

ULTRASONOGRAPHIC DIAGNOSIS OF HEPATIC HYDATID CYSTS IN DONKEYS

ABU-SEIDA, A.M.

* Dept. of Surgery Anaesthesiology and Radiology,
Faculty of Vet. Med., Cairo Univ.

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SUMMARY

Hepatic ultrasonography was carried out on 45 donkeys (used for educational purposes) for the presence of hydatid cysts. Eight donkeys representing 17.8% of the total examined animals had hepatic hydatid cysts. The age of the affected donkeys was 5-10 years. Hepatic hydatid cysts were imaged through only one intercostal space in 5 donkeys, through two adjacent spaces in one, through three scattered spaces in one and through 8 scattered spaces in another donkey. Ultrasonographically, the cyst appeared as unilocular rounded, oval or pear-shaped anechoic sac with a distinct thin echogenic capsule and strong distant enhancement. The diameter of the cysts was between 2.15cm and 10cm and their number was 1-15 cysts. In every affected animal, the diagnosis was confirmed by centesis and aspiration of the hydatid fluid under ultrasonographic guidance.

The fluid was colourless, odourless and transparent containing protoscolices microscopically. After ultrasonic examination, the all examined donkeys were euthanized and hepatic hydatid cysts were found in eight animals with 100% positive predictive value. The results of serum biochemical tests and histopathological findings were also discussed.

INTRODUCTION

Echinococcosis or hydatid disease is a cyclozoonotic infection caused by the larval stages of several species of the genus *Echinococcus*. The final definitive host is a carnivore (dog, fox,...etc.) and the larval form (hydatid cyst) occurs in one or more species of herbivorous and omnivorous vertebrate animals and human (Gordo and Bandera, 1998).

Hydatid disease today constitutes a major public health and economic problem in all continents (Schantz and Schwabe, 1969; Dixon et al., 1973; Gelberg et al., 1984; Miyauchi et al., 1984; Fahmy et al., 1989; Gathura and Kamiya, 1990; Bin-hazim et al., 1992; Himonas et al., 1994; Hosseini and Eslami, 1998; Sobeih et al., 1998 and Eckert 2007). In most Mediterranean countries, the disease is hyperendemic in sheep, camels, goats and donkeys (Laila , 1991; Hoida et al., 1998; Mukbel et al., 2000 and Haridy et al., 2006).

Published records of the incidence of equine hydatid cysts are scarce and the majority are based on post-mortem finding (Andrews and Lancaster, 1990; Miller et al., 1993; McGorum et al., 1994 and Gonenc et al., 1998). In an abattoir survey, Dixon et al. (1973) found hepatic and pulmonary hydatid cysts in 61.7% of the examined horses. On the other hand, Mukbel et al. (2000) observed hydatid cyst in the liver and lungs of 16.9% of the necropsied donkeys.

Equine hydatid cysts are usually unilocular and located in the liver and/or lungs (Edwards, 1981; Rezabek et al., 1993 and Gordo and Bandeira, 1998). Miyauchi et al. (1984) recorded a case of multilocular hydatid cyst in a horse.

Since clinical manifestations of hydatid disease are rare, ante-mortem diagnosis is still difficult depending on serodiagnostic techniques (Mousa and Aida , 1999).

Hydatid cysts have been visualized on equine and caprine thoracic radiograph as circular homogeneous radiodensities (Gelberg et al., 1984 and Ramadan and Al-Bihari, 1987).

Ultrasonography was used by Maxson et al. (1996) for diagnosis of hepatic and pulmonary hydatidosis in sheep and goats with 82.1% positive predictive value. In addition, Lahmar et al. (2007) used ultrasonography for detection of viable and dead hydatid cysts in the liver of the Tunisian sheep while Kalinbacak et al. (2002) diagnosed hepatic hydatid cyst in a cow by the ultrasound.

