DOI : 10.5958/2277-8934.2018.00031.0 *Vol 25 No 2, p 237-244*

CYSTIC ECHINOCOCCOSIS IN DROMEDARY CAMEL: BIOCHEMICAL, HISTOPATHOLOGICAL AND PARASITOLOGICAL STUDIES

F.A. Al-Hizab[1], M.A. Hamouda[1], O.H. Amer[2], A.M. Edris[3], W.R. El-Ghareeb[3,4], S.M. Abdel-Raheem[3,5], Najoua Hawas[2], A.M. Elmoslemany[6] and A.M Ibrahim[1,7]

[1]Department of Pathology, [3]Department of Veterinary Public Health and Animal Husbandry (Meat Hygiene), College of Veterinary Medicine, King Faisal University, Box 400 hofof 31982 Saudi Arabia

[2]Department of Clinical Laboratory Science, College of Applied Medical Sciences, University of Hail, Saudi Arabia

[4]Food Control Department, Faculty of Veterinary Medicine (Meat Hygiene), Zagazig University, Egypt

[5]Department of Animal Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Assiut University, Egypt

[6]Hygiene and Preventive Medicine Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh 35516, Egypt

[7]Department of Pathology, College of Veterinary Medicine, Suez Canal University, Egypt

ABSTRACT

This study was conducted on 600 dromedary camels from March 2017 to December 2017 at Al Omran (n=330) and Al Ahsa (n=270) abattoirs (Al Ahsa Province, Saudi Arabia). The study was designed to determine the prevalence, cyst fertility and viability, biochemical and electrolyte analysis of fertile and infertile hydatid cyst as well as histopathological findings. In these 600/2600 (23.07%) camels were randomly selected for antemortem examintation. Total 171 (28.5%) were found infected with hydatid cyst, i.e. 91 (27.6%) from Omran and 80 (29.6%) from Al-Ahsa abattoirs, respectively. Furthermore, 171 hydatid cysts were examined for fertility and viability. In Al Omran, 4/91 (4.4%) were found to be fertile and viable, 1/91 (1.1%) was fertile non viable, 23/91 (25.3%) were sterile and 63/91 (69.2%) in Al Ahsa, 2/80 (2.5%) were found to be fertile and viable, whereas, 33/80 (41.2%) were sterile and 45/80 (56.2%) were calcified. Biochemical and electrolyte analysis of hydatid cysts showed significant increase in iron, total protein and alkaline phosphatase in fertile cyst. Whereas, sterile cysts showed significant increase in triglyceride, chloride and sodium. In conclusion, infertile cysts were either sterile or calcified in the dromedary camel and were predominant. The biochemical and electrolyte parameters of cyst fluid could help us to recognise different kind of cysts.

Key words: Biochemical, cystic echinococcosis, dromedary camel, fertility

Cystic echinococcosis (CE), formerly known as hydatid disease (HD) or hydatidosis, is considered an important worldwide disease that has economical and zoonotic impact. Several studies have documented the endemic and zoonotic properties of cystic echinococcosis in various provinces of Saudi Arabia (Al Mofleh *et al,* 2000; Fadaladdin *et al,* 2013; Toulah *et al,* 2017). However, this disease has public health importance as it is one of the neglected diseases (Ahmed *et al,* 2011; Ahmadic and Meshkehkar, 2012). Cystic echinococcosis remains to be a considerable cause of morbidity and mortality in many countries. It required strict control measure that may take around 20 years of sustained efforts to eliminate such disease (Craig *et al,* 2007). Liver is the main site of hydatid cysts, followed by the lungs. Other organs, like kidneys, spleen, abdominal and pelvic cavity, heart, brain and spinal cord (Geramizadeh, 2013) can be infected. Diagnosis of hydatid disease in living animals is very difficult with no routine reliable test. Detection of cysts during meat inspection or at post-mortem examination is considered the most reliable diagnostic tool (Collins and Huey, 2015). Many attempts were made to apply serological tests for diagnosis of the disease using crude Hydatid cyst fluid (HCF) (Lightowlers, 1990; Craig *et al,* 1996). Due to the great difference in antibody titre in natural CE infections, these tests show reduced sensitivity as well as cross-reactions with *T. hydatigena* (Yong and Heath, 1984; Soliman *et al,* 2014). Other techniques like a western blot showed higher sensitivity and specificity for the ovine disease (Gatti *et al,* 2007). Proteomic characterisation was used to highlight the complexity and heterogeneity of a wide range of

SEND REPRINT REQUEST TO F.A. ALHIZAB email: falhizab@kfu.edu.sa

proteins originated from the host and the parasites, found in HCF collected from sheep, cattle and humans (Aziz *et al,* 2011). The present study was designed to estimate the prevalence, cyst fertility and viability rates, histopathological findings and electrolyte profile and biochemical parameters in fertile and sterile cysts of cystic echinococcosis.

Materials and Methods

Study animals

The present study was conducted on local breed one humped camel of different ages and both sexes. The samples were collected from Al- Omran and Al-Ahsa abattoirs. The period of this survey was from March 2017 to December 2017.

