Taenia multiceps: a rare human cestode infection in Israel

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Summary

Brain infestation caused by the metacestode of *Taenia multiceps* is a rare phenomenon in humans, but is fairly common among sheep in Mediterranean countries. No more than 150 human cases have been reported. In this present study, we report an unusual case of a huge intra-parenchymal cyst in a four-year-old girl caused by *T. multiceps*. No cross-reactivity between *Echinococcus granulosus* and *T. multiceps* antigens was demonstrated. After surgical removal of the cyst, followed by chemotherapeutic treatment with albendazole combined with praziquantel, the child recovered completely.

Keywords

Coenurosis, Dog, Life cycle, Neurosurgery, Public health, Sheep, *Taenia multiceps*, Zoonosis.

Taenia multiceps: una rara infezione umana da cestodi in Israele

Riassunto

L'infestazioni celebrale da metacestodi di Taenia multiceps *è un evento che raramente si verifica nell'uomo mentre è abbastanza comune tra le pecore nei paesi mediterranei. Infatti, nell'uomo sono noti non più di 150 casi. In questo lavoro viene presentato un caso atipico di estesa cisti intraparenchimale causato da* T. multiceps *in un ragazzo di 14 anni. Non è stata provata una reattività crociata tra* Echinococcus granulosus *e* T. multiceps. *Dopo la rimozione chirurgica della cisti e il successivo trattamento chemioterapico con albendazolo e praziquantel, il paziente è completamente guarito.*

Parole chiave

Cane, Cenurosi, Ciclo vitale, Neurochirurgia, Pecora, Sanità pubblica, *Taenia multiceps*, Zoonosi.

Introduction

Coenurosis is a zoonotic cestode infection of humans and animals caused by the larval stage (metacestode) of *Taenia* species (*T. multiceps*, *T. serialis*, *T. brauni*, *T. glomerata*). The parasite is a two-host zoonotic cestode and the definitive host is a carnivore (e.g. dog, fox) while most of the intermediate hosts are herbivores (e.g. sheep, rabbits, rodents and cows) (36). The disease is mainly distributed in sheep-raising areas (2, 3, 8, 12, 41) and is also moderately common in sheep in countries of the Mediterranean and Middle East, including Turkey (0.85%) (28), Jordan (3%) (1, 9), Iran (9.8%) (26) Italy (36, 37) and France (10) (Table I). The disease is also considered to be endemic in Israel. Most cases in sheep were reported approximately 50 years ago (20, 33)

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Table I
Representative cases of *Taenia multiceps* infections among humans and animals

Country	Prevalence in sheep (%)	Prevalence in dogs (%)	No. of human cases	Ref.
Caucasia	–	–	1	4
Ethiopia	2.3-4.5	–	–	2
France	+	–	–	10
India	+	–	–	41
Iran	9.8	–	–	26
Ireland	0.35-1	–	–	8
Israel	+	–	1	5, 20, 33
Italy	0.35	–	4 + 1	30, 36, 37
Jordan	3.0	3.8	–	1, 9
Russia	+	–	–	3
Turkey	0.85	–	–	28
Wales	+	–	–	12
Uganda	–	–	1	6
United States	–	–	1 + 6	16, 18

and several new cases in sheep and lambs in central Israel were reported recently by the Israeli Ministry of Agriculture (J. El-On, personal communication). In sheep, the metacestode (previously known as *Coenurus cerebralis*) inhabits the central nervous system, including the brain and spinal cord, causing circling ('gid'), ataxia, incoordination, drowsiness, hind leg paralysis, head pressing, blindness and coma.

