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A CASE OF HEPATIC CYSTICERCOSIS IN A DROMEDARY CALF

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ABSTRACT

A camel calf died shortly after it was translocated together with its mother from Pakistan to a farm in Dubai in the United Arab Emirates. Necropsy revealed severe alterations in the liver caused by bladder-like structures which appeared to be parasitic in nature and measured between 3-6 mm in size. Parasitological and histological examination showed structures resembling cysticerci at early stages of development. DNA sequencing of fragments amplified within the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) and the NADH dehydrogenase subunit 1 (*ND1*) revealed that the infection had been caused by *Cysticercus tenuicollis*, the metacestode larval stage of *Taenia hydatigena*. The majority of the metacestode cysts of *C. tenuicollis* (30/36) observed at necropsy were in a stage of caseous degeneration which suggested that the dromedary may be an aberrant host of this parasite.

Key words: *Cysticercus tenuicollis*, DNA sequencing, dromedary, *Taenia hydatigena*

The thin-necked bladderworm (*Cysticercus tenuicollis*) is the metacestode larval stage of *Taenia hydatigena*, an intestinal tapeworm of dogs and wild canids. Mature *C. tenuicollis* are usually found on the omentum, mesentery and peritoneum and less frequently on the pleura and pericardium of domestic and wild ruminants as well as pigs. Migrating *C. tenuicollis* larvae can mostly be found in the liver parenchyma causing traumatic hepatitis in young animals (Blažek *et al,* 1985). Most infections are chronic and asymptomatic and are usually diagnosed at slaughter (Christodoulopoulos *et al,* 2008; Scala *et al,* 2015). In heavy infections, the migrating larvae destroy the hepatic cells with eosinophilic infiltration and severe inflammation that may be fatal (Scala *et al,* 2014).

Camels are known to serve as hosts of other metacestoda such as those that cause cystic echinococcosis with prevalence rates in the Middle East and North Africa ranging between 6 and 100% (Sadjjadi, 2006). In addition, *Cysticercus dromedarii*, the larval stage of *Taenia hyaenae* was described from the muscle tissue and internal organs of dromedaries and cattle in Egypt and Eritrea (Nomani, 1920; Pellegrini, 1947; El Badri *et al,* 2010). Dromedaries are also listed as hosts of *C. tenuicollis* and *Coenurus cerebralis* in many reviews and textbooks (Abuladze, 1964; Dakkak and Ouheli, 1987; Fassi-Fehri, 1987; Troncy *et al,* 1989; Kaufmann, 1996; Parsani *et al,* 2008; Abu-Samra, 2015), yet no recent original reports of either of the parasite in camels were found in the literature. Moreover, although the occurrence of *C. tenuicollis* in ungulate intermediate hosts such as sheep and goats is well documented (Mekuria *et al,* 2013), no similar data is available on the occurrence of this parasite in camels. At the Central Veterinary Research Laboratory (CVRL) in Dubai, the United Arab Emirates (UAE), more than 800 adult and sub-adult camels and nearly 900 camel calves were necropsied during the period between 2005 and 2014. Hydatid cysts of sheep *Echinococcus granulosus* (G1 genotype) and camel *E. canadensis* (G6 genotype) were the only metacestode larvae found in 50 (= 6.2%) of the necropsied adult dromedaries (Schuster *et al,* 2015). In a recent slaughterhouse-study carried out in Iran only 0.01% (11/60,792) of camel livers were condemned as a result of cysticercosis although the species involved was not confirmed (Khaniki *et al,* 2013). The present report is a morphological and biomolecular study of cysticercosis due to *C. tenuicollis* in a dromedary. A three month old camel calf that was translocated together with its mother to a farm in Dubai from Pakistan in April 2015 died two weeks after arrival and was subsequently sent for necropsy to the Central Veterinary Research Laboratory.

