

***Cysticercus tenuicollis* Infection in Captive Vervet Monkeys (*Chlorocebus pygerythrus*) in Iran**

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Abstract

In this article we described necropsy findings of *Cysticercus tenuicollis* infection among a group of 65 wild-caught imported vervet monkeys from Tanzania into “Razi vaccine and serum research institute Karaj-Iran” for assessment of oral polio vaccine neurovirulence test (MNVT). The methods of diagnosis were based on observation of the *C. tenuicollis*, morphological characters of scolex as well as the shape, size and the number of the rostellar hooks and histopathological examination. Totally 7 female monkeys were detected positive (10.8%) for *C. tenuicollis*. Our results highlighted that, most cases of *C. tenuicollis* infection in vervet monkeys detected incidentally and cause mild or no disease, but well developed cysts could be organized in abdominal cavity causing peritoneal cysticercosis. It was suggested to set up more measures such as ultrasound imaging and serological tests, on next import shipment of the vervet monkeys, to prevent entrance of infected animals to the post quarantine holding areas.

Key words: *Taenia hydatigena*, *Cysticercus tenuicollis*, Vervet monkey

Introduction

Taenia hydatigena is a member of cestodes distributed in all over the world. The adult tape worm is found in the small intestine of the definitive hosts, dog or wild carnivores. Metacestode (or larval cestodes) of *T. hydatigena* (*Cysticercus tenuicollis*) is characterized in part by its occurrence in a long list of hosts and also its unusually broad geographical distribution. Adults are 75-500 cm long and have two rows of 26 and 46 rostellar hooks. The gravid proglottids are 12 by 6 mm and the uterus

has six to ten lateral branches. The eggs are oval and pass through feces of the definitive hosts (Soulsby 1982). The intermediate hosts infected with cysticercus by ingesting foods contaminated with eggs of *Taenia hydatigena* (Sweatman & Plummer 1957). In the small intestine of the intermediate hosts the eggs hatch and the oncosper reach the liver via the blood, where the embryo getaway of the portal vessels and migrate in the liver. The developing cysticerci migrate into peritoneal cavity, where, they found

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attached to the serosal surface of organs, intestinal mesentery and the greater omentum. Mature *Cysticercus tenuicollis* are large (from 1 cm up to 6–7 cm) and the scolex has a long neck. *Cysticercus tenuicollis* infection have been reported from different intermediate hosts including: squirrels, cattle, wild ruminants, sheep and goat, swine, and non-human primates (NHPs) (Cogwell 2007, Murrell et al. 2005, Dorny et al. 2005, Jones et al. 1997, Tsubota et al. 2009). According to Kunts and Myers (1967), a Japanese researcher (Sugimoto) in 1939 presented an extensive list of hosts in which the parasite occurred in the Orient. A few reports of wild intermediate hosts are as follows: Sgroi et al. (2019) reported a massive infection due to *T. hydatigena* cysticercosis in a wild boar from southern Italy (Sgroi et al. 2019). Filip et al. (2019) reported Identification of *Taenia hydatigena* in Wild Ungulates in Poland (Filip et al., 2019). Chege et al. (2016) described incidental findings of *Cysticercus tenuicollis* metacestodes in five oryx species in the United Arab Emirates (Chege et al., 2016). In sheep and goats migration of the cysticerci in the liver may cause hemorrhagic and fibrotic tracts where viable, caseated or calcified cysticerci may be present. This may result in condemnation of the infected livers. Furthermore, in infected lambs, it may be the reason of insufficient growth. However, heavy infection of young lambs may cause traumatic hepatitis leading to death. Diagnosis of *Cysticercus tenuicollis* infection is based on the finding of the cysts during necropsy procedure or meat inspection. Morphological characters of scolex, rostellar hooks and histopathological examination can be used for characterization of the cyst. However, in cases where the key morphologic features cannot be detected, molecular approaches are useful tools to define species of parasites (Utuk & Piskin 2012). Other methods such as ELISA, biochemical and hematological tests can be useful for diagnosis of infection

in live animals (Radfar et al. 2014; Chege et al., 2016). In laboratory monkeys, parasitic foci were frequently found during the period when captured wild-caught monkeys had been used (Abbott & Majeed 1984, Tsubota et al. 2009). *Cysticercus tenuicollis* is grossly visible at post mortem and/or during meat inspection, but light infections are often missed (Dorny et al. 2005, Murrell 2005). In this article while reporting detected cases of *T. hydatigena* cysticercosis in a group of imported wild-caught vervet monkeys from Tanzania into Razi institute, the significance and pathogenesis of *Cysticercus tenuicollis* for vervet monkeys has been reviewed.

