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Parasitological and Pathological Studies on Hepatic Hydatidosis in Donkeys from Egypt

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Abstract

Eighty three slaughtered donkeys (*Equus asinus*), 3-33 years old at Giza Zoo slaughter house, were examined for the presence of hydatid cyst from May 2014 to December 2015. Seventeen donkeys out of 83 (20%) had hydatid cysts in their livers. Clinical signs were almost asymptomatic. Different sized thick-walled hydatid cysts, each 6–8 cm in diameter in the liver. Incised cysts contained a turbid, amber liquid mixed with hydatid sand. Fertility of the cysts was assessed by examining the cysts' fluid for the presence of protoscoleces and the viability of the protoscoleces was determined using staining with an aqueous solution of 0.1% eosin stain. Seven out of 17 examined cyst (41%) were fertile where 4 out of 7(57%) were viable. Prevalence and intensity of infection increased with age. Microscopically, the tri-layered cyst walls consisted of an outer adventitial layer, a laminated acellular intermediate layer, and an inner germinal membrane. The result revealed hepatic hydatidosis in 20% of donkeys indicated the contamination of the environment in Egypt with *Echinococcus granulosus*.

Key words: Hydatid cyst, fertility, donkeys, viability, Egypt

INTRODUCTION

Hydatidosis is an important worldwide zoonotic disease caused by larval stages (metacestodes) of tapeworm parasites of the genus *Echinococcus*.

These cyclophyllidean cestodes have an indirect, 2-host life cycle, with carnivores (mostly wild or domestic canids) as definitive hosts and herbivorous or omnivorous mammals as

intermediate hosts. Adult tape worms inhabit the small intestine of the definitive host and release gravid cestode segments (proglottids) containing eggs into the environment via feces. Upon accidental ingestion of these eggs, intermediate hosts become infected with larvae of *Echinococcus* spp. The developing metacestodes are fluid-filled hydatid cysts, in which protoscoleces are produced as the next generation of tapeworms. The diagnosis of hydatidosis was based on characteristic morphological criteria of cyst architecture and morphology of protoscoleces according to (Chiou et al., 2001 and Thompson & McManus, 2002). The hydatids mainly develop in liver and lung. Consumption of viable protoscoleces by a definitive host completes the life cycle of the parasite (Rausch, 1995; Eckert, 2000; Jenkins et al., 2005). Infection of animals or human with larval stages of the tape worm *Echinococcus granulosus* result in significant economic and public health problems in many temperate and tropical areas of the world (Andersen et al., 1991; Nahmias et al., 1991). Hydatid disease was considered endemic in the Mediterranean regions including all countries of the Middle East (Andersen et al., 1997). The present report described fertility of hydatid cyst and viability of their protoscoleces, as well as, the pathological changes in the infected liver of donkeys.

MATERIALS AND METHODS

Natively reared donkeys from Fayoum Governorate aged between 3-33 years of age slaughtered at Giza zoo, Egypt were examined for presence of hydatid cysts from May 2014 to December 2015. Individual cysts were grossly examined for any evidence of degeneration and calcification. Cysts were examined for fertility, where hydatid fluid and wall fragments were examined under the light microscope for the presence of protoscoleces. Cysts which contained

no protoscoleces as well as heavily suppurative or calcified were considered infertile. Protoscoleces viability was assessed with staining with a 0.1% aqueous eosin solution according to (Smyth & Barrett, 1980). Specimens from parasitic cysts and surrounding liver tissue were taken then fixed in buffered neutral formalin (10%). Subsequently, formalin fixed specimens were processed for H&E and Periodic acid Schiff (PAS) staining according to (Bancroft and Gamble, 2007). Morphometric characterization of scoleces was performed on photographic images of fresh hydatid sand with the use of a morphometric image analysis system (image J = <http://imagej.en.softonic.com>).

RESULTS

Seventeen out of 83 donkeys (20%) harboured hydatid cysts in their livers. All infected donkeys had no clinical signs before slaughter. No hydatid cysts were found in the lungs. Prevalence and intensity of infection increased with age started with 7 years. All infected donkeys have no clinical signs of disease before slaughter. Different sized thick-walled hydatid cysts, each 6–8 cm in diameter was observed in the liver. The rate of infection was 1-3 cysts. The incised cysts contained a turbid, amber liquid mixed with hydatid sand (Fig. 1). Interior wall showed numerous broad capsules (Fig. 2A). Wet mount preparation showed scoleces and their rostellum (Fig. 2 B&C). Identification of dead scolices was done by mixing 1% aqueous eosin solution; living protoscoleces did not take up the stain, unlike the dead ones (Fig. 2D). The rate of fertile cysts was found to be 7 out of 24 (29%) in donkeys while 13 (54%) were sterile cysts and 4 (16.7%) cysts was calcified. By investigation of their viability, 5 cysts were viable (71%) (Table 1).

