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PATHOLOGICAL AND SEROLOGICAL STUDIES ON CYSTIC EXHINOCOCCOSIS IN NATURALY INFECTED CAMEL CALVES

***AMER, H.A.; *NIBAL, A.H. ;**DALAL, S. Mostafa and HASSAN, H.M.**
*Pathology of Reproduction Dept. **Biology of Reproduction Dep.
Immunology Unit. Animal Reproduction Research Institute, El- Haram, Giza.

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SUMMARY

Hydatid cyst infected lung and liver specimens (60 and 10 respectively) of camel calves (about 12-18 months old) were collected from Kerdasa abattoir, Giza governorate during the period from January to August 2004. These specimens were subjected to parasitological and pathological examinations. Blood sample was also taken from each camel calf for serological study.

Parasitological results revealed that, the infection rate with hydatid cysts in lungs was more than liver and the fertility of these cysts was 75% in lungs and 60% in liver. Serological results proved that, there were variable degrees of positivity that parallel correlate with gross findings of infected lungs and liver with hydatid cysts indicating different stages of parasitic infection. Pathological findings showed a significant host tissue response in the form of mononuclear cell infiltration and fibroblastic proliferation (medium sized cysts). In addition, the tissue surrounds the fertile cysts revealed hyalinization.

The study concluded that, histopathological and serological examinations must be applied in hydatid cyst control programmes particularly in young animals.

INTRODUCTION

Cystic Echinococcosis (CE) or cystic hydatid disease, caused by infection with the larval stage (metacestode) of *Echinococcus granulosus*, is considered to be one of the most important helminthes zoonoses. It is a widespread infection throughout the world and is found to occur in all domestic livestock

including camels (Craig et al., 1996 and Ibrahem and Gusbi, 1997). Older camels had higher infection rates (number and size of the cysts) than younger ones (Alyaman et al., 1985). On the other hand, the most predicated site of cystic infection in camels was the lung followed by the liver while the infection in other organs was rarely seen (Haridy et al., 1998 and Dyab et al., 2005). In contrast, the liver was the predominant infection site in sheep and goats (Alyaman et al., 1985; Ibrahem and Craig, 1998 and Mehrabani et al., 1999). Ibrahem et al. (2002) recorded that, ELISA based on serum antibody detection to Agb could be developed for immunodiagnosis of CE in camels. Hassan (1982) stated that, hydatid cyst causes an inflammatory reaction and compresses the surrounding tissue.

The aim of the present study is to estimate pathological and serological responses of the host to the cystic echinococcal infection in camel calves.

MATERIALS AND METHODS

A total of 60 hydatid cyst infected lung specimens (50 infected lungs only and 10 infected lungs and livers) of slaughtered camel calves were collected (during the meat inspection) from Kerdasa abattoir, Giza governorate during the period from January to August 2004. The age of the animals ranged from 12-18 months old. Blood samples were taken from each camel calf for serological study. The gross pathological lesions were recorded and representative portions from each infected lung and liver were taken in 10% formol saline for parasitological and pathological examinations.

Parasitological examination:

The discovered hydatid cysts were freed from the surrounding tissues and subjected to laboratory investigations to determine the fertility rate. The fertility of the cysts was determined by microscopic examination of a portion of hydatid fluid for the presence of broad capsules, daughter cysts and protoscolices (Zayed and Derbala, 1993; Lotfi et al., 1994; Saeed et al., 2000 and Mohamed, 2005)

Serological studies:

Serum samples were separated from the collected blood samples and stored in 1ml aliquots at -20°C. Each serum sample was numbered and labeled for CE according to the postmortem examination results.

Antigen preparation:

Crude hydatid cyst fluid antigen:

Hydatid cyst fluid was aspirated and collected from lung hydatid cyst under sterile conditions and examined microscopically for the presence of protoscolices. All hydatid cyst fluids were clarified by centrifugation at 2000 g for 15 minutes to remove the protoscolices and any other solid material and the supernatant was then stored in aliquots at -20°C until used.