Study design and sample size

This cross-sectional study was proposed to estimate the prevalence of CE. A representative random sample size was calculated using the formula: $N= (Z^2xP)(1-P)/e^2$ as described by Dohoo *et al* (2010), where the N = Total number of sample size, Z=1.96 for 95%confidence interval. Based on the criteria, a total of 197 samples per locality (abattoir) was required to get an accurate estimation of the prevalence. Hence, 330 and 270 were examined from Al Omran and Al Ahsa abattoirs, respectively. The samples were collected with the intention of maximising the sample size to increase precision. The sampling interval was computed based on to study period, the whole number of animals slaughtered and the required sample size. Therefore, the sampling interval was 5 (1600/330) in Al-Omran abattoir and 4 (1000/270) in Al Ahsa abattoir. The first animal was selected randomly (Thrusfield, 2005).

Abattoir survey

Visceral organs were subjected to a thorough visual inspection, palpation and systematic incision. The total number of cysts was counted and recorded. Cyst was cautiously removed with circular incisions around it and was separately collected in clean containers for further cyst characterisation. The cysts were subsequently subjected to a systematic size measurement using Vernier caliper and classified as small (<4cm), medium (4-8 cm) and a large cyst (>8cm) (Schantz, 1990).

Biochemical and electrolyte analysis

The cystic fluids were aspirated aseptically, centrifuged at 1500 rpm at 4°C for 30 min and the supernatants were analysed for various biochemical parameters including glucose, total protein, triglycerides, cholesterol, alkaline phosphatase (ALP) and electrolyte profiles using commercial kits.

Parasitological examination

Following sample collection, the individual cysts were carefully incised and its contents evacuated into a sterile test tube for microscopic examination of protoscolices.

A. Examination of cysts fertility

The cysts were classified into fertile and infertile based on the presence of either free protoscolices, appeared as white sand like material on the germinal layer. Further, the infertile cysts were subsequently categorised as sterile (fluid filled cysts), or calcified cysts which has a gritty sound sensation upon incision (McPherson, 1985; Assefa *et al,* 2015).

B. Examination of cyst viability

Viability test was applied to figure out whether they are alive or dead. Briefly, one drop of the residue containing the protoscolices added to a microscope slide then covered with a cover slip and examined microscopically to detect ameboid-like peristaltic movements of protoscolices. Identification of flame cells in the anterior and the posterior portion covered with a knob-like projection were characteristic for the invaginated protoscolices. To confirm the viability of cyst, one drop of hydatid fluid was mixed with one drop of aqueous solution 0.2% eosin (W/V) and examined microscopically (40×) (Dalimi *et al,* 2002). If the protoscolex is unable to gain the stain it considered live, whereas the dead one is stained uniformly. Several other vital stains like fast green were also used.

Histopathological examination

For histopathological examination, one cubic cm of the infected organ (liver or lung), including a part of the cyst wall and a part of the surrounded tissue, was collected from specimens, then fixed at 10% neutral formalin. The fixed tissue was prepared through paraffin section technique and 5 µ paraffin sections were stained with either haematoxylin and eosin (H&E) (Kim *et al,* 2013).

Statistical Analysis

The collected data from camels were recorded and analysed via SPSS 16.0. A statistically significant association among variables considered to exist if P value ≤ 0.05.

Results and Discussion

Distribution, Size, Shape and Nature of cystic echinococcosis

Gross examination revealed presence of cystic echinococcosis in the liver and lung. Liver possessed the vast majority of the cysts. The number of cysts ranged from only one detectable cyst to numerous cysts. Cyst's size was measured as 1-2 cm in diameter and reached up to 3 cm (<4cm). Some cysts were thin walled and contained clear, watery fluid (Fig 1A), others had a thick whitish wall with viscous fluid containing small sand like material (Fig 1B). Calcified cysts were also seen as firm whitish calcified nodules (Fig 1C). Cysts that were detected in the lung were large, with opaque wall and contained turbid sand like materials (Fig 1D)

Biochemical and electrolyte profile

Biochemical analysis revealed a significant increase in glucose, total protein, cholesterol, alkaline phosphatase, potassium, phosphorus, calcium and iron in fertile cysts, compared to sterile ones. Whereas, there was a significant increase of sodium, chloride and triglyceride in sterile cysts as compared to fertile ones (Table 1).

Cyst fertility and viability

The fertile cysts were examined directly without stains (wet amount) (Figs 2A, B, C). To confirm

