Table I. Comparisons of SVL, mean intensity (MI), and time until death of juvenile American toads, *Bufo americanus*, and juvenile wood frogs, *Rana sylvatica*, infected with maggots of *Bufolucilia* species from southeastern Wisconsin.

<table>
<thead>
<tr>
<th>Variable</th>
<th><em>B. americanus</em> (n = 9*)</th>
<th><em>R. sylvatica</em> (n = 3)</th>
<th>Statistics</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVL (cm ± 1 SD)</td>
<td>4.1 ± 0.23</td>
<td>2.6 ± 0.28</td>
<td></td>
<td>8.78</td>
<td>0.0001</td>
</tr>
<tr>
<td>MI (±1 SD)</td>
<td>10.5 ± 7.2</td>
<td>24 ± 9</td>
<td></td>
<td>2.86</td>
<td>0.017</td>
</tr>
<tr>
<td>Time until death (hr ± 1 SD)</td>
<td>114.33 ± 100.84</td>
<td>26 ± 20</td>
<td></td>
<td>2.48</td>
<td>0.035</td>
</tr>
</tbody>
</table>

* Data from Bolek and Coggins (2002).

LITERATURE CITED


Biologic and Molecular Characteristics of *Toxoplasma gondii* Isolates From Striped Skunk (*Mephitis mephitis*), Canada Goose (*Branta canadensis*), Black-Winged Lory (*Eos cyanogenia*), and Cats (*Felis catus*)

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ABSTRACT: *Toxoplasma gondii* isolates can be grouped into 3 genetic lineages. Type I isolates are considered virulent to outbred mice, whereas Type II and III isolates are not. In the present report, viable *T. gondii* was isolated for the first time from striped skunk (*Mephitis mephitis*), Canada goose (*Branta canadensis*), and black-winged lory (*Eos cyanogenia*). For the isolation of *T. gondii*, tissues were bioassayed in mice, and genotyping was based on the SAG2 locus. *Toxoplasma gondii* was isolated from 3 of 6 skunks, 1 of 4 Canada geese, and 2 of 2 feral cats (*Felis catus*) from Mississippi. All donor animals were asymptomatic. Viable *T. gondii* was also isolated from 5 of 5 lories that had died of acute toxoplasmosis in an aviary in South Carolina. Genotypes of *T. gondii* isolates were Type III (all skunks, lories, and the goose) and Type II (both cats). All 5 Type III isolates from birds and 2 of the 3 isolates from skunks were mouse virulent.

*Toxoplasma gondii* infections are widely prevalent in human beings and animals worldwide (Dubey and Beattie, 1988; Tenter et al., 2000). Postnatally, humans become infected by ingesting tissue cysts from undercooked meat or consuming food or drink contaminated with oocysts. However, only a small percentage of exposed adult humans develop clinical signs after exposure. It is not known whether the severity of
The isolation of Toxoplasma gondii from animals

<table>
<thead>
<tr>
<th>Host Location</th>
<th>MAT titer</th>
<th>Tissue for bioassay</th>
<th>No died (day of death)</th>
<th>No positive for T. gondii*</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skunk 1</td>
<td>Mississippi</td>
<td>ND†</td>
<td>Brain</td>
<td>0</td>
<td>III</td>
</tr>
<tr>
<td>Skunk 2</td>
<td>Mississippi</td>
<td>ND</td>
<td>Brain</td>
<td>5 (14±16)</td>
<td>5</td>
</tr>
<tr>
<td>Skunk 3</td>
<td>Mississippi</td>
<td>ND</td>
<td>Brain</td>
<td>4 (17±1, 17, 18, 20)</td>
<td>5</td>
</tr>
<tr>
<td>Goose</td>
<td>Mississippi</td>
<td>ND</td>
<td>Brain</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Cat 1</td>
<td>Mississippi</td>
<td>≥1:80</td>
<td>Brain</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Cat 2</td>
<td>Mississippi</td>
<td>ND</td>
<td>Brain</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Lory 1</td>
<td>South Carolina</td>
<td>1:40</td>
<td>Muscle</td>
<td>4 (2§, 2§, 24±, 40)</td>
<td>3</td>
</tr>
<tr>
<td>Lory 2</td>
<td>South Carolina</td>
<td>1:80</td>
<td>Muscle</td>
<td>5 (2§, 20±, 26, 27)</td>
<td>4</td>
</tr>
<tr>
<td>Lory 3</td>
<td>South Carolina</td>
<td>&lt;1:25</td>
<td>Muscle</td>
<td>5 (1, 14, 17, 17)</td>
<td>4</td>
</tr>
<tr>
<td>Lory 4</td>
<td>South Carolina</td>
<td>1:800</td>
<td>Liver, lung</td>
<td>5 (2*, 2*, 5*, 21±, 30)</td>
<td>2</td>
</tr>
<tr>
<td>Lory 5</td>
<td>South Carolina</td>
<td>1:40</td>
<td>Liver, lung</td>
<td>4 (18±, 21, 28)</td>
<td>4</td>
</tr>
</tbody>
</table>

