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Prevalence and Histopathological Studies on Hydatidosis among Sheep Carcasses at Al-Ahsa, Saudi Arabia

Waleed R. El-Ghareeb [1, 2], Abobakr M. Edris [2], Ahmed E. Alfifi [2] and Abdelazem M. Ibrahim [3,4]
[1] Food Control Department, Faculty of Veterinary Medicine, Zagazig University, 44519, Egypt
[2] Department of Veterinary Public Health and Animal Husbandry, College of Veterinary Medicine, King Faisal University, Saudi Arabia
[3] Department of Pathology, College of Veterinary Medicine, Suez Canal University
[2] Department of Pathology, College of Veterinary Medicine, King Faisal University, Saudi Arabia

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Correspondence to:
welsaid@kfu.edu.sa

ABSTRACT

The study was carried out to determine the prevalence of hydatidosis among the sheep carcasses at Al-Ahsa region, Saudi Arabia. The study included also histopathological tissue evaluation. The results of the present study showed that the prevalence of hydatidosis among sheep carcasses was 6.60 % (98/1485); meanwhile among the native sheep breed was 7.70 %, and 5.8 % among the imported ones. Concerning the effect of age, the prevalence of hydatidosis increased significantly ($p \leq 0.0001$) in old aged sheep. On the other hand, the prevalence in liver, lung, heart and muscles was 66.3%, 23.4%, 7.1% and 3.0%, respectively. Regarding the cyst state in sheep, the percentages of viable, non-viable, sterile and calcified one were 24.4 %, 12.2 %, 7.14 % and 56.1 %, respectively. Meanwhile, results of histopathological examination had shown that most of hydatid cysts in sheep were fertile.

1. INTRODUCTION

Hydatidosis is an economic problem facing the Kingdom of Saudi Arabia (KSA) because of many condemnations for infested organs. In addition, it results in a public hazard effect because of the possible transmission to the human beings. The World Health Organization and International Epizootic Bureau included echinococcosis in the list of the diseases, which are subjected to radical eradication (Borji *et al.*, 2012). Infection occurs due to ingestion of food or water contaminated with eggs of the dog tapeworm, *Echinococcosis granulosus* (Devi and Parija, 2003). Meat inspection is conducted in the abattoir for the purpose of screening and removing abnormal pathological lesions that unfit for human consumption to produce wholesome meat (Gracey *et al.*, 1999). Detection of hydatidosis in slaughtered animals is based on post-mortem inspection. The most common sites of hydatid cysts are the liver, lungs and spleen of domestic animals (Kaplan and Baspinar, 1998).

The infection rate differs significantely according to different locality; at Salalah, Oman was 0.07% (Al-Kitani *et al.*, 2015), while in south Sudan, Erneo *et al.* (2016) revealed that the mean prevalence in sheep was 6.99%. 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 2.5% in Al- Qassim (Sobeih et al., 1998), 12.61% in Al-Baha (Ibrahim *et al.*, 2008 & Ibrahim, 2010), 6.8% in Najran (Al-Qurashi and Bahnass, 2012), 13.5% in Al-Taif (Firas et al., 2014) and 2.83 % in Dammam (El-Ghareeb, 2017).

Few studies have been conducted on the impact and prevalence of cystic echinococcosis in slaughtered sheep in Al-Ahsa region, Saudi Arabia. Therefore, the main objectives of the present study were to determine the prevalence rate and histopathological studies on hydatidosis among sheep carcasses at Al-Ahsa, Saudi Arabia.

2. MATERIAL AND METHODS

2.1. Study Area

This study was carried in Al-Ahsa, located at 25º 15' to 25º 40' N and 49º 30' to 49º 45' E. It lies in

the Eastern region of Saudi Arabia, about 60 Km west of the cost of the Arabian Gulf.

2.2. Sampling

A total of 1485 sheep carcasses were inspected and recorded at Al-Ahsa abattoirs. According to Collins et al. (2015), postmortem examination had been done to all slaughtered animals on edible organs such as liver, spleen, lung, kidney and heart as well as muscles for detection of hydatid cysts from the beginning of October 2015 until the end of March 2016. Sample size to estimate prevalence was based on the following criteria:

a) Expected prevalence of 20%. b) Confidence level of 95%. c) Desired precision of 5%. Sample size is calculated using the formula: $n = (Z^2 \times P (1 - P))/e^2$ as described by Dohoo et al. (2010), where: (Z = 1.96 for 95% CI), (P is expected true prevalence), and (e is desired precision).