History

Ethics statement

This investigation was performed within the facilities of Razi Vaccine and Serum Research Institute (RVSRI), Karaj, Iran. The animals were housed nearly under standard condition and treated in compliance with the guidelines of animal welfare committee of the research deputy of RVSRI and National Ethical Framework for Animal Research in Iran, the Ministry of Health and Medical Education (TUMS, reference code: 91-01-159-18022).

The parasites described and pictured in this report had been found between 65 imported wild-caught vervet monkeys from Tanzania into “Razi vaccine and serum research institute Karaj-Iran” for assessment of oral polio vaccine neurovirulence test (MNVT). These monkeys obtained from a native animal dealer who had procured the animals for exportation. The monkeys were purchased at 2- 5 years of age. After arrival of the monkeys into the NHPs quarantine of Razi institute, initially the animals were kept in large cages, each, consisted of 3-4 monkeys for adaptation to new environment. Three months after the arrival, they moved into individual indoor-cages, for MNVT. During quarantine and testing, the animals were fed with a balanced diet consisted of washed

fruits and vegetables and other nutrients. Moreover, supplementary vitamins and minerals was regularly added to the diet. The animals were observed daily by an expert veterinarian and, if necessary, they were closely examined. During that period, medical checkup including microscopic and feces-smear examination for parasites (*Giardia*, *Strongyloides*, and *Entamoeba* and worms eggs) were implemented twice (Balansard et al. 2019). The results of medical tests except to a mild to moderate microcytic anemia (Iron deficiency) in most of the animals revealed absence of infectious diseases. Anemia was treated by supplementary vitamins and minerals. During daily observation in quarantine, except detection of sickness in one of the monkeys (no. 65) in the form of progressive anorexia, lethargy and weight loss and moderate bulging of abdomen, all other animals showed no abnormal clinical signs. The sick monkey was decided to euthanize by veterinary adviser.

Method of diagnosis

At the last step of the MNVT all the monkeys, except one which has been already euthanized for health problems, were sacrificed and their central nervous systems removed for histopathological examination. Postmortem examinations revealed, 7 females of 65 vervet monkeys (10.8%) were infected with *Cysticercus tenuicollis*. One of the monkeys, which has already euthanized (no. 65), had only one well develop large cyst (7 cm) containing nearly 100 ml of pale yellow fluid. The cyst was in the posterior aspect of the stomach in the peritoneal cavity that was wrapped in fatty tissues and folds of the omentum. One pedunculate scolex hung from the inner wall of the cyst was seen (Fig. 3-5). Another four vervet monkeys had two cysts each and

two other monkeys had only one cyst each. The size of these cysts was 2-3 cm in diameter, and loosely attached to greater omentum or to the liver surface (moderately rigid) (Fig. 1-2). Loose connective tissue occupied the stroma surrounding the scolex. The bladder wall of these cysts was almost different in thickness and rigidity, color and the size. To examine the rostellar hooks, the larval stage scolex was isolated from the cyst by cross-section. To clarify the samples, lactophenol was used on the slide with lamellar coating. After clearance of rostellar hooks, examination was performed on cysts with complete hooks. In order to measure and determine the morphometric properties, a light microscope with a calibrated ocular lens was used. In microscopic examination of the specimens, after counting the number of hooks in each scolex, five large hooks and five small hooks were studied in terms of morphometric characteristics by a calibrated microscope (Radfar et al. 2014). The total number of hooks were measured ranging from 28 to 32. The size of large and small hooks have been measured 202 to 212 (μm) and 138 to 142 (μm), respectively (Fig. 4-5). Furthermore, for histological examination, samples of tissues and cysts were kept in 10% formalin solution then, after completion of fixation, the resected samples processed and embedded in paraffin then tissue sections, 5 μm in thickness, were cut and stained with hematoxylin and eosin (H&E) (Fig. 6-7). Mild to moderate presence of hemorrhagic and fibrotic tracts in the liver parenchyma, due to migration of *C. tenuicollis*, were the most obvious microscopic lesion. In these regions the hepatic cells were mostly degenerated and focally destructed. No other organs showed obvious lesions.



Fig. 1: Cysticercosis, vervet monkey- a *cysticercus tenuicollis* attached to the liver surface is seen (arrow).



Fig. 2: Cysticercosis, vervet monkey- a *cysticercus tenuicollis* attached to the greater omentum is seen (arrow).



Fig. 3: Cysticercosis, vervet monkey- an unusually large cyst of *cysticercus tenuicollis* containing pale yellow fluid in the peritoneal cavity is seen (Asterisk).