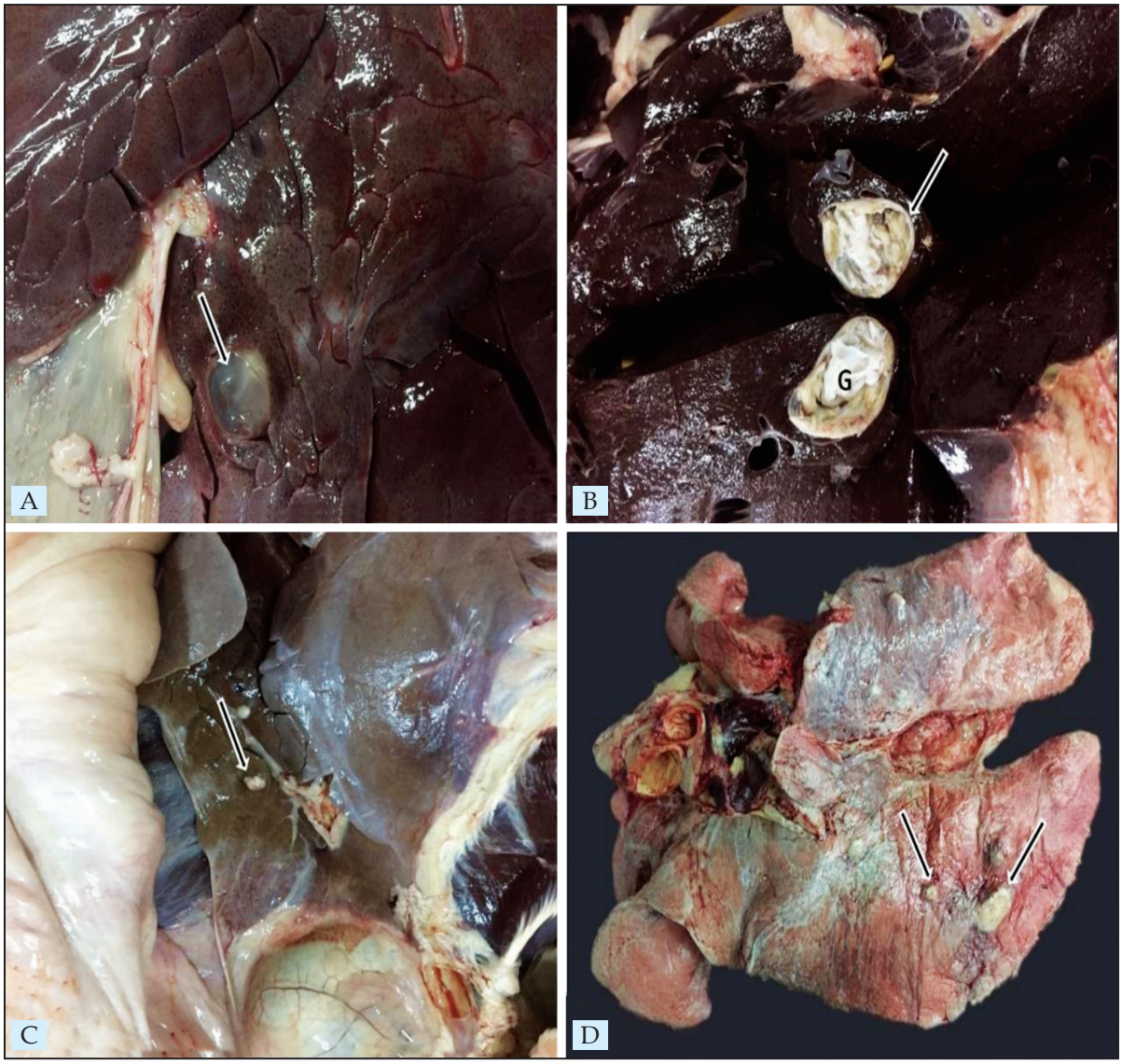

Fig 1. **A**, liver of a camel contains Cystic Echinococcosis with a thin walled and clear fluid (arrow). **B**, Large cyst (arrow) is seen embedded in the hepatic tissue containing germinal layer and sandy like materials (G). **C**, 1-2 cm diameter whitish, calcified cyst is located on the visceral surface of the liver (arrow). **D**, Multiple Cystic Echinococcosis were seen within the lung tissue (arrows).

Table 1. Comparison between various electrolyte and biochemical profiles based on fertility and sterility of cyst.

Item	Unit	Fertile (n=10) Mean ± SE	Sterile (n=10) Mean ± SE	P
Glucose, mmol/L	mmol/L	0.84±0.1**	0.12±0.00	<0.01
Total Protein	g/L	38.56±0.71***	3.04±0.39	<0.001
Cholesterol	mmol/L	1.11±0.02***	0.93±0.01	<0.001
Triglycerides	mmol/L	0.18±0.004***	0.25±0.01	<0.001
Alkaline phosphatase	U/L	2.37±0.23***	0.07±0.02	<0.001
Sodium	mmol/L	161.1±0.82*	165.2±1.49	0.02
Chloride	mmol/L	141.2±2.65***	159.75±1.19	<0.001
Potassium	mmol/L	6.12±0.16**	5.51±0.07	<0.01
Calcium	mmol/L	2.18±0.08***	1.68±0.02	<0.001
Phosphorus	mmol/L	0.7±0.24*	0.1±0.01	0.03
Iron	µmol/L	28.63±1.47***	0.85±0.37	<0.001

* = significant at p <0.05; ** = significant at p<0.01; *** = significant at p<0.001.

fertility, the slides stained with 0.1% eosin, Live cyst refuse eosin staining (Fig 3A) and stained with special stain (Fast green) (Fig 3B). Dead cysts take eosin stain (Fig 3C). Out of 171/600 (28.5%) cysts, the fertile cysts were examined for viability. In Al Omran, 4/91 (4.4%) were found to be fertile and viable, 1/91 (1.1%) was fertile non viable, 23/91 (25.3%) were sterile and 63/91 (69.2%) were calcified. In Al-Ahsa, 2/80 (2.5%) were found to be fertile and viable, 33/80 (41.2%) were sterile and 45/80 (56.2%) were calcified (Table 2).

