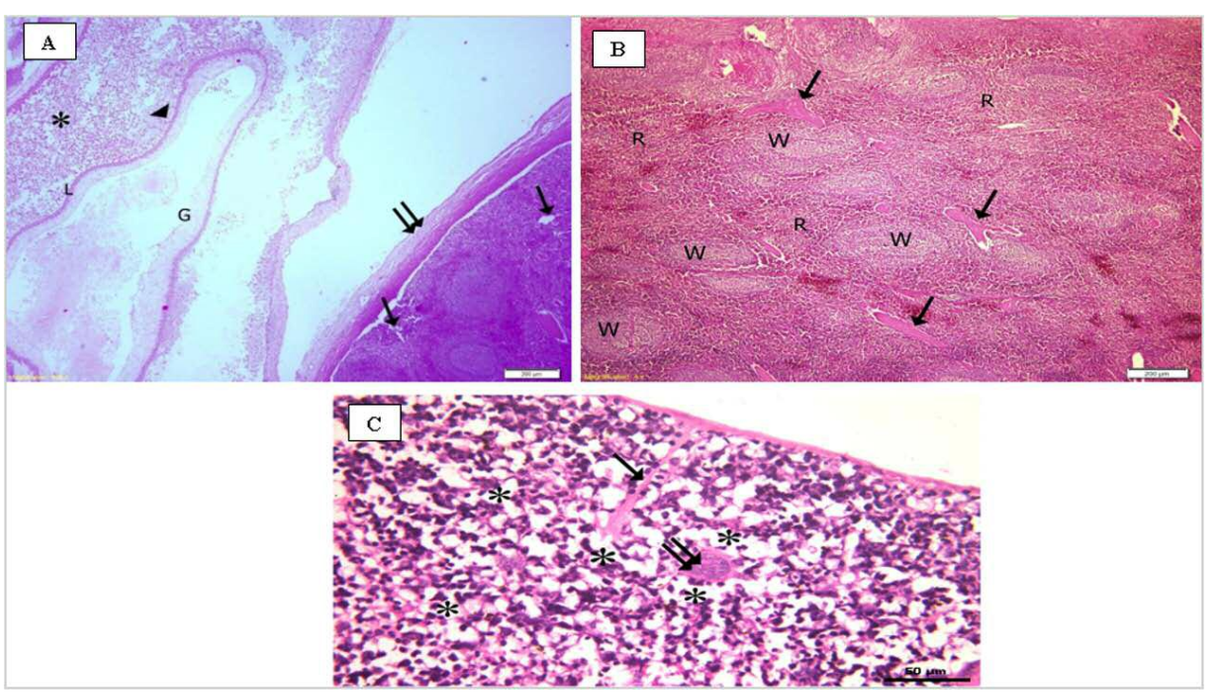


Fig. 11. H&E stained sections from spleen of goat showing: A: Hydatid cyst (▲) with active geminal layer (G). Outer fibrous capsular layer (*) is heavily infiltrated with inflammatory cells and middle eosinophilic acellular laminated layer (L). Spleen shows thick capsule (↑↑) and areas of depletion of the lymphocytes in the white pulp (↑). B: Disrupted parenchymal architecture with loss of white pulp in some areas and other with irregular arrangement of white pulp (W) scattered irregularly in a background of red pulp (R). Thick connective tissue septa are detected (↑). C: Thick connective tissue septa (↑), red pulp of spleen with megakaryocytes (↑↑) and dilated splenic sinusoids (*) are detected.

