Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

CASE REPORT

Toxoplasmosis in the Eastern Grey Kangaroo, *Macropus giganteus* and the Cape Hyrax, *Procavis capensis* in Japan

Khaled Mohamed El-Dakhly[1,4], Nagwan El-Habashi[2], El-Shaymaa El-Nahass[3,4], Hiroki Sakai[4] and Tokuma Yanai[4,*]

[1]Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt; [2]Department of Veterinary Pathology, Faculty of Veterinary Medicine, Kafr-El-Sheikh University, Egypt; [3]Department of Veterinary Pathology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt; [4]Laboratory of Veterinary Pathology, Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193 Japan

*Corresponding author: yanai@gifu-u.ac.jp

ARTICLE HISTORY

Received: January 21, 2013
Revised: April 23, 2013
Accepted: May 05, 2013

Key words:
Hyraxes
Japan
Kangaroos
Toxoplasma gondii
Zoo animals

ABSTRACT

Toxoplasmosis was investigated in an eastern grey kangaroo, *Macropus giganteus*, and four cape hyraxes, *Procavia capensis*, in a Japanese zoo. Clinically, the kangaroo showed neurological signs, emaciation, diarrhea, elevated AST and CK, and subjected to coma before death. One young cape hyrax had severe anorexia, while the other three died without exhibiting clinical signs. Grossly, lungs of the kangaroo were dark red in color, while hyraxes, besides, showed hepatic multifocal white foci, and intestinal multifocal hemorrhages. Histologically, the kangaroo had frequent *Toxoplasma gondii* pseudocysts in brain, heart and skeletal muscles. All hyraxes had multifocal necrosis with cysts containing numerous bradyzoites in liver and spleen, along with necrotic gastroenteritis and intestinal hemorrhages. Immunohistochemically, cysts showed positive reaction to anti-*T. gondii* antibodies. These findings indicate possible outbreaks of toxoplasmosis in eastern grey kangaroos and cape hyraxes, zoo habitants; therefore, they could be susceptible intermediate hosts for *T. gondii* in terms of zoonosis. This is the first report of toxoplasmosis in eastern grey kangaroos and cape hyraxes in Japanese zoos.

To Cite This Article: El-Dakhly Kh M, N El-Habashi, E El-Nahass, H Sakai and T Yanai, 2013. Toxoplasmosis in the eastern grey kangaroo, *Macropus giganteus* and the cape hyrax, *Procavis capensis*, in Japan. Pak Vet J, 33(4): 529-531.

INTRODUCTION

Toxoplasmosis is an acute and frequently fatal zoonotic disease caused by *Toxoplasma gondii*, an obligate intracellular coccidian protozoan with a global distribution in a wide range of warm-blooded mammals, birds and reptiles (Lehrer *et al.,* 2010). Wallabies and marsupials are considered among the most susceptible species. Their vulnerability to toxoplasmosis is explained by increased stress factors and immunosuppression due to zoo conditions and/or their possibly infrequent contact with feline species (Kabak *et al.,* 2011). Some literatures reported at least five species of mammals/birds that were serologically positive to *T. gondii* in Japanese zoo animals (Murata, 1989).

Toxoplasmosis is considered to be an opportunistic infection, and spontaneous cases of fulminating fatal toxoplasmosis have been reported in immuno-compromised animals (Hermosilla *et al.,* 2010). It was often fatal for captive wilds, and accompanied by acute multifocal necrotizing lesions with *T. gondii* cysts and tachyzoites. Captivity act as stressor and, therefore, tend to increase the chance of reactivated infection (Parameswaran *et al.,* 2009).

Reports on various parasitic diseases of kangaroos and hyraxes in veterinary literatures are limited, and detailed investigations on protozoal infections in these wilds are uncommon (Stern, 2010). The purpose of this case report is to describe the macroscopic and microscopic features of an eastern grey kangaroo and four cape hyraxes found dead in a Japanese zoo as a result of toxoplasmosis.

History and clinical examination: From a zoo located in the western Japan, one male eastern grey kangaroo around one year in age, weighing 12 kg, was submitted

because of a previous history of neurological signs, reduced appetite and activity, anorexia, emaciation, arrhythmia, and diarrhea (on the second day). Hemogram indicated hemoglobin 27%, CK>3000 and elevated AST. Subsequently, the animal had a normal appetite but emaciation progressed, followed by mydriasis, coma and death within 24 days. The body weight at death was 8 kg.

