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ORIGINAL ARTICLE

Prevalence and bacterial isolation from hydatid cysts in dromedary camels (*Camelus dromedarius*) slaughtered at Sharkia abattoirs, Egypt

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Abstract Cystic echinococcosis (CE) is a severe neglected zoonotic parasitic disease caused by the larval stage of the dog tapeworm, *Echinococcus granulosus*. The objectives of this study were to determine the prevalence of hydatid cysts in dromedary camels (*Camelus dromedarius*) at Sharkia province, Egypt and investigate the occurrence of bacteria in hydatid fluid. A total of 6416 dromedary camels slaughtered in five abattoirs in Sharkia province, Egypt during the period from January and December 2018 were investigated for the presence of hydatid cysts. Furthermore, the bacterial species in 10 hydatid fluid isolated from lungs and livers was identified. The current findings revealed that the prevalence of hydatid cysts was 3.7%. Among those, the infection rate in lungs was 78.2%, which was significantly higher than hepatic infections (21.8%). The prevalence of hydatid cysts was the highest in winter (7.4%) and the lowest in spring (1.5%). The most common bacterial species found inside hydatid fluid collected from lungs were *Salmonella* spp., *Staphylococcus* spp., Enterococci and *Pseudomonas* spp. Meanwhile, *Staphylococcus* spp. were isolated from hepatic hydatid fluid. In conclusion, hydatid cysts infection is prevalent in dromedary camels in Sharkia province, Egypt as well as various aerobic and anaerobic bacterial species were isolated from hydatid fluid from camel lungs and livers.

Keywords Prevalence · Hydatid cyst · *Salmonella* · *Staphylococcus* · Camel · Egypt

Introduction

Cystic echinococcosis (CE) caused by *Echinococcus granulosus* is one of the most important zoonotic parasitic diseases in the Middle East, North Africa and other regions of the world (Eckert and Deplazes 2004; Sadjjadi 2006). CE affects humans and livestock species including cattle, sheep, goats and camels, which act as intermediate hosts and harbor the larval stage (hydatid cysts) of *E. granulosus*. However, dogs and other canids are the definitive hosts of *E. granulosus* and get infected when ingestion of hydatid cysts (da Silva 2010). In animals, CE causes high economic losses through decreasing the meat, wool and milk production in addition to the condemnation of infected organs (Torgerson et al. 2001; Umur 2003; Jahed Khaniki et al. 2013); whereas the economic losses in humans were due to the elevated cost for therapy and surgery (Torgerson and Dowling 2001).

In Egypt, camels (*Camelus dromedarius*) in Egypt used mainly for meat production (Kadim et al. 2013; Yel

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Badawi 2018). In previous studies, the prevalence of CE was 31% in Cairo abattoir (Rahman et al. 1992) and 6% in Assuit province abattoirs (Haemaei et al. 2017). Furthermore, in camels, the prevalence of hydatid cyst was 6.8% in China (Qingling et al. 2014), 23% in Ethiopia (Debela et al. 2015) and 14.6% in Iran (Mirzaei et al. 2016). However, the prevalence of CE infection in humans was high and is considered to be endemic in Egypt (Abdel Aaty et al. 2012; Amer et al. 2015).