Akhan et al. (1993) and Deger et al. (2000) treated abdominal hydatid cysts in sheep under ultrasonic guidance. Guarnera et al. (2000) used ultrasound for control of hydatidosis.

Unfortunately no proper studies have been carried out to determine the role of donkeys in the hydatid disease cycle in Egypt. Therefore, the present study is carried out to record- for the first time- the incidence of hepatic hydatid cysts in donkeys by using ultrasound and to assess ultrasonography as a method of diagnosis.

MATERIALS AND METHODS

During the period between May 2003 and May 2006, 45 donkeys (used for educational purposes) were randomly selected for hepatic ultrasonography at surgery clinic in Faculty of Veterinary

Medicine, Cairo University. The animals' age ranged from 2 to 10 years and they were of both sexes (23 males and 22 females).

The ultrasound examinations were carried out by the technique described by Smith (2002).

The examinations were made with a 3.5 MHz convex transducer connecting to Toshiba ultrasound just vision system.

The hair was clipped and the skin shaved between the right 7th intercostal space and a hand's breadth behind the last rib. After the application of the ultrasound coupling gel to the transducer, the donkeys were examined in the standing position beginning caudal to the right last rib and ending at the right 7th intercostal space. Each intercostal space was examined dorsally to ventrally with the transducer held parallel to the ribs. All the hepatic structures and texture were examined.

In donkeys with hepatic cysts, the cysts were aspirated under local analgesia and ultrasonographic guidance to confirm the diagnosis. A puncture needle with trocar stylet (17G x 150mm) was introduced and guided ultrasonographically through the abdominal wall towards the cyst. The end of the needle within the cyst was usually seen in the ultrasonographs. The stylet was removed and 10-20ml of the cystic fluid was aspirated with a syringe (Fig.1) for direct microscopic examination.

Serum biochemical examinations were carried out on the affected animals to determine albumin, globulin, total bilirubin and gamma-glutamyl transferase (GGT) values according to Durham et al. (2003).

After euthanasia of the examined animals, post-mortem examinations were made to compare between the ultrasonographic findings and post-mortem findings.

For histopathological examination, multiple specimens from the cysts and adjacent hepatic tissue were fixed in 10% neutral buffered formalin, processed by conventional paraffin embedding technique, sectioned and stained with haematoxylin and eosin.

RESULTS

On hepatic ultrasonographic examination of 45 donkeys, eight donkeys representing 17.8% of the total examined animals had hepatic hydatid cysts. The affected animals were five males and three females and their age ranged between five and ten years. All data concerning animal's description and cysts characteristics were listed in table (1).

The affected donkeys had decreased appetite and loss of weight and three of them had various degrees of icterus.

Ultrasonographically, the cyst appeared as unilocular anechoic rounded (Fig. 2), oval or pear-shaped sac (Fig.3b) inside the hepatic parenchyma (7 cases) or protruding from it (one case). All hepatic hydatid cysts showed strong distant enhancement (Fig. 3a). The cyst had thin echoic capsule and its diameter ranged between 2.15cm and 10cm (Fig. 3). The number of the cysts was one (3 cases), two (two cases, Fig. 4), three (one case), eleven (one case, Fig. 5) and fifteen (one case, Fig. 6).

The hepatic hydatid cysts could be imaged in one intercostal space in five donkeys and in two, four and eight adjacent or separated intercostal spaces in one donkey each.

Additional abnormal hepatic ultrasonographic finding included liver cirrhosis in two donkeys with hepatic hydatid cysts (Fig. 2) and in one she-donkey without hydatid cysts.

In all donkeys with hepatic cysts, the diagnosis was confirmed by percutaneous centesis and aspiration of hydatid fluid under ultrasonographic guidance. The fluid was odourless, colorless, transparent and under pressure (Fig. 1). Some

cysts had brownish hydatid sand. Microscopically some cysts were fertile containing protoscolices (Fig. 7) while others were sterile.