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Methods and Results

The main finding at necropsy conducted on the camel calf was severe hepatic alterations. At macroscopical examination several bladder-like structures ranging in size from 3 to 6 mm were observed under the hepatic capsule (Fig 1) as well as deep within the liver tissue. In total 36 cysts were isolated; of which 30 were in varying stages of caseous degeneration and were surrounded by a capsule formed by host tissue. Six of these cysts were intact, contained a small quantity of clear liquid and had a white coloured body that showed a slight movement when placed in warm normal saline at 37°C. Microscopical examination revealed a membranous structure with a serrated surface and a conspicuous 100 µm deep invaginated canal which was interpreted as a developing scolex although neither suckers nor hooklets were observed (Fig 2, Fig 3). The suspect parasite was encircled by necrotic liver tissue and a host-derived capsule composed of macrophages, myofibroblasts and T-lymphocytes (Fig 4). These observations suggested that metacestode larvae were responsible for the observed hepatic alterations.

Genomic DNA was extracted from individual putative larval metacestodes using the High Pure PCR Template Preparation kit (Roche Diagnostics, Mannheim, Germany). DNA was used to amplify a fragment within two mitochondrial genes, cytochrome c oxidase subunit 1 (cox1) (~391 bp) with primers JB3 (5′-TTTTTTGGGCATCCTGAGGTTTAT-3′) and JB4.5 (5′-AAAGAAAGAACATAATGAAAATG-3′) and NADH dehydrogenase subunit 1 (ND1) (~471 bp) using primers JB11 (5′-AGATTCGT AAGGGGCCTAATA-3′) and JB12 (5′-ACCACT AACTAATTCACTTTC-3′) as previously described (Bowles *et al,* 1992; Bowles & McManus, 1993a; 1993b). PCR products were purified using the High Pure PCR product purification kit (Roche Diagnostics, Mannheim, Germany) and commercially sequenced by MWG-Biotech (Ebersberg, Germany) using the PCR primers. Nucleotide sequences were compared to those available in GenBank® through the use of the basic local alignment search tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST/). We analysed a 324bp and 393bp fragment within the *cox 1* and *ND1* mitochondrial genes, respectively using DNA extracted from the bladder-like cysts removed from the liver of the infected camel calf. A BLAST search revealed that the infection was caused by *C. tenuicollis*, the larval metacestode stage of *T. hydatigena*.

These *cox1* and *ND1* camel *C. tenuicollis* nucleotide sequences were also used to construct a parsimony haplotype network. A total of 96 *cox 1* (*C. tenuicollis* n = 76; *T. hydatigena* adult tapeworm n=20) and 68 *ND1* (*C. tenuicollis* n = 55; *T. hydatigena* adult tapeworm n=13) nucleotide sequences from ungulate intermediate and canid definitive hosts from various geographical locations, which were used in a previous study were re-analysed together with sequences generated in this study using published methodology (Boufana *et al*, 2015).

Results obtained from the generated cox1 parsimony haplotype network (data not shown) showed that the camel calf *C. tenuicollis* haplotype clustered closely with *C. tenuicollis* haplotypes originating from a pig from China (Accession number JN831308) and a sheep from Iran (Accession number JQ710615) and was separated from the main central *T. hydatigena* haplotype (Hap 33) by 9 mutational steps (Boufana *et al*, 2015) (Fig 5). A similar picture was depicted for the *ND1* haplotype network where the camel calf *C. tenuicollis* haplotype was closely associated with a *C. tenuicollis* haplotype from a pig from China (Accession number JN831284) (Hap 43) (Boufana *et al*, 2015) (Fig 6).

Discussion

In this study 83.3% (30/36) and 16.7% (6/36) of molecularly confirmed *C. tenuicollis* removed from the liver of a 3 month old camel calf were caseous or degenerated, respectively. In addition, 6 of the camel-derived *C. tenuicollis cysticerci* had begun to degenerate before the scolex was fully developed. These findings as well as the failure to recover *C. tenuicollis* from at least 1,700 camel carcasses analysed in Dubai during the past 10 years, suggests that camels may be poor hosts of *C. tenuicollis*. This assumption however, would need to be verified through the experimental infection of camels using *T. hydatigena* eggs derived from various intermediate hosts. Parsimony analysis conducted in this study has shown the camel-derived *C. tenuicollis* sample to be closely related to *C. tenuicollis* from Chinese pigs. A recent study on the genetic variation of *T. hydatigena* isolates has indicated to the possible existence of variants within *T. hydatigena* with *C. tenuicollis* isolates from pigs and goats being genetically differentiated to those from sheep hosts (Boufana *et al,* 2015).