Enzyme Linked Immunosorbent Assay (ELISA):

Echinococcus granulosus antigen was optimally diluted with 0.05 M bicarbonate/ carbonate buffer (BCB), Ph 9.6, and used to coat (100 µl/well) polystrene microtitre plates (Immulon, 1,Dynatech, USA), incubated at 4°C overnight and for 2hours at 37°C. Unbound antigen was removed by three washes with 0.1% phosphate buffer saline (PBS), Ph 7.4, mixed with 0.05% Tween 20(T 20) and locked with 100 µl/well of 0.3% PBS/ T 20 at room temperature (RT) for 1 hour. The blocking solution was removed and 100 µl/well of camel sera be tested optimally diluted with 0.3% PBS/T20, were added (in a single or duplicate) and incubated for two hours at RT. When camel sera were used and after incubation and three washes as above, 100µl/well of rabbit polyclonal anticamel IgG optimally diluted (10µg/ml) with 0.3% PBS/T20 were added for one hour incubation at RT, followed by goat antirabbit IgG (all molecules) alkaline phosphatase conjugate (Sigma) optimally diluted (2.3 mg/ml) with 0.3% PBS/T20 for 1.5 hour incubation at RT. After conjugation treatment, all plates were washed and developed by addition of 100 µl/well of the substrate p- nitrophnyl phosphate (P-NPP, Sigma) at 1mg/ml with 1M diethanolamine buffer, Ph 9.8, for 15 – 30 minutes in the dark. The optical density (OD) values were measured at 405 nm using an ELISA automatic microplate reader (Dynatech MR 5000, USA).

Enzyme Linked Immunosorbent Assay standardization (ELISA):

ELISA standardization included determination of optimal working dilution (and concentration) for antigen, sera and anti-species immunoglobulins or conjugates using checkerboard titration from serial dilutions with known positive and negative serum samples (Voller et al., 1976). The reference serum samples used in this standardization were obtained from camel calves confirmed to have infection with CE as assessed at post-mortem examination. In addition, negative control serum samples were obtained from animals confirmed not to have any gross signs of infection as assessed at post-mortem

examination (Essentially helminthes free). ELISA cut off values for each antigen preparation was based on the mean OD values of all serum samples from camels that did not exhibit any gross infection plus three standard deviations above the mean.

Histopathological examination:

The fixed collected lung and liver specimens were processed by conventional paraffin embedding technique, sectioned (3-4 µm) and stained by hematoxylin and eosin (Harris, 1898) for histopathological examination.

RESULTS

Parasitological results:

Gross examination of the collected hydatid cyst infected lungs and livers of the camel calves revealed that, the infection rate was more in lungs than the livers (60 and 10 respectively). Concerning the fertility and sterility of the collected cysts, they were 75% and 25% respectively for lungs and 60% & 40% respectively for livers (Table).

Table (1): Condition of the collected hydatid cysts in lungs and livers of camel calves.

condition / organ	fertile		sterile	
	No.	%	No.	%
lungs(60)	45	75	15	25
livers(10)	6	60	4	40

Serological results:

ELISA positive-negative cut off values were calculated (0.32) and sera were recorded as positive or negative by comparing the ELISA optical density (OD) regarding with cut off absorbance values.

The result revealed that, the collected samples (60) were positive with ELISA technique. The positive samples displayed variable values of absorbance at 405nm (Figure 1).