Biochemical serum tests revealed marked increase in globuline (50-70 g/L - normal, 42), gammaglutamyl transferase (GGT) (60-80 IU/L - normal, 54), total bilirubin (4.8-9 mmol/L - normal, 2.9) and decreased albumine value (18-21 g/L - normal, 27.4).

Histopathologically, the cyst had a thick fibrous connective tissue capsule and the adjacent hepatic tissue showed necrosis and atrophy of the hepatocytes together with diffuse hepatic fibrosis (Fig. 8). The fibrous capsule contained multifocal areas of infiltration with eosinophils, lymphocytes and plasma cells.

Subsequent post mortem examination of all donkeys confirmed the ultrasonographic findings with 100% positive predictive value. The cysts were either superficial or embedded in the hepatic tissue. They were spherical, oval or pear-shaped and with thick yellowish white pericystic capsule. A thick white germinal membrane and dark brown hydatid sand were identified.

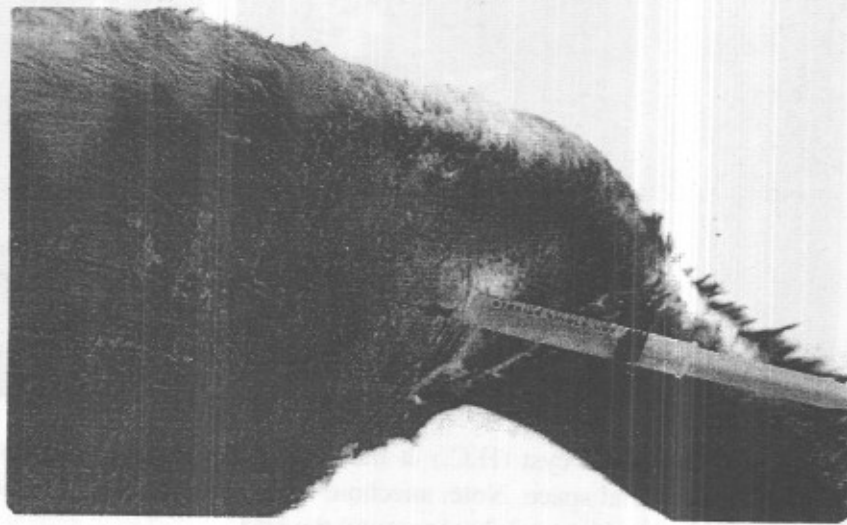


Fig.(1): Percutaneous centesis and aspiration of the hepatic hydatid cyst under ultrasonographic guidance. Note the colourless hydatid fluid.

Table (1): Description of the affected donkeys and characteristics of the hepatic hydatid cysts.

Animal's Description	Hepatic hydatid cyst's characteristics			
	Number	Shape	Diameter (cm)	Site
7-year-old donkey	1	Rounded	2.15	Dorsal third of 13 th ICS*
5-year-old donkey	1	Pear-shaped	10	Middle and ventral thirds of 13 th ICS
10-year-old donkey	1	Rounded	7.5	Ventral third of 12 th ICS
10-year-old donkey	2	Rounded+half rounded	7.3 and 8.1	Dorsal and middle thirds of 13 th ICS.
5-year-old she donkey	2	Rounded	3.7 and 4.3	Middle and ventral thirds of 13 th ICS
7-year-old she donkey	3	Rounded+oval	3.8,5.1,6.2	Middle and ventral thirds of 13 th and 14 th ICS
10-year-old donkey	11	Rounded+oval +pear shaped	3.4-10	Dorsal, middle and ventral thirds of 14 th , 11 th , 10 th and 8 th ICS.
10-year-old she donkey	15	Rounded+oval +pear shaped	4.5-10	Dorsal, middle and ventral thirds of 15 th – 8 th ICS.