According to Blazek *et al* (1985) who described the development of *C. tenuicollis* in piglets, a "scolex anlage" was said to appear 13 days post infection with the rostellar cone forming on day 21 and rostellar hooks appearing on day 35 post-infection. Cysticerci were seen to reach 3.6 - 4.8 mm in length on day 15

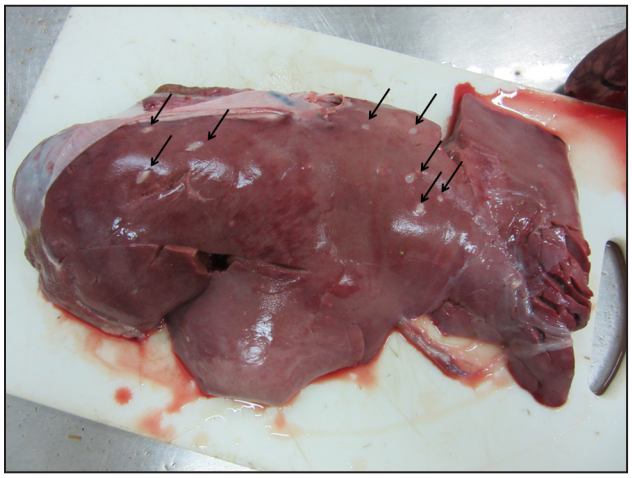

Fig 1. Liver of a dromedary calf showing multiple cyst like structures (arrows) under the capsule.

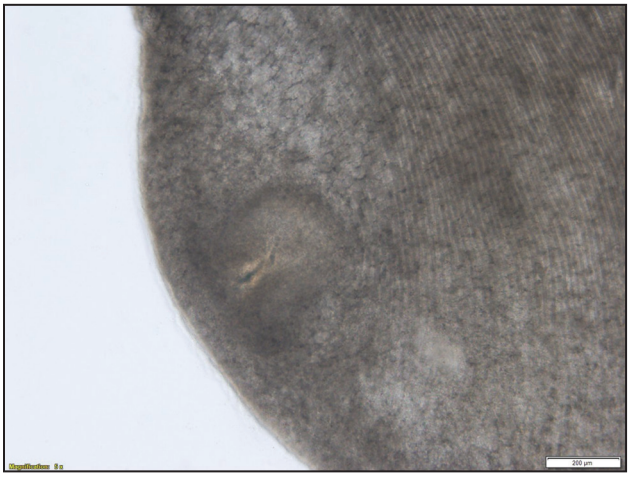

Fig 3. Fraction of the metacestode showing the invagination and the serrated surface.

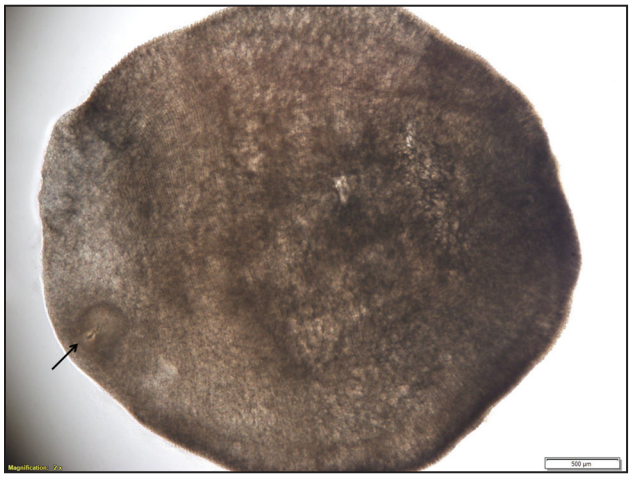

Fig 2. Isolated early stage of a metacestode removed from an intact liver cyst. A short invaginated canel is present at one pole.

