Ante-Mortem Diagnosis, Diarrhea, Oocyst Shedding, Treatment, Isolation, and Genetic Typing of Toxoplasma gondii Associated with Clinical Toxoplasmosis in a Naturally Infected Cat

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ABSTRACT: Toxoplasma gondii infections are common in humans and other animals, but clinical disease is relatively rare. It is unknown whether the severity of toxoplasmosis in immunocompetent hosts is due to the parasite strain, host variability, or to other factors. Recently, attention has been focused on the genetic variability among T. gondii isolates from apparently healthy and sick hosts. Whether T. gondii genetic makeup plays a part in the pathogenesis of clinical feline toxoplasmosis is uncertain because little is known of genetic typing of strains associated with clinical feline toxoplasmosis. A 6-mo-old domestic male cat was hospitalized because of lethargy, anorexia, fever, and diarrhea. Numerous (6 million in 1 sample) T. gondii oocysts were found in feces of the cat and antibodies to T. gondii (titer 1:800) were found in its serum by the modified agglutination test. The cat was medicated orally with Cldamycin for 10 days; it became asymptomatic after 10 days and was discharged from the hospital. Viable T. gondii (designated TgCatUs9) was isolated from feces (oocysts) by bioassays in mice. Genetic typing using the DNA fragments length polymorphism (RFLP) markers revealed Type II allele at the L358 and Apico loci; therefore, this isolate belongs to the ToxoDB PCR-RFLP genotype no. 4, which is grouped into the Type 12 lineage that is dominant in wildlife from North America. To our knowledge, this is the first T. gondii isolate characterized genetically from a sick cat in the USA.

Cats are considered as the key host in the transmission of Toxoplasma gondii to humans and other animals because they are the only hosts that can excrete the environmentally resistant oocysts in their feces (Dubey, 2010). Humans become infected post-natally by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts, or by accidentally ingesting oocysts from the environment. However, only a small percentage of exposed adult humans or other animals develop clinical signs of disease. It is unknown whether the severity of toxoplasmosis in immunocompetent hosts is due to the parasite strain, host variability, or to other factors. Recently, attention has been focused on the genetic variability among T. gondii isolates from apparently healthy and sick hosts. Severe cases of toxoplasmosis that have been reported in immunocompetent patients are considered to be due to infection with atypical T. gondii genotypes (Ajzenberg et al., 2009; Elbez-Rubinstein et al., 2009; Grigg and Sundar, 2009; Demar et al., 2011; Pomares et al., 2011). Little is known of the association of genotype and clinical disease in animals.

Approximately 30% of cats have antibodies to T. gondii, but clinical disease is rarely reported (Dubey, 2010). The reason why some cats develop clinical toxoplasmosis is not fully understood, although the age of the cat, concurrent infections, and immunosuppression are known to be factors. Toxoplasmosis is most severe in neonatal cats; however, there is no evidence that the primary toxoplasmosis in aged cats is more severe than in younger mature cats (Dubey and Carpenter, 1993a). Of the 100 cats with clinical toxoplasmosis, only 10 were 10- to 16-yr-old (Dubey and Carpenter, 1993a). Concurrent infections were noted in 13 cats, but there was no case of overwhelming toxoplasmosis associated with concurrent infections (Dubey and Carpenter, 1993a).

DOI: 10.1645/GE-3257.1
Clindamycin hydrochloride, Antirobe® Drops, Pfizer Animal Health, 25 mg/ml at a dose of 25–50 mg/kg per day in divided doses (0.5 ml, 3 times a day). The kitten was also medicated for Capillaria sp. infection with fenbendazole (Panacur® granules), 50 mg/kg daily for 10 days (Intervet, Summit, New Jersey) for 10 days. The kitten became well and was discharged after 10 days of hospitalization. The cat was neutered when 1-yr-old and was not seen again.