* ICS = intercostal space

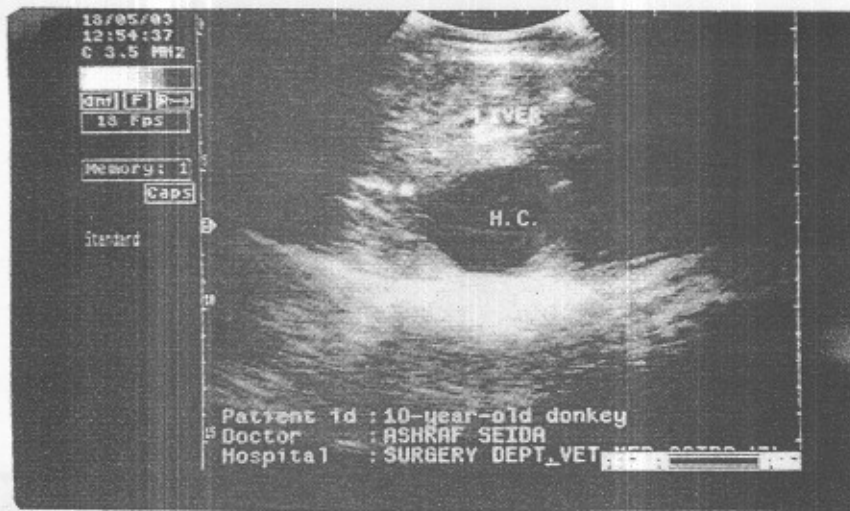


Fig.(2): Hepatic hydatid cyst (H.C.) at the level of the ventral third of the 12th intercostal space. Note, anechoic rounded cyst, strong distant enhancement and liver cirrhosis around the cyst.

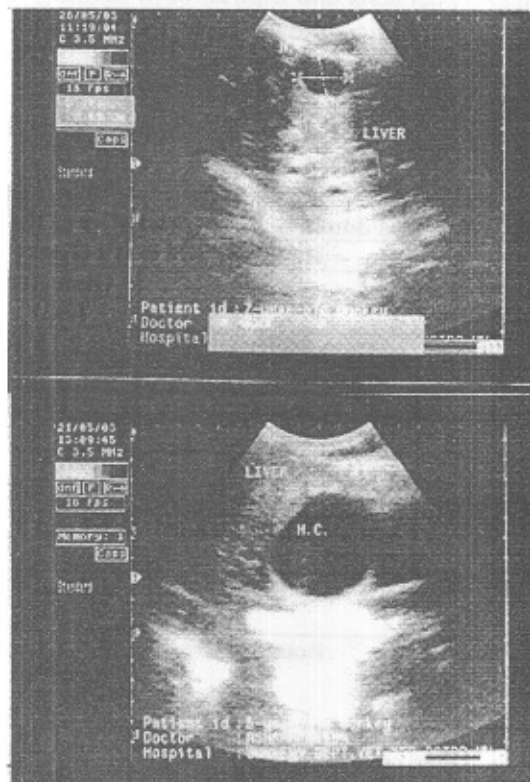


Fig.(3): (a) A small-sized (2.15cm) hepatic cyst imaged at the dorsal third of the 13th intercostal space.
(b) A large-sized (10cm), pear-shaped hepatic hydatid cyst at the level of the middle and ventral thirds of the 13th intercostal space.