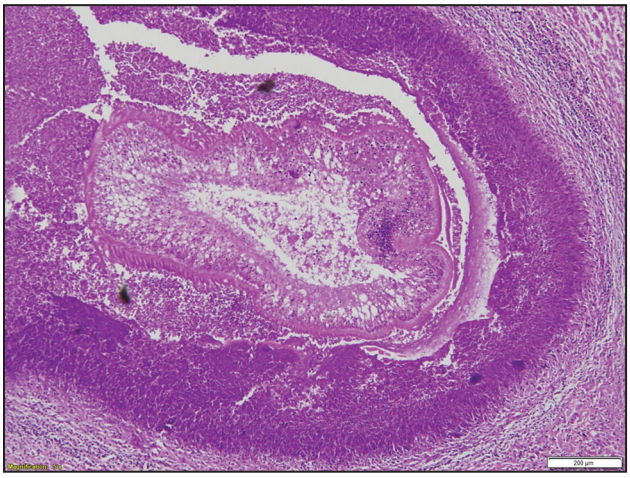

Fig 4. Histological section of the liver showing the metacestode surrounded by a host-derived capsule.

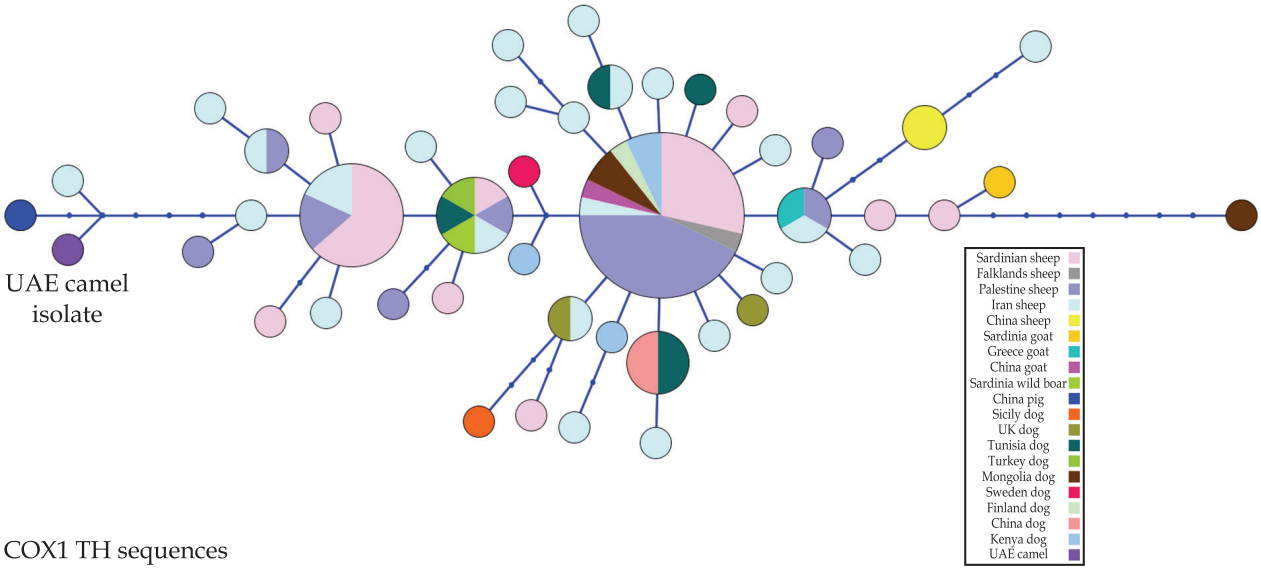

Fig 5. Position camel calf *C. tenuicollis* haplotype within the cox1 haplotype network.