2.3. Cyst Viability:

Cyst viability was examined according to Daryani *et al.* (2006), individual cysts were grossly examined for degeneration, hydatid cysts in sheep were selected for fertility study. To reduce intracystic pressure, the cyst wall was penetrated using sterile needle and it was cut with scalpel and scissors. The contents were then transferred into a sterile container. The cysts were classified as sterile (fluid filled cyst without protoscolices), calcified, non viable cysts (dead protoscolices). To determine viability of the protoscolices, a drop of cyst fluid was placed on a microscopic glass slide and cover slip was applied and observed for the motility of flame cells activity like peristaltic movement, on doubtful, a drop 0.1% aqueous eosin solution was added and examined under a light microscope for dye taking (living protoscolicesdid not take the dye, while dead one did) (Getachew *et al.*, 2012).

2.4. Histopathology

In order to evaluate the morphology of the hydatid cyst as well as the secondary tissue alterations, specimens were examined microscopically. The obtained specimens were fixed in 10% neutral buffer, formalin solution. After complete fixation, gradual dehydration of samples was done using different concentration of ethyl alcohol. Then, the samples were cleared in xylene and embedded in paraffin. Section of 5 micron thickness were prepared and stained with Haematoxilyne and Eosin (H&E) stain and covered with glass cover using canda balsam (Bancroft and Stevens, 1990).

2.5. Statistical Analysis:

Effect of sheep origin, age, weight, type and sex on hydatid infestation were analyzed by the General Linear Model (GLM) procedure (SAS, 2004). Means ± standard errors were calculated and tested for significance using the "t" test. Prevalence for hydatidosis was analyzed by the Proc Frequency procedure (SAS, Institute, Inc, 2004). The Pearson's χ^2 (Chi-square) statistics were calculated according to Steel and Torrie, (1960). The corresponding histograms were graphed using Microsoft Office Excel program (2007).

3. RESULTS and DISCUSSION

Table (1): The overall prevalences of hydatidosis among sheep carcasses.

Normal		Infested	
No.	%	No.	%
(1387/1485)	93.40	(98/1485)	6.60

Table (2): Pevalence of hydatidosis in sheep origin with regard to their sex.

Sheep Origin	Sex	Normal		Infested		Total	
		No	%	No	%	No	%
Native	Male	533	95.01	28	4.99	561	91.96
	Female	30	61.22	19	38.78	49	8.04
	Total	563	92.30	47	7.70	610	100.00
Imported	Male	792	94.06	50	5.49	842	96.23
	Female	32	96.97	1	3.03	33	3.77
	Total	824	94.17	51	5.83	875	100.00
Total		1387	93.40	98	6.60	1485	100.00

Chi Squares for the effect of sex on hydatid prevalencein native breeds (Value = 71.2444) and imported breeds (Value= 0.3400) is very high significant ($P \leq 0.0001$).

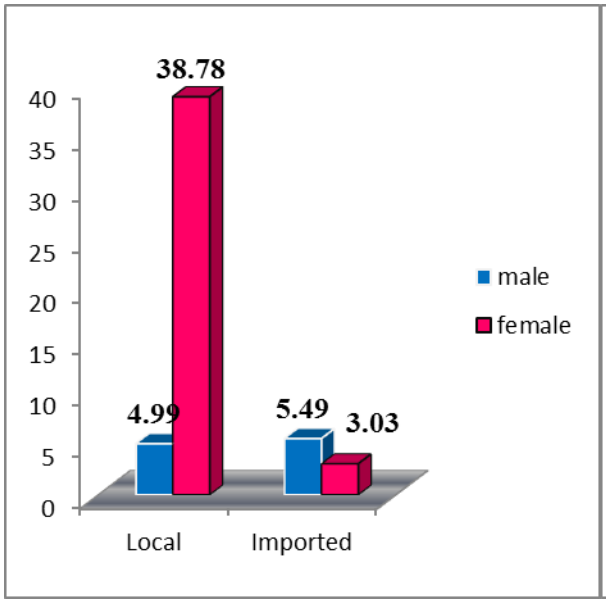

Fig 1. Effect of sheep sex on the prevelance of hydatidosis (%)