* Of 5 mice inoculated.
† ND, no data.
§ Mice used for genotyping.
‡ Died of bacterial infection.

Toxoplasmosis in animals is due to the parasite strain, host variability, or other factors.

Overall, there is low genetic diversity among T. gondii isolates so far examined. Toxoplasma gondii isolates have been classified in 3 genetic Types (I, II, and III) based on restriction fragment length polymorphism (Howe and Sibley, 1995; Howe et al., 1997; Grigg, Bonnefoy et al., 2001). It has been suggested that isolates of Type I and II are more likely to result in clinical toxoplasmosis in humans, but genetic characterization has been limited essentially to isolates from patients ill with clinical toxoplasmosis (Howe et al., 1997; Fuentes et al., 2001; Grigg, Gantar et al., 2001; Aspinall et al., 2003). The prevalence of T. gondii types in asymptomatic humans is unknown. In 1 study, all 86 isolates of T. gondii obtained from women (or their fetuses), who acquired infection during pregnancy, were genotyped: 73 were Type II, 2 were Type III, 4 were atypical, and 7 were Type I (Ajzenberg et al., 2002). Most reported isolates of T. gondii from domestic animals from the United States and Europe were Type II or Type III (Howe and Sibley, 1995; Mondragon et al., 1998; Owen and Trees, 1999; Dubey, Gamble et al., 2002; Jungersen et al., 2002; Lehmann et al., 2003), irrespective of clinical status. Recently, 70% of isolates of T. gondii obtained from asymptomatic free-range chickens from Brazil were Type I (Dubey, Graham et al., 2002; Dubey, Graham, Silva et al., 2003; Dubey, Navarro et al., 2003).

Little is known of the prevalence and distribution of genotypes of T. gondii in wildlife species. Recently, we reported genotypes of T. gondii isolates from white-tailed deer, raccoons, coyotes, black bears, and foxes (Dubey et al., 2004). In this study, we describe the genetic and biologic characteristics of isolates of T. gondii from striped skunks (Mephitis mephitis), a Canada goose (Branta canadensis), and black-winged lories (Eos cyanogenia). This is the first report of isolation of T. gondii from skunks, geese, and lories.

The 6 skunks and the 2 feral cats (Table I) were obtained from northeastern Mississippi in July 2003. They were trapped around human dwellings in Starkville, Mississippi, by the United States Department of Agriculture Wildlife Service at the request of owners. The 4 Canada geese were killed by hunters in September 2003. They were from the Tennessee–Tombigbee Waterway, in the vicinity of Columbus, Mississippi.

Tissues from 5 lories from an aviary in South Carolina were obtained in 2 batches in October 2001 (Table I). The birds had died of acute toxoplasmosis (P. G. Parnell, unpubl.). Tissue fluids were obtained from these birds for serology because serum was not available. In the first batch, livers from 3 lories (nos. 1–3) were sent from South Carolina to Maryland for confirmation of diagnosis. When it was realized that the livers had been frozen for 6 days at −20 C, the eviscerated carcasses of the animals were retrieved from the cold storage and muscle tissue was removed for parasite isolation. Fluid for serology assays was obtained by compression and partial maceration of liver tissue. In the second batch, livers and lung tissue from 2 lories were obtained; fluid was taken from lung tissue for serology.