The hydatid cyst consists of three layers filled with clear yellow fluid, which contains many protoscolices (Eckert and Deplazes 2004; Hijjawi et al. 2018). Protoscolices ingested by canids and develops into adult worms; which significantly contribute in the life cycle of *Echinococcus* (Eckert and Deplazes 2004). Different types of proteins (albumin and globulin), minerals (sodium, potassium and zinc) and free amino acids have been detected in hydatid fluid (Juyi et al. 2013; Yakhchali et al. 2017). Furthermore, the occurrence of different bacterial species as *Citrobacter freundii, Aeromonas hydrophila, Staphylococcus* species, *Salmonella* species, *Escherichia coli and Proteus vulgaris* have been isolated from fluid of hydatid cysts collected from cattle, sheep and goats (Ziino et al. 2009; Sevimli et al. 2014). Removal of the cyst-infested organ during meat inspection may lead to leakage of hydatid fluid and possibly contaminate the carcass with bacterial species from cyst contents (Ziino et al. 2009). CE is considered an important neglected parasitic diseases, and little is known about the public health significance in livestock (da Silva 2010; Amer et al. 2015; Tigre et al. 2016). Therefore, understanding how hydatid cysts can evolve in the body with regard to cyst fluid contents can help in control strategies and reduce the risk of disease transmission (Rahdar et al. 2008). Routine monitoring of CE in Egypt is a crucial requirement to provide update information about the disease in animals and humans (El-Dakhly et al. 2019). The objectives of the present study were to determine the prevalence of hydatid cysts in dromedary camels slaughtered in Sharkia province abattoirs, Egypt and identify the the occurrence of bacteria in hydatid fluid.

Materials and methods

Study area

This study was carried out in Sharkia province, which is located in the northern part of Egypt at 30.7° N, 31.63° E, at 120 km from Cairo (Fig. 1). Sharkia province is considered the third most populous province in Egypt and it has a strong agriculture industry. It also has a high density of dromedary camels, which are mainly used for meat production.

Study design and sampling

A cross-sectional study was carried out in five abattoirs (Zagazig, Belbies, Abu Hammad, Minya El-Qamh and Faqous abattoirs) in Sharkia province during the period from January and December 2018. Abattoirs were selected based on the number of dromedary camels slaughtered each year (> 500 camels) and the geographical range from which camels are sourced. In total, 6416 camels were slaughtered at the focus abattoirs. During routine meat inspection, inspectors examine organs for defects including but not limited to: abscess, liver fluke, hydatid cysts and nephritis. Only camels and organs infected with hydatid cysts were included in the present study. Hydatid cysts were collected in sterile phosphate buffer saline (PBS) and transported on ice to the laboratory for later examinations. The study protocol was approved by the Committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine, Zagazig University, Egypt. However, camels were slaughtered as a part of normal abattoir process; thus, ethical approval was not required.

Parasitological examination

Collected hydatid cysts were carefully washed with sterile PBS and the surface was sterilized with 70% ethyl alcohol. The diameter of hydatid cysts was determined using a ruler and they were classified into large (diameter > 10 cm), medium (diameter ranged from 5 to 10 cm) and small (diameter < 5 cm) (Kebede et al. 2009; El-Dakhly et al. 2019). Hydatid fluid was collected using a sterile syringe and transferred to sterile microcentrifuge tubes. The cysts wall was incised and the germinal layer and internal protoscolices were collected. A small portion of collected fluid was examined under a light microscope to investigate the fertility of each cyst with regard to the presence or absence of protoscolices (Abbas et al. 2016).

Bacteriological examination

Hydatid fluid samples (n = 10) were collected from camels infested organs (eight from lungs and two from livers). Three samples of eight lung cysts were fertile (Sample 2, 4, 5), while, others were degenerated (Table 3). However, both liver samples (9, 10) were degenerated (Table 3). Bacterial culture was performed using techniques described in Bailey and Scott's Diagnostic Microbiology (Forbes et al. 2002). Briefly, hydatid fluid was inoculated on Plate Count Agar (PCA) for the detection of *Staphylococcus* spp., Baird-Parker agar base with egg yolk tellurite emulsion for detecting *Staphylococcus* spp., *Pseudomonas* agar base for detecting *Pseudomonas* species, Bile esculin agar base for identifying *Enterococcus* species, and Eosin