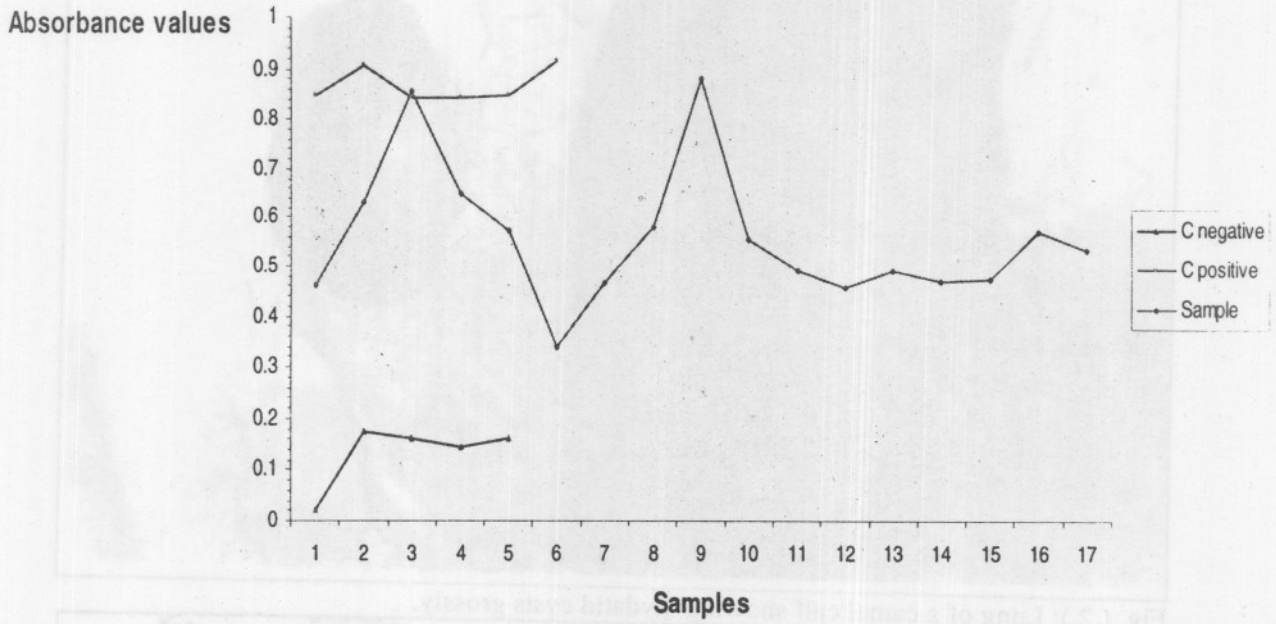

Fig. (1): Distribution curve of different serum samples by ELISA

Pathological results:

Gross findings:

The infected lungs and livers showed mild to moderate congestion with the presence of very small to moderate sized hydatid cysts scattered throughout pulmonary and hepatic tissues. The tissue surrounds the cysts appeared firm and dark blue in color (Fig. 2).

Histopathological findings:

Lungs:

Lung tissue close contact with the hydatid cyst wall (the outer layer was homogenous, structurless and laminated) showed infiltration of mononuclear inflammatory cells mostly of lymphocytes (Fig. 3) in addition, fibroblastic proliferation was seen in medium sized hydatid cyst infected lungs (Fig. 4). The alveoli were compressed into scarcely recognizable slits lying parallel in a direction of the adjacent pressure (Fig. 5) in few lungs with fertile cysts meanwhile, compensatory emphysema was noticed near the areas of lung collapse (Fig. 6). Blood vessels and capillaries revealed severe congestion (Fig.7) and areas of diffuse hemorrhage were also observed in few lungs (Fig.8).

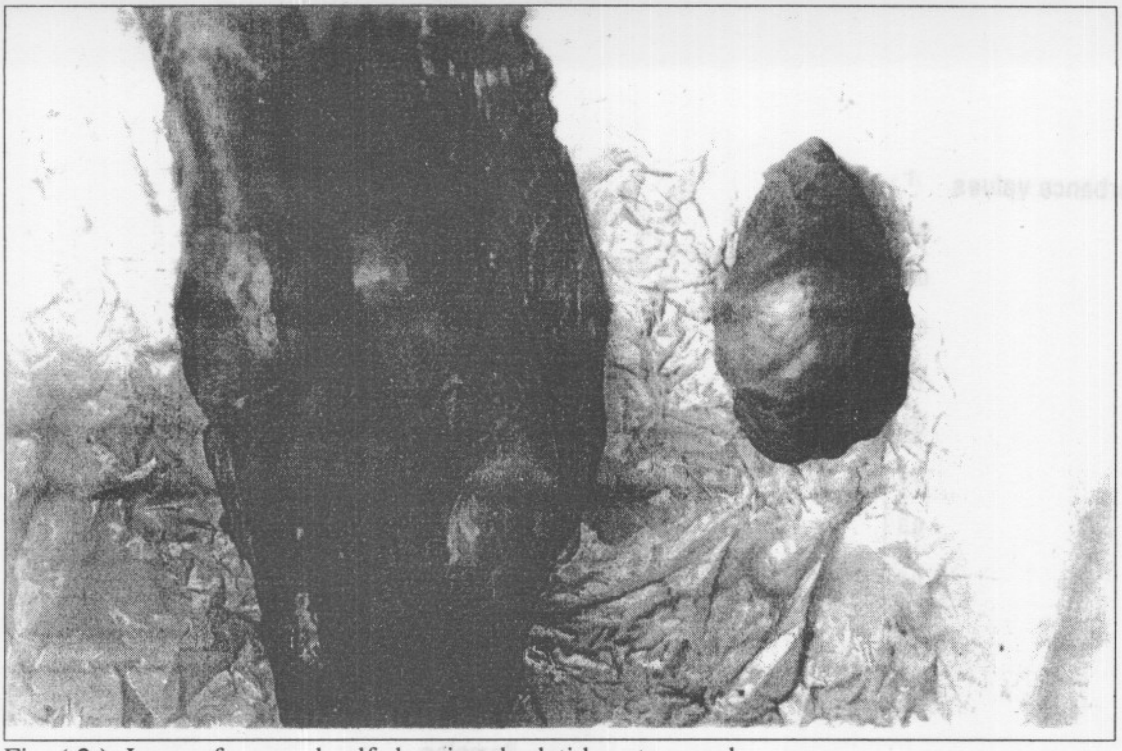

Fig. (2): Lung of a camel calf showing hydatid cysts grossly.

