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Isolation and molecular characterization of *Toxoplasma gondii* from captive slender-tailed meerkats (*Suricata suricatta*) with fatal toxoplasmosis in Argentina

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ABSTRACT

In this study, the diagnosis of fatal disseminated toxoplasmosis in three captive slender-tailed meerkats (*Suricata suricatta*) in the zoo of La Plata, Argentina and the *in vitro* isolation and molecular characterization of *Toxoplasma gondii* are reported. The animals showed depression, dyspnea and hypothermia, and also ataxia in one case, and died within 1–5 days. The main histopathological lesions included interstitial pneumonia, non-suppurative inflammatory changes and focal necrosis in liver, spleen, kidney and brain. Tachyzoites or tissue cysts were present in lung, liver, spleen, brain, striated muscle, kidney, intestine and mesenteric lymph node sections, and stained strongly with *T. gondii* antiserum in immunohistochemical analysis. *T. gondii* was isolated in Swiss mice and in bovine monocytes cultures from tissues of one of the meerkats. The isolate was cryopreserved and it was named TG-Suricata-1. *T. gondii* DNA was demonstrated in tissues of all three animals and in tachyzoites isolated in cell cultures. The PCR-RFLP analysis of markers based in the loci 3′-SAG2, 5′-SAG2, BTUB, GRA6, SAG3, c22-8, L358, PK1, c29-2 and Apico of *T. gondii* produced patterns corresponding to the clonal type III. Type III strains of *T. gondii* possess no or only little virulence in the mouse model, however their association with virulence in other animal species is uncertain. In the present case, *T. gondii* of the clonal lineage III was responsible for fatal cases in *S. suricatta*. To our knowledge, this is the first report of isolation and genotyping of *T. gondii* from *S. suricatta*.

1. Introduction

Toxoplasma gondii infection occurs in a broad range of warm-blooded animals including humans and is frequently asymptomatic; however, it can be severe or even fatal to some hosts. Most *T. gondii* isolates from North America and Europe have been classified into one of three clonal lineages (I, II, III) based on PCR-RFLP analyses (Howe and Sibley, 1995; Howe et al., 1997). These lineages have different virulence phenotypes in mice. Type I strains are uniformly lethal in outbred mice, while type II and III strains are usually significantly less virulent in these animals (Sibley and Boothroyd, 1992). It is not known however, if the virulence phenotype observed in the mouse

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model is associated with virulence in some other animal species. Genotypes that do not belong to the three main clonal lineages were found predominantly in other continents (Beck et al., 2009). Some species like New World monkeys (Dietz et al., 1997; Inoue, 1997; Epiphanio et al., 2003), lemurs (Spencer et al., 2004), Pallas' cats (Dubey et al., 1988a; Kenny et al., 2002; Basso et al., 2005) and some Australian marsupials (Patton et al., 1986; Dubey et al., 1988b; Basso et al., 2007) are considered highly susceptible to clinical toxoplasmosis. A high susceptibility has also been suggested for slender-tailed meerkats (*Suricata suricatta,* Herpestidae) (Juan-Sallés et al., 1997). These small-sized carnivorous are widely distributed in Southern Africa and are bred in captivity in many zoos around the world (van Staaden, 1994). They have a primarily insectivorous diet in the wild, but they become omnivorous in captivity and prey easily upon small mammals (van Staaden, 1994).

In this study, the diagnosis of fatal disseminated toxoplasmosis in captive slender-tailed meerkats (*S. suricatta*) in the zoo of La Plata, Argentina and the *in vitro* isolation and molecular characterization of *T. gondii* from *S. suricatta* are reported.

2. Materials and methods

2.1. Animals

Between January and April 2007 six of eighteen slender-tailed meerkats died at the zoo of La Plata, Argentina. Samples from three dead animals (in the following referred to as MK-1, MK-2 and MK-3) were obtained and further investigated. The colony derived from a group of six young animals that had arrived in October 2004 from the Bester Birds & Animal Zoo Park, South Africa and was kept in an outdoor–indoor system enclosure. The diet at the beginning of the outbreak consisted mainly in commercial cat food, raw chicken from commercial farms, and flour beetles (Tenebrionidae). The meerkats showed dyspnea, depression, hypothermia and one of them (MK-2) displayed also ataxia. Despite intensive care, the animals died within 1 and 5 days after the onset of clinical signs.

