Fatal *Toxoplasma gondii* infection in the giant panda

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Abstract – *Toxoplasma gondii* can infect nearly all warm-blooded animals. We report an acute fatal *T. gondii* infection in the endangered giant panda (*Ailuropoda melanoleuca*) in a zoo in China, characterized by acute gastroenteritis and respiratory symptoms. *T. gondii* infection was confirmed by immunological and molecular methods. Multilocus nested PCR-RFLP revealed clonal type I at the *SAG1* and *c29-2* loci, clonal type II at the *SAG2*, *BTUB*, *GRA6*, *c22-8*, and *L358* loci, and clonal type III at the alternative *SAG2* and *SAG3* loci, thus, a potential new genotype of *T. gondii* in the giant panda. Other possible pathogens were not detected. To our knowledge, this is the first report of clinical toxoplasmosis in a giant panda.

Key words: *Toxoplasma gondii*, Giant panda, Genotype, PCR.

Introduction

Toxoplasmosis, caused by the obligate intracellular protozoan *Toxoplasma gondii*, is an important zoonosis worldwide. It is a major public health concern, mainly because of congenital disease, infection of immunocompromised patients, and an emerging severe form of acquired toxoplasmosis in immunocompetent patients [1]. The lifecycle of *T. gondii* includes sexual multiplication within cats and asexual multiplication within nearly all warm-blooded animals, including humans [6]. Humans and animals become infected by eating undercooked or raw meat containing cysts, or by ingesting food or water contaminated with sporulated oocysts.

The giant panda (*Ailuropoda melanoleuca*) is an emblematic endangered species and regarded as a national treasure and “living fossil” in China [16]. Its population is estimated at approximately 1600 in the wild, and the captive population is more than 300 [7, 13]. The health of giant pandas has attracted...
global attention. Here, we report an acute fatal *T. gondii* infection in the giant panda in China.

**Case presentation**

In February 2014, a seven-year-old giant panda named Jin Yi was found dead at Zhengzhou Zoo, Henan Province, China. The panda did not eat at noon on February 7. On February 8, the panda was found lying in the room with head buried in the abdomen. Treatment measures included intramuscular administration of cephalosporin and intravenous infusion of glucose. The animal had difficulty breathing overnight and was found dead in the morning of February 9.

A complete necropsy was conducted. Severe pathologic lesions were found, localized to the gastrointestinal tract and lungs. The gastrointestinal tract contained little or no ingesta, had multifocal mucosal hemorrhage, and dry, hard-packed digesta in the duodenum. Lungs were congested and chyme blocked the respiratory tract. Histologically, macrophages containing *T. gondii* tachyzoites were seen in the alveoli (Fig. 1). Other lesions included congestion in the intestinal lamina propria and submucosa, gastric epithelial necrosis, and sloughing.

Serum and tissue samples were collected for examination of potential pathogens that may cause hemorrhagic gastroenteritis. The animal had an antibody titer for *T. gondii* of 200 by the modified agglutination test [5]. *T. gondii* DNA was detected in the liver, spleen, lungs, kidneys, and small and large intestines by nested PCR targeting the B1 gene [12]. The immunofluorescence assay (IFA) revealed *T. gondii* tachyzoites present in the lung and small intestine tissues (Fig. 2), suggesting acute orally acquired toxoplasmosis in the giant panda, probably occurring 7–10 days before signs.

The positive DNA samples were directly typed by multilocus nested PCR-RFLP (Mn-PCR-RFLP) using 10 genetic markers (*SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico*), and the reference strains, including GT1, PTG, CTG, MAS, TgCgCa1, TgCatBr5, TgCatBr40, TgCatBr64, and TgRscCr1, were used as positive controls. The results revealed clonal type I at the *SAG1* and c29-2 loci, clonal type II at the *SAG2*, *BTUB*, *GRA6*, c22-8, and L358 loci, and clonal type III at the alternative *SAG2* and *SAG3* loci, showing a potential new atypical genotype of *T. gondii* in the giant panda. Other potential pathogens, including viruses and bacteria that cause acute gastroenteritis, or respiratory disease, were not detected. These results demonstrated that the giant panda died from acute toxoplasmosis due to a *T. gondii* strain of an atypical genotype.

**Discussion**

*Toxoplasma gondii* is considered to be one of the most successful eukaryotic pathogens, based on the number of host species and percentage of animals infected worldwide. The consequences of infection with *T. gondii* are associated with the host species and parasite genotypes. Primary infections in adults are mostly asymptomatic, but severe, acute, disseminated toxoplasmosis can occur in immunocompetent hosts when infected with some isolates [11]. Many *T. gondii* genotypes identified in animals and humans show high genetic diversity of *T. gondii* in China [10]. In addition to the atypical ToxoDB#9, there are several other atypical *T. gondii* genotypes identified in animals and humans in China 1 [2, 17, 14]. Atypical *T. gondii* strains have been shown to cause severe clinical disease in immunocompetent hosts [4, 9]. Despite its taxonomic classification as a carnivore, the giant panda has a diet that is primarily herbivorous, almost exclusively bamboo. The panda still retains decidely ursine teeth and will eat meat when available [15]. In addition to bamboo, the captive panda is given some formulated biscuits or other dietary supplements. There are a number of stray cats and small rodents in the zoo, and these animals can freely roam in the habitat of the giant panda. The infection may be obtained by consuming food or water contaminated with sporulated oocysts, or by ingestion of rodents infected with *T. gondii* [3]. Treatment
of the disease should include pyrimethamine plus sulfadiazine. Avoiding consumption of raw or undercooked meat is the main measure recommended to prevent *T. gondii* infection [8].