Humans, especially young children, become intermediate hosts by ingesting eggs passed in the excrement of a definitive host. The ingested eggs release oncospheres in the intestine of the host that penetrate the intestinal wall, passing through the blood stream towards target organs, usually the brain, spinal cord and eyes (6, 16, 22, 23). Human disease caused by *T. multiceps (Coenurus)* is a rare phenomenon that has been reported from Africa (39), Europe (30, 31, 34, 35), Asia (4, 5) and the Americas (16, 17, 18, 25, 38, 40) (Table I). The most common symptoms of brain coenurosis in humans include headaches, vomiting and pappiloedema that are related to the increased intracranial pressure caused by the mass effect of the cystic lesion. Focal neurological deficit, such as cranial nerve palsy and motorical disturbances are also frequent.

Until recently, in the southern part of Israel which is mostly arid desert, zoonotic cestode infections were very rare. It has been suggested that the desert conditions in this area were unsuitable for the development of the eggs of the parasite (10, 44) (Fig. 1). This area has approximately 500 000 inhabitants, 23% of whom are Bedouin (Muslim). An increasing prevalence of echinococcal (*Echinococcus granulosus*) infection has recently been revealed in the Bedouin community of southern Israel (44). It has been suggested that this rate of infection could be the result of the recent movement of the Bedouin populations into towns where only limited ground is available for animals. It should be noted that sheep and goat breeding is the Bedouin's traditional occupation. Over 1 395 flocks are officially registered; they are mainly low in numbers and apparently intended for domestic use. The Bedouins have an estimated total of 260 000 sheep and goats with an unknown number of dogs. Furthermore, many of the Bedouin tribes that have recently settled in permanent towns and villages still maintain a semi nomadic lifestyle, where dogs play an essential role in the protection of livestock which leads to increased and close daily contacts with dogs and, consequently, the occurrence of increased zoonotic diseases (Fig. 2). In this study, an unusual case of *Coenurus* cyst in the brain of a four-four-year-old Bedouin child is presented.

Figure 1
Traditional lifestyle of Bedouins in the Negev desert area in southern Israel, prior to movement into new towns
Dogs and sheep are kept far from living areas

Figure 2
Traditional lifestyle of Bedouins in the Negev desert area in southern Israel after their movement into new towns
Sheep are reared in close proximity to the living areas

Materials and methods

Computed tomography and magnetic resonance imaging

Imaging methods, including computerised axial tomography (CT) and magnetic resonance imaging (MRI) were performed using standard procedures.

Serological examinations

Serological tests, including the enzyme-linked immunosorbent assay (ELISA), agarose gel immunodiffusion (ID), immunoelectrophoresis (IEP) and immunoblot (IMB), using *E. granulosus* hydatid cyst fluid as antigen, were performed prior to and after surgery. The ELISA-immunoglobulin G (IgG) test was performed using a commercial kit (r-biopharm, Darmstadt). Goat anti-human antibody conjugated with peroxidase was used to determine IgG activity. ID and IEP (6 volts/cm) were performed in 0.09% agarose (Sigma) prepared in veronal buffer, pH 8.6 (19). Results were recorded after 24 h incubation at room temperature (immuno-electrophoresis) or after 72 h at 4°C (immuno-diffusion), before and after staining with amido black. Precipitation of antigen A (arc 5) was indicative of echinococcal infection (29). The IMB was prepared in sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Kharebov *et al.* (19). Antigens were subjected to electro-phoresis in stacking gel (5%) and separating gel (16%) under reduced conditions. Pre-stained molecular weight standards (7-208 kDa, Bio-Rad) were included in all gels. Resolved antigens were electrophoretically transferred to nitrocellulose paper (Schleicher & Schuell, Dassel) using a transblot cell (Hoffer Scientific Instruments, San Francisco). Separated proteins in slab gel were either stained with Coomassie blue or ponceau S. Goat anti-human fragment, antigen binding (Fab)-IgG conjugated with horseradish peroxides (Bio Makor, Rehovot) was used to detect the antigens. The reaction was visible using 3-3′-diaminobenzidine/H_2O_2 as a sub-strate. The presence of antibodies against the 20 kDa and 39 kDa (antigen A) molecules and particularly against the 12 kDa, 16 kDa and 24k Da (antigen B) molecule indicated echinococcal infection.