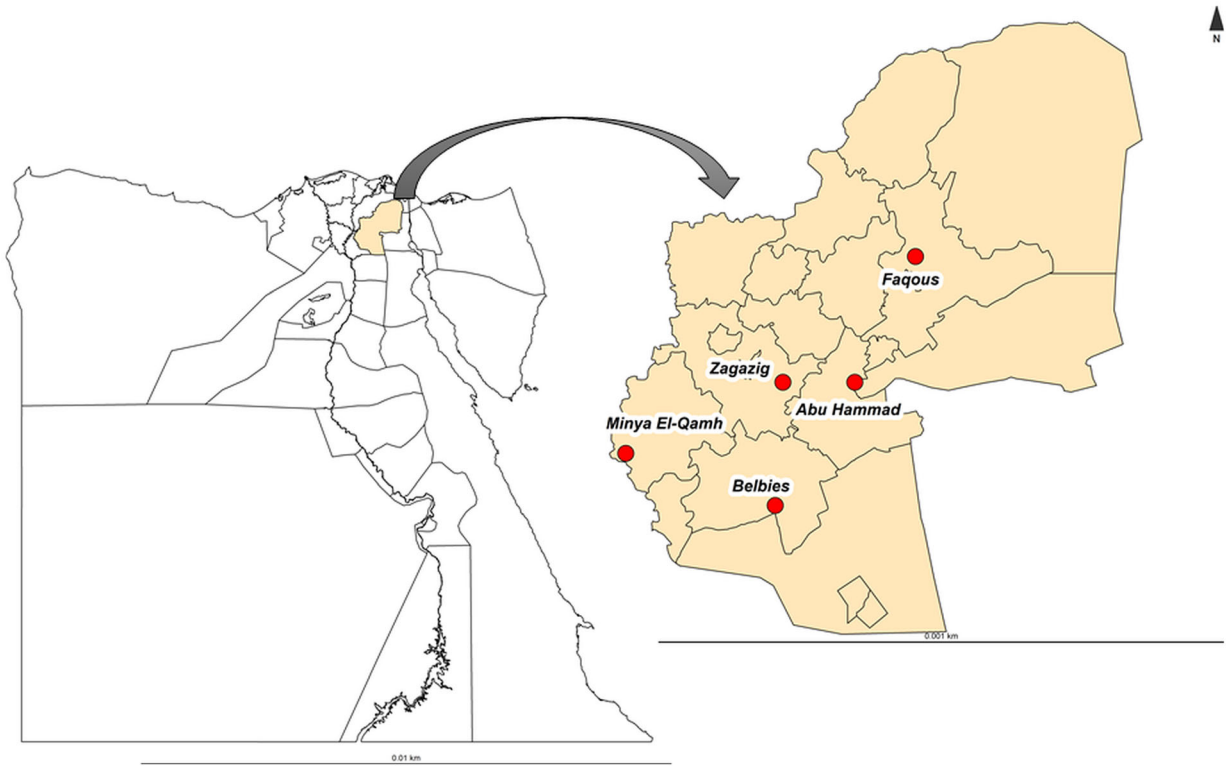

Fig. 1 The location of Sharkia province in Egypt and the location of the abattoirs involved in the study

methylene blue (EMB) for isolation of *E.coli*. All media for bacteriological analyses were purchased from HiMedia Laboratories, Mumbai, India. Bacteria were identified at the genus level. A volume of 100 µL of hydatid fluid was streaked under aseptic conditions using a bacteriological loop and the plates were incubated at 37 °C for 24 h. For isolation of *Salmonella* spp., 1 mL of each sample of hydatid fluid was added to Rappaport–Vassiliadis *Salmonella* Enrichment Broth in a separate test tube, and the culture incubated at 42.5 °C for 48 h. Subsequently, the enriched Rappaport Vasiliadis *Salmonella* Enrichment Broth culture was sub-cultured on Xylose Lysine Deoxycholate Agar (XLD) plates for isolation of *Salmonella*. The XLD plates were incubated at 37 °C for 24 h (ISO 2002). The bacterial species were identified based on colony morphology on selective media.

Statistical analysis

Data were analyzed with Chi square (χ^2) tests using IBM SPSS Statistics for Windows software version 21. P values < 0.05 were considered statistically significant.