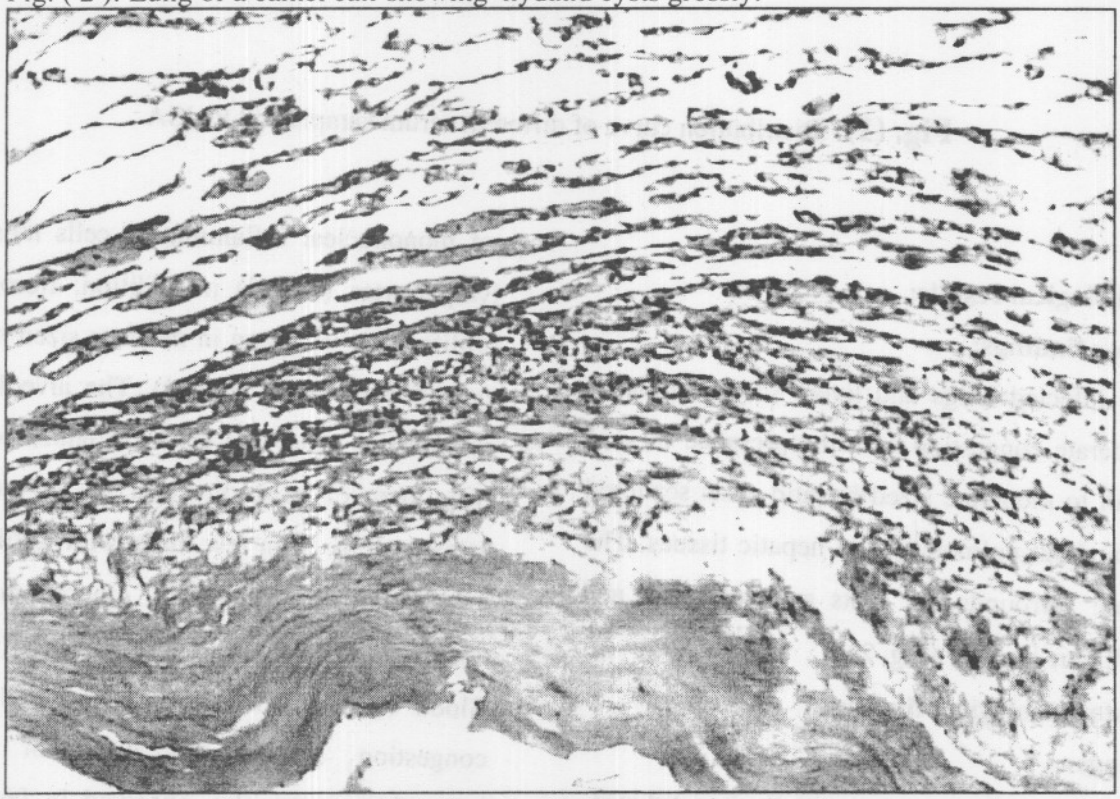

Fig. (3): Lung of a camel calf infected with hydatid cysts showing mononuclear cell infiltration mostly of lymphocytes close contact with the hydatid cyst wall (H&E; X100).