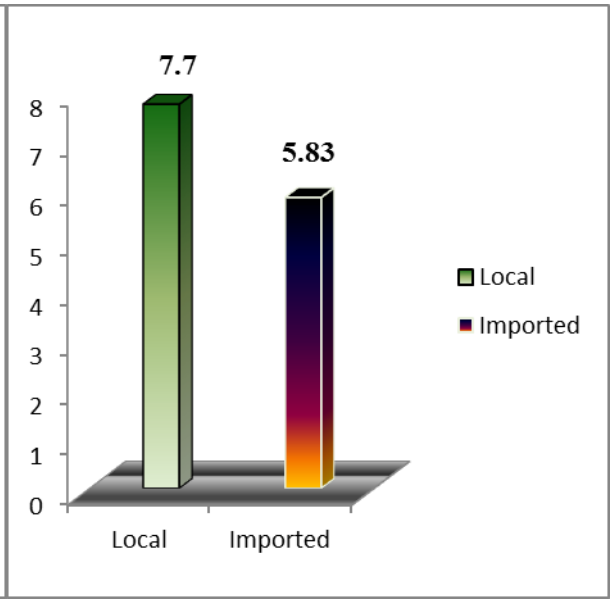

Fig 2. Effect of sheep origin on the prevalence of hydatidosis (%)

Table (3): Pevalence of hydatidosis in sheep with regard to their breed.

Sheep Origin	Age	Normal		Infested		Total	
		No	%	No	%	No	%
Native	< 1 y.	484	96.41	18	3.59	502	82.30
	1-2 y.	70	77.78	20	22.22	90	14.75
	> 2 y.	9	50.00	9	50.00	18	2.95
	Total	563	92.30	47	7.70	610	100.00
Imported	< 1 y.	770	95.18	39	4.82	809	92.46
	1-2 y.	53	84.13	10	15.87	63	7.20
	> 2 y.	1	33.33	2	66.67	3	0.34
	Total	824	94.17	51	5.83	875	100.00
Total		1387	93.40	98	6.60	1485	100.00

Chi Squares for the effect of age on hydatid prevalencein native breeds (Value = 83.9310) and imported breeds (Value= 33.3068) is very high significant (P ≤0.0001).

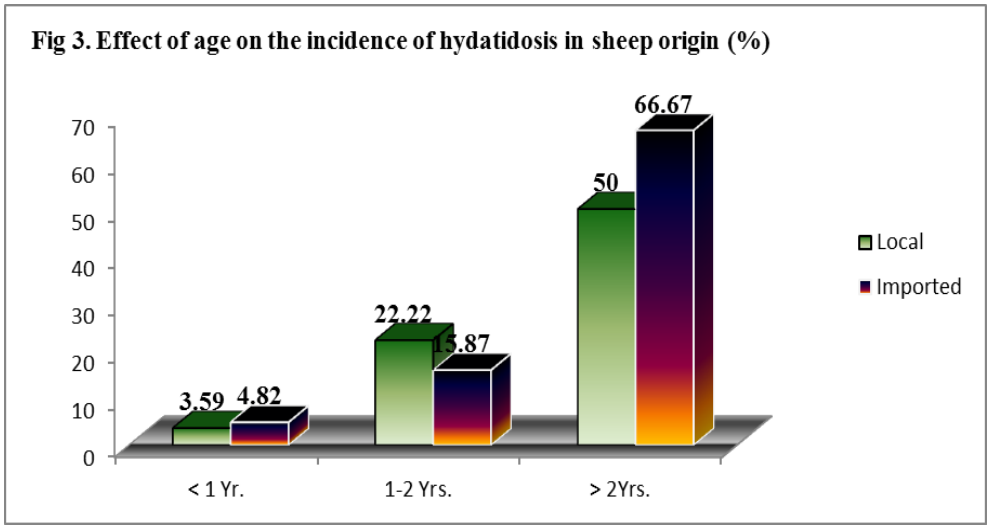

Table (4): The prevalence of hydatidosis in both native and imported sheep organs.