The animal was submitted to the hospital suffering from anorexia, loss of/decreased movement and frequent daze states while in its barn. It was treated with the aim of improving nutritional status; therefore, fecal examination was done to detect parasitic eggs and/or oocysts. There was no evidence of parasites, but the animal, however, was administered vermicides as a protective measure. In addition, a mild degree of arrhythmia developed. Finally, the kangaroo subjected to a coma and died. The herd to which this eastern grey kangaroo had belonged was kept in yard surrounded by metal fences, feeding on vegetations, with access to water *ad libitum*.

The cape hyraxes were bred in separate breeding groups, and were fed vegetables and commercial diets for rodents, along with water mixed with vitamins and minerals. Annual health assessment was done, including detailed clinical examination, biometry, skin testing and routine laboratory tests (hemogram, serum chemistry and parasitological examinations). Hyraxes were not subjected to experimental procedures. Necropsy of the grey kangaroo and cape hyraxes was done immediately after death.

Histopathological and immunohistochemical techniques: For histopathology, tissues (brain, lungs, liver, and other organs) from all necropsied cases were fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin wax, sectioned at 5 µm and stained with hematoxylin and eosin (H&E) (Antoniassi *et al.*, 2011). Paraffin embedded sections of the liver (kangaroo) or the skeletal muscle and brain (cape hyraxes) were immunolabelled with *T. gondii* rabbit antiserum by the avidin-biotin-complex (ABC) immunoperoxidase method, as described previously (El-Nahass *et al*., 2012), using ABC kits (Vector Laboratories, Burlingame, CA, USA), a mouse-monoclonal (primary) antibody specific for *T. gondii* (1 in 128: Biogenesis Ltd., Poole, UK), and biotinylated anti-mouse immunoglobulin G (secondary) (DAKO Cytomation, Fort Collins, CO, USA). Visualization of the reaction was done by using 3,3'-diaminobenzidine (DAB) substrate chromogen system (DAKO Cytomation), and counterstained with hematoxylin.

Postmortem, histopathological and immune-histochemical findings: At necropsy (Fig. 1a), lungs of the grey kangaroo were dark red. The left salivary gland exhibited a cystic dilatation. Microscopically, the presence of *Toxoplasma* cysts in the brain (Fig. 1b), heart and tongue was predominant. There were frequent focal infiltrations of mononuclear cells, mainly lymphocytes, plasma cells and macrophages. Also, lungs exhibited varying degrees of interstitial pneumonia with hyperplastic epithelium of the bronchi. The spleen showed atrophy of lymphoid follicles with lymphoid depletion, and an increased thickness of the splenic capsule associated with hemosiderosis. Liver sinusoids were severely congested, with increased frequency of apoptosis. Immunohistochemically, *T. gondii* cysts could be clearly detected in the brain tissue and heart muscle (Fig. 1c, d).

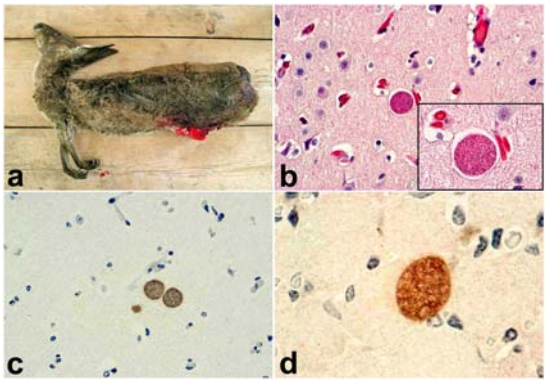

Fig. 1: a. An eastern grey kangaroo, *Macropus giganteus*, found dead as a result of toxoplasmosis. **b.** Brain tissue of infected kangaroo with distinct pseudocysts of *Toxoplasma gondii.* H&E (20x). Inset: Higher magnification. H&E (40x). **c.** Immunolabelling with *Toxoplasma gondii* antibody in the brain tissue. Avidin-Biotin Complex method, counterstained with Mayer's hematoxylin (20x). **d.** Immunolabelling with *Toxoplasma gondii* antibody in the heart muscle. Avidin-Biotin Complex method, counterstained with Mayer's hematoxylin (40x).