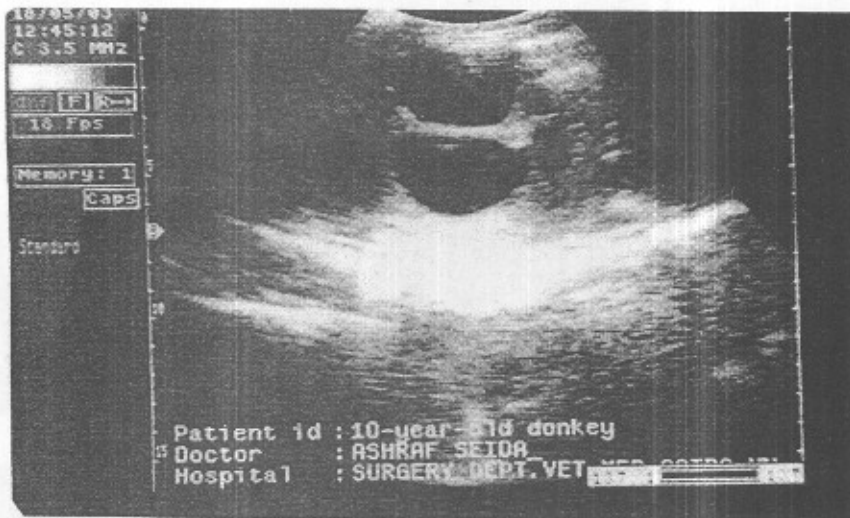


Fig.(4): Two hepatic hydatid cysts with distant enhancement at the level of the dorsal and middle thirds of the 13th intercostal space.

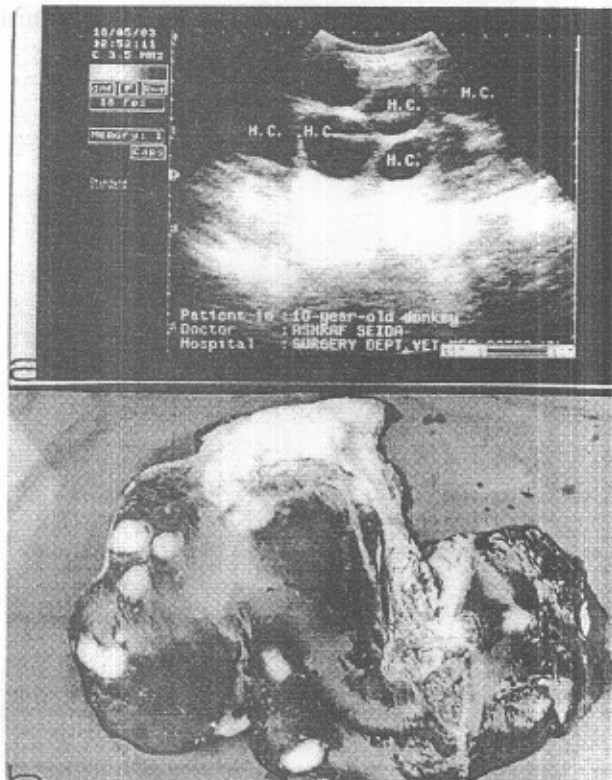


Fig.(5): (a) Multiple hepatic hydatid cysts (H.C.) of different sizes and shapes in a 10-year-old donkey.
(b) Liver specimen of the same animal showing multiple embedded hepatic hydatid cysts.

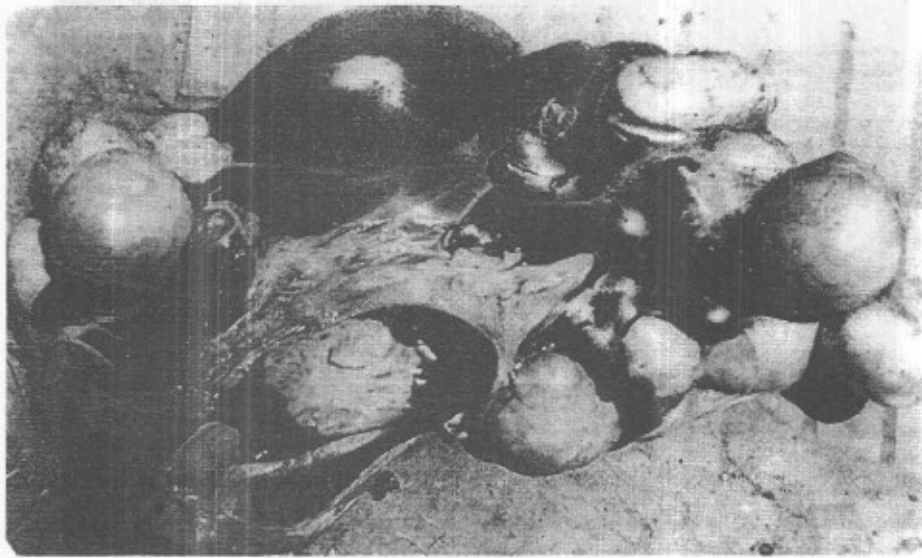


Fig.(6): Liver specimen showing multiple embedded and protruded hydatid cysts.