SheepOrigin	Liver		Lung		Heart		Muscle	
	No.	%	No.	%	No.	%	No.	%
native n = 47	33	70.21	10	21.28	3	6.38	1	2.13
Imported n =51	32	62.75	13	25.49	4	7.82	2	3.92
Total	65	66.32	23	23.46	7	7.14	2	3.06

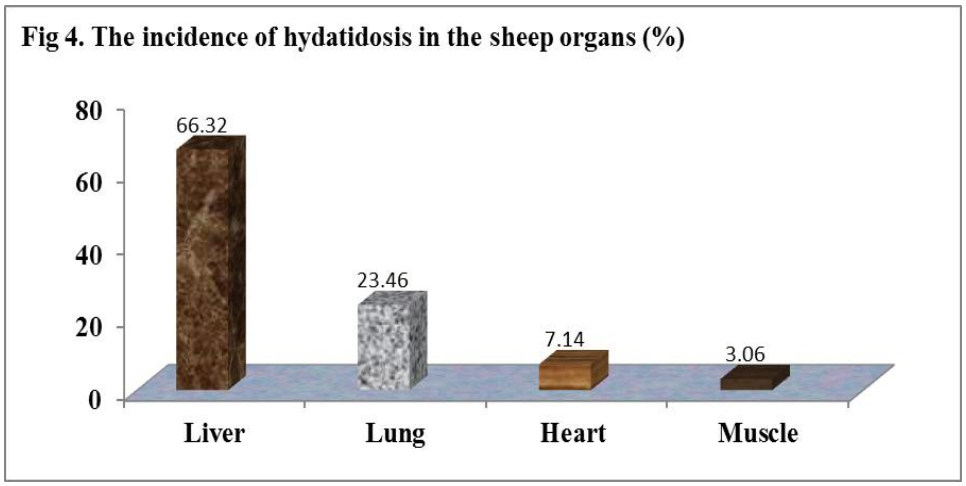

Table (5): Viability of hydatid cysts from sheep slaughtered at Al-Hasa abattoirs.

Sheep Origin	Cyst state	Cyst location									
		Liver		Lung		Heart		Muscle		Total	
		No.	%	No.	%	No.	%	No.	%	No.	%
	Viable	7	53.8	5	38.4	1	7.6	0	0.0	13	27.6
	Non-Viable	5	83.3	1	16.6	0	0.0	0	0.0	6	12.7
	Sterile	3	100	0	0.0	0	0.0	0	0.0	3	6.38
Native	Calcified	18	72.0	4	16.0	2	8.0	1	4.0	25	53.1
	Total	33	70.2	10	21.2	3	6.3	1	2.1	47	100
	Viable	6	54.5	4	36.3	1	9.0	0	0.0	11	21.5
	Non- Viable	3	50.0	3	50.0	0	0.0	0	0.0	6	11.7
	Sterile	2	50.0	2	50.0	0	0.0	0	0.0	4	7.84
Imported	Calcified	21	70.0	4	13.3	3	10.0	2	6.6	30	58.8
	Total	32	62.7	13	25.4	4	7.8	2	3.9	51	100
	Viable	13	54.1	9	37.5	2	8.3	0	0.0	24	24.4
	Non- Viable	8	66.6	4	33.3	0	0.0	0	0.0	12	12.2
Overall	Sterile	5	71.4	2	28.6	0	0.0	0	0.0	7	7.14
	Calcified	39	70.9	8	14.5	5	9.0	3	5.4	55	56.1
	Total	65	66.3	23	23.4	7	7.1	3	3.0	98	100

Histopathological Findings:

Histopathological examination had been done not only to evaluate tissue changes that related to hydatid cyst infesteion, but also to see the detailed structure of hydatidosis as it is shown in following figures:

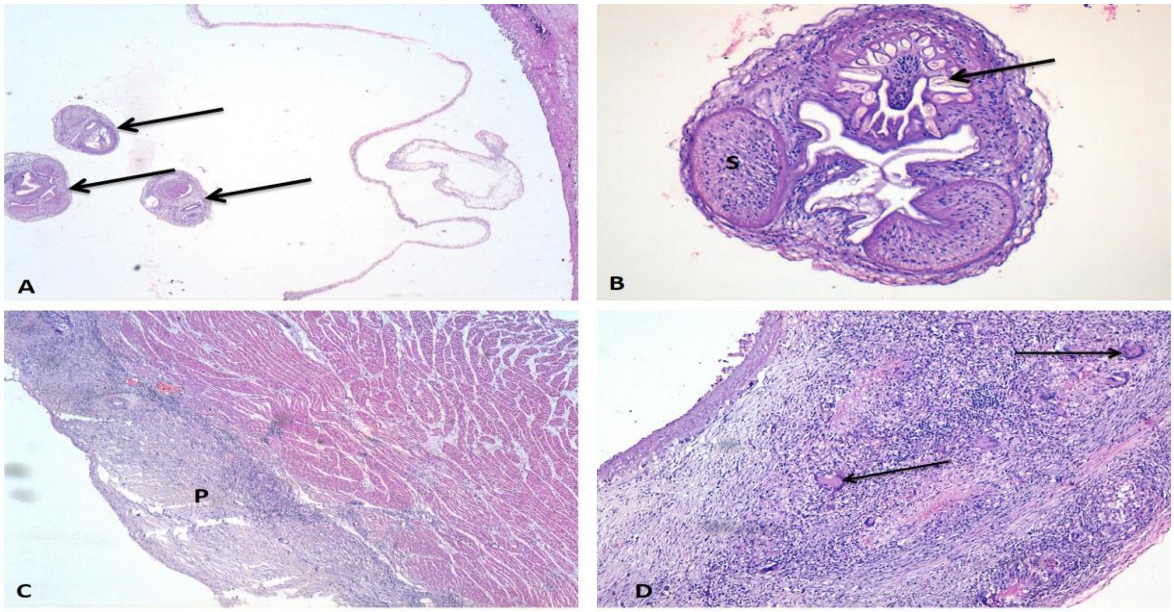

Fig 5: H&E-stain, sections from heart of sheep infested with hydatidosis.
A) Three protoscolices are seen inside the cyst, 4 X.
B) Higher magnification show the structre of protosclex, sucker (s) rostrellum with birefringent hooks (arrow), 40 X.
C) Thick pericyst layer is heavily infiltrated with inflammatory cells (p), 4 X.
D) The inflammatory cells contain many giant cells (arrow), 20X.

The results recorded in (tables 1 & 2 and Fig. 1&2) revealed that the overall prevalence of infested sheep at Al-Ahsa abattoirs was 6.6% regardless of sex or sheep origin.The prevalence was 7.70 % in native sheep breed, while in the imported one was 5.83%, the significant difference may be due the difference in the environmental conditions between the different areas of the country along the season (EL-Majdoub and Rahman, 2015). The prevalence of hydatidosis in male native sheep was 4.99%, but in male imported sheep was 5.40%, while the prevalence in female native sheep was 38.78 and in female imported sheep was 3.03% (table 2 & Fig. 1). 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 less number of slaughtered female sheep than males, which are needed more for slaughtering. Highly significant difference was observed for the effect of sex on the prevalence of hydatidosis in native and imported sheep breeds at ($P < 0.0001$). The above mentioned results were nearly similar to those reported by (Sabri *et al.*, 2005; El-madowy *et al*, 2011; Salih *et al.*, 2011; Salem *et al.*, 2011; Al-Qurashi and Bahnass ,2012; Muqbil *et al.*, 2012; Kaseem *et al.*, 2013, Al-Shaibani *et al.*, 2015 and Ochi *et al.*, 2015).

The results recorded in (table 3 & Fig. 3) revealed the prevalence of hydatid cysts in relation to the age stages, less than 1 year, 1-2 years and more than 3 years in native sheep where the percentages were 3.59%, 22.22% and 50%, while 4.82%, 15.87% and 66.67% for imported, while the overall prevalence of hydatidosis in native breeds was 7.70%, meanwhile the percentage was 5.83% for imported one. The results recorded in table (3) were nearly similar to those obtained by Ibrahim *et al.* (2011), Kumso and Mahammed (2012), Khan *et al*. (2013), Al-Shaibani *et al.* (2015) and Ochi *et al.* (2015). Highly significant difference was recorded for the prevalence of hydatidosis between the age of sheep and between both native and imported sheep breeds ($P < 0.0001$). These findings could be attributed to the longer exposure of infested sheep to *E-granulosus*. Moreover, Khan *et al.* (2013) stated that there is a positive relation between the infection rate of the disease and the animal age.