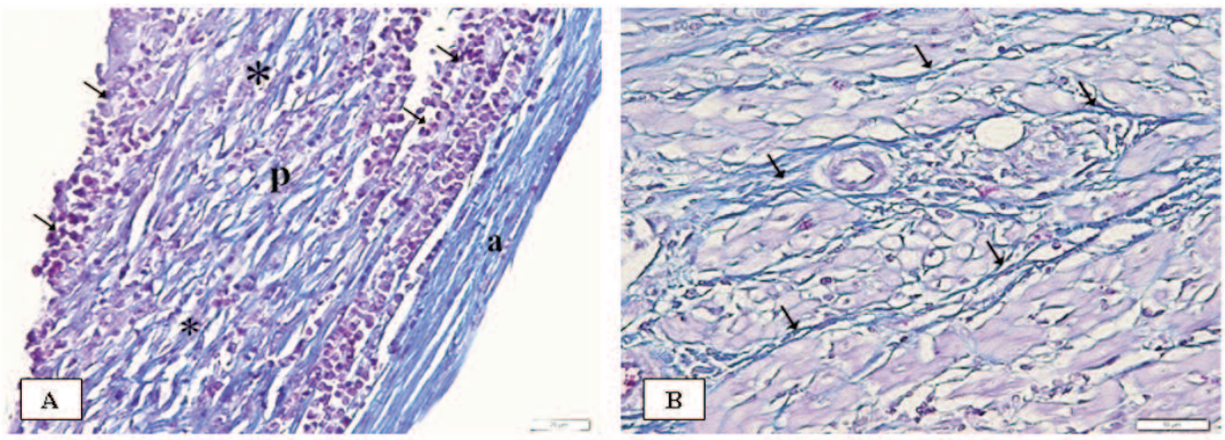


Fig. 12. Masson trichrome stained sections of the goat heart with hydatid cyst. A: laminated acellular layer (a) and thick fibrous capsular layer (p) with heavy infiltrated inflammatory cells (↑) and collagen fibers (*); B: Marked fibrosis between the cardiac muscles (↑).

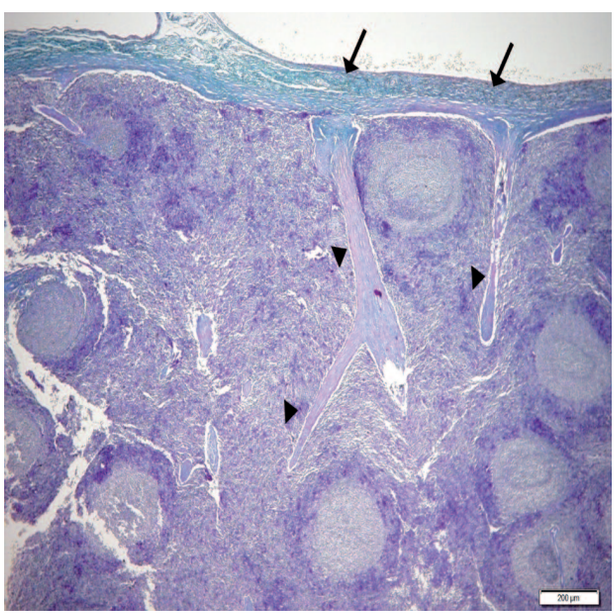


Fig. 13. Masson trichrome stained sections from spleen goat showing thick capsule (↑) and thick connective tissue septa (▲)

lowest prevalence recorded in goats.

In the current study investigated also the frequency and percentage of hydatosis on both infected imported and local animals (heart and spleen). It detected that those local animals were 16972. Infected Hearts were 40.35% and the infected spleen was 48.48% subsequently. On the other hand, the imported animals were 2124, the infected animals in heart were 59.64% and the spleen was 51.51%. The above-mentioned results were nearly similar to those reported by El-Ghareeb et al. [1].

In our study we found that sex had significant effect on the prevalence of hydatidosis in goats. Our findings agreed with El-Ghareeb et al. [1] reported that female sheep had higher infection rate than males within the local area and this may be explained by the fact that female sheep is usually kept for a longer period for production and breeding purposes. Moreover, the imported female sheep revealed low prevalence due to a smaller number of slaughtered female sheep than males, which are needed more for slaughtering. Furthermore, previous studies from Jordan, Saudi Arabia and Libya Ibrahim, [8], Almalki et al. [9], Al-Yaman et al. [21], Elmajdoub and Rahman [22] who reported that male have higher prevalence than the female. On the other hand, Godara et al. [19] who stated that the prevalence of hydatidosis in goats did not

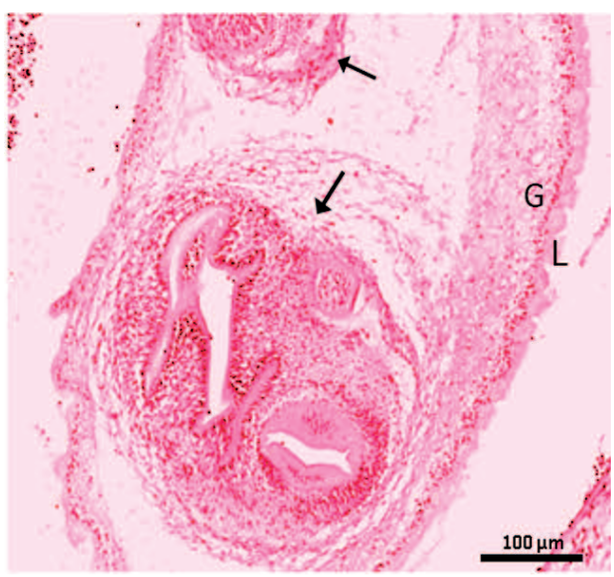


Fig. 14. PAS stained sections of the goat heart with hydatid cyst showing intact laminated membrane (L), germinal layer (G) and protoscolices (↑) PAS positive