Results

The current study revealed that hydatid cysts were detected in 234 (3.7%) camels. Hydatid cysts were detected in the lungs of 183 (78.2%) infected camels, which significantly higher ($P < 0.05$) than 51 (21.8%) detected in livers (Table 1). Furthermore, the prevalence of hydatid cysts was significantly higher in winter (7.4%) than in spring (1.5%). Concerning the relationship between camel age and the infection rate, it was observed that young camels ($\leq$ 5 years) had an infection rate of 3.4%, which increased to be 4.6% in older camels (> 5 years) (Table 2).

By visual examination, palpation and aspiration, our findings showed that the size of hydatid cysts collected from lungs ranged from 3 cm (small) to 7 cm in diameter (medium sized cysts) (Fig. 2a). While, the laminated membranes in an incised hydatid cyst demonstrated in Fig. 2b. Out of 183 cysts found in the lungs 128 were filled with clear yellow fluid, while 55 cysts appeared to be degenerated. Microscopic examination revealed that the fertility rate of lung cysts as determined by the presence of protoscolices was 70% (Fig. 3a and b). The size of hydatid cysts collected from livers ranged from 2 cm (small) to medium sized cysts of 5 cm in diameter and the majority appeared to be degenerated as the fertility rate was 9.8%.

Bacterial species were isolated from 7 out of 10 hydatid cysts collected from the lungs and livers of slaughtered

Table 1 Seasonal prevalence and infected organs of cystic echinococcosis in slaughtered camels

Season	Examined camels	Infected camels (%)	No. of infected organs (%)	
			Lungs	Livers
Winter	1333	99 (7.4)	63 (63.6)	36 (36.4)
Spring	1836	27 (1.5)	27 (100)	0 (0.0)
Summer	1425	42 (2.9)	42 (100)	0 (0.0)
Autumn	1822	66 (3.6)	51 (77.3)	15 (22.7)
Total	6416	234 (3.7)	183 (78.2)	51 (21.8)

Table 2 Prevalence of cystic echinococcosis in slaughtered camels according to age

Age	Examined camels	Infected camels (%)	No. of infected organs (%)	
			Lungs	Livers
$\leq$ 5 years	5016	169 (3.4)	130 (76.9)	39 (23.1)
$>$ 5 years	1400	65 (4.6)	53 (81.5)	12 (18.5)
Total	6416	234 (3.7)	183 (78.2)	51 (21.8)

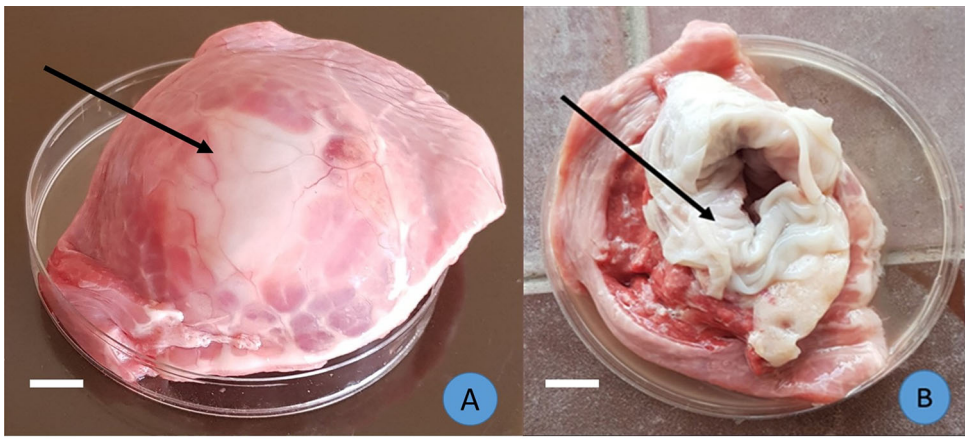

Fig. 2 Hydatid cysts from lungs of infected camels. **a** Camel lung with intact medium size hydatid cyst (arrow) and **b** Opened hydatid cysts in camel lung revealing laminated membranes (arrow). Scale bar, 5 mm