Histopathological examination was carried out on paraffin sections of hydatid cysts. The cysts displayed a three-layered wall architecture, with an

outer, host-derived adventitial layer, an acellular intermediate laminated layer, and an inner germinal membrane of parasitic origin. The laminated and germinal layers of the cyst walls were PAS positive and formed low interlacing folds, among which brood capsules with viable protoscolexes were evident. Free intact scolexes were seen in hepatic tissue due to opening of the cyst wall (Figs. 3&4). The outer adventitial layer consisted of a chronically inflamed fibrous granulation tissue with vigorous attendance of lymphocytes, accentuated fibrosis, and scattered small foci of calcification (Fig. 5). Scolexes measured approximately 167.27µm. Other pathological lesions were detected in hepatic tissue including hydropic degeneration, focal steatosis, congestion, follicular aggregation of lymphocytes, periductal and periportal fibrosis with aggregation of lymphocytes (Figs. 6&7).

DISCUSSION

Several reported cases of hydatidosis in donkeys provided data on the prevalence and fertility of cysts (Abou-Eisha 1999; Dyab et al., 2005; Haridy et al., 2008; El-Kattan 2012). Depending on the geographical situation, host, site, size and type of cyst; cysts may have different rates of fertility. The liver is the most common site of hydatid cysts in equids (Eckert, 2000). This is explained by the fact that livers and lungs possess the first great capillaries sites encountered by the migrating *Echinococcus* oncosphere (hexacanth embryo) which adopt the portal vein route and primarily negotiate the hepatic and pulmonary filtering system sequentially before any other peripheral organ is involved (Kebebe et al., 2010). Lungs and less commonly other organs or locations, including cranium, brain, pericardium,

pleura, spleen, kidneys, muscle, uterus, and ocular structures, may also be affected (Barnett et al., 1988; Le et al., 2002). No hydatid cysts were found in the lungs or other organs of slaughtered donkeys.

Our investigation showed that 20% of donkeys harboured hydatid cysts in their livers in agreement with (Aboelhadida et al., 2013), who revealed that the majority of infected donkeys (70%) harbored hydatid cyst in their livers at Beni-Suef, Egypt. The host tissues produce a rigid fibrous capsule around larvae. The exocyst is an elastic acellular layer elaborated by the parasite. It is permeable to nutrients from the hosts, but not to bacterial organisms (Sun et al., 1988). The endocyst or inner membrane is a germinal layer which protrudes into the cyst cavity and forms multiple vesicular structures known as brood capsules (Lewal & McCorkell, 1985). The latter contain protoscolices which float in the highly antigenic cyst fluid or “eau de roche” (Lewis et al., 1975). Protoscolices have a head invaginated from the endocyst with four suckers and a rostellum with two rows of hooklets (Sun et al., 1988). Under certain circumstances, multiple septations form, leading to the formation of “daughter cysts.” It has been suggested that the production of daughter cysts may be part of a natural aging process (Lewal & McCorkell 1985). Brood capsules and protoscolices that break away from the germinal layer form “hydatid sand” settles at the dependent portions of the cyst (Lewal & McCorkell, 1985). In This study; mural calcification occurs in up to 10 percent of examined cysts . Densely calcified cysts contain dead organisms and are usually asymptomatic (Lewis et al., 1975). Here we reported the fertility, viability and histopathology of hepatic cystic hydatidosis in donkeys

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Table 1: Frequencies of infected donkeys by age and viability

Age (years) group	No. of infected donkeys	No. of Hydatid cyst	Fertile	Sterile	Calcified	Viable	Nonviable
<7	1	1	1	0	0	1	0
7-20	3	4	1	0	0	1	0
>20	13	19	5	13	4	3	2
	17	24	7	13	4	5	2

Fig. 1: Gross picture shows (A) 3 whitish thick walled cysts on the surface of the liver and (B) cut section of formalin fixed hydatid cyst shows turbid, amber liquid with hydatid sand.

