RESEARCH NOTES

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Isolation of *Toxoplasma gondii* From the Keel-Billed Toucan (*Ramphastos sulfuratus*) From Costa Rica

J. P. Dubey, G. V. Velmurugan, J. A. Morales*, R. Arguedas†, and C. Su‡, United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Animal Parasitic Diseases Laboratory, Building 1001, Beltsville, Maryland 20705-2350; *Departamento de Patología, Escuela Medicina Veterinaria, Universidad Nacional Autonoma 3000 Heredia, Costa Rica; †Zoológico Nacional Simón Bolivar, San José Costa Rica; ‡Department of Microbiology, University of Tennessee, Knoxville, Tennessee 37996-0845.
e-mail: jitender.dubey@ars.usda.gov

ABSTRACT: Pectoral muscles from a captive keel-billed toucan (*Ramphastos sulfuratus*) from Costa Rica were fed to a *Toxoplasma gondii*–free cat, and the cat shed oocysts. Laboratory mice fed these oocysts developed antibodies to *T. gondii* in their sera and *T. gondii* tissue cysts in their brains. The DNA extracted from the brains of infected mice was characterized using 10 polymerase chain reaction–restricted fragment length polymorphic markers (SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico). The isolate designated TgRsCr1 was found to be non-clonal with Type I, II, and III alleles at different loci. This is the first isolation of *T. gondii* from this host.

Toxoplasma gondii infections are common in many species of warm-blooded animals, including humans and birds worldwide (Dubey and Beattie, 1988; Tenter et al., 2000). Historically *T. gondii*–like organisms were first found in birds, before its discovery in 1908 in mammals, but these were probably hemoprotozoans (reviewed in Dubey, 2002, 2007). In the present report we isolated a *T. gondii* strain from a keel-billed toucan (*Ramphastos sulfuratus*) from Costa Rica for the first time.

A keel-billed toucan from the Costa Rican National Zoo died on 10 July 2007. A necropsy was performed on the day of death. Unfixed muscles tissues were sent by air to the Animal Parasitic Diseases Laboratory (APDL), United States Department of Agriculture, Beltsville, Maryland, for characterization of a species of *Sarcocystis* (Dubey et al., 2008).

Unfixed muscles heavily infected with sarcocysts were fed to a 3-mo-old laboratory-raised cat (Dubey, 1995). Feces of the cat were examined microscopically by fecal flotation daily for coccidian oocysts. The cat shed *T. gondii*–like oocysts 4 days later. After sporulation in 2% sulfuric acid at room temperature for 1 wk, sporulated oocysts were washed in water by centrifugation and fed to 2 Swiss Webster (SW) mice (Dubey et al., 2002). The mice fed oocysts became comatose 4 days later and were killed. Protozoan tachyzoites were found in smears of their mesenteric lymph nodes, and tachyzoites were cryopreserved in liquid nitrogen and inoculated into new SW mice. The inoculated mice remained asymptomatic. Antibodies to *T. gondii* were detected in a 1:25 dilution of sera of the mice 3 mo post-inoculation (PI) by the modified agglutination test (MAT) (Dubey and Desmonts, 1987), and tissue cysts were found in the brains of both mice killed 129 days PI. The isolate was designated TgRsCr1. Serial 10-fold dilutions of oocysts were fed to groups of 4 mice, which were then observed for 2 mo PI. Mice fed 1,000, 10,000, or 100,000 died of acute toxoplasmosis between 8 and 11 days PI, and tachyzoites were found in their mesenteric lymph nodes. Mice fed fewer oocysts became infected, and tissue cysts were found in their brains 2 mo PI.

Toxoplasma gondii DNA was extracted from tissues of positive mice using DNeasy kit (Qiagen, Valencia, California) and genotyped using the genetic markers SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico (Dubey, Paticucci et al., 2006; Su et al., 2006). The TgRsCr1 had a Type I allele with 5 markers, Type III with 3 markers, Type II with new SAG2, and unusual alleles at 2 markers (Table I). The genotype of TgRsCr1 is different from 32 chicken isolates from Costa Rica reported previously (Dubey, Su et al., 2006) but is identical to 2 of 44 Nicaragua chicken isolates (TgCkNi12 and TgCkNi32) at all 10 loci (Dubey, Sundar et al., 2006; data not shown). This result indicates that TgRsCr1 may be present in other countries in Central America.

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TABLE I. Genetic characterization of the *Toxoplasma gondii* isolate from the toucan.

Isolate source	SAG1	5′ + 3′ SAG2*	New SAG2†	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	Apico	
Reference	I	I	I	I	I	I	I	I	I	I	I	RH88
Reference	II or III	II	II	II	II	II	II	II	II	II	II	PTG
Reference	II or III	III	III	III	III	III	III	III	III	III	III	CTG
Reference	I	II	II	III	II	II	II	u-1	I	u-2§	I	TgCgCa1
Reference	u-1‡	I	II	III	III	III	u-1	I	I	III	I	MAS
Reference	I	III	III	III	III	III	I	I	I	u-1	I	TgCatBr5
Toucan	u-1	I	II	III	I	III	u-2	I	I	III	I	TgRsCr1

* SAG2 marker based on 5′- and 3′-end DNA sequence polymorphisms of SAG2 gene (Howe et al., 1997).
† The SAG2 marker developed recently based on 5′-end DNA sequence of SAG2 gene is able to identify additional alleles often seen in atypical *T. gondii* strains (Su et al., 2006).
‡ Unique 1, different from Type I, II, III.
§ Unique 2, different from Type I, II, III.

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