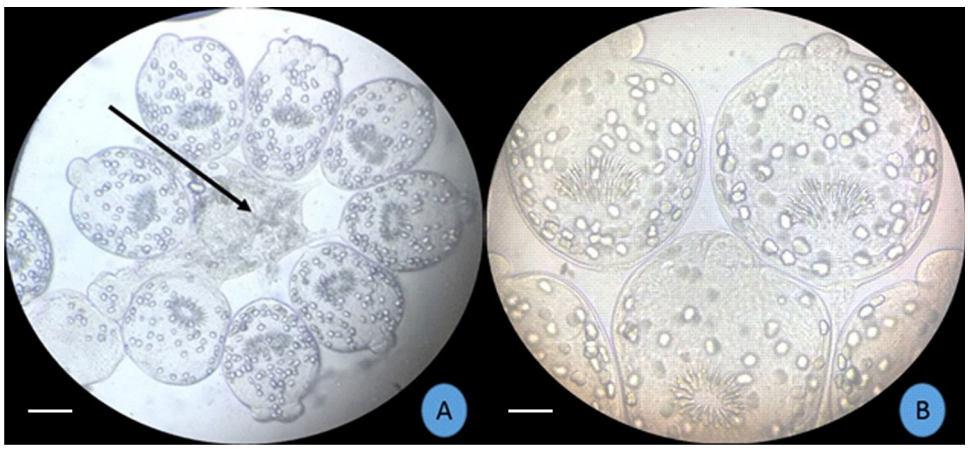

Fig. 3 Hydatid fluid. **a** Unstained wet preparation, brood capsule with protoscolices and germinal layer (arrow). Original magnification ×150X and **b** unstained hydatid sand. Original magnification ×400X. Scale bar, 100 µm

camels. Our investigations showed that out of the eight hydatid cysts collected from lungs, three cysts contained *Salmonella* spp., five contained *Staphylococcus* spp., three Enterococci and four samples were infected with *Pseudomonas* spp. However, *E. coli* was not detected in any of the hydatid fluid samples originating from cysts found in the lungs. *Staphylococcus* was isolated only from one hepatic hydatid fluid (Table 3).

Discussion

Cystic echinococcosis (CE) is a chronic infection of medical and veterinary importance caused by the parasitic cestode *Echinococcus granulosus* (McManus et al. 2003; Eckert and Deplazes 2004; Zeghir-Bouteldja et al. 2009). In the present study, the prevalence of hydatid cysts in slaughtered dromedary camels in Sharkia province was 3.7%. This finding is consistent with the prevalence of hydatid cysts infection (3.5%) reported previously in camels in Egypt (Gab-Allah and Saba 2010). The current result was slightly lower than the overall annual prevalence reported in Egypt in 1992 (5.5%), 1993 (6.1%), 1994 (6.7%), 1995 (8.2%) and 1996 (4.3%) (Haridy et al. 1998). Moreover, Dyab et al. (2005) and El-Dakhly et al. (2019) demonstrated that CE in camels in Egypt was 7.67% and 10.82% respectively. However, Amer et al. (2018) reported a prevalence of CE (0.51%) in camels in Saudi Arabia and the infection rate of CE in camels in Oman was 5.3% (Al Kitani et al. 2015). Furthermore, the prevalence estimated in this study was significantly lower than the 35.9% in Libya (Gusbi et al. 1990), the 29.7% in Sudan (Ibrahim et al. 2011) and the 30.8% reported in Iran (Elham et al. 2014). The lower prevalence reported in the current study may be attributed to the distinctive husbandry and feeding management of camels, which depend on raising camels in farms under strict feeding system for high-quality meat production, milk and breeding as well as the interaction between camels and stray dogs is less frequent (Haemaei et al. 2017; Abdel-Baki et al. 2018). However, the variation in prevalences might be referred to difficulty to control stray dogs and lack of shepherd awareness regarding the life cycle of that parasite (El-Dakhly et al. 2019).