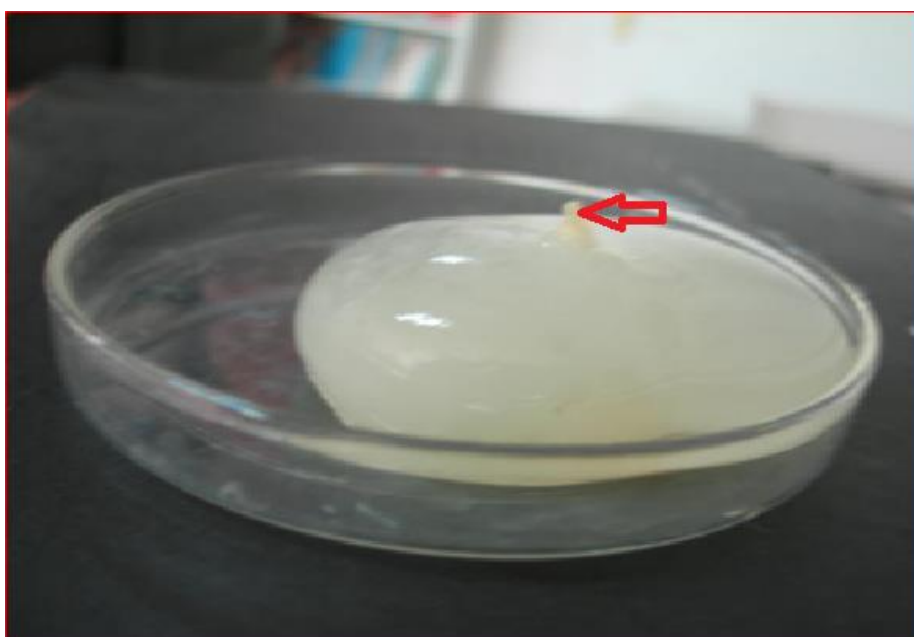


Fig. 4: *Cysticercus tenuicollis*, vervet monkey- intact translucent cyst with a fluid-filled bladder and a long neck (arrow) is seen.

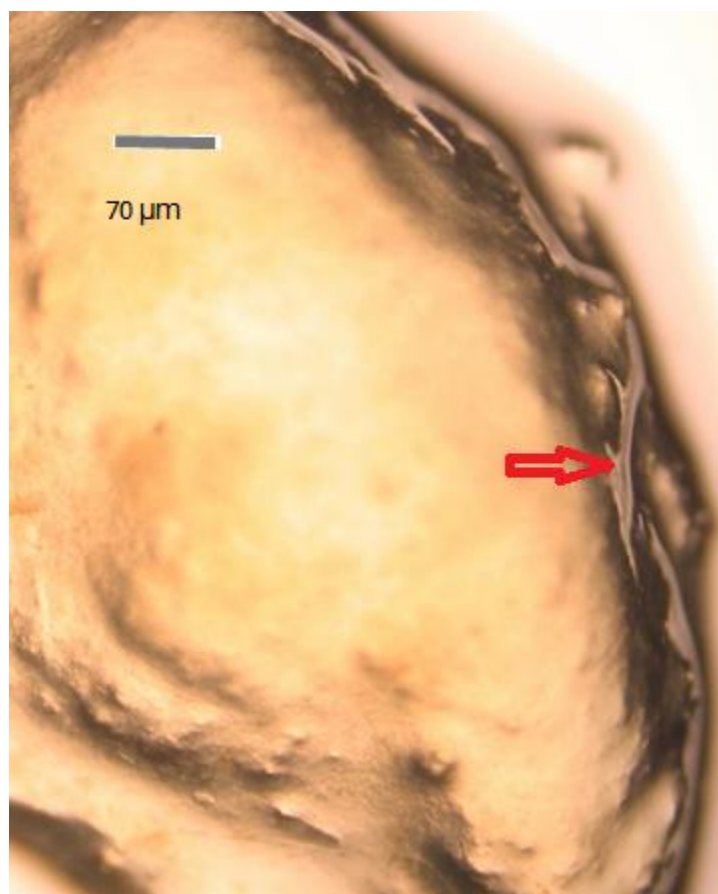


Fig. 5: Cysticercosis, vervet monkey- lateral view of scolex of larva *C. tenuicollis*. Arrow points to a large hook.