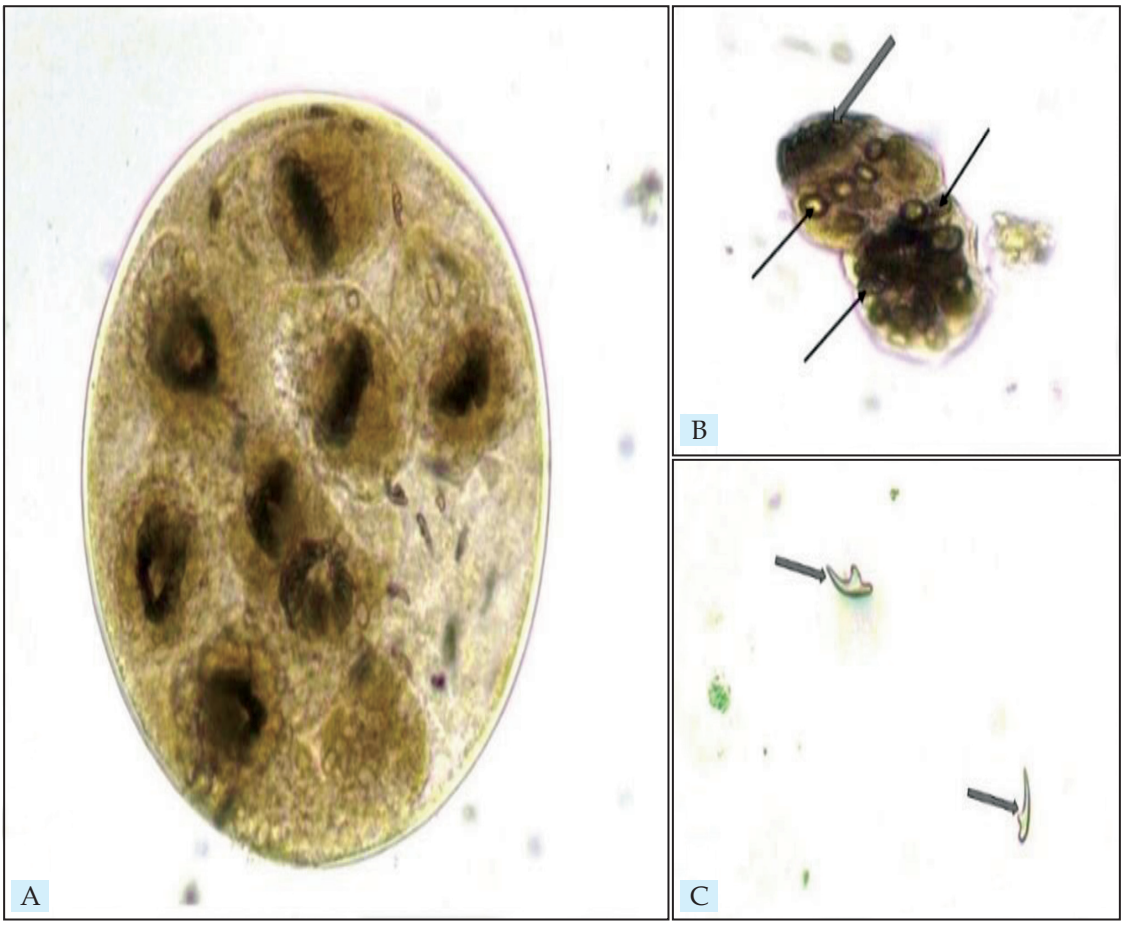

Fig 2. Wet mount - unstained fertile Cystic Echinococcosis: A, Invaginated scolices. B, Evaginated scolices with double row rostellar hooklets (Thick arrow) and calcareous corpuscles (Thin arrows). C, Note the hooks, Large hooks: 25.0 (µm), Small hooks: 17.5 (µm).