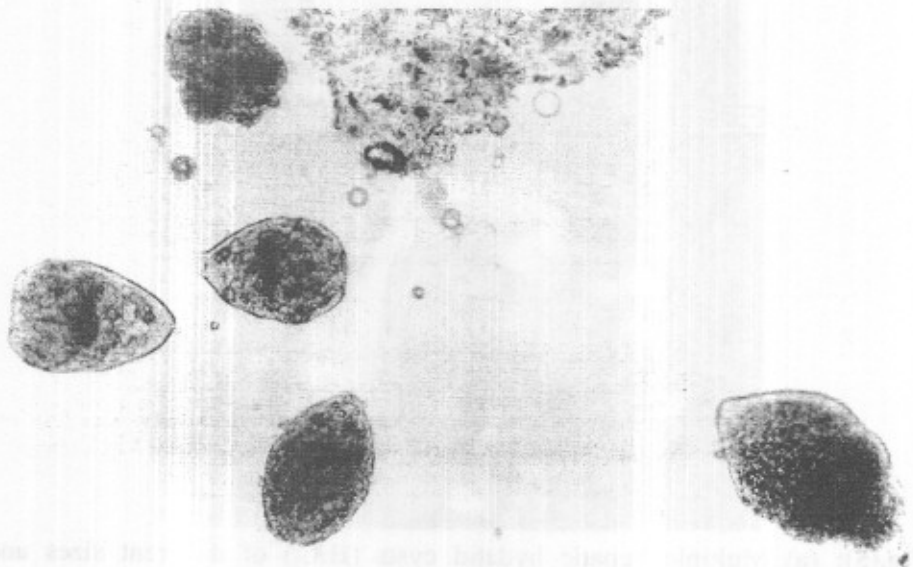


Fig.(7): Protoscolices recovered from hepatic hydatid cyst in a she-donkey (x100).

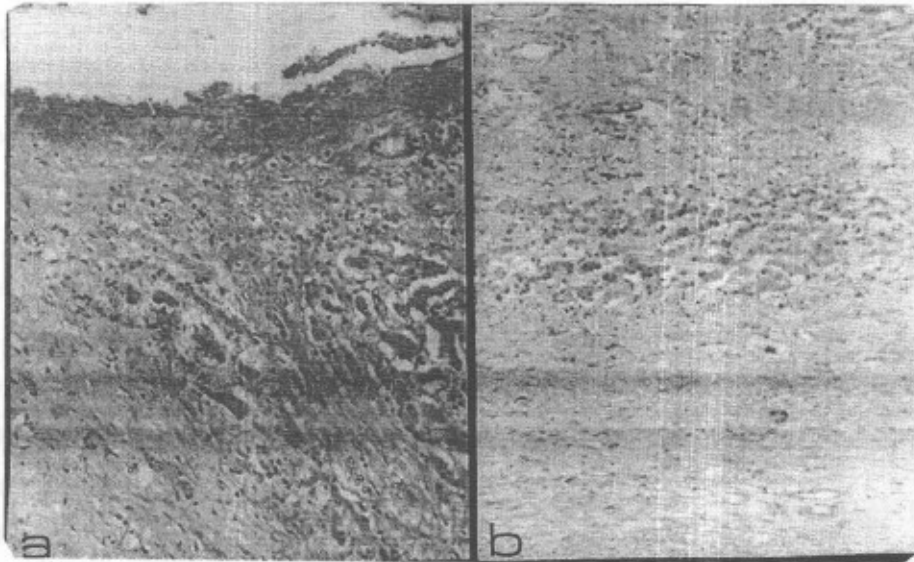


Fig.(8): (a) Photomicrograph of the hepatic hydatid cyst showing thick fibrous connective tissue capsule surrounded by necrosis and atrophy of hepatocytes (H & E x 200).
 (b) Photomicrograph of the affected liver showing diffuse hepatic fibrosis (H& E X 200).