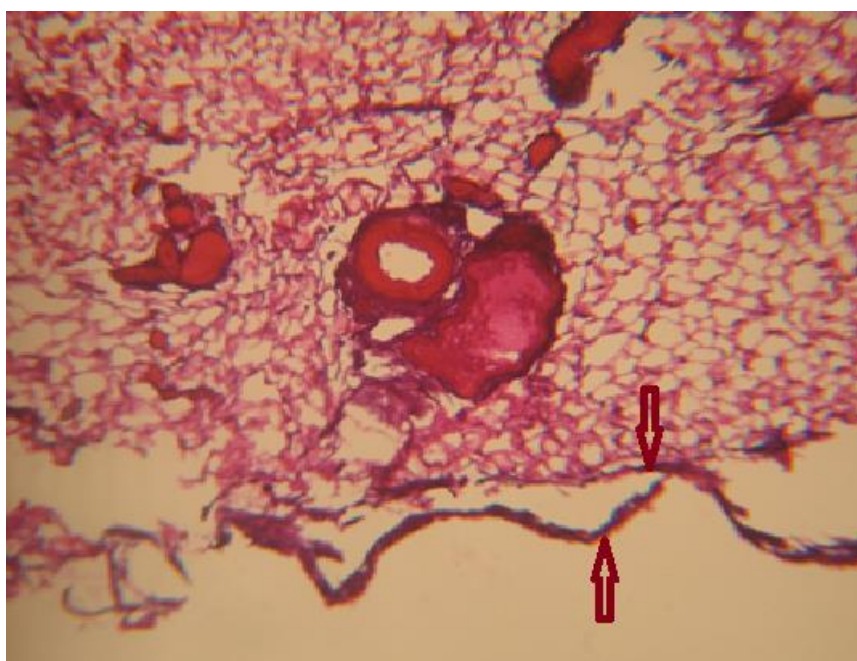


Fig. 6: Cysticercosis, vervet monkey- double layered *cysticercus tenuicollis* wall; greater omentum and cyst wall (Arrows) is seen (H&E $\times 200$).

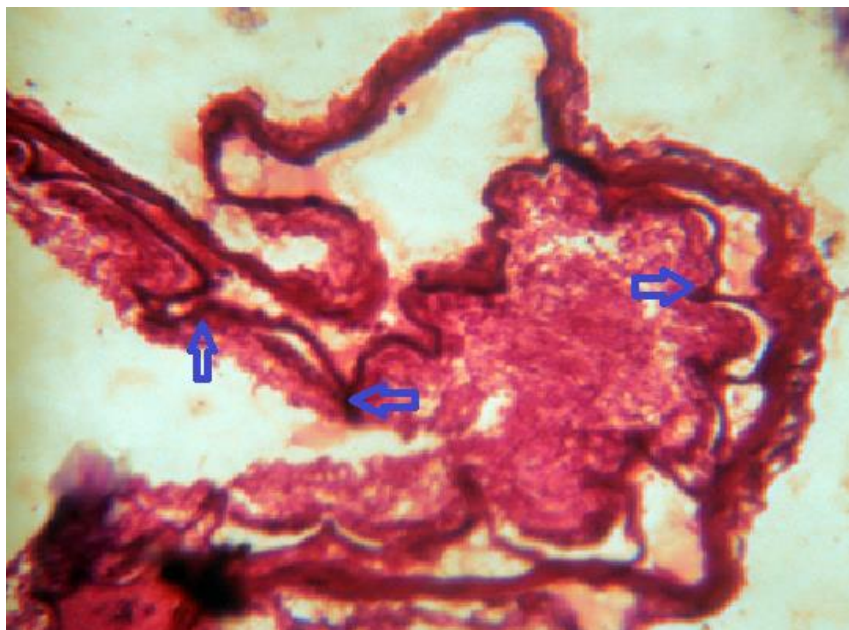


Fig. 7: Cysticercosis, vervet monkey- cross section through the invaginated scolex of *Cysticercus tenuicollis*; A number of small and large hooks are seen (arrows) (H&E $\times 400$).

Discussion

Vervet monkey is an important animal model for biomedical research. Although these animals are accompanied by fewer health and safety risks (Jasinska et al. 2013), but, all NHPs supplied for use in biomedical research should be of known health status. Meanwhile, agents which may or may not have any pathogenic or opportunistic capacity but which has the potential to interfere with research outcomes should be monitored in selection of laboratory NHPs (Balansard et al. 2019).