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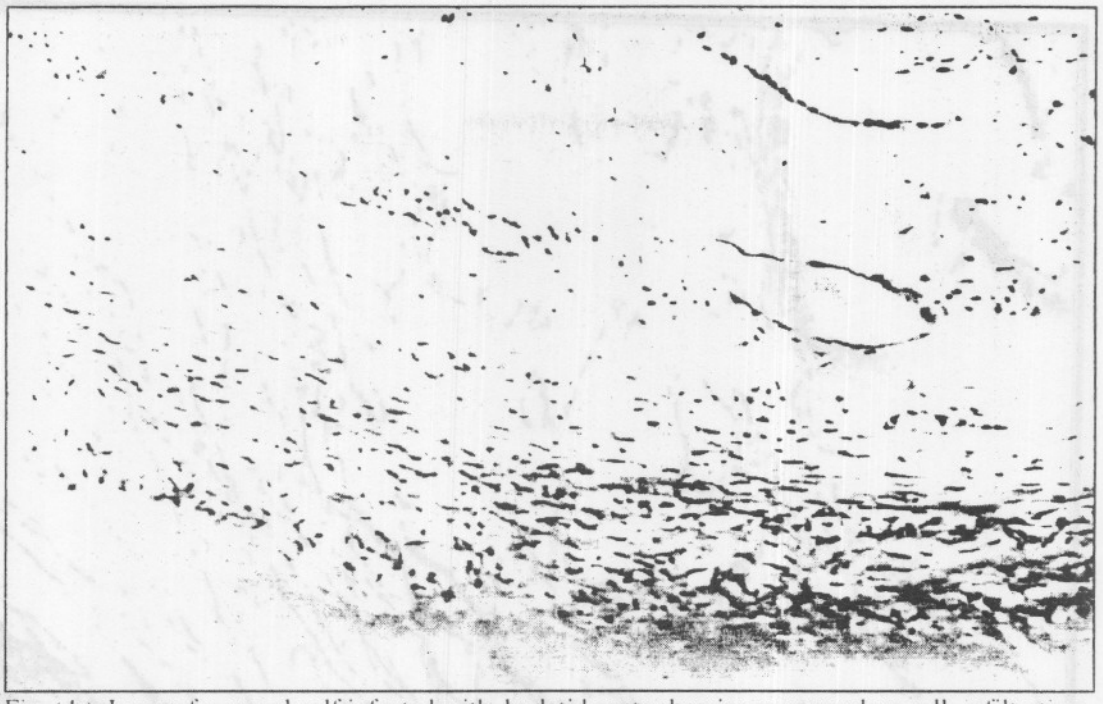

Fig. (4): Lung of a camel calf infected with hydatid cysts showing mononuclear cell infiltration and fibroblastic proliferation close contact with the hydatid cyst wall (H&E; X100).

Fig.(5): Lung of a camel calf infected with hydatid cysts showing collapsed alveoli (notice the scarcely recognizable slits lying parallel in a direction of the adjacent pressure) - (H&E; X100).

Fig. (6): Lung of a camel calf infected with hydatid cysts showing compensatory emphysema (notice the alveoli have been stretched and burst) - (H&E; X100).

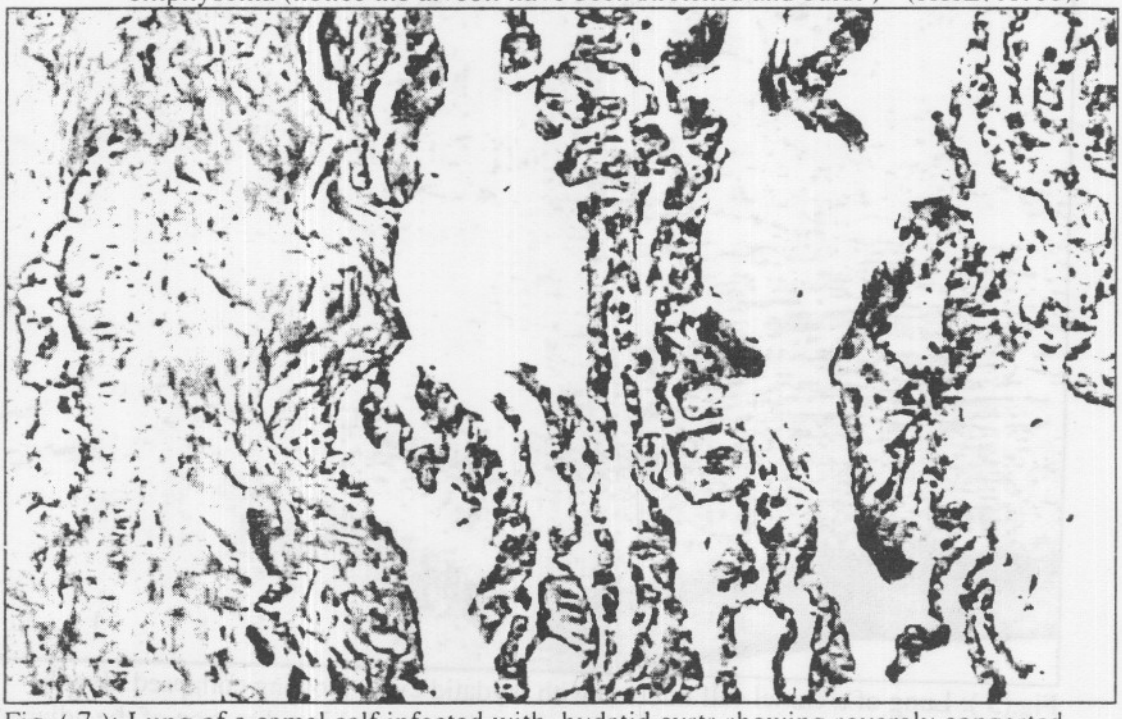

Fig. (7): Lung of a camel calf infected with hydatid cysts showing severely congested blood capillaries (H&E; X100).