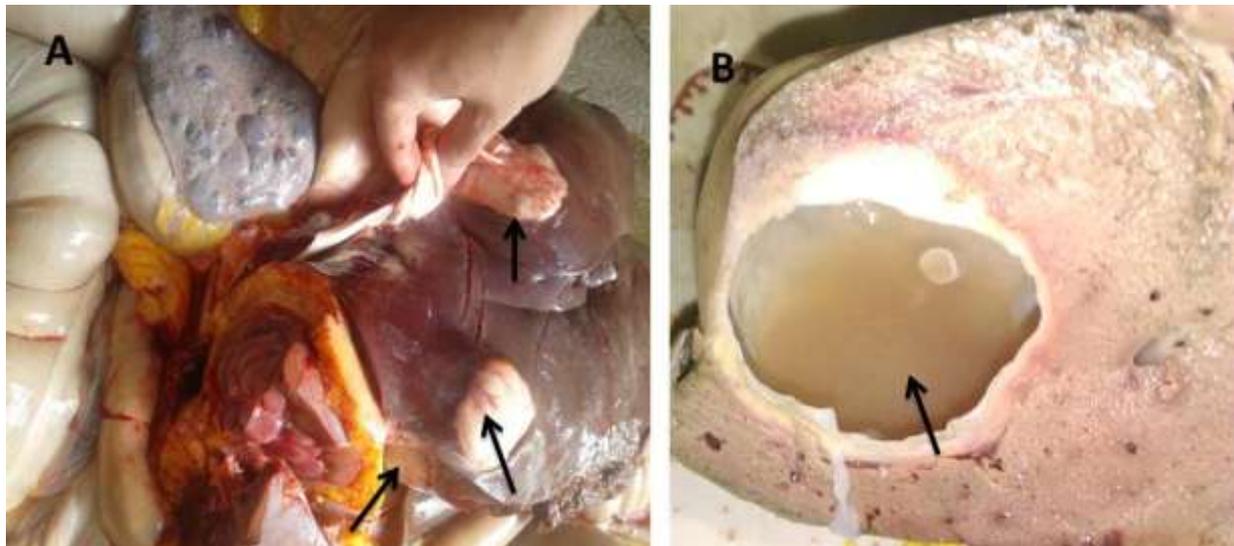
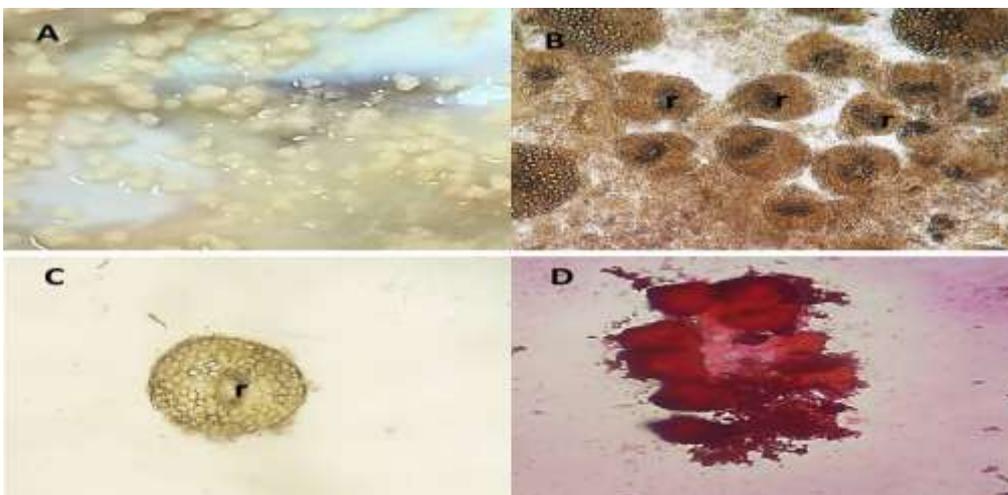


Fig. 2: Gross picture shows (A) interior wall of the cysts shows multiple whitish daughter cysts. (B&C) protoscolex with rostellum (r) (Wet mount X: 200). (D) eosinophilic stained dead scoleces (0.1% eosin stain, X: 200)