Tapeworm infections have significant human and veterinary disease implications as well as economic effects. If tapeworm larvae have migrated out of intestines and formed cysts in other tissues, they can eventually cause organ and tissue damage. The effect of this infection upon the hosts depends largely on the degree of parasitism, the organ involved and the existence of other concurrent infections (Urquhart et al. 1996). Presence of cysticerci in primates is not an uncommon finding. Pathologists who frequently necropsy laboratory monkeys are figure out that the incidences of cysticercosis is rare but probable (Kuntz & Myers 1967, Hobbs et al. 2003, Tsubota et al 2009). In NHPs usually there is very little

host inflammatory reaction to presence of viable cysts. However dead cysts could provoke an intense chronic inflammatory reaction (Myers 1972, Vickers & Penner 1968). In current study all the infected animals were part of a group of 65 vervet monkeys (7/65, 10.8%) import shipment from Tanzania. It is more likely that, the animals had been infected in wildlife of Tanzania by ingestion of contaminated foods with wild carnivore's feces. Because, the possibility of infection in the quarantine of the Razi institute is very low, since the monkeys were kept under nearly strict sanitary control. To the best of authors' knowledge, there are few reports of monkeys' infection with *Cysticercus tenuicollis*. In one report, Kunts and Myers (1967) reported three cases of *Cysticercus tenuicollis* in 3 of 240 imported vervet monkey from Kenya into United States and also between 2 of 57 Taiwan macaques examined for survey of parasites of vertebrate in Taiwan. The number of cysts, their size and morphological characteristic of rostellar hooks in present study, considerably were in agreement to the report of Kunts and Myers (1967). The extracting data from current study suggests

that in most instances *C. tenuicollis* has no considerable effects on infected vervet monkeys. However, well developed cysts could be organized in abdominal cavity causing peritoneal cysticercosis and its consequences like as anorexia, weight loss and lethargy (Corda et al. 2020). In fact, as the cysts enlarge there may be compression of adjacent tissues leading to deep tissue and organ injury and dysfunction which in turn may cause interference with research outcomes. So, it is better including monitoring these kind of infection, during quarantine period of imported monkeys. All infected animals in this study were females. This may be due to the greater susceptibility

of female monkeys to *C. tenuicollis* infection. More detailed studies are required to identify gender as a risk factor in *C. tenuicollis* infection of vervet monkeys.

Conclusion

Because of relatively high prevalence of *C. tenuicollis* infection (10.8%) between wild-caught imported vervet monkeys from Tanzania, it was suggested to set up more measures such as ultrasound imaging and serological tests on next import shipment of the vervet monkeys from potentially infected areas of Africa, to prevent entrance of infected animals to the post quarantine holding areas.

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Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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آلودگی سیستمی سرکوس تنیاکولیس در میمون‌های وروت (*Chlorocebus pygerythrus*) محبوس در قفس در ایران

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چکیده

در این مقاله یافته‌های کالبدگشایی عفونت سیستمی سرکوس تنیاکولیس در میان گروهی متشکل از ۶۵ سر میمون وروت صید شده از حیات وحش کشور تانزانیا که به منظور ارزیابی آزمون نوروویرولانس واکسن فلج اطفال خوراکی خریداری و به مؤسسه‌ی تحقیقات واکسن و سرم‌سازی رازی-ایران وارد شده بودند، توصیف گردیده است. روش‌های تشخیصی مورد استفاده در این مطالعه بر اساس مشاهده سیستمی سرکوس تنیاکولیس، خصوصیات مورفولوژیکی اسکولکس و همچنین شکل، اندازه و تعداد قلاب‌های رستلومی و بررسی هیستوپاتولوژیک بود. در مجموع ۷ میمون ماده (۱۰/۸ درصد) آلوده به سیستمی سرکوس تنیاکولیس تشخیص داده شدند. نتایج به دست آمده از این تحقیق نشان داد که در اکثر موارد عفونت‌های ناشی از سیستمی سرکوس تنیاکولیس در میمون‌های وروت به طور اتفاقی شناسایی گردیده و باعث ایجاد بیماری خفیفی در میزبان خود شده و یا بیماری ایجاد نمی‌نمایند. اما کیست‌های که در حفره‌ی شکمی به خوبی رشد کرده باشند، می‌توانند باعث ایجاد سیستمی سرکوز صفاقی بشوند. جهت جلوگیری از ورود حیوانات آلوده به مکان‌های نگهداری پس از دوران قرنطینه، پیشنهاد گردید که بر روی میمون‌های که در آینده به مؤسسه‌ی رازی وارد می‌گردند، اقدامات تشخیصی بیشتری همانند تصویربرداری اولتراسوند (سونوگرافی) و آزمایش‌های سرمی صورت پذیرد.

کلمات کلیدی: تنیا هیداتیدئوز، سیستمی سرکوس تنیا کولیس، میمون وروت

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