Detection of echinococcal antigens in the cyst fluid

Anti-*E. granulosus* antibodies against proto-scoleces and crude hydatid cyst fluid (HCF) were raised in rabbits as described by Peiser *et al.* (32). Briefly, 2 ml (4-5 mg) of antigen mixed with an equal volume of Freunds complete adjuvant were inoculated intracutaneously into 20-25 sites on the abdomen. An additional seven inoculations of equal quantities of antigen in adjuvant were administered subcutaneously into the neck and intramuscularly into the rump every 10 days. Sera from immunised rabbits were collected and kept frozen at –20°C. Elimination of contaminating antibodies directed against common host components was achieved by immunoabsorption of immune sera with sheep sera.

Preparation of tissues for histological examination

Fresh material and pathological preparations stained with hematoxyline/eosin were analysed. Tissue samples taken from the outer and inner cysts were fixed in 10% neutral buffered formalin at 4°C for 48 h, dehydrated in increasing concentrations of ethanol, cleared in xylol and impregnated with and embedded in paraffin wax (Paraplas Plus) with a melting point of 56°C. Tissue sections 4 µm thick were mounted on microscope slides (Menzel-Gläser SuperFrost® Plus, Braunschweig), dried overnight at 37°C, stained with Mayers haematoxylin and with eosin (0.5 g % hematoxylin crystals, 5% vol. ethanol, 10 g % $NH_4Al(SO_4)_2$, 0.037 g % NaIO) (Finkelman, Yehud), and after dehydration and clearing, coverslipped with Eukitt mounting medium (Electron Microscopy Sciences, Hatfield, Pennsylvania).

Results

A four-year-old girl who was previously healthy, presented to the emergency room

with two months history of morning headaches and nausea. The parents reported direct contact with dogs and possible contact with the stool of dogs. Her neurological test revealed an irritable child with hemiparesis in her right arm (3/5) and leg (4/5). A fundoscopic examination revealed bilateral papilloedema. The complete blood count, including the eosinophilic count, was within the normal limits. A head CT followed by a brain MRI were performed and revealed a very large left hemispheric cystic lesion that measured 13 cm in diameter and had no solid component or enhancement in the medial aspect of the surrounding capsule (Fig. 3). Surgical removal of the cyst was performed showing a very thick, grey-brownish capsule that was ruptured during the procedure with no clinical change (Fig. 4). On histological examination of the cyst, the specimen revealed the typical components of a cerebral chronic abscess represented by collagen fibres, dense acute and chronic inflammatory cells and a marked reactive proliferation of glial cells. The inflammatory infiltrate was rich in plasma cells and lymphocytes but eosinophils were absent. Inside the fibrous capsule, another paper-thin transparent cyst was detected floating within it (Fig. 4). The inner cyst that was eventually removed intact contained multiple white protoscoleces of few millimetres in length that were attached to the cyst membrane. The inner cyst was unilocular, about 6-7 cm at its largest diameter, with a thin, transparent and