The prevalence of hydatid cysts detected in the lungs (78.2%) of infected camels was significantly higher than that (21.8%) in livers. Similar findings have been reported in Iran (Elham et al. 2014), where the rate of lung and liver infection reached 72.5% and 12.6%, respectively. Furthermore, a previous study has reported that lungs were more frequently positive for cysts than livers (56% vs. 33.9%) (Debela et al. 2015). The possible explanation for higher infection rates in lungs than livers may be due to the oncosphere is more likely to stay in any organ it encounters first as well as the size of the *E. granulosus* oncosphere with respect to the venules and lymphatic lacteal of the villus in various animals. In ruminant, the lymphatic lacteal of the villus is large, and lung cysts are more common. However, in non-ruminant, the lymphatics are quite small, and liver cysts are more often encountered (Tenhaeff and Ferwerda 1935; Heath 1971). Other researchers have reported that livers were more frequently infected with hydatid cysts (75%) than lungs (17%) (Haemaei et al. 2017) and might be due to the liver being the first organ in which the large metacestode remains after penetrating the mucosa of the intestine and entering the bloodstream (Al-Khayat 2019).

Our study revealed that the hydatid cyst infection rate in older camels (> 5 years) was significantly higher than that in the youngest ones. This finding is in agreement with the results of Mirzaei et al. (2016) who reported a higher prevalence of CE (5.6%) in older animals (between 5 and 10 years) than young ones (2.02%). Age variation can be attributed to the difference in exposure, as older livestock may have been potentially subjected to more infective

Table 3 The occurrence of bacteria in hydatid fluid isolated from lungs and livers of infected camels

Sample	Organ	Isolated bacteria
1	Lungs	*Staphylococcus* spp.
2	Lungs	No bacteria identified
3	Lungs	*Staphylococcus* spp., *Salmonella* spp.
4	Lungs	No bacteria identified
5	Lungs	*Pseudomonas* spp., Enterococci
6	Lungs	*Staphylococcus* spp., *Pseudomonas* spp., *Salmonella* spp.
7	Lungs	*Staphylococus* spp., *Pseudomonas* spp., Enterococci
8	Lungs	*Staphylococcus* spp., *Pseudomonas* spp., Enterococci, *Salmonella* spp.
9	Livers	*Staphylococcus* spp.
10	Livers	No bacteria identified

stages (Ibrahim et al. 2008). In fact, the prevalence of infection depends upon age, but we should take into consideration that younger animals were more slaughtered in the abattoirs when we consider age as a substantial risk or infection factor (Torgerson and Heath 2003; Craig et al. 2015; Abbas et al. 2016). The highest prevalence of CE in this study was reported in winter in contrast with Ibrahim (2010) study, who reported a higher prevalence in spring. The seasonal variation in prevalences between studies may be attributed to the sources and/or age of slaughtered camels (Daryani et al. 2007; Ibrahim 2010).

Regard the occurrence of bacteria in hydatid fluid; our study revealed that the most common bacterial species in hydatid fluid collected from the lungs are *Salmonella* spp., *Staphylococcus* spp., Enterococci and *Pseudomonas* spp.. However, *Staphylococcus* spp. was the only bacterial species isolated from hepatic hydatid cysts. It is now well recognized that *Echinococcus* eggs are excreted in feces of infected carnivores and ingestion of eggs by intermediate hosts, like camels, leads to the development of the oncosphere which migrates through the intestinal mucosa and develops in hydatid cysts within lungs or livers (Eckert and Deplazes 2004; Thompson and Jenkins 2014). The direct contact between eggs and the external environment during the life cycle could lead to the contamination of hydatid fluid with bacteria (Ziino et al. 2009). This hypothesis is consistent with results obtained with other parasites, which demonstrated that the nematode parasites *Nematospiroides dubius* may act as a vector for pathogenic species of *Salmonella typhimurium* during infection (Bottjer et al. 1978). In addition, *Salmonella enterica Typhimurium* possibly attached to the outer coating of eggs of *Ascaridia galli* causes *Salmonella* infections in chicks infected with those eggs (Chadfield et al. 2001). Furthermore, it was observed that *Pasteurella multocida* had an impact on the establishment of *Ascaridia galli* infection in free-range chickens in Denmark (Dahl et al. 2002).