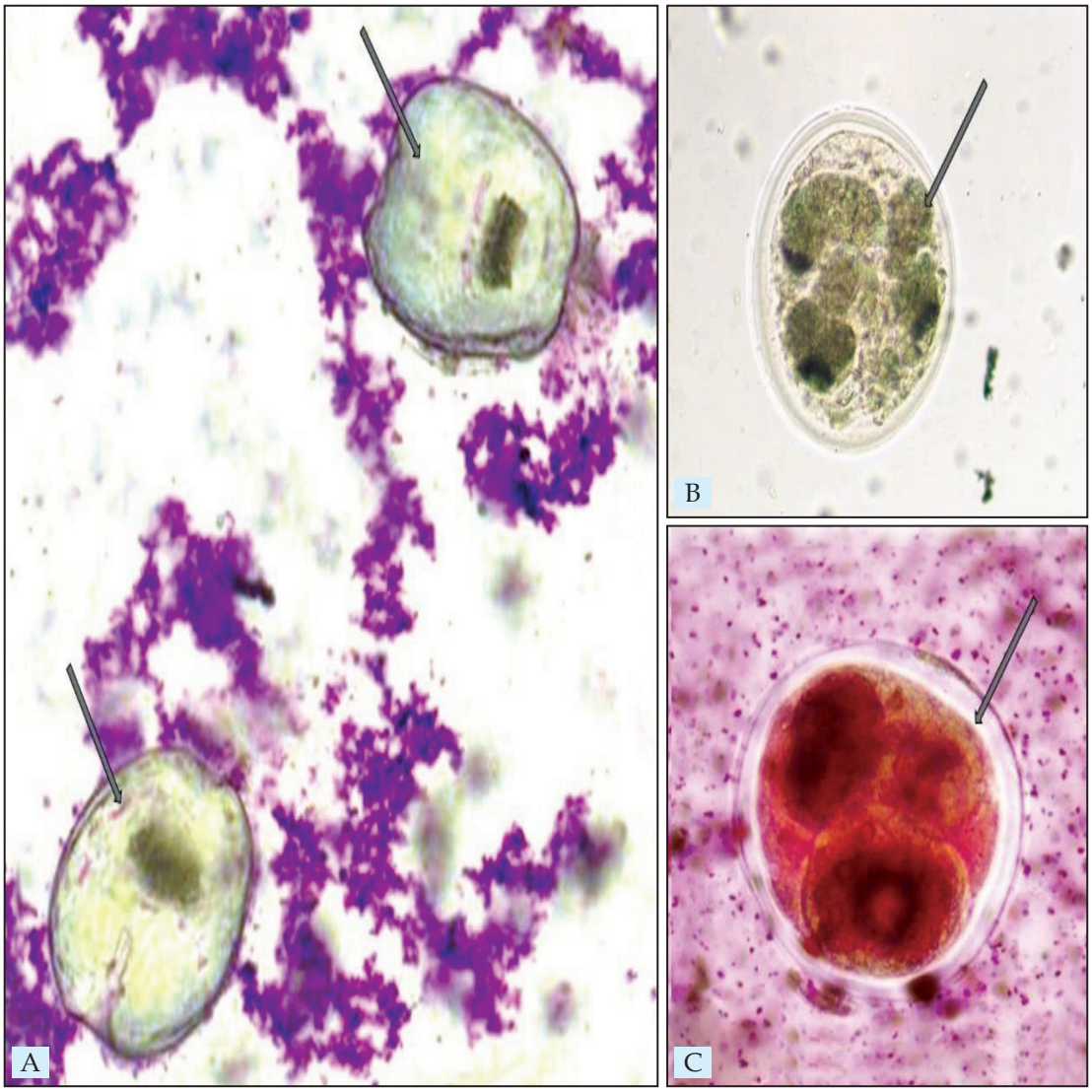

Fig 3. Wet mount - stained Cystic Echinococcosis: A, live invaginated protoscolices refuse staining with 0.1% eosin (arrows). B, Fast green stained viable cyst (arrow). C, Dead invaginated protoscolices staining with 0.1% eosin (arrow).

Table 2. Characterisation of cystic echinococcosis in two abattoirs.

Province	Abattoir	Total	Fertile (%)		Sterile (%)	Calcified (%)
			Viable (%)	Dead (%)		
Al Ahsa	Al Omran	91	4 (4.4%)	1 (1.1%)	23 (25.3%)	63 (69.2%)
	AL Ahsa	80	2 (2.5%)	0	33 (41.2%)	45 (56.2%)
Total		**171**	**6 (3.51%)**	**1 (0.58%)**	**56 (32.7%)**	**108 (63.16%)**

Microscopic findings

Cystic Echinococcus was effacing and replacing approximately 20-50% of the organ parenchyma. The majority of cysts had one chamber (unilocular). Some cysts were empty and others were filled with a cellular homogenous eosinophilic material. The cyst consisted of 3 layers, germinal, laminated, pericyst layer (P), (Fig 4 A,B). The fertile cyst was evident with the presence of multiple protoscolices, 200-250µm in diameter either free or attached to the germinal layer (protoscolices are absent in sterile cyst). Each protoscolex had thick tegument and contain suckers calcareous corpuscles and rostellum with birefringent hooks (Fig 4C). In calcified cysts, there was evidence of a large necrotic area with basophilic calcium mineralisation.The cyst was surrounded with a thick layer of fibrous connective tissue infiltrated with lymphocytes, plasma cells, macrophages, eosinophils and few multinucleated giant cells. The surrounding