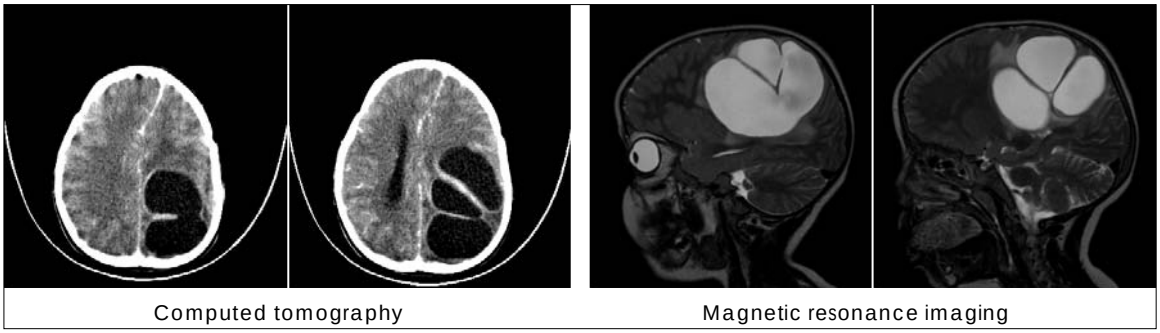


Figure 3
Head computed tomography followed by brain magnetic resonance imaging demonstrating a very large left hemispheric cystic lesion with no solid component and enhancement in the medical aspect of the surrounding capsule
Mild vasogenic oedema was noted, contributing to the severe mass effect with signs of subfalcine and transtentorial herniation

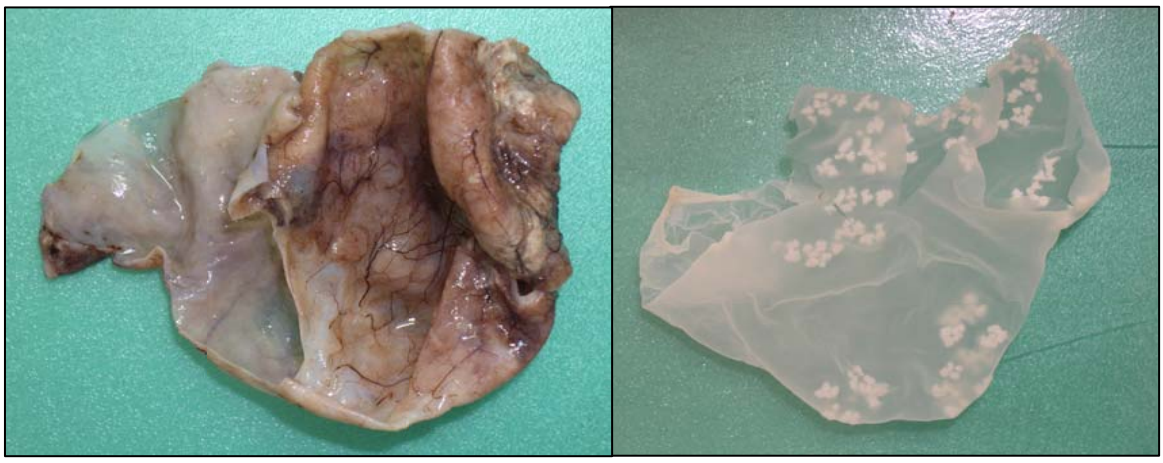
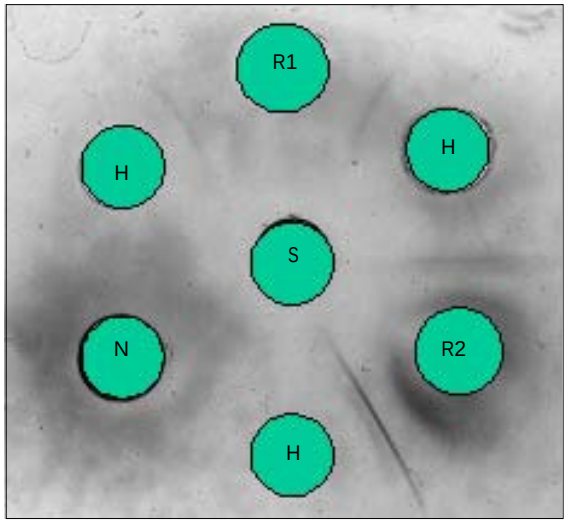


Figure 4
Coenurus cysts removed from the patient by surgery
Outer (left) (13 cm in diameter)
Inner (right) (6-7 cm in diameter)
The outer cyst is characterised by a very thick, fibrotic, grey-brownish capsule while the inner cyst is characterised by a thin transparent wall with several white protoscoleces clumped on the inner surface

gelatinous wall. The germinative wall was thin, non-laminated and was composed of a thin layer of tegumental cells in an amorphous pink tegument. The absence of laminated thick wall, typically seen in hydatid cysts, excluded the diagnosis of echinoccocal infection. Neither daughter cysts nor protoscoleces or hooklets floating in the cyst fluid of both the external and the internal cysts were observed.