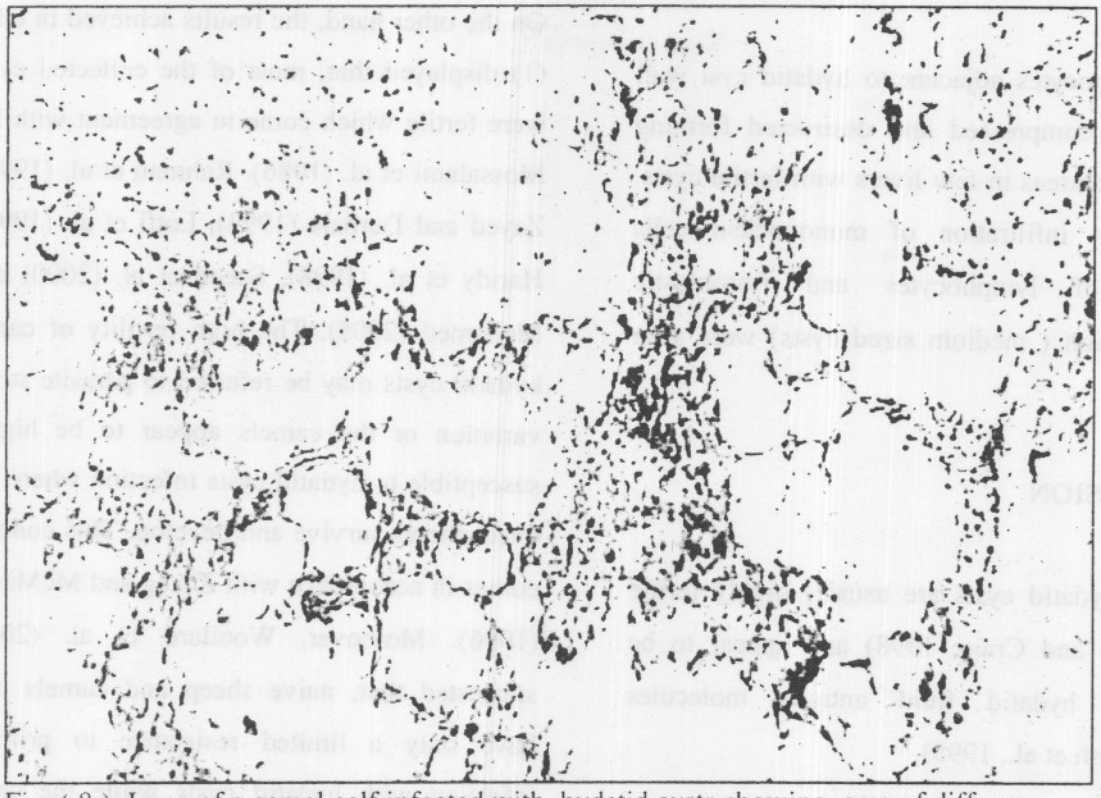

Fig. (8): Lung of a camel calf infected with hydatid cysts showing area of diffuse hemorrhage (H&E; X100).