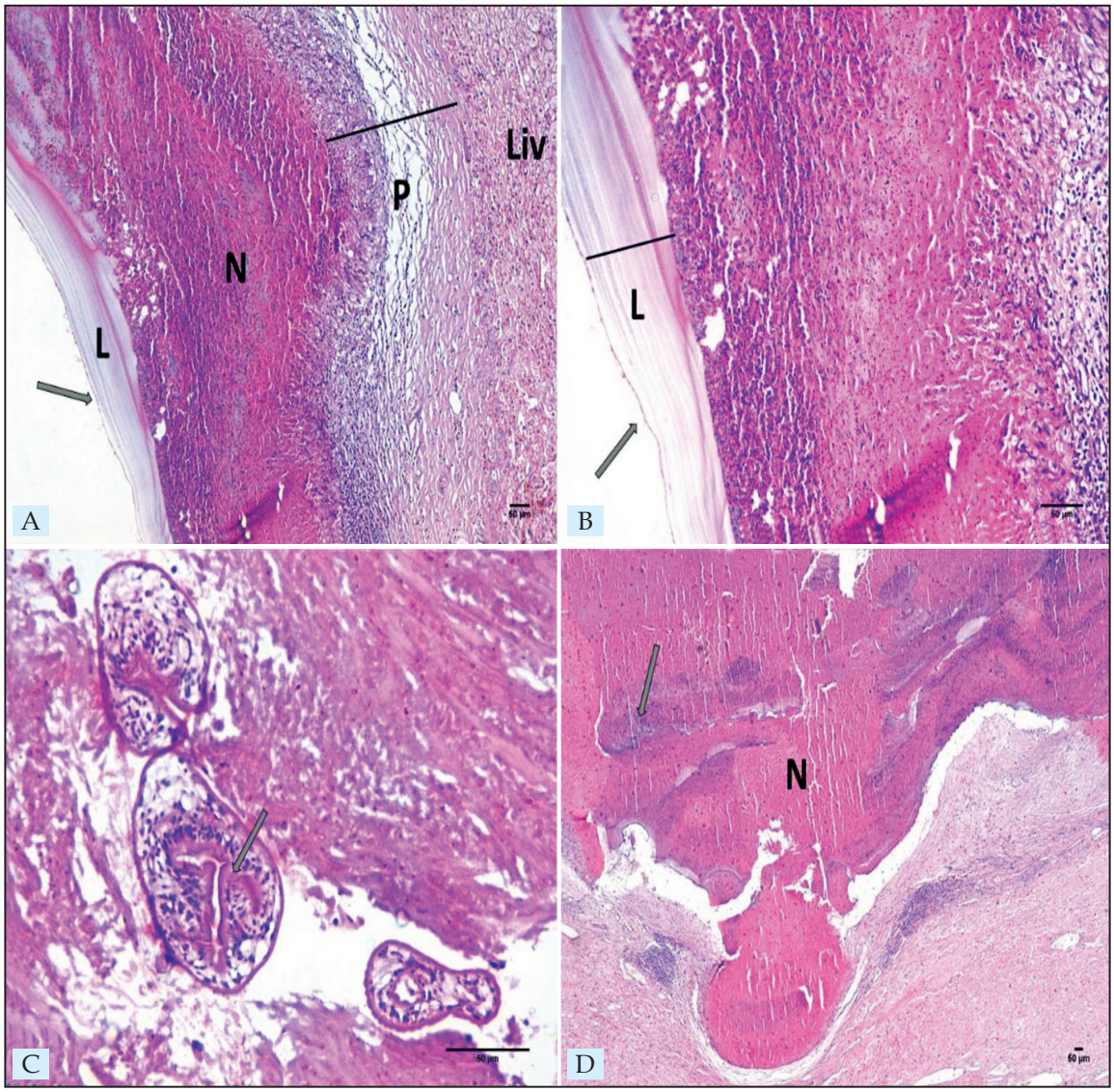

Fig 4. H&E- stained sections of camel's liver infested with cystic echinococcosis (Bar= 50µm): A, Hepatic tissue (Liver) is effaced by a parasitic cyst formed of 3 layers; germinal layer (arrow), laminated layer (L), pericyst layer (P), in addition to area of necrosis (N), B, Higher magnification of the previous image illustrates the germinal layer lined with single layer of cells (arrow) and laminated layer (L). C, Three protoscolices are seen inside the cyst containing rostrellum with birefringent hooks (arrow). D, A calcified cyst contains large necrotic area (N) with mineralisation (arrow).

hepatocytes showed necrotic cells associated with massive inflammatory cells.

Cystic echinococcosis is among the most neglected public health problems in humans and animals and causes serious socioeconomic effects throughout the world. The present study provides assessment of the magnitude of the disease in dromedary camels slaughtered in Al-Ahsa Province. The overall prevalence of cystic echinococcosis was 28.5%. Our findings were comparable with previous results in different localities in KSA, 34.64%, 16%, 32.85% and 6.86% in El-Madinah, Meka, Al-Baha and Jeddah cities, respectively (Fadaladdin *et al,* 2013; Haroun *et al,* 2008; Ibrahim, 2010; Toulah *et al,* 2017). This variation could be related to pastoralism practice, high dog population, inadequate medication and veterinary services, low hygiene and education standards, importing of animal from infected endemic areas are a continuous risk of re-introducing a disease and maintain its zoonotic life cycle (Daryani *et al,* 2007).

The fertility of cysts is an important factor that can influence the life cycle of a disease. The study showed that 3.5% of the cysts were fertile and viable, 32.7% was sterile and 63.2% was calcified. The fertility rate was lower than those observed in camels (51.57%) and sheep (18.18%) (Moghaddas *et al,* 2014; Hasona *et al,* 2017). This variation could be attributed

to the geographical situation, the nature of infected hosts, the sites of infection and genotype dependant (McManus, 2006). The high proportion of sterile cysts in camel may generally imply that most of the cysts in camel are infertile and this underscores the role of camel in maintaining the life cycle of a disease. Also, a high number of calcified cysts may be due to end stage to hydatid cysts or in the liver may be due to abundant fibrous tissue and reticuloendothelial cells (Haftu and Kebede, 2014). Histology of hydatid cyst in camel and its tissue damage were quite similar to those observed by Singh *et al* (2016) who reported that hydatid cyst results in a considerable damage to the affected organ. Hydatid cyst fluid is a mixture of chemical components arise from the parasite and host (Vuitton and Gottstein, 2010). There were variations in the biochemical contents of hydatid fluids inside intermediate hosts (Radfar and Iranyar, 2004; Osman *et al,* 2014). The variations of components play an important role in all biochemical reaction inside a hydatid cyst (Li *et al,* 2013; Osman *et al,* 2014). In the present study, biochemical analysis showed a significant increase in alkaline phosphatase in fertile cysts compared to sterile cysts. Alkaline phosphatase (ALP) is an enzyme which plays a critical role in biochemical reactions within hydatid cyst (Shaafie *et al,* 1999). Karibozorg *et al* (2014) reported that hydatid infection stimulates biliary cells to excrete ALP. So, an increase in ALP activity could be considered as a pathological biomarker in hydatid disease. Also the present results revealed a significant increase in calcium, phosphorus and iron in fertile cysts compared to sterile cysts. This finding was consistent with Radfar and Iranyar (2004) and Shaldoum *et al* (2017). There was a significant difference between the level of sodium, potassium and chloride in fertile and sterile one. The inflow of electolytes in the cyst has depended on parasite requirement and selective permeability (Rahdar *et al,* 2008).