DISCUSSION

Hydatidosis is a worldwide distributing disease. This global distribution is partly due to the ability of the parasite to adapt to a wide variety of domestic and wild intermediate and definitive hosts.

In Egypt, the existence of a significant reservoir of the parasite must consequently remains a matter of concern in the fields of both veterinary and public health. Several abattoir studies were carried out on hepatic hydatid cysts in slaughtered animals. Meanwhile no reports-concerning the incidence of hepatic hydatid cyst in donkeys in Egypt or on ante-mortem diagnosis of this liver disease-were found in the available literatures.

In the present study, hepatic hydatid cysts were diagnosed ultrasonographically-for the first time - in 17.8% of the total examined donkeys. No donkeys of four years old or less were infected. In Jordan, Mukbel et al. (2000) mentioned nearly the same results in 130 necropsied donkeys. In contrast in Turkey, Gonenc et al. (1998) found only one horse with hepatic hydatid cysts among 128 horses and 112 donkeys slaughtered to feed carnivores in Ankra Zoo.

This high incidence of hepatic hydatidosis in donkeys could be explained by the close contact between infected dogs and foxes and donkeys. The present increase in the prevalence of hydatid disease in donkeys in Egypt has been correlated with the increasingly popular practice of feeding dogs

row offal from infected animals. The adult cestodes that develop in the dog's bowel after ingesting offal pass eggs in the dog's feces which contaminate donkeys' feeding stuff, completing the cycle.

In this study, the recorded hepatic hydatid cysts were unilocular, rounded, oval or pear-shaped and of 2.15-10 cm diameter. Most of them were sterile and few were fertile. Similar findings were mentioned by Gelberg et al. (1984), Binhazim et al. (1992) and Gordo and Bandera (1998) in horses.

The observation that the liver is the predilection site of hydatidosis in equine is in accordance with other studies (Miller et al., 1993; Rezabek et al., 1993 and Mukbel et al., 2000) and may be explained by the liver serving as a primary barrier in the body after the penetration of the intestinal wall. Similar explanation was mentioned by Saeed et al. (2000).

There is little information available on the growth and development rates of equine hydatid cyst but Edwards (1981) recovered cysts of up to 36 x 15 x 20mm 15 months after infection. This suggests that most cysts described in the present study were from infections since more than 15 months. This may explain that all the affected donkeys in this study were more than 5 years old. This fact, together with the finding of small cysts in the presence of large ones in the same donkey, points

to the absence of either a protective age immunity to infection or significant levels of acquired resistance to infection and suggests that donkeys may become re-infected.

Diagnosis of hepatic hydatidosis was difficult and based mainly on serodiagnostic techniques as ELISA (Mousa and Aida, 1999) and on post-mortem examination (Mukbel et al., 2000). The clinical findings of the affected donkeys were non-specific as has been described by other authors (Binhazim et al., 1992 and Durham et al., 2003). These findings included moderate disturbances of the general condition, reduced food intake and loss of weight. Icterus as a sign of hepatic malfunction was seen only in three donkeys indicating that the flow of bile was impaired.

In agreement with Durham et al. (2003), the results of biochemical examinations showed hyperglobulinaemia, hypoalbuminaemia, increased gammaglutamyl transferase (GGT) and increased total bilirubin. These results may not be directly applicable to diagnose hepatic hydatidosis as they appear in most liver diseases. Similar result was recorded by Binhazim et al. (1992).

