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Isolation and genetic characterization of *Toxoplasma gondii* from mute swan (*Cygnus olor*) from the USA

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ABSTRACT

Little is known of the genetic diversity of *Toxoplasma gondii* circulating in wildlife. In the present study, antibodies to *T. gondii* were determined in serum samples from 632 mute swans (*Cygnus olor*) collected from different areas of the USA. Sera were tested by *T. gondii* modified agglutination test; 54 (8.5%) of 632 samples were seropositive with titers of 25 in 28 sera, 50 in 22 sera, 100 in three samples, and 200 or higher in one swan. Hearts from 14 seropositive swans were bioassayed in mice and viable *T. gondii* (designated TgSwanUs1–3) were isolated from the hearts of three. These three *T. gondii* isolates were further propagated in cell culture, and DNA isolated from culture-derived tachyzoites was characterized using 11 PCR-RFLP markers (SAG1, 5′- and 3′-SAG2, alt.SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and Apico). Results of genotyping revealed that two strains (TgSwanUs1, TgSwanUs2) were Type III (ToxoDB PCR-RFLP genotype #2), and TgSwanUs3 was a new genotype designated here as ToxoDB PCR-RFLP genotype #216. Pathogenicity of oocysts derived from these three strains was determined in Swiss Webster (SW) outbred mice. All mice infected with oocysts and tachyzoites of the atypical isolate (TgSwanUs3) died of acute toxoplasmosis, irrespective of the dose. Oocysts of the remaining two isolates were less pathogenic but differed from each other; 10 oocysts of the TgSwanUs1 killed all inoculated mice whereas 1 million oocysts of the TgSwanUs2 were needed to kill all infected SW mice. Isolation of *T. gondii* from mute swan indicates that the local waters were contaminated by *T. gondii* oocysts, and that mouse *T. gondii* virulent strains are circulating in wildlife. Mute swan is a new host record for *T. gondii*.

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1. Introduction

The protozoan parasite *Toxoplasma gondii* infects virtually all warm-blooded animals, including birds, humans, livestock, and marine mammals (Dubey, 2010). In the USA, various surveys have found that 10–50% of the adult human population has been exposed to this parasite (reviewed in Dubey and Jones, 2008). Humans become infected post-natally by ingesting tissue cysts from undercooked meat, or by consuming food or drink contaminated with oocysts. 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

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Table 1
Occurence of *T. gondii* in mute swan in the USA.

State	Number of swans			
	Total tested	% seropositive	Bioassayed	Bioassay positive
Indiana	7	1	1	0
Michigan	291	12	9	2
New Jersey	187	15	2	0
New York	11	1	0	0
Rhode Island	127	25	2	1
Wisconsin	9	0	0	0
Total	632	54 (8.5%)	14	3

variability, or to other factors. Recently, attention has been focused on the genetic variability among *T. gondii* isolates from apparently healthy and sick hosts (Grigg and Sundar, 2009). Severe cases of toxoplasmosis have been reported in immunocompetent patients in association with atypical *T. gondii* genotypes in certain countries (Ajzenberg et al., 2004; Demar et al., 2007; Elbez-Rubinstein et al., 2009; Grigg and Sundar, 2009; Vaudaux et al., 2010). A variant of Type II (NE-II) was recently found associated with prematurity and severe disease at birth in congenitally infected children in the USA (McLeod et al., 2012). Little is known of the association of genotype and clinical disease in animals (Dubey, 2010). Type II strains are the most prevalent in Europe and the USA (Su et al., 2012).

Historically, *T. gondii* was considered to be clonal with low genetic diversity and grouped into three types I, II, III (Dardé et al., 1992; Howe and Sibley, 1995; Ajzenberg et al., 2002a,b, 2004; Lehmann et al., 2006; Aubert et al., 2010). However, recent studies have revealed a greater genetic diversity of *T. gondii*, particularly isolates from domestic animals in Brazil and wildlife in the USA (Dubey et al., 2010, 2011a,b; Khan et al., 2011; Dubey et al., 2012; Su et al., 2012). Most intriguing are findings that some genotypes such as Type 12 predominantly found in wildlife in the USA (Dubey et al., 2011a,b; Khan et al., 2011). Though Type 12 has also been identified from pigs and sheep in the USA, the frequency is low, and the dominant genotype in these domestic animals is the Type II (Dubey et al., 2008; Velmurugan et al., 2009). Also, it is not clear how specific genotypes become established in a particular host, because all strains are transmitted by oocysts shed by felids or by ingestion of infected tissues and studies involving *T. gondii* in wildlife are time consuming, expensive, and difficult. Additionally, permission is needed to collect tissues from certain wildlife, including swans. In the present study we had an opportunity to genetically characterize three isolates of *T. gondii* from mute swan (*Cygnus olor*).

2. Materials and methods

2.1. Naturally infected swans

Mute swans (*C. olor*) are native to Eurasia, and were introduced from Europe into the United States in the late 19th and early 20th centuries for use in ornamental ponds and lakes, zoos, and aviculture collections (Maryland Mute Swan Task Force, 2001; Ciaranca et al., 1997). Feral breeding is believed to have first started among escaped birds in the lower Hudson Valley in 1910 and on Long Island in 1912 (Atlantic Flyway Council, 2003). Since that time mute swans have expanded their range to many Eastern states several Midwestern states and portions of the western USA and Canada. Mute swans are a non-native species in North America that can have adverse impacts on aquatic habitats and compete with native waterfowl for food and resources and can damage property and agriculture (Atlantic Flyway Council, 2003; MDNR 2002, 2012, Craves and Susko, 2010). Mute swans are also a hazard to human health and safety because of aggressive behavior by territorial or food-habituated birds, and fecal contamination of water sources and areas with high recreational use. Because of these reasons, the United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services program (WS) manages mute swan populations in several states in the Great Lakes Region and the Atlantic Coast.

From March 2011 through September 2012, 632 mute swans were removed by Wildlife Services program from the Great Lakes region and in other northeastern states for damage management purposes. The swans were shot legally by USDA personal trained in the safe use of firearms. Samples were opportunistically collected from the mute swans post-mortem and submitted for various disease testing including *T. gondii*. Blood was collected from a jugular vein by making a small cut in the jugular vein and then lowering the head below the body to allow the blood to flow into a blood collection tube. The tube was identified with a barcode number unique to each swan and then the tube was placed in a cooler with ice packs. The blood was allowed to clot for at least 4 h and then centrifuged at 1500 rpm for 15 min. The serum was separated using a disposable pipette and stored refrigerated until shipping.

The entire heart was collected by making a 10–12 cm cut in the abdomen directly below the sternum and subsequently pulling the heart back out the cavity. The heart was placed in a Ziploc bag and assigned the same barcode number as the serum sample. It was then placed in a cooler with ice packs and stored in a refrigerator until shipping to the testing laboratory. Collection site-specific data including GPS coordinates, county, state (Table 1), and date were recorded for each site and a unique barcode number for each swan on a standardized datasheet. Samples were shipped within 3 days of collection to the Animal Parasitic Diseases Laboratory, United States Department of Agriculture in Beltsville, Maryland for *T. gondii* examination.

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