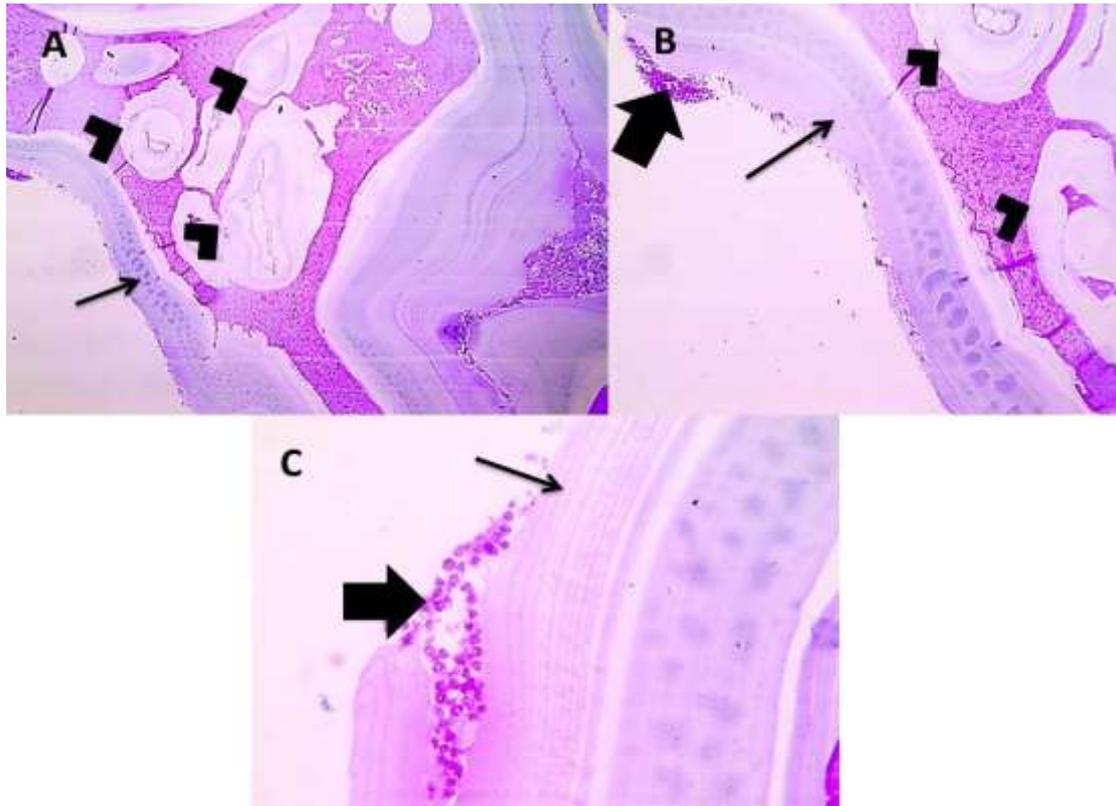


Fig. 3(A-C): Histopathology of daughter hydatid cyst. Wall of the cyst (thin arrows), germinal epithelium (thick arrows), protoscolices (arrowheads) (H&E, X: A: 50, B: 100 and C: 200).