Although radiological examination could be used for diagnosis of pulmonary hydatidosis in a goat (Ramadan and Al-Bihari, 1987), it is so difficult to use in donkeys with hepatic hydatidosis due to their large sizes.

In contrast to Binhazim et al. (1992) ,who mentioned that necropsy remains the only method for diagnosis of hydatidosis in equine, ultrasonography was found to be a rapid, safe and accurate method for diagnosis of hepatic hydatidosis in this study. Simillar finding was recorded by McGorum et al. (1994) in the lungs of a horse, Sage et al. (1998) in the liver of sheep and goats and Kalinbacak et al. (2002) in the liver of a cow.

In ultrasonography, the cyst appeared as unilocular anechoic sac with strong distant enhancement as the result of its fluid content. It had echogenic wall due to its fibrous connective tissue capsule. Kalinbacak et al. (2002) mentioned simillar findings in a cow. The hydatid cysts had no predilection site inside the liver as it could be diagnosed between the 15th and 8th intercostal spaces either on dorsal, middle or ventral third.

Although percutaneous centesis of the hepatic hydatid cysts and aspiration of hydatid fluid under ultrasonic guidance was easy and rapid method for diagnosis, it is important that the needle used for the centesis should be closed with a stylet, not only during its introduction, but also during its withdrwal so that the hydatid fluid does not escape, thereby preventing possible metastasis.

Histopathological examination was a confirmatory method for diagnosing hepatic hydatidosis. The cyst has thick fibrous connective tissue capsule which is surrounded by liver cirrhosis as a

result of pressure atrophy. These are in agreement with that mentioned by McGorum et al., (1994).

In conclusion, hepatic hydatidosis is a prevalent liver disease in donkeys and ultrasonographical examination is a useful screening tool for its diagnosis *in vivo*. Future goals are to use ultrasonography in the epidemiological survey, therapy and formulation and evaluation of control programmes for hydatidosis.

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التشخيص بالموجات فوق الصوتية للاكياس المائية الكبدية فى الحمير

أشرف محمد عبدالرحمن أبوسعدة

* قسم الجراحة والتخدير والأشعة - كلية الطب البيطرى - جامعة القاهرة

فى هذه الدراسة تم إجراء فحص كبدى بالموجات فوق الصوتية لعدد ٤٥ حمار تستخدم لأغراض تعليمية. وذلك بهدف تشخيص الاكياس المائية.

وقد وجدت هذه الاكياس فى ثمانية حمير مثلث ١٧.٨٪ من مجموع الحيوانات التى تم فحصها. هذا وقد تراوحت أعمار الحمير المصابة بين ٥ إلى ١٠ أعوام.

وقد تم تصوير الاكياس المائية الكبدية من خلال مسافة بين ضلعين (٥ حالات)، من خلال مسافتين متجاورتين بين الضلوع (حالة)، من خلال ثلاثة مسافات متفرقة (حالة)، ومن خلال ثمانية مسافات متفرقة بين الضلوع (حالة).

ظهرت الاكياس المائية خلال الفحص بالموجات فوق الصوتية كحويصلات وحيدة التجويف ، عديمة الصدى ودائرية أو بيضاوية أو كمترية الشكل ذات جدار واضح الصدى مع إزدياد الصدى أسفل الاكياس.

وقد تراوح قطر الاكياس بين ٢.١٥ - ١٠ سم وعددها من ١-١٥ كيس.

وقد تم تأكيد التشخيص فى كل حيوان وذلك بيزل الاكياس وشفط السائل الموجود بها وذلك تحت توجيه الموجات فوق الصوتية. هذا وقد كان السائل عديم اللون والرائحة وتحت ضغط ويحتوى على رؤوس أولية تحت الميكروسكوب.

تم إعدام جميع الحيوانات التى تم فحصها ووجد أن ثمانية حيوانات كان بها أكياس مائية كبدية بقيمة تنبئية إيجابية ١٠٠٪.

كما تم مناقشة نتائج الفحوص العملية والتسجيرية.