Serological examinations performed prior to surgery, using ELISA, IEP and IMB gave negative results. Furthermore, after surgery, using anti-*E. granulosus* HCF and anti-scoleces rabbit sera, no echinococcal antigens were demonstrated in either the internal or external cyst fluids (Fig. 5). The presence of many scoleces with very long hooks attached to the surface of the inner membrane demonstrated by microscopic examination of fresh material (Figs 6 and 7) and pathological preparations (Fig. 8) established the diagnosis of coenurosis in this child. A combined treatment was



H hydatid cyst fluid
S patient's cyst fluid
N normal rabbit serum

Figure 5
Immunodiffusion using sera from rabbits immunised against *Echinococcus granulosus* scoleces (R1) and hydatid cyst fluid (R2), indicating no cross-reactivity between *Taenia multiceps* and *E. granulosus* antigens

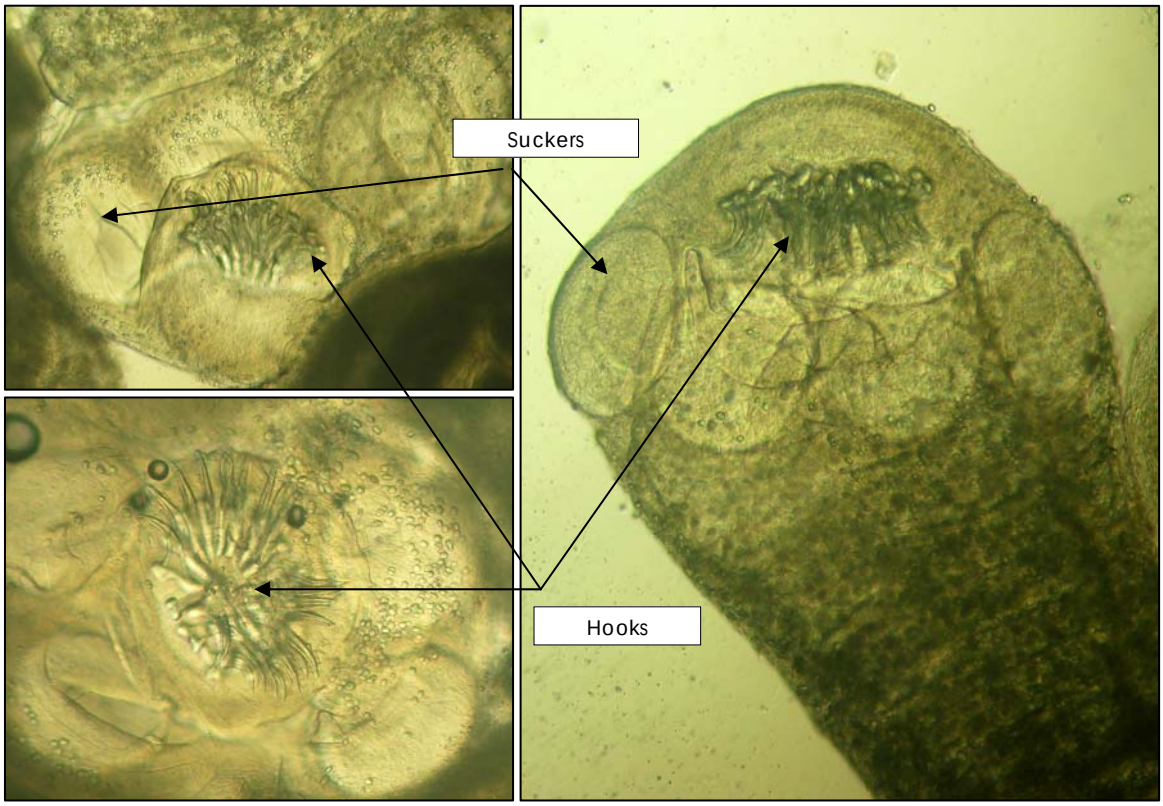


Figure 6
Fresh, unstained *Taenia multiceps* protoscoleces, showing typical hooks and suckers