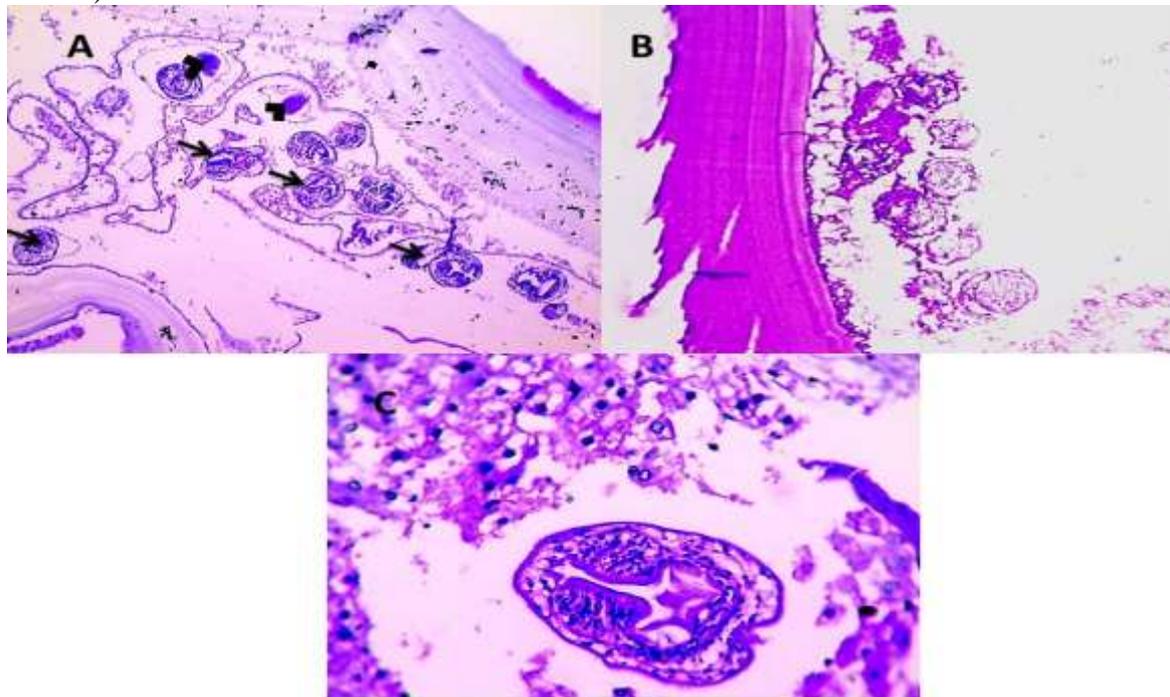


Fig. 4 (A&B): Histopathology of hydatid cyst. **A.** Broad capsule containing viable (arrows) and calcified scoleces (arrowheads) (H&E, X: 100). **B.** PAS positive laminated and germinal layers (PAS stain, X: 100). Scolex is seen in hepatic parenchyma due to ruptured cyst (H&E, X: 200).

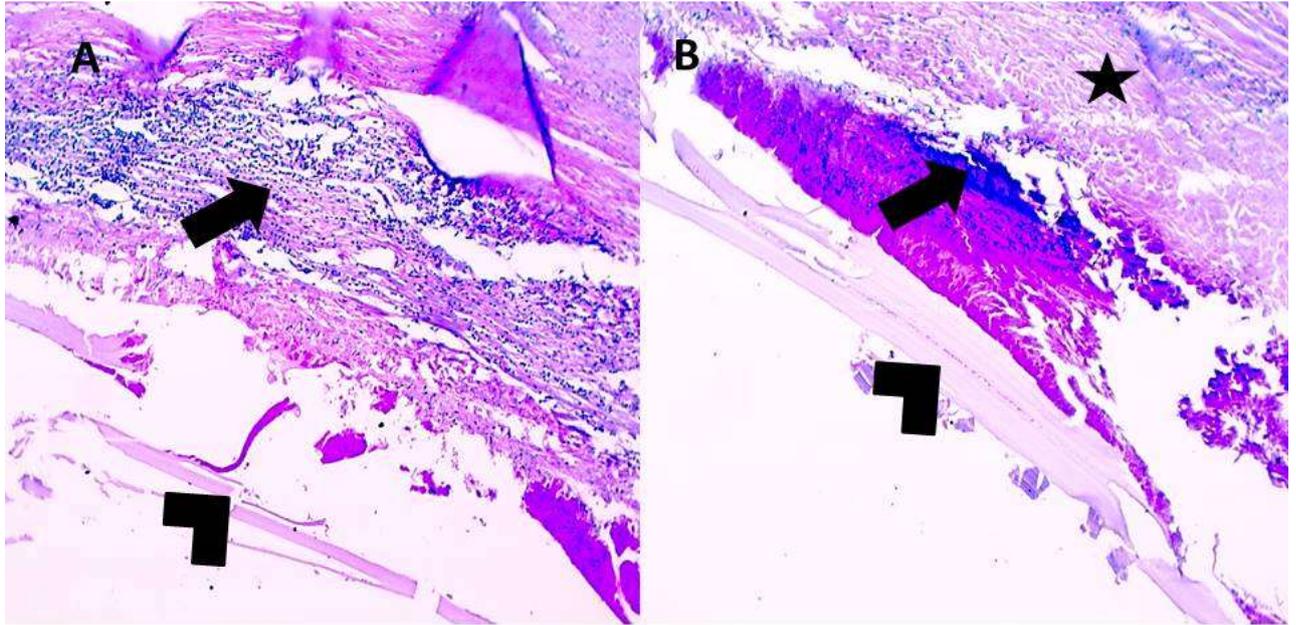


Fig. 5 (A&B): Histopathology of hydatid cyst. **A.** Wall of dead cyst (arrowhead) surrounded by chronic inflammation with diffuse infiltration of lymphocytes and accentuated fibrosis. **B.** Wall of the dead cyst (arrowhead) surrounded by hyalinised fibrous tissue with deep blue calcification foci (arrow) followed by fibrous tissue (asterisk) (H&E, X: 200).