Serum samples were tested for antibodies to T. gondii with the modified agglutination test (MAT) as described (Dubey and Desmons, 1987). Sera were diluted 2-fold starting at 1:25 or 1:5 dilutions.

Squash preparations were made from the brains of the skunks and cats and examined microscopically for the presence of tissue cysts.Brains from cats, skunks, and geese were bioassayed in outbred female Swiss Webster mice obtained from Taconic Farms, Germantown, New York, as reported previously (Dubey, Graham et al., 2002). Each brain was homogenized, digested in acidic pepsin, washed, and the homogenate inoculated subcutaneously into 5 mice (Dubey, 1998). Avian tissue fluids were homogenized in 0.85% NaCl aqueous solution (saline), and the homogenates were inoculated into mice without digestion in pepsin.

Tissue imprints of mice that died were examined for T. gondii tachyzoites or tissue cysts. Survivors were bled 5 wk postinoculation (PI), and a 1:25 dilution of serum from each mouse was tested for antibodies with the MAT. Mice were killed 62 days PI, and their brains and a 1:25 dilution of serum from each mouse was tested for antibodies with the MAT. Mice were killed 62 days PI, and their brains were examined for tissue cysts as described (Dubey and Beattie, 1988). Mice were considered infected with T. gondii when tachyzoites or tissue cysts were found in murine tissues.

Samples of lung tissue from dead mice as well as from those killed were frozen at −70 C for DNA characterization as described (Lehmann et al., 2000). Polymerase chain reaction–restriction fragment length polymorphism genotypes of the SAG2 locus were used to genetically characterize the isolates (Howe et al., 1997).

Toxoplasma gondii was isolated from the brains of 3 of 6 skunks (Table I). Mice inoculated from the brains of 2 skunks died from acute toxoplasmosis with demonstrable tachyzoites. These isolates of T. gondii also killed subinoculated mice. Tissue cysts of T. gondii were found by direct microscopic examination of brain smears from skunk no. 3 as described (Sreekumar et al., 2003) and inoculated subcutaneously into 3 mice, 1 tissue cyst per mouse. The mice inoculated with individual tissue cysts died from acute toxoplasmosis 13 days PI with numerous demonstrable tachyzoites in lungs. All isolates of T. gondii from skunks, including clones were Type III.

Antibodies to T. gondii were found in 1 feral cat examined, and T. gondii was isolated from the brains of both cats bioassayed; both isolates were Type II (Table I). Toxoplasma gondii was isolated from the brain of 1 of the 4 geese sampled, and this isolate was Type III.

Viable T. gondii was isolated from the tissues of 5 of 5 lories; all 5 isolates were Type III and were virulent for mice (Table I). A few mice inoculated with these avian tissues died 1–5 days PI because of bacterial infection.
infections and were discarded (Table I). Numerous *T. gondii* tachyzoites or tissue cysts were found in tissues of dead birds. No viable *T. gondii* was isolated from frozen livers, confirming earlier findings that freezing kills *T. gondii* (Dubey and Beattie, 1988). Antibodies to *T. gondii* were found in fluids obtained from avian tissues (Table I).

Isolation of viable *T. gondii* in the present study from the skunk, black-winged lory, and goose is a new host record. Walton and Walls (1964) found *T. gondii* antibodies in 1 of the 5 skunks from Georgia but were unable to isolate the parasite from the brains of 4 skunks bioassayed in mice. Ditters and Nielsen (1978) histologically diagnosed toxoplasmosis in a skunk that was immunosuppressed due to concurrent distemper virus infection. Dubey, Hamir et al. (2002) found a *T. gondii*-like tissue cyst in tongue of 1 of 4 skunks. However, the diagnosis could not be confirmed in either of these 2 previous reports.