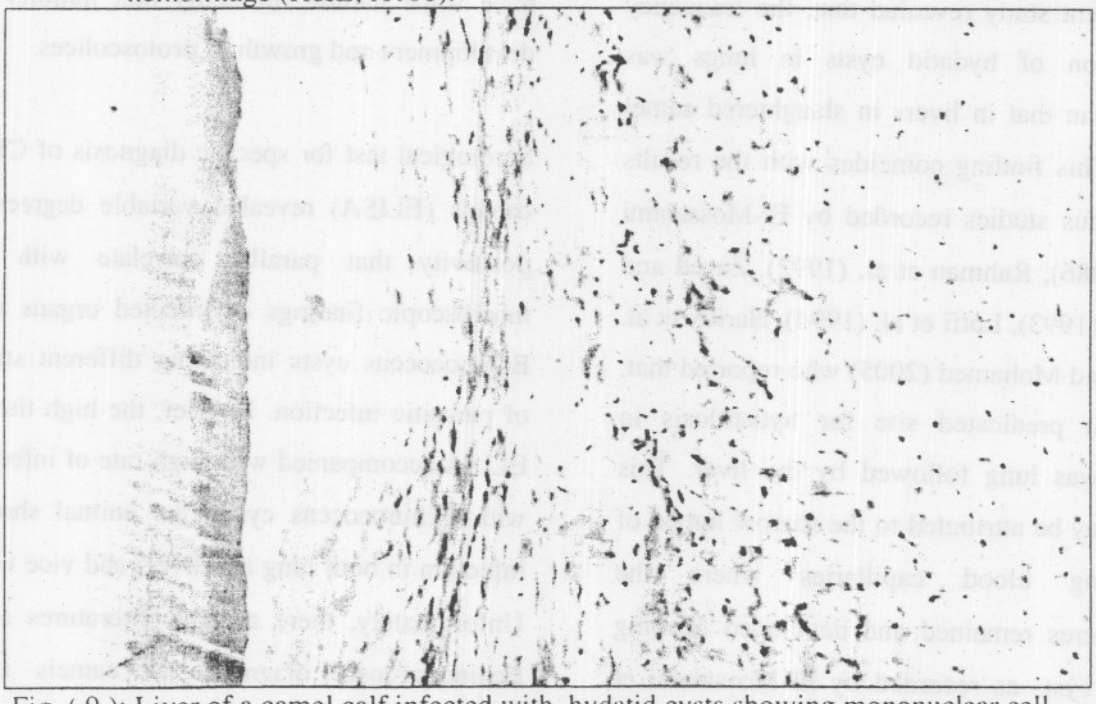

Fig. (9): Liver of a camel calf infected with hydatid cysts showing mononuclear cell infiltration, fibroblastic proliferation and hyalinized areas close contact with hydatid cyst wall (H&E; X100).

Liver:

The hepatocytes adjacent to hydatid cyst wall appeared compressed and destructed forming hyalinized areas in few livers with fertile cysts. Moreover, infiltration of mononuclear cells mostly of lymphocytes and fibroblastic proliferation (medium sized cysts) were seen (Fig.9).

DISCUSSION

Camel hydatid cysts are usually highly fertile (Ibrahem and Craig, 1998) and appear to be rich in hydatid fluid antigen molecules (Shambesh et al., 1995).

The present study revealed that, the frequency distribution of hydatid cysts in lungs was higher than that in livers in slaughtered camel calves. This finding coincides with the results of previous studies recorded by El-Mossalami et al. (1986), Rahman et al. (1992), Zayed and Derbala (1993), Lotfi et al. (1994), Haridy et al. (1998) and Mohamed (2005) who reported that, the most predicated site for hydatidosis in camels was lung followed by the liver. This result may be attributed to the narrow lumen of the lung blood capillaries where the oncospheres remained and developed forming hydatid cysts as recorded by El-Mossalami et al. (1986).

On the other hand, the results achieved in table (1) displayed that, most of the collected cysts were fertile which come in agreement with El-Mossalami et al. (1986), Rahman et al. (1992), Zayed and Derbala (1993), Lotfi et al. (1994), Haridy et al. (1998), Saeed et al. (2000) and Mohamed (2005). The high fertility of camel hydatid cysts may be referred to parasite strain variation or the camels appear to be highly susceptible to hydatid cysts infection where the oncospheres survive and develop. This concept comes in accordance with Zhang and McManus (1996). Moreover, Woollard et al. (2000) suggested that, naive sheep and camels may have only a limited resistance to primary infection with hydatid cysts while the cattle have some natural immunity that inhibits the development and growth of protoscolices.

Serological test for specific diagnosis of CE in camels (ELISA) revealed variable degrees of positivity that parallel correlate with the macroscopic findings of infected organs with Echinococcus cysts indicating different stages of parasitic infection. In brief, the high titer of ELISA accompanied with high rate of infection with Echinococcus cysts (the animal showed infection in both lung and liver) and vice versa. Unfortunately, there are few literatures about Echinococcosis diagnosis in camels using ELISA technique.