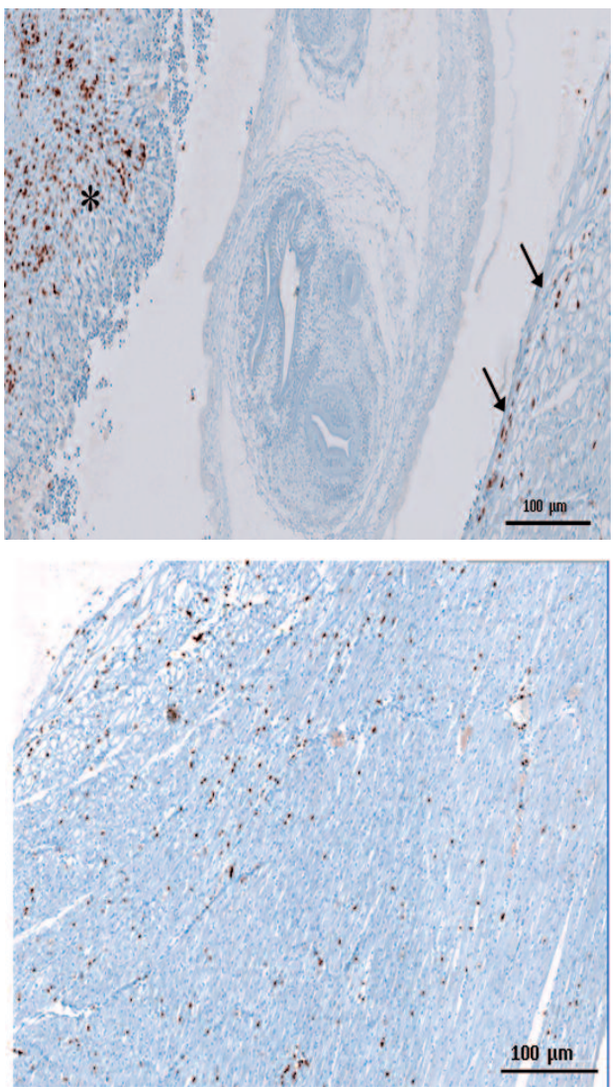


Fig. 15. Stained section of the goat heart. A: hydatid cyst with scattered positive brown stained cells reaction for CD3 Marker in the cyst wall (*) and parenchyma in cardiac tissue (↑); B: scattered positive brown stained cells reaction for CD3 Marker in the parenchyma of the cardiac tissue.

depend on the sex difference.

Therefore, it can be concluded that the important feedback of these results in the slaughterhouse to the different farms is very important in the field of preventive medicine in order to decrease and prevent the risk of acquiring the most important zoonotic diseases as hydatidosis [1].

The histologic differential diagnosis for hydatid cyst includes cysticerci (*Cysticercus bovis, Cysticercus ovis*, *Cysticercus tenuicollis),* and *Coenurus cerebralis*. Cysticerci usually show fluid filled thick-walled cyst (bladder worm), which is containing a single scolex. On the other hand, hydatid cyst contains multiple scolices. Moreover, the scolex of *Cysticercus bovis* does not contain hooklets [23,24]. Although *Coenurus cerebralis* cyst holds many scolices, it is mostly found within CNS and rarely within the internal organs [25].

The wall of hydatid cyst, as we shown in our results, consists of three layers. The outermost one is the fibrous capsular layer made up of fibrous connective tissue in addition to inflammatory cells such as eosinophils and lymphocytes [25]. The middle layer of the cyst consists of acellular hyaline lamellated membrane, so called laminated layer. The inner layer (germinal layer) is made up of a single cell layer which is responsible for formation of other layers, cyst fluid, and broad capsule which may be attached to the germinal layer by a stalk or freely floated within the fluid (hydatid sand) [26]. Broad capsule is characteristic for hydatid cyst if detected [1].