2.2. Necropsy, histopathological and immunohistochemical analysis

The first dead meerkat (MK-1), a male with a weight of 1.2 kg, was necropsied at the zoo. Samples from spleen, mesenteric lymph nodes, kidney, lung and liver were submitted in 10% formalin to the Faculty of Veterinary Medicine from La Plata. Necropsies of two female meerkats (MK-2 and MK-3) with weights of 1 and 0.55 kg, respectively, were performed at the Faculty. Samples of brain, lungs, heart, striated muscle, liver, intestine, spleen, and kidney from both animals were fixed in 10% neutral buffered formalin for histological and immunohistochemical studies. All formalin-fixed tissue samples were routinely processed for histology, sections cut to 5 μm thickness and stained with hematoxylin & eosin (H&E), and immunohistochemically for *T. gondii* with the LSAB+ System HRP using a commercial kit according to the manufacturer's instructions (DakoCytomation, Carpinteria, USA). A rabbit anti-*T. gondii* hyperimmune antiserum (kindly provided by Dr. J.P. Dubey, USDA, Beltsville, MD, USA) was used at a 1:2000 dilution (overnight at 4 °C) as the primary antibody. Sections of brain from a *T. gondii*-experimentally infected mouse and heart sections from a *Neospora caninum*-naturally infected calf were used as positive and negative controls, respectively.

2.3. Isolation of T. gondii in mice and cell cultures

Samples of brain from MK-2, and samples of brain, striated muscle and abdominal fluid from MK-3 were homogenized in saline containing antibiotics (penicillin 1000 IU/ml and streptomycin 100 μg/ml). Each sample was inoculated subcutaneously in two and intraperitoneally in one N:NIH Swiss mice. At 7 days post inoculation, peritoneal exudates of the mice were examined for tachyzoites of *T. gondii*. At 60 days post infection (dpi), one mouse (07/14R) inoculated with striated muscle from MK-3 was euthanized and brain homogenates were examined for *T. gondii* cysts.

After parasites had been detected, peritoneal exudate or brain homogenate from inoculated mice were inoculated into bovine monocytes (BM) cultures, and incubated with RPMI 1640 medium and 3% fetal calf serum at 37 °C and 5% CO_2 until tachyzoite growth was observed. Positive cultures were cryopreserved in RPMI 1640 medium with 20% fetal calf serum and 5% DMSO.

2.4. Serologic studies

The inoculated mice were bled at 48 dpi. Sera were tested for antibodies to *T. gondii* beginning at a 1:25 dilution by the indirect fluorescent antibody test (IFAT) using culture-derived tachyzoites of the RH strain and a goat-anti mouse-IgG-FITC-conjugate (Sigma Bio Sciences, St. Louis, MO, USA) at a dilution of 1:50, and by the modified agglutination test (MAT) of Desmonts and Remington (1980).

2.5. DNA extraction

DNA was extracted from fresh samples of brain and hind-limb muscles from MK-2 and MK-3, from paraffin-embedded lung tissues of all three meerkats, and from tachyzoites grown in cell cultures, with a commercial DNA-extraction kit (DNeasy® Tissue Kit QIAGEN, Hilden, Germany), according to the manufacturer's recommendations. In the case of paraffin-embedded tissues, previous steps of xylol–ethanol treatment (xylol for 5 min [twice], ethanol 100% for 5 min [twice], ethanol 96% for 5 min, ethanol 70% for 5 min, ethanol 50% for 5 min, distilled water for 5 min) were performed.

2.6. Polymerase chain reaction (PCR) for T. gondii

The amplification was performed with the specific primer pair B22/B23 (Bretágne et al., 1993) for the B1 gene of *T. gondii*. One microliter of genomic DNA from each sample was added to tubes of a PCR master mix containing 2.5 μl of 10× PCR buffer (Fermentas Life Sciences, Hanover, USA), 200 μM each of dATP, dTTP, dGTP and dCTP, 0.75 U of