Moreover, bacteria were carried to the lungs by migrating *Ascaris* larvae in piglets (Adedeji et al. 1989). *Salmonella Typhimurium* excretion was protracted and increased during *Oesophagostomum* spp. infection in pigs (Steenhard et al. 2002), while, *Mycobacterium avium* subspecies *paratuberculosis* was cultured from ovine trichostrongylid larvae (Lloyd et al. 2001). Additional reports found that fascioliasis enhanced the susceptibility of cattle to the lethal impacts of *Salmonella Dublin* (Aitken et al. 1978), and Melhem and LoVerde (1984) suggested prolonged infections of *Salmonella* in schistosome infected patients due to contamination of *Schistosoma* worms with *Salmonella* and that pili producing were necessary for attachment to the surface of Schistosome tegument.

Our findings show some differences in bacterial species isolated from the lungs. These variations in the level of contamination are probably due to differences in the parasite's route of migration through tissues and organs and the periods they stay in the external environment (Ziino et al. 2009). Other studies suggest that the infection of hydatid cyst with bacterial flora from the bile or bronchial tree is usually due to communicating rupture as tearing of the pericyst and evacuation of cyst contents into the biliary tract or bronchioles (Wani et al. 2010; Fallah et al. 2014). As the most important route of infection of hepatic hydatid cysts is via entry from biliary passages, whereas the bloodstream is a less critical route and that breakage of the cyst is necessary for the entrance of organisms (Mills 1927; Dew 1928). Moreover, Hsu et al. (1986) isolated anaerobes bacterial microflora from the intestine of female *Ascaris suum* using culture methods. In addition, the body surface and internal organs of *Ascaris lumbricoides* were found to be contaminated with human gut microflora (Adedeji and Ogunba 1986).

Furthermore, a study by Blenkharn et al. (1987) indicated that although bile flora could be a source of hepatic hydatid cyst infection, *Haemophilus influenza,* which is rare in bile flora, was isolated from two human cases infected with liver hydatid cysts. A case of hepatic hydatid cyst infected with a Gram-negative Bacillus, *Morganella morganii* that is commonly found in the environment and normal intestinal microflora in humans (Hakyemez et al. 2012). In the present study, we isolated a few bacterial species from a hepatic hydatid cyst. This observation is consistent with previous reports that bacterial infection of hydatid cyst in the liver is relatively rare (Saidi 1976; Barros 1978). However, the mechanism of bacterial infection of hydatid fluid remains unclear and depends on a complication of erosion and communication with adjacent structures (Blenkharn et al. 1987). Further studies are needed to investigate the occurrence of microbes in hydatid fluid using molecular markers to understand how bacteria infect hydatid cysts. This will help us to restrict the problem through finding new avenues for treatment of parasites before removal of the cyst.

Conclusion

This study demonstrates that hydatid cysts infections are common in dromedary camels in Sharkia province, Egypt, and this observation is of public health and economic relevance, particularly in the meat industry. We observed different bacterial species in hydatid fluid. The source of these bacteria is unclear and their growth of protoscolices during cyst development remains to be investigated. This will be helpful in the strategy of treatment especially in human cases as a pre-operative medication is required to prevent anaphylactic shock from leakage of hydatid fluid in

peritoneum. As well, during meat inspection to avoid contamination of carcasses with bacteria during removal of cysts and subsequently, it affects public health.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval Samples were collected from legally slaughtered animals and no experiments were done on live animals by any of the authors.

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