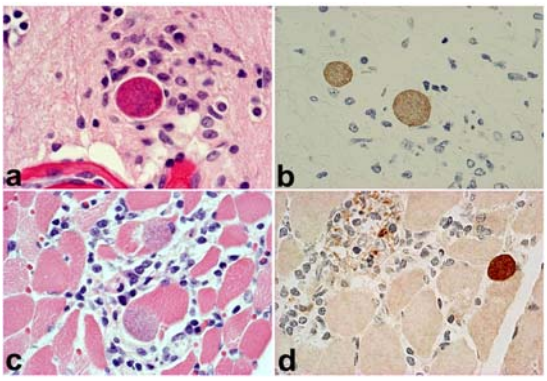

Fig. 2: Histopathology and immunohistochemistry of brain tissue and heart muscle of the cape hyraxes, *Procavis capensis*, infected with *Toxoplasma gondii* cysts. **a.** Brain tissue with mild inflammatory reactions. H&E (40x). **b.** Immunolabelling with *Toxoplasma gondii* antibody in the brain tissue. Avidin-Biotin Complex method, counterstained with Mayer's hematoxylin (40x). **c.** Heart muscle with *Toxoplasma gondii* cysts. H&E (40x). **d.** Positive reaction by immunolabelling in the heart muscle using *Toxoplasma* antibody. Avidin-Biotin Complex method, counterstained with Mayer's hematoxylin (40x).

The cape hyraxes (three adult males/females and a young male) showed similar abnormalities in the liver, spleen, small intestine and lungs. There were numerous white foci measuring 1 to 5 mm scattered on the hepatic surface and parenchyma. The entire jejunum and ileum had multifocal and coalescing zones of hemorrhages on the serosa and on the slightly thickened mucosa. The lungs were dark red. Using Giemsa stain for specimens from the liver and spleen, frequent pseudocysts and tachyzoites of *Toxoplasma gondii* were seen. Histopathologically, all four animals had similar lesions varied in severity. *Toxoplasma* cysts could be easily

detected in the brain tissue and heart muscle (Fig. 2a, c). The liver exhibited a severe degree of multifocal necrosis associated with *Toxoplasma gondii* pseudocysts containing few to many bradyzoites. Also, there was multifocal necrosis with various pseudocysts, marked eosinophilic infiltration and abundant macrophages in the alveolar wall with frequent tachyzoites in the alveolar space. The stomach showed severe necrotic gastritis, with large numbers of tachyzoites in the mucosa. The entire small intestine showed moderate to severe necrotic enteritis, and multifocal hemorrhages in the mucosa and submucosa. Immunohistochemically, pseudocysts were intensively positive in brain, heart (Fig. 2b & d), liver and spleen. Numerous organisms in the hepatic cytoplasm outside the necrotic foci were positively stained in the liver.

DISCUSSION

Toxoplasmosis is a considered as a zoo management problem due to dissemination of *T. gondii* oocyst in feces of wild cats, and to the presence of feral cats inside the zoos. The shedded oocysts can find their way into appropriate hosts. Macropods (wallabies and kangaroos) are known to be highly susceptible, with high mortality rates (Adkesson *et al.,* 2007). Infection occurs in many free-ranging and captive wild species. Moreover, toxoplasmosis is a common cause of death in captive and wild Australian marsupials (Canfield *et al.,* 1990). In the present study, histopathological lesions of the kangaroo were compatible with those of Australian marsupials and macropods. An epizootic of toxoplasmosis among the captive black-faced kangaroos (*Macropus fuliginosus melanops*) has been reported with 32% (8/25) had antibodies to *T. gondii*. Tachyzoites were found in several organs in one infant kangaroo, and numerous cysts were seen in skeletal muscles of the other infant. Adult kangaroos exhibited subclinical infections. Furthermore, the presence of *Toxoplasma* cysts antigen provided a strong evidence for the etiological role of the protozoan cysts as a cause of death. In immunocompetent animals, the infection is asymptomatic, as the protozoan evades from the immune system. In immunodeficient animals and animals under stress, toxoplasmosis may be fatal. Moreover, *Toxoplasma* is considered to be an opportunistic infection. Reduction of lymphoid follicles possibly indicates immune suppression, causing a fulminating toxoplasmosis and subsequently death of animals (Djurkovic-Djakovic, 1998).

As toxoplasmosis widely occurs in various animal species, serologically positive reactions have been detected in zoo animals worldwide (Minervino *et al.,* 2010). Since infected animals have opportunities to come in contact with visitors (directly or indirectly), it is greatly important that zoo staff should be sufficiently qualified for monitoring *T. gondii* infection in terms of zoonosis. Seroprevalence of toxoplasmosis revealed high infection levels in carnivores (Murata, 1989). Meanwhile, new world monkeys, wallabies and kangaroos are highly susceptible to infection which could be easily detected histologically using hematoxylin and eosin, or immunohistochemical stains (Antoniassi *et al.,* 2011).