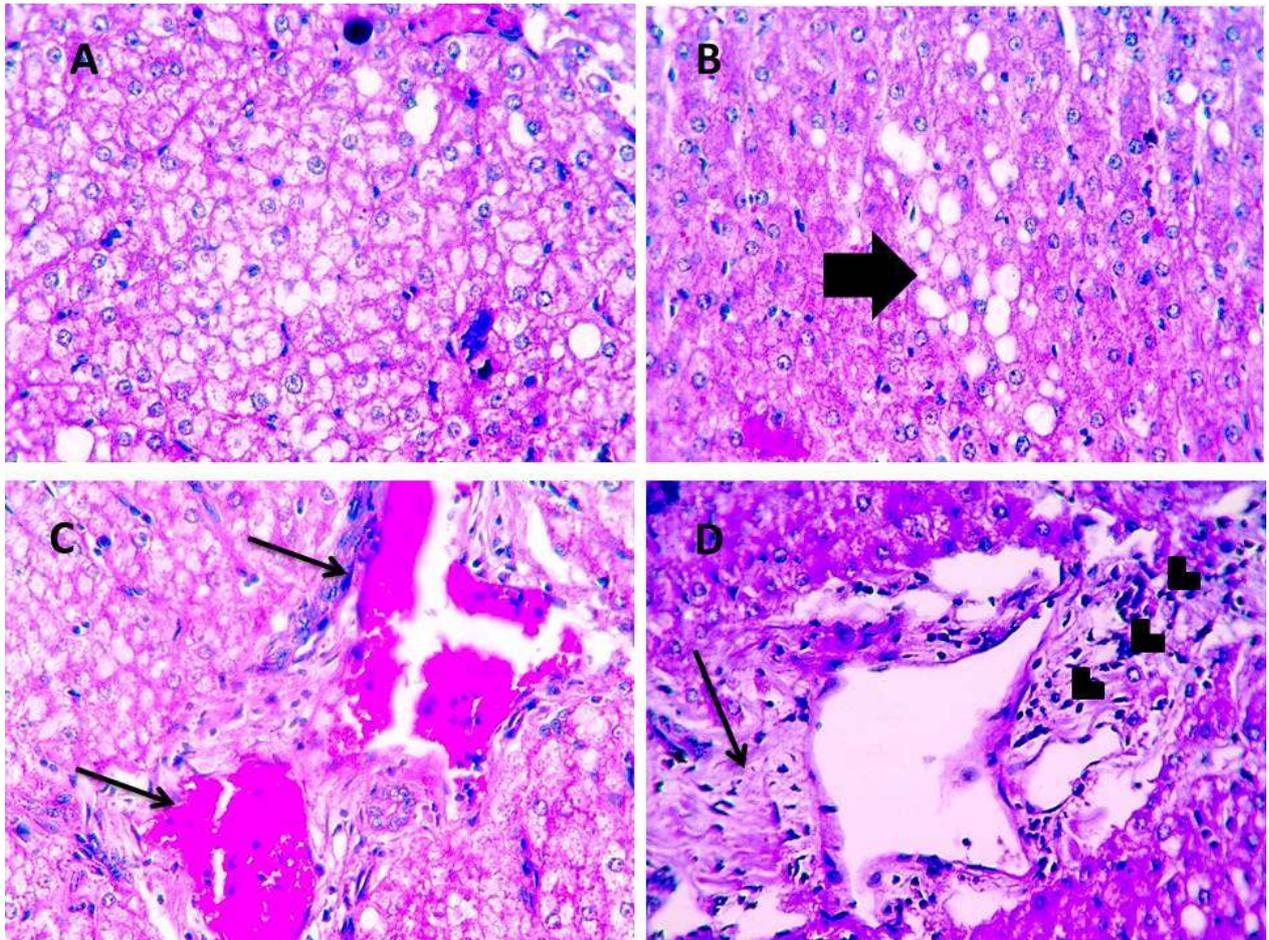


Fig. 5(A-D): Liver of donkey shows (A) hydropic degeneration, (B) focal steatosis (arrow), (C) congested portal blood vessels (arrows), (D) periportal fibrosis (arrow) with lymphocytic infiltration (arrowheads) (H&E, X : 200).