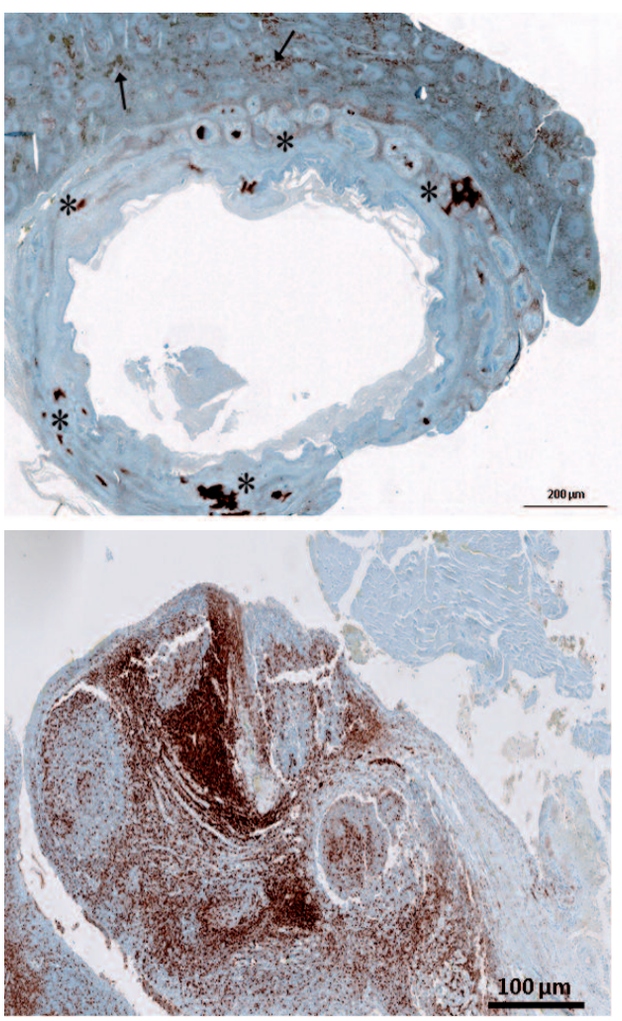


Fig. 16. Stained section of the goat spleen with hydatid cyst showing scattered positive brown stained cells reaction for CD3 marker. A: in the cyst wall (*) and parenchyma in splenic tissue (↑); B: in the splenic tissue.

The cysts surrounded by infiltration of mononuclear cells creating granulomatous reaction which surrounded by fibrous tissues capsule. Then calcification occurred on top of the chronic infections which lead to focal pressure at the site of infection [20].

According to the current study, Masson trichrome stained sections were superior in demonstrating the fibrous tissue capsule. Similar results were detected by Ibrahim and Gamel [16] who reported that liver and lung sections stained green with Masson's trichrome. This result concomitant with Rashed et al. [27] who stated that the thickness of layers stained with Masson trichrome stain appeared green in color and varied in the thickness due to glycogen and mucopolysaccharide content.

In the current study, PAS stained sections of the of the cyst consisted of three layers with positive PAS magenta color. This result explained by Khalifa et al. [28] and Ibrahim and Gamel [16] who reported that the cyst wall layers were stained positive with PAS. They explained that this occurred due to the higher content of mucopolysaccharides and glycoproteins in the cyst wall. furthermore, they mentioned that the PAS staining was much superior to Masson trichrome stain in showing the cyst wall layers.

CD3 marker was used in the current study to detect infiltrated tissue with lymphocytes. In the current study, immunohistochemical stained sections showed a positive reaction for CD3. This finding in agreement with Ibrahim and Gamel [16] who reported that the inflammatory cells in all sections of the liver and the lung showed a positive reaction for CD3 but negative reaction for CD20. Moreover, Keir et al. [29]; Naji et al. [30] reported that positive CD3 is associated with the T cell receptors in both CD4 and CD8 cells. Also, Pearce et al. [31] mentioned that CD4, CD8 T lymphocytes play a role in the immune response in case of human infection with cystic hydatidosis.

In the present study, H&E examination of the spleen tissue were correlated with the lesions described in cystic hydatosis of spleen pig Singh [32] and sheep Vural et al. [33]. Moreover, Sreedevi et al. [34] reported that spleenic hydatidosis was high prevalence in cattle, sheep, and goat than in buffaloes.

In the present work, light microscopic examination of sections of the spleen displayed disrupted parenchymal architecture with loss of white pulp in some areas and other with irregular arrangement of white pulp scattered irregularly in a background of red pulp. This result explained by Elmore [35] who mentioned typical cellular changes that can be observed after exposure to an immunomodulatory agent are an alteration in the size and density of the PALS and/or marginal zone, and a change in the number of follicles with germinal centers. Red pulp of spleen with megakaryocytes were also detected in some specimens tis finding correlated with Raafat et al. [36] who explained that megakaryocytes appeared in the spleen as a defense mechanism as the platelets can play important roles in host defense against parasitic infection by directly damaging the parasites.

In the current study, cardiac muscles sections showed irregular arranged widely separated cardiac muscle fibers with perinuclear vacuolization, sarcoplasmic vacuolation, area of pale acidophilic sarcoplasm and congestion and dilatation blood vessels. This result explained by Dispersyn and Borgers [35], Dove, [36] who mentioned that the nuclear changes could be due to hypoxia. Sarcoplasmic vacuolation was due to intracellular fluid and electrolytes redistribution with loss of selective permeability of the cell membrane.

Acknowledgements

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