Oocysts had been collected from feaces of the cat by sugar flotation (Dubey, 2010), sporulated in 2% sulfuric acid on a shaker for 1 wk, and then stored at 4°C for 4 mo. Six-million oocysts were counted in the sample using a hemacytometer. For definitive diagnosis, oocysts were bioassayed in Swiss Webster (SW) mice. For this procedure, the oocyst suspension was neutralized with NAOH and diluted 10-fold from 10^3 to 10^7 dilutions. Aliquots (0.5 ml) from each dilution were orally inoculated into 2 SW mice (Dubey, 2010). The mice were bled 5 wk later and a 1:25 dilution of their sera was tested for T. gondii antibodies by MAT. Impression smears of mesenteric lymph nodes or lungs were examined for T. gondii tachyzoites. Survivors were bled 2 mo later and their brains were examined for tissue cysts as described (Dubey, 2010).

For genotyping, the DNA extracted from the brains of mice was genotyped using 10 PCR-restriction fragment length polymorphism (RFLP) markers, SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico (Su et al., 2010). The mice fed undiluted and a 10^1 dilution of oocysts died of acute toxoplasmosis 9 days later and tachyzoites were found in smears of their lymph nodes. Tissue cysts and antibodies to T. gondii were found in mice fed the 10^2 dilution and in 1 of 2 mice fed the 10^3 dilution. Thus, there were 1,000 infectious oocysts in the inoculum used for bioassay. Toxoplasma gondii from mouse brain was cryopreserved in liquid nitrogen. Five years later, the strain was revived in mice. For this, 2 SW mice were inoculated subcutaneously with the contents of a cryopreserved vial; both mice remained asymptomatic but became infected, and tissue cysts were found in their brains 3 mo post-inoculation.

Genotyping revealed that the strain from the present cat (designated TgCatUs9) had Type II alleles at the SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, and PK1 loci and Type I alleles at the L358 and Apico sites. This isolate belongs to the ToxoDB RCR-RFLP genotype no. 4, which is grouped into the Type 12 lineage that is dominant in wildlife from North America (Rajendran et al., 2012) and was previously recoded from a feral cat in Illinois (Dubey et al., 2011).

The present case was diagnosed as toxoplasmosis based on clinical signs, seropositivity to T. gondii, and isolation of viable T. gondii oocysts. The present case is also unusual because the diagnosis was made ante-mortem and the cat was treated with anti-T. gondii medicine. Clindamycin, Clindamycin hydrochloride (25–50 mg/kg, orally or intramuscularly, every 8 hr) has been used to treat cats experimentally (Dubey and Yeary, 1977) or naturally infected cats (Dubey and Lappin, 2006). Clindamycin administration can depress or stop oocyst shedding in cats (Dubey and Yeary, 1977).

Cats can shed very large numbers of T. gondii oocysts in a matter of few days (Dubey and Frenkel, 1972; Dubey, 2010) without any clinical signs. At any given time, only 1% of cats have been found shedding oocysts and, as indicated earlier, there are no previously documented cases of diarrheaa in cats during the time of oocyst shedding. Perhaps the greatest concern is the management of cats that might be treated to prevent shedding oocysts in veterinary clinics. In hospitalized situations, cats are usually housed in cages with newspapers as bedding. Toxoplasma gondii oocysts are shed sporulated (not infective) in feces and it takes about a day for sporulation and infectivity to be attained. Therefore, feces, including bedding, should be removed daily to prevent sporulation. Cleaning and disinfection of infected cat cages is also problematic because none of the commonly employed disinfectants kill T. gondii oocysts. However, T. gondii oocysts are killed by exposure to temperatures higher than 70°C. Although steaming the infected cage is an option, there is also the danger of aerosolization of fecal matter and oocysts and of spreading infection on the floor. Use of hot air (hair dryer or flame) is one of the options to kill oocysts in the infected cage. Pouring boiling water in the infected cage is another option. During these cleaning operations, the operator should wear gloves, goggles, and protective clothing. The persons attending infected sick cats, and the owners of the cat, should be informed of the consequences of T. gondii infection so that they can seek medical advice.

We would like to thank Dr. Chunlei Su, University of Tennessee, Knoxville, Tennessee, for advice regarding genotyping.

LITERATURE CITED