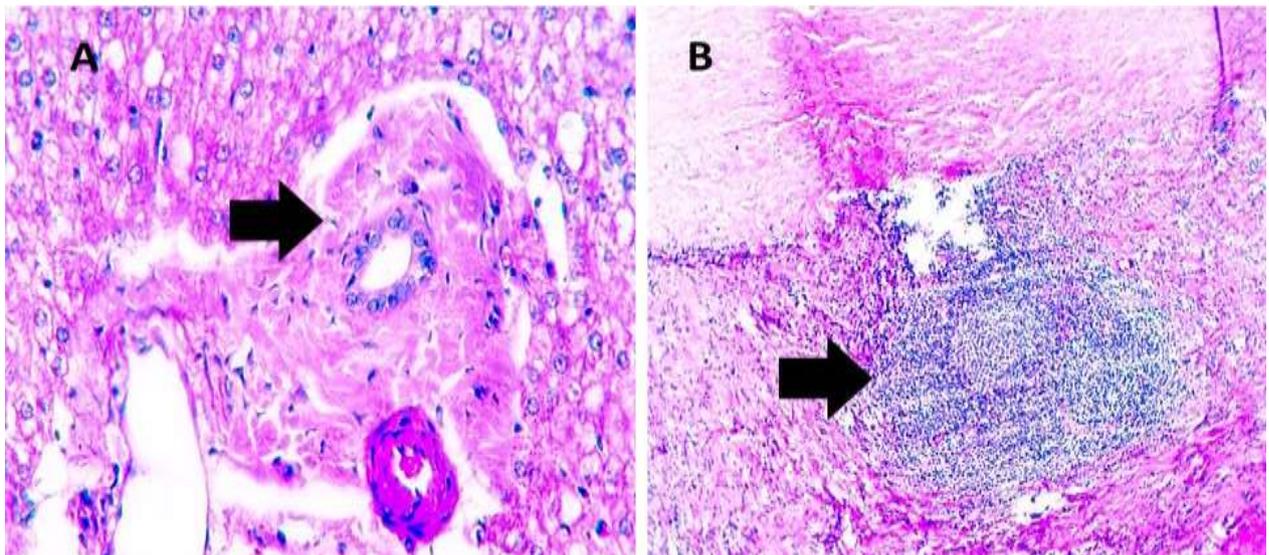


Fig. 6(A&B): Liver of donkey shows (A) periductal fibrosis (arrow) and (B) follicular lymphocytic aggregation (arrow) (H&E, X: 200).

دراسات طفيلية و باثولوجية على اكياس الدودة الشريطية بالكبد في الحمير في مصر

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قسمى الطفيليات والباثولوجيا – كلية الطب البيطري- جامعة المنصورة – مصر

تم فحص عدد ٨٣ حمار (٣-٣٣ سنة) مذبوح في مجزر حديقة الحيوان بالجيزة لتحري وجود اكياس الدودة الشريطية بالكبد في الفترة من مايو ٢٠١٤ الى ديسمبر ٢٠١٥. وجد ان ١٧ حمار من اصل ٨٣ مصاب باكياس الدودة الشريطية في الكبد. الاعراض الظاهرية كانت غائبة. الاكياس كانت سميكة الجدار و مختلفة الاحجام بمتوسط قطر ٦-٨سم. بفتح الاكياس وجد انها تحوى سائلا معكرا و يحتوى على رمل الدودة الشريطية. قيمت نسبة الخصوبة بفحص السائل لتحري وجود الرنوس الاولى للطفيل و قيمت حيويتها بصيغ تلك الرنوس بمحلول مائى لصبغة الايوسين ١ ، ٠% . وجد ان ٧ اكياس كانت خصبة من اصل ١٧ كيس تم فحصهم (٤١%) و ٤ اكياس كانت حية من اصل ٧ (٥٧%). و تبين ان نسبة الاصابة و شدتها زادت بزيادة عمر الحيوان. بالفحص المجهرى تبين ان جدار الاكياس مكون من ٣ طبقات: طبقة خارجية و طبقة متوسطة مصفحة لاخلوية و داخلية هى غشاء جرثومى. اوضحت نتائج البحث ان النسبة المرتفعة للاصابة باكياس الدودة الشريطية فى الكبد هى ٢٠% فى الحمير و هى تشير الى تلوث البيئة فى مصر بطفيل الدودة الشريطية (الايكينوكوكاس جرانيلوزا).