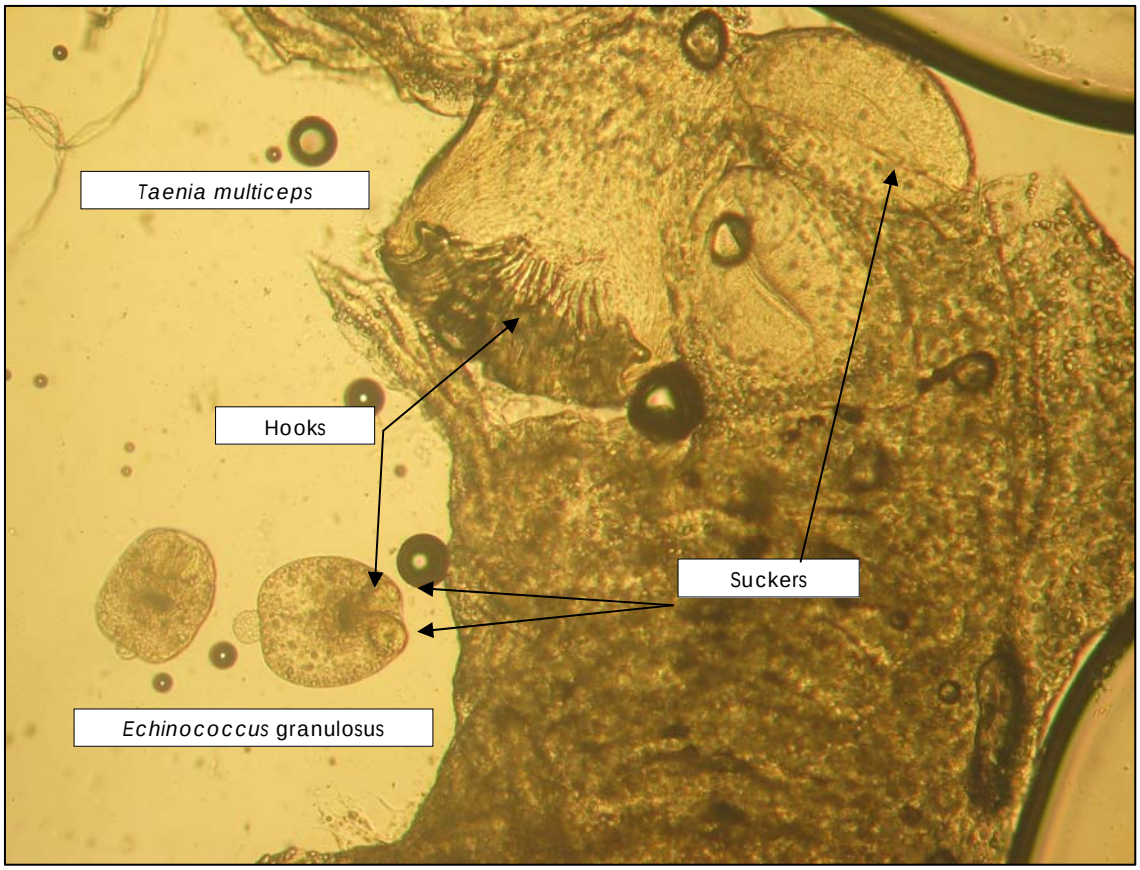


Figure 7
An artificial mixture of *Echinococcus granulosus* and *Taenia multiceps* protoscoleces, indicating the very large size difference between these two biological forms

administered using albendazole over a period of eight weeks and praziquantel for three weeks. The child had fully recovered six months after surgery, with no neurological disorders or impairment or papilloedema. No evidence of residual lesions was detected by MRI.