The obtained results are in accordance with findings of Ibrahem and Gusbi (1997); they recorded that the serological tests for specific diagnosis of CE in camels would be useful for screening hydatid infection in commercial livestock production or epidemiological studies. To explain these data, Woollard et al. (2000) and Thompson and McManus (2001) found that the earliest detectable immunoglobulin G for response to hydatid cyst infection antigen occur after 2-11 weeks in mice and sheep respectively. The early infection may be associated with a significant cellular inflammatory response (Richards et al., 1983 and Liu et al., 1993) that may cause pathologic changes (Allen & Maizels, 1996 and Ferreira et al., 1995) since there is an increased leucocytosis. With oncospheres, necrosis of surrounding cells is followed by infiltration of neutrophils and macrophages 3-5 days after infection in sheep (Pearce et al., 1991). Experiment in vitro have shown also that neutrophils, in association with antibody, can bring about the killing of *E. granulosus oncospheres* (Rogan, 1988), suggesting a possible role for antibody-dependent cell-mediated cytotoxicity reactions. At the early stages of disease, there is a marked activation of cell-mediated immunity to the parasite (Finkelman et al., 1991).

Concerning pathological results, the histopathological findings observed in this study (mononuclear cell infiltration and fibroblastic proliferation) represent host tissue response against hydatid cyst infection. This view was supported by Richards et al. (1983), Dixon (1997) and Ferreira et al. (2000) who reported that, early infection with hydatid cyst may be associated with a significant cellular inflammatory response that may cause pathological alterations. In addition, Ramzy et al. (1999) and Rigano et al. (1999) recorded that in the establishment phase of the infection, cellular infiltration and fibrocytes were noticed. On the other hand, hyalinization observed in the surrounding tissue of the fertile cysts may be attributed to the presence of oncospheres as reported by Pearce et al. (1991).

Hydatid cyst fluid toxins play a great role in the development of these responses. This suggestion come in harmony with De Rycke et al. (1991), Janssen et al. (1992), Janssen et al. (1993) and Janssen et al. (1997).

Early diagnosis of CE can result in significant improvements in the quality of the management and treatment of the disease. In most cases, the early stages of infection are asymptomatic, so that methods that are cheap and relatively easy to use are required for large scale screening of

populations at high risk. Immunodiagnosis provides such an approach and can also confirm clinical findings. This hypothesis coincides with that of Wenbao Zhang et al. (2003).

CONCLUSION

Abattoir data are important particularly in the surveillance of hydatid cyst control programmes, but it can be very difficult to identify specifically small lesions in the lungs and / or liver of young animals without additional histological examination. Moreover, the ante-mortem diagnosis of CE in livestock would also be useful where abattoir data are absent or difficult to obtain. Therefore, a specific serological test would be useful.

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دراسات باثولوجية وسيرولوجية على صغار الجمال المصابة بحويصلات الايكينوكوكاس

هانىء عبد الخالق عامر –نيبال عبد العليم حسن – دلال سعد الدين مصطفي - هانى محمد حسن

معهد بحوث التناسليات الحيوانية –الهرم –الجيزة

تم جمع عدد ٦٠ رئة و ١٠ كبد مصابة بحويصلات الهيداتيد من صغار الجمال (عمر حوالى ١٢ – ١٨ شهرا) مذبوحة فى مجزر كرداسة التابع لمحافظة الجيزة فى الفترة من شهر يناير الى أغسطس ٢٠٠٤.

اجريت الفحوص الطفيلية والباثولوجية لتلك العينات كما أخذت عينة دم من كل جمل مصاب للفحص السيرولوجى، وقد أوضح الفحص الطفيلى أن معدل الاصابة بحويصلات الهيداتيد فى الرئات كان أكبر من الكبد وأن نسبة خصوبة هذه الحويصلات (الحيوية) فى الرئات كانت ٧٥% بينما كانت فى الكبد ٦٠%.

أثبت الفحص السيرولوجى أن هناك علاقة متوازية بين معدل الإصابة بالحويصلات ومستوى الأجسام المضادة فى مصل هذه الجمال، كما بين الفحص الهيستوباثولوجى للأنسجة المصابة بالحويصلات أن هناك رد فعل التهابى من الجسم فى النسيج الملاصق للحويصلات متمثلا فى وجود ارتشاح للخلايا الالتهابية وحيدة النواه وخصوصا "الليمفوسايت" وكذلك وجود بعض التليفات فى حالة الإصابة بالحويصلات متوسطة الحجم، أيضاً ظهر إضمحلال بللورى (hyalinization) فى النسيج المحيط بالحويصلات.

خلصت الدراسة الى أنه يجب تطبيق الفحص "الهيستوباثولوجى والسيرولوجى" فى برامج السيطرة على حويصلات الهايداتيد فى الجمال وخصوصا فى الأعمار الصغيرة.