Among the avian hosts of *T. gondii*, passeriform birds are most susceptible for clinical toxoplasmosis (Dubey, 2002). There are a few reports of clinical toxoplasmosis in birds of the Psittaciformes. These species are susceptible for clinical toxoplasmosis (Dubey, 2002). There are a few reports of toxoplasmosis in birds of the Psittaciformes. These reports include toxoplasmosis in 2 budgerigars (*Melopsittacus undulatus*) from Switzerland (Galí-Valerio, 1939). 1 Swanson's Lorikeet (*Trichoglossus moluccanus*) from the Netherlands (Poema and Zwart, 1972), 1 regent parrot (*Polytelis anthopeplus*), 1 superb parrot (*P. swainsonii*), and 1 crimson rosella (*Platycercus elegans*) from Australia (Hartley and Dubey, 1991). There is 1 report of clinical toxoplasmosis in a red lory (*Eos bornea*) from the United States (Howert et al., 1991). A *T. gondii*-like parasite was found histologically in sections of tissues of this bird. Serum and unfixed tissues were not available for confirmation in any of the reports in Psittaciformes.

Both antibodies to *T. gondii* and viable organisms were found in black-winged lories. A few of the mice died of bacterial infection 1–5 days PI with avian tissues. The tissues had been stored in a cooler for 7 days before bioassays were performed. The lories had died of *T. gondii*-associated hepatitis and pneumonia, and therefore muscle tissue was not collected initially for *T. gondii* examination.

Before molecular genotyping (Howe and Sibley, 1995), *T. gondii* isolates were grouped as mouse virulent or mouse avirulent based on infectivity in outbred mice. However, mouse virulence has no correlation with disease in humans or domestic animals (Dubey and Beattie, 1988). For example, the strains of *T. gondii* isolated from tissues of aborted ovine fetuses were not mouse virulent. Initial studies by Howe et al. (1997) indicated that all Type I strains of *T. gondii* were lethal for mice, irrespective of the dose, whereas Types II and III were relatively avirulent for mice. However, data are now accumulating that indicate that based on the SAG2 locus the phenotypic and genetic characteristics of 1 regent parrot (*P. anthopeplus*), 1 superb parrot (*P. swainsonii*), and 1 crimson rosella (*P. choglossus moluccanus*) found in fluids obtained from avian tissues (Table I).

LITERATURE CITED


Molecular and Biological Characterization of Hammondia heydorni–Like Oocysts From a Dog Fed Hearts From Naturally Infected White-Tailed Deer (Odocoileus virginianus)

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ABSTRACT: Neospora caninum and Hammondia heydorni are morphologically and phylogenetically related coccidians that are found in dogs. Although there is serological evidence of N. caninum infection in the white-tailed deer (Odocoileus virginianus), the parasite has not been yet isolated from the tissues of this host. In an attempt to isolate N. caninum from deer, studies from 4 deer with antibodies to N. caninum were fed to 2 dogs. One of these dogs shed unsporulated oocysts 12–14 μm in diameter. Sporulated oocysts were not infective to Mongolian gerbils (Meriones unguatus), and DNA isolated from these oocysts was not amplified using N. caninum–specific primers. However, positive amplification with the H. heydorni–specific first internal transcribed spacer (ITS-1) primers and common toxoplasmatiid ITS-1 primers confirmed the presence of H. heydorni DNA in the samples. The oocysts were considered to be H. heydorni on the basis of their morphology, biology, and molecular characteristics. This is the first record of a H. heydorni–like parasite in the white-tailed deer.

Neospora caninum is a parasite of livestock and companion animals and is an important cause of bovine abortion in dairy cattle worldwide (Dubey, 2003). It is transmitted transplacentally, by the ingestion of infected tissues and by the ingestion of food and water contaminated with oocysts excreted in the feces of dogs. The domestic dog is the only known definitive host for N. caninum (McAllister et al., 1998). The role of the dog in the epidemiology of N. caninum is currently unclear because experimentally infected dogs usually excrete only a few oocysts, and the parasite has been isolated only a few times from naturally infected dogs (Basso et al., 2001; Gondim et al., 2002; Slapeta, Modry et al., 2002; McGarry et al., 2003). Furthermore, N. caninum oocysts morphologically resemble the oocysts of a related coccidian, Hammondia heydorni, and there is no simple method to distinguish them.