In conclusion, infertile cysts in the dromedary camel, either sterile or calcified, were predominant and biochemical and electrolyte parameters of cyst fluid can help us to recognise the different kinds of cysts and also to promote distribution of the drug to the cyst.

Acknowledgement

This work was financed by King Abdulaziz City for Science and Technology (KACST) through a grant number (Arp-35-169), Saudi Arabia.

References

Ahmadic NA and Meshkehkar M (2012). An abattoir-based study on the prevalence and economic losses due to cystic echinococcosis in slaughtered herbivores in Ahwaz, southwestern Iran. Journal of Helminthology 85:33-39.

Ahmed ME, Mohamed IA and Fatima MA (2011). Hydatid disease, a morbid drop needs awareness. Sudan Medical Journal 47(1):56-64.

Al Mofleh IA, Al Rashed RS, Ayoola EA, Al Faleh F, Al Amri SM, Al Rikabi AC, Al Sohaibani MO and Reyes AH (2000). Hepatic granulomas in an Arab population: a retrospective study from a teaching hospital in Riyadh. Saudi Journal of Gastroenterology 6:41-46.

Assefa H, Mulate B, Nazir S and Alemayehu A (2015). Cystic echinococcosis amongst small ruminants and humans in central Ethiopia. Journal of Veterinary Research 82(1):1-7.

Aziz A, Zhang W, Li J, Loukas A, McManus DP and Mulvenna J (2011). Proteomic characterisation of *Echinococcus granulosus* hydatid cyst fluid from sheep, cattle and humans. Journal of Proteomics 74:1560-72.

Collins DS and Huey RJ (2015).Gracey's Meat Hygiene, 11th Edn. Wiley-Blackwell, West Sussex, UK.

Craig PS (1993). Immunodiagnosis of *Echinococcus granulosus*. In: Compendium on cystic echinococcosis with special reference to the Xinjiang Uygur Autonomous Region. Andersen FL,Chai J, Liu CH (Ed.). The People's Republic of China. Brigham Young University Print Services, Provo, Utah, USA. pp. 85–118.

Craig PS, McManus DP, Lightowlers MW, Chabalgoity JA, Garcia HH, Gavidia CM and Nieto A (2007). Prevention and control of cystic echinococcosis. The Lancet: Infectious Diseases 7(6):385-394.

Craig PS, Rogan MT and Allan JC (1996). Detection, screening and community epidemiology of taeniid cestode zoonoses: cystic echinococcosis, alveolar echinococcosis and neurocysticercosis. Advances in Parasitology 38:169-250.

Dalimi A, Motamedi G, Hosseini M, Mohammadian B, Malaki H, Ghamari Z and Ghaffari F (2002). Echinococcosis/ hydatidosis in western Iraq. Veterinary Parasitology 105:161-171.

Daryani A, Alaei R, Arab R, Sharif M, Dehghan MH and Ziaei H (2007). The prevalence, intensity and viability of hydatid cysts in slaughtered animals in the Ardabil province of Northwest Iran. Journal of Helminthology 81:13-17.

Dohoo IR, Martin W and Stryhn H (2010): Veterinary Epidemiologic Research. AVC Inc., University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada.

Dore F, Varcasia A, Pipia AP, Sanna G, Pinna Parpaglia ML, Corda A, Romig T and Scala A (2014). Ultrasound as a monitoring tool for cystic echinococcosis in sheep. Veterinary Parasitology 203:59-64.

Fadaladdin YA, Alsaggaf AI and Wakid MH (2013). Comparative epidemiological studies on cystic echinococcosis of local and imported livestock in Al-Madina Al-Munawwarah in Saudi Arabia. The Egyptian Journal of Hospital Medicine 50:108-126.

Gatti A, Alvarez AR, Araya D, Mancini S, Herrero E, Santillan G and Larrieu E (2007). Ovine echinococcosis. I. Immunological diagnosis by enzyme immunoassay. Veterinary Parasitology 143:112-121.

Geramizadeh B (2013). Unusual locations of the hydatid cyst: a review from Iran. Iranian Journal of Medical Sciences 38(1):2-14.

Haftu B and Kebede T (2014). Study on prevalence and economic significance of bovine hydatidosis in Bako Muncipal Abattoir, West Shoa Zone, Oromiya Regional State. Journal of Veterinary Science Technology 5(5): 1-5.

Haroun EM, Omer OH, Mahmoud OM and Draz A (2008). Serological studies on hydatidosis in camels in Saudi Arabia. Research Journal of Veterinary Sciences 1(1):71-73.