In hyraxes, seropositivity for *Toxoplasma* was detected in African free-living rock hyraxes, and sporadic cases were reported in previous literatures (Olubayo and Karstad, 1981). In the present study, about half of the animals were lost due to toxoplasmosis in a herd, and all dead animals exhibited dissemination of the protozoan in various organs, including the gastrointestinal tract. Felids, including wild species, are considered the most probable source of infection and the only definite natural host through shedding oocysts in soil or water (Dubey, 2010). Therefore, *T. gondii* antigen has frequently been detected in cats caught at zoos.

In conclusion, the case described was diagnosed as toxoplasmosis in an eastern grey kangaroo, based on clinical signs, histopathological findings, and the detection of *Toxoplasma* antigen by immune-histochemistry. This study raises red flags regarding cape hyraxes and marsupials, including wallabies and kangaroos, as potential intermediate hosts for transmitting toxoplasmosis, thus, throw light on the role played by these hosts for eradication and control of toxoplasmosis in terms of hygiene. Improving the health of captive hyraxes and kangaroos must be offered by veterinarians, owners, and caretakers of such wild animals.

REFERENCES

Adkesson MJ, ME Gorman, V Hsiao, JK Whittington and JN Langan, 2007. *Toxoplasma gondii* inclusions in peripheral blood leukocytes of a red-necked wallaby (*Macropus refuogriseus*).Vet Clin Pathol, 36: 97-100.

Antoniassi NAB, FM Boabaid, RL Souza, L Nakazato, MFA Pimentel, JOX Filho, CA Pescador, D Driemeier and EM Colodel, 2011. Granulomatous meningoencephalitis due to *Toxoplasma gondii* in a black-headed night monkey (*Aotus nigriceps*). J Zoo Wildl Med, 42: 118-120.

Canfield PJ, WJ Hartley and JP Dubey, 1990. Lesions of toxoplasmosis in Australian marsupials. J Comp Pathol, 103: 159-167.

Djurkovic-Djakovic O, 1998. Toxoplasmosis and immunosuppression. Srp Arh Celok Lek, 126: 197-203.

Dubey JP, 2010. Toxoplasmosis of animals and humans. 2nd Ed. CRC Press.

El-Nahass E, N El-Habashi, AA Abdelaziz, M Nayel, S Kasem, H Fukushi, H Tuji, A Hirata, H Sakai and T Yanai, 2012. Kinetics and pathogenicity of oral infection by equine herpesvirs-9 in mice and suckling hamsters. J Comp Pathol, 146: 211-222.

Hermosilla C, N Pantchev, N Gies and A Taubert, 2010. Presumptive acute neural toxoplasmosis in a captive red-necked wallaby (*Macropus rufogriseus*). Res Vet Med Int, 2010: Article ID 561212, doi:10.4061/2010/561212.

Kabak YB, T Guvenc, O Kul, A Deniz and MY Gulbahar, 2011. Systemic toxoplasmosis in a kangaroo (*Macropus* sp.). Ankara Üniv Vet Fak Derg, 58: 209-212.

Lehrer EW, SL Fredebaugh, RL Schooley and NE. Mateus-Pinilla, 2010. Prevalence of antibodies to *Toxoplasma gondii* in woodchucks across an urban–rural gradient. J Wildl Dis, 46: 977-980.

Minervino AHH, HS Soares, RA Barrêto-Júnior, KAL Neves, HF Pena, EL Ortolani, JP Dubey and SM Gennari, 2010. Seroprevalence of *Toxoplasma gondii* antibodies in captive wild mammals and birds in Brazil. J Zoo Wildlife Med, 41: 572-574.

Murata K, 1989. A serological survey of *Toxoplasma gondii* in zoo animals and other animals. Jpn J Vet Sci, 51: 935-940.

Olubayo RO and LH Karstad, 1981. Fatal toxoplasmosis in a tree hyrax (*Dendrohyrax arboreus*). Bull Anim Health Prod Afr, 29: 262-264.

Parameswaran N, RM O'Handley, ME Grigg, SG Fenwick and RCA Thompson, 2009. Seroprevalence of *Toxoplasma gondii* in wild kangaroos using an ELISA. Parasitol Int, 58: 161-165.

Stern AW, 2010. A retrospective study of disease in captive kangaroos from Oklahoma State University, 1995-2009. J Vet Diagn Invest, 22: 324-326.