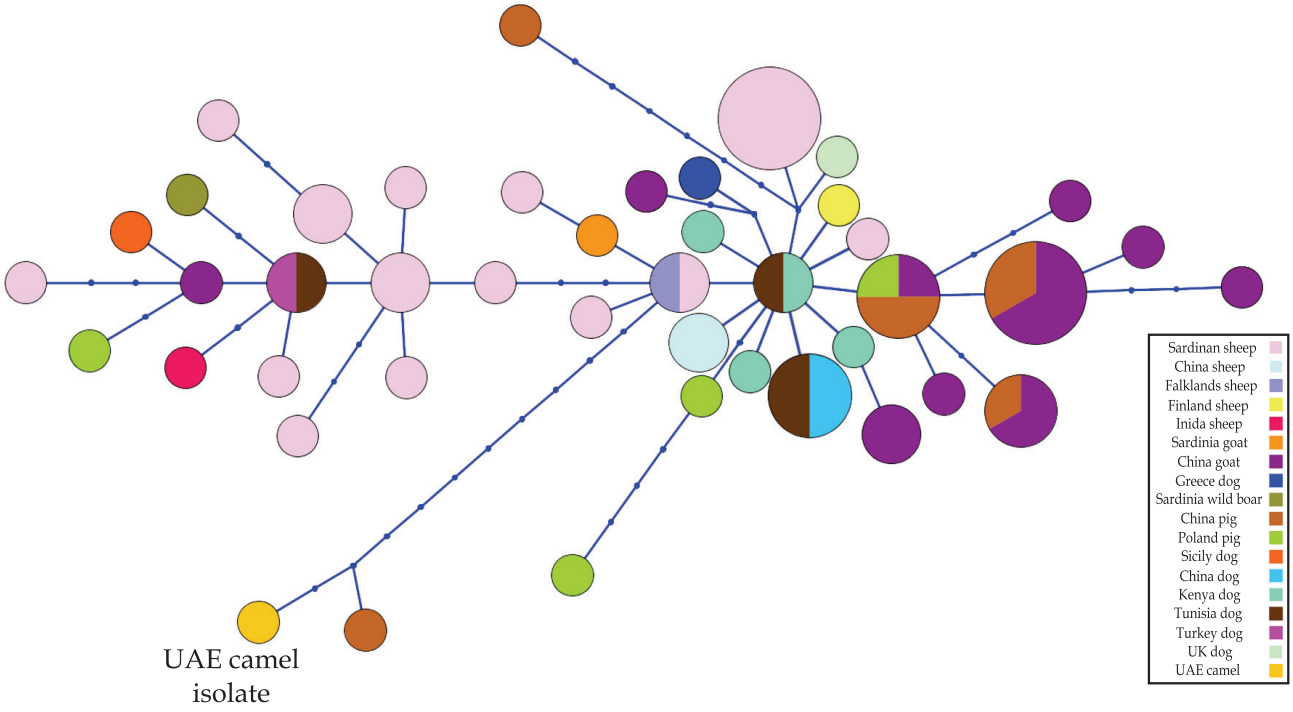

Fig 6. Camel calf *C. tenuicollis* haplotype within the *ND1* haplotype network.

and 16 post-infection and are thus comparable in size to the camel *C. tenuicollis* cysticerci observed in the present study. This suggests that the early development of *C. tenuicollis* in the camel calf may have a similar time course to that observed in pigs and that infection of the camel calf was probably acquired in the country of origin (Pakistan). There are limited data available on parasites in livestock in Pakistan. Despite numerous abattoir surveys on small ruminant parasites (Iqbal *et al,* 2004) *C. tenuicollis* was reported only from sheep and goats with prevalences of 18.8 and 17.3%, respectively in the North-West Frontier Province (Rayaz, 1990). In the Punjab province of Pakistan these prevalences were 11.7 and 8.2%, respectively (Iqbal *et al,* 2012). However, adult camels slaughtered in various abattoirs in the Punjab province of Pakistan were infected with hydatid disease with prevalence ranging between 17 and 76% (Hussain *et al,* 1992; Anwar and Khan, 1998; Lativ *et al,* 2010). The examination of 200 dogs in Karachi (Sindh province of Pakistan) showed these definitive hosts to have a 23% and 7% prevalence for *T. hydatigena* and *E. granulosus* respectively (Saleh & Ahmed, 1965).

In summary, results obtained in this study indicate that camels may serve as aberrant and/or poor hosts for *C. tenuicollis*. Although as such they would not contribute to the epidemiology of *T. hydatigena* yet cysticercosis due to *C. tenuicollis* may be responsible for pathological changes and early death in camel calves as seen in the present case.

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