Hasona NA, Amer OH, Morsi A and Azza Raef (2017). Comparative biochemical, parasitology and histo-pathological studies on cystic echinococcosis in infected sheep. Comparative Clinical Pathololgy 26(4):805-810

Ibrahem MM, Craig PS, McVie A, Ersfeld K and Rogan MT (1996). *Echinococcus granulosus* antigen B and sero reactivity in natural ovine hydatidosis. Research in Veterinary Science 61:102-106.

Ibrahim MM (2010). Study of cystic Echinococcosis in slaughtered animals in Al Baha region, Saudi Arabia: interaction between some biotic and abiotic factors. Acta Tropica 113(1):26-33.

Karibozorg M, Ali F, Mohammad B, Taghi G and Mohammad R (2014). Assessment of alkaline phosphatase activity in hydatid cyst protoscolices and liver tissue as a pathological biomarker. Journal of Medical Microbiology and Infectious Diseases 2(2):68-70.

Kim S, Christopher L and John DB (2013). Bancroft's Theory and Practice of Histological Techniques. 7th edn. Churchill Livingstone.

Li J, Yan J, Xiufang W, Zhaoqing Z, Junliang L, Mingxing Z and Wei Z (2013). Analysis of the chemical components of hydatid fluid from *Echinococcus granulosus*. Revista Da Socieda deBrasilrira DeMedicina Tropical 46(5):605-610.

Lightowlers MW (1990). Cestode infections in animals: immunological diagnosis and vaccination. Scientific and Technical Review – OIE 9:463-487

McManus DP (2006). Molecular discrimination of taeniid cestodes. Parasitology International 55(1):31-37.

McPherson CN (1985). Epidemiology of hydatid disease in Kenya: a study of the domestic intermediate hosts in Masailand. Transactions of The Royal Society of Tropical Medicine and Hygiene 79(2):209-217.

Moghaddas E, Borji H, Naghibi A, Razmi G and Shayan P (2014). Epidemiological study of hydatidosis in the dromedaries (*Camelus dromedarius*) of different regions of Iran. Asian Pacific Journal of Tropical Biomedicine 4(1):148-151.

OIE (2008). Echinococcosis/hydatidosis, In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. pp 175-189.

Osman F, Mohamad M and Gadee H (2014). The prevalence and biochemical characters of hydatid cyst in sheep and goats slaughtered at El-Karhga, New-Valley governorate, Egypt. Sky Journal of Agriculture Research 3(1):017-024.

Radfar M and Iranyar N (2004). Biochemical profiles of hydatid cyst fluids of Echinococcus granulosus of human and animal origin in Iran.Veterinarski Arhiv 74(6):435-442.

Rahdar M, Maraghi S, Rafei A and Razijalali M (2008). Comparison of some electrolytes in hydatid cyst fluid and serum of liver hydatidosis of sheep. Jundishapur Journal of Microbiology 1(1):10-14.

Ris DR, Hamel KL and Mackle ZM (1987). Use of two polysaccharide antigens in ELISA for the detection of antibodies to *Echinococcus granulosus* in sheep sera. Research in Veterinary Science 43:257-263.

Sage AM, Wachira T, Zeyhle EE, Weber EP, Njoroge E and Smith G (1998). Evaluation of diagnostic ultrasound as a mass screening technique for the detection of hydatid cysts in the liver and lung of sheep and goats. International Journal of Parasitology 28:349-353.

Schantz PM (1990). Parasitic zoonoses in perspective. International Journal of Parasitology 21(2):165-166.

Shaafie I, Khan A and Rambabu K (1999). Biochemical profiles of hydatid cyst fluids of *E. granulosus* of human and animal origin in Libya. Journal of Helminthology 73:253-258.

Shaldoum FM, Wafaa FA, Hanan TH and Shahin MS (2017). Comparative study on copper, zinc, magnesium and iron in hydatid cyst fluid (supernatant and residue) in sheep and camel in Egypt. The Egyptian Journal of Hospital Medicine 66:40-45.

Singh B, Sharma R, Sharma J, Mahajan V and Gill J (2016). Histopathological changes associated with *E. granulosus* echinococossis in food producing animal in Punjab (India). Journal of Parasitic Diseases 40(3):997-1000.

Soliman M G, Farid AA, Shalash IR, Abo Elqasem AA and El-Amir AM (2014). Evaluation of sandwich elisa with dot-elisa as an immunodiagnostic assay for cystic hydatosis using *E. granulosus* protoscoleces antigens. Global Veterinaria 13(2):150-158.

Thrusfield M (2005). Veterinary Epidemiology, 3rd edn. Blackwell Science Ltd, London. pp 182-198.

Toulah FH, El Shafi AA, Alsolami MN and Wakid MH (2017). Hydatidosis among imported animals in Jeddah Saudi Arabia. Journal of Liver and Clinical Research 4(1):1031.

Vuitton D and Gottstein B (2010). Echinococcus multilocularis and its intermediate host: a model of parasite-host interplay. Journal of Biomedicine and Biotechnology 2010:1-14.

Yong WK and Heath DD (1984). Comparison of cestode antigens in an enzyme-linked immunosorbent assay for the diagnosis of *Echinococcus granulosus, Taenia hydatigena* and *T. ovis* infections in sheep. Research in Veterinary Science 36:24-31.