Conerning the organ preavalence in this study (Tab.4&Fig.4), liver was found to be the most commonly affected organ followed by lung. Higher liver infestation may be a reflection of the parasite route in great capillaries as portal vein (Kebede *et al.*, 2009a). Furthermore, the prevalence of hydatidosis in visceral organs and muscles of infested native and imported sheep were 70.21%, 21.28%, 6.38% and 2.13% in liver, lung, heart and carcass muscle respectively of native breed and

62.75%, 25.49%, 7.84% and 3.92% in the same organs and muscles for imported one, respectively.

The prevalence of hydatidosis in relation to its location in animals was higher in liver; the higher incidence in liver may be attributed to the fact that liver is the first organ where the blood flows through it after leaving the intestine and then filtered in liver parenchyma with more chances of infection. On the other hand, ova are not trapped in the liver, pass to the lung and then to other organs (Soulsby, 1982).

Concerning the results recorded in table (5) the prevalence of hydatid cyst viability collected from Al-Hasa abattoirs revealed that the non-viable and calcified cysts had higher percentage 83.33% and 72%, respectively in liver of native breeds, while in lung the viable cyst was more prevalent 38.43% then calicifeied one 16%. Meanwhile, calicified cyst was recorded in heart and muscles of the same breeds at 8% and 4%, respectively. Furthermore, in imported breeds calicified and viable cysts were 70% and 54.53%, respectively in liver tissues, while in lung non-viable and sterile were prevalent at 50% and 50%. The muscle and heart revealed only calicified cysts. The fertility of cysts in sheep liver was higher than in the lung (Dalimi *et al.*, 2002). These results recorded in table (4&5) were nearly similar to those reported by Fokhar and Sadjiodi (2007), Latif *et al.* (2010), Ibrahim (2010), Salem *et al.* (2011), Tappe *et al.* (2011) and Ochi *et al.* (2015).

Therefore, it can be concluded that the important feedback of these results in the slaughterhouse to the different farms is of great value in the field of preventive medicine in order to minimize the risk of acquiring the most important zoontic diseases as hydatidosis are of paramount importance (Abunna *et al.*, 2012).

The gross and 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 contain multiple scolices. Moreover, the scolex of *Cysticercus bovis* does not contain hooklets (Bowman, 2009 and Gardiner *et al.*, 1999). The cysts that we have investigated in our study had more than one scolex and possess hooklets. Although *Coenurus cerebralis* cyst holds many scolices, it is mostly found within CNS and rarely within the internal organs (Golzari *et al.*, 2014).

The wall of hydatid cyst is unique among aforementioned cysts. The wall of hydatid cyst, as we shown in our results, consists of three layers. The outermost one is the pericystlayer, known as ectocyst or adventitial layer, made up of fibrous connective tissue in addition to inflammatory cells such as eosinophils and giant cells (Golzari *et al.*, 2014). The middle layer of the cyst consists of a cellular 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) (Uzal *et al.*, 2016). Broad capsule is characteristic for hydatid cyst if detected. We did not recognize the broad capsule in our cases, but scolices were founded individually within the cyst.

Hydatid cyst of *Echinococcus granulosus* is usually unilocular whereas that of Echinococcus *multilocularis*is, multilocular (alveolar hydatid cyst).We have detected both cysts in our study.The release of the broad capsule or protoscolices out the cyst, following cyst rupture, may lead to develop a new cyst with extensive tissue reaction. Some old cysts may be calcified and it is considered dead cyst (Bowman, 2009 and Meyers *et al*., 2000).

CONCLUSION

The abattoir survey evidence of the present investigation showed that hydatidosis is prevalent in sheep population of Al-Ahsa area located in Eastern Saudi Arabia and may be a public health concern. The prevalence of hydatidosis increased significantly with the age of infested animals. High infestation rate of hydatidosis in liver and lung rather than other organs and was higher in liver. Histopathologically, hydatid cysts were detected in liver, lung and tissues. Other cysts showed ruptured empty cell wall. However, some cysts were filled with a cellular homogenous eosinophilic material.

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