Discussion

In humans, cerebral coenurosis caused by *T. multiceps* (also referred to as *Multiceps multiceps*) is a rare phenomenon that is reported mainly in developing countries (13, 15, 16, 17, 34) (Table I). Dogs (the final host) and sheep (the intermediate host) are the natural common hosts and humans become intermediate hosts by ingesting eggs, resulting in cerebral multilocular coenuri, which are rarely intraparenchymal (13).

Diagnosis

The diagnosis of *T. multiceps* infection in humans is extremely difficult since the clinical symptoms are non-specific, the imaging procedures (CT, MRI) are not pathognomonic and no serological test is available. In the differential diagnosis of intra-parenchymal brain cysts, neurocysticercosis and echinococcosis are considered to be the most common parasitic infections. However, only 2% of hydatidosis patients will develop cerebral cysts; these will generally be unicellular, with a spherical shape and no contrast enhancement (42). In addition, a cyst of <5 cm in diameter might develop in both *T. solium* and *T. racemose*, in comparison with >10 cm in *T. multiceps* infection. However, the size of the cyst described in this case was ~13 cm in diameter; to date, no report of *T. solium* infection has ever been made in Israel.

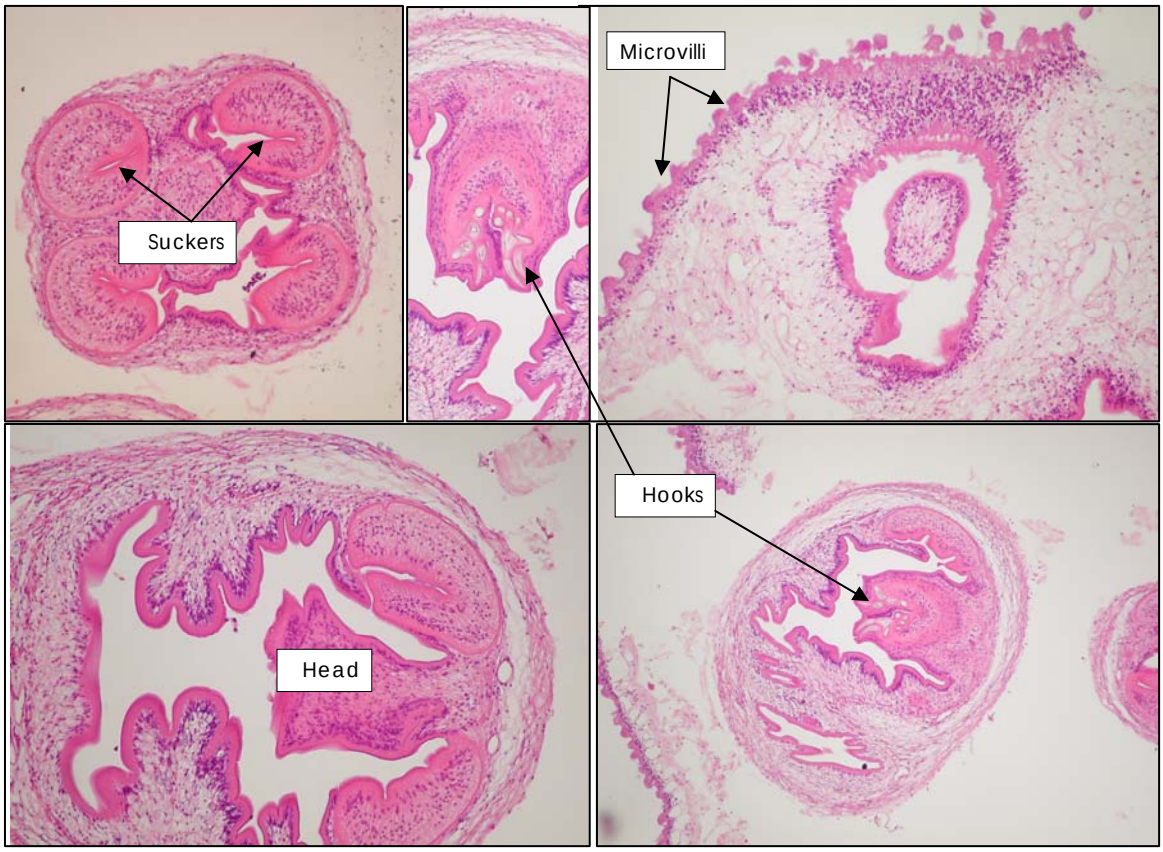


Figure 8
Hematoxyline eosin stained sections of a protoscolex of *Coenurus* showing the principal anatomic details (head, suckers, hooks and microvilli)
The protoscoleces develop from invagination of the cystic wall (germinative layer)

The differential diagnosis of supratentorial, intra axial brain cystic lesions includes low- and high-grade neoplastic lesions, abscesses, neuroglial cysts and parasitic cysts (27). Hence, when the possibility of a parasitic infection involving the brain exists, coenurosis should be considered after echinococcosis and cysticercosis infections have been excluded. Generally, the final diagnosis of *T. multiceps* is possible after surgical removal and pathological examination. Serological examinations are not very effective in the diagnosis of the disease, even if *E. granulosus* and *T. multiceps* do share a common antigen (antigen A) (7). However, in the present case study, anti-echinococcal antibody activity was not demonstrated in the patient's sera by ELISA, ID, IEP or IMB. Furthermore, no echinococcal antigens (antigens A and B) were demonstrated in the patient's cyst fluid, using hyperimmune sera from rabbits immunised against *E. granulosus* HCF and scoleces.