Little is known about the life cycle of H. heydorni or whether additional Hammondia species that use dogs as their definitive host occur (Dubey et al., 2002; Schaeres et al., 2002; Slapeta, Modry et al., 2002). Hammondia heydorni–like oocysts were found in the feces of dogs that were fed naturally infected tissues from cattle (Bos taurus), water buffalo (Bubalus bubalis), sheep (Ovis aries), goats (Capra hircus), moose (Alces alces), and camels (Camelus dromedarius) (reviewed in Dubey et al., 2002). In addition, red foxes (Vulpes vulpes) fed tissues from sheep, cattle, roe deer (Capreolus capreolus), mountain gazelle (Gazella gazella), and reindeer (Rangifer tarandus) shed H. heydorni–like oocysts in their feces (Dubey et al., 2002). Until recently, all these oocysts excreted in feces of dogs and foxes were considered 1 species. However, studies of Schaeres et al. (2002, 2003) indicated the parasite in foxes is different morphologically and biologically from the parasite in the domesticated dog. Furthermore, molecular studies indicate that there are more than 1 genetic variant at present designated as H. heydorni (Sreekumar et al., 2003). We report isolation of another H. heydorni–like parasite from the white-tailed deer (Odocoileus virginianus) for the first time from this host. During studies on the genetic characterization of Toxoplasma gondii isolates from wildlife (Dubey et al., 2004) in March 2003, tissues from 4 white-tailed deer from Mississippi were also examined for antibodies to N. caninum using the N. caninum–agglutination test (NAT; Romand et al., 1998). The NAT titer was 1:25 in 2 and 1:50 in 2 deer. Heart tissue from these 4 deer were pooled and fed to 2 laboratory-raised dogs. The dogs had not ingested uncooked meat products before feeding on deer tissues. Feces of these dogs were examined daily for 3 wk for oocysts. Oocysts were collected from feces, sporulated in 2.5% potassium dichromate aqueous solution at room temperature for 7 days, and then stored at 4°C until used. Sporulated oocysts were washed with water to remove potassium dichromate by centrifugation, treated with 5.25% sodium hypochlorite solution, washed with water to remove potassium chromate by centrifugation, and stored in 2.5% potassium dichromate aqueous solution at 4°C until used. Sporulated oocysts were washed with water to remove potassium dichromate by centrifugation, treated with 5.25% sodium hypochlorite solution (Clorox), washed, and divided into aliquots for bioassay, in vitro cultivation, and polymerase chain reaction (PCR) studies. Aliquots were fed to 2 gerbils (Meriones unguatus) and to 5 interferon gamma–gene knockout (KO) mice (Dubey and Lindsay, 1998). For in vitro cultivation, 1 aliquot was vortexed for 5 min with 500-μm glass beads (Microbeads, Ferro Corporation, Cleveland, Ohio) and subsequent incubation in an excystation medium (sodium taurocholate 250 mg, sodium deoxycholic acid 400 mg, trypsin [1:250] 25 mg in 100 μl saline, pH 7.5) at 37°C. After excystation, the suspension was washed with growth medium and layered over each of the 2 CV1 (African Green monkey [Cercopithecus aethiops] kidney cells) and equine dermal cell monolayers grown over coverslips in multiwell plates. The coverslips were removed at intervals, fixed with Bouin fixative and stained with Giemsa.

For obtaining DNA, the oocyst suspension was ruptured by 2–3 freeze–thaw cycles, followed by grinding of the pellet in small volumes (about 30 μl) in a 0.2-ml microtissue grinder (Wheaton, Fischer Scientific, Pittsburgh, Pennsylvania). The DNA was extracted from the homogenized suspensions using DNAzol (MRC, Cincinnati, Ohio) according to the manufacturer’s instructions. Five sets of primers, Neospora-specific NP6/NP21 (Yamage et al., 1996), H. heydorni–specific first internal transcribed spacer (ITS-1) RAPD primers JS5/JS5 (Slapeta, Koudela et al., 2002), H. heydorni–specific HhAP7 and HhAP10 primers, and common toxoplasmatiid ITS-1 primers CT1/CT2 (Sreekumar et al., 2003), were used for PCR amplification of the DNA, according to previously described protocols. The PCR products were electrophoresed in a 2% agarose gel, and the gel-cleared PCR products were directly sequenced in both directions using the Big Dye terminator system, version 3.1 (Applied Biosystems, Foster City, California) using an ABI 377 sequencer. The sequence chromatograms were edited using Sequencher 4.1 software (Genecodes Corp., Ann Arbor, Michigan).