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**References**

1. Carme B, Demar M, Ajzenberg D, Dardé ML. 2009. Severe acquired toxoplasmosis caused by wild cycle of *Toxoplasma gondii*, French Guiana. Emerging Infectious Diseases, 15, 656–658.

2. Chen ZW, Gao JM, Huo XX, Wang L, Yu L, Halm-Lai F, Xu YH, Song WJ, Hide G, Shen JL, Lun ZR. 2011. Genotyping of *Toxoplasma gondii* isolates from cats in different geographic regions of China. Veterinary Parasitology, 183, 166–170.

3. Elmore SA, Jones JL, Conrad PA, Patton S, Lindsay DS, Dubey JP. 2010. *Toxoplasma gondii*: epidemiology, feline clinical aspects, and prevention. Trends in Parasitology, 26, 190–196.

4. Fernandez-Aguilar X, Ajzenberg D, Cabezon O, Martinez-Lopez A, Darwich L, Dubey JP, Almeria S. 2013. Fatal toxoplasmosis associated with an atypical *Toxoplasma gondii* strain in a Bennett’s wallaby (*Macropus rufogriseus*) in Spain. Veterinary Parasitology, 196, 523–527.

5. Liu Q, Wang ZD, Huang SY, Zhu XQ. 2015. Diagnosis of toxoplasmosis and typing of *Toxoplasma gondii*. Parasites & Vectors, 28, 292.

6. Liu Q, Singla LD, Zhou H. 2012. Vaccines against *Toxoplasma gondii*: status, challenges and future directions. Human Vaccines & Immunotherapeutics, 8, 1305–1308.

7. Liu X, He T, Zhong Z, Zhang H, Wang R, Dong H, Wang C, Li D, Deng J, Peng G, Zhang L. 2013. A new genotype of *Cryptosporidium* from giant panda (*Ailuropoda melanoleuca*) in China. Parasitology International, 62, 454–458.

8. Montoya JG, Liesenfeld O. 2004. Toxoplasmosis. Lancet, 363, 1965–1976.

9. Qian W, Wang H, Su C, Shan D, Cui X, Yang N, Lv C, Liu Q. 2012. Isolation and characterization of *Toxoplasma gondii* strains from stray cats revealed a single genotype in Beijing, China. Veterinary Parasitology, 187, 408–413.

10. Shwab EK, Zhu XQ, Majumdar D, Pena HF, Gennari SM, Dubey JP, Su C. 2014. Geographical patterns of *Toxoplasma gondii* genetic diversity revealed by multilocus PCR-RFLP genotyping. Parasitology, 141, 453–461.

11. Su C, Shwab EK, Zhou P, Zhu XQ, Dubey JP. 2010. Moving towards an integrated approach to molecular detection and identification of *Toxoplasma gondii*. Parasitology, 137, 1–11.

12. Sun H, Wang Y, Zhang Y, Ge W, Zhang F, He B, Li Z, Fan Q, Wang W, Tu C, Li J, Liu Q. 2013. Prevalence and genetic characterization of *Toxoplasma gondii* in bats in Myanmar. Applied and Environmental Microbiology, 79, 3526–3528.

13. Wang Y, Zhao PX. 2012. Habitat assessment of giant panda in Qingmucuang Nature Reserve, Shaanxi Province of Northwest China. Journal of Applied Ecology, 23, 206–212.

14. Zhang XX, Huang SY, Zhang YG, Zhang Y, Zhu XQ, Liu Q. 2014. First Report of genotyping of *Toxoplasma gondii* in free-living *Microtus fortis* in Northeastern China. Journal of Parasitology, 100, 692–694.

15. Zhao H, Yang JR, Xu H, Zhang J. 2010. Pseudogenization of the umami taste receptor gene Tas1r1 in the giant panda coincided with its dietary switch to bamboo. Molecular Biology and Evolution, 27, 2669–2673.

16. Zhao S, Zheng P, Dong S, Zhan X, Wu Q, Guo X, Hu Y, He W, Zhang S, Fan W, Zhu L, Li D, Zhang X, Chen Q, Zhang H, Zhang Z, Jin X, Zhang J, Yang H, Wang J, Wang J, Wei F. 2013. Whole-genome sequencing of giant pandas provides insights into demographic history and local adaptation. Nature Genetics, 45, 67–71.

17. Zhou P, Nie H, Zhang LX, Wang HY, Yin CC, Su C, Zhu XQ, Zhao JL. 2010. Genetic characterization of *Toxoplasma gondii* isolates from pigs in China. Journal of Parasitology, 96, 1027–1029.
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