Vaccination

Approximately 30 years ago Verster and Tustin (43), using oncosphere secretory antigens were able to protect lambs against *T. multiceps* infection. Recently, a recombinant vaccine (EG95) has been developed which was found to be highly effective against *T. ovis* in sheep, *T. saginata* in cattle, *T. solium* in pigs and *E. granulosus* in livestock (11, 14, 21). *E. granulosus* and *T. multiceps* share common antigens (7) and therefore the vaccine developed against *E. granulosus* might also be useful against *T. multiceps* infection. Very recently, a recombinant antigen, designated 'Tm16 and Tm18', prepared from the onchosphere of *T. multiceps* protected sheep successfully from *T. multiceps* infection (14). These results are extremely encouraging for the development of effective and useful vaccines against cestodes, including *T. multiceps* infections.

Treatment

In most cases, surgical intervention for patients with brain *Coenurus* is indicated to establish a diagnosis and for symptom relief. In the past, surgery was associated with high mortality and morbidity rates (13, 24, 38), but the situation improved with the introduction of the modern neuroradiological techniques (CT and MRI) (30, 31). To date, no useful information is available regarding the effectiveness of chemotherapeutic treatment against *T. multiceps* infection. In most cases, the regimen applied in hydatidosis is adopted in *T. multiceps* infection, namely: the administration of albendazole either alone or combined with praziquantel post surgery to prevent the dissemination of disease (35), as performed in this study.

Conclusions

We report here the first case of intracranial *T. multiceps* infection in Israel. In countries that are considered to have an endemic status in regard to both echinococcosis and coenurosis, cerebral coenurosis, although rare, should be considered in the differential diagnosis of a brain cyst. No serological cross-reactivity between *T. multiceps* and *E. granulosus* antigens was demonstrated using standard methods, including ELISA, IEP and IMB. Complementary treatment with albendazole combined with praziquntel should be administered before and after surgery to eliminate the possibility of dissemination of the disease.

Conflict of interest/competing interests

The authors have no conflict of interest or financial investments to disclose.

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