



Toxoplasmosis in Sand cats (*Felis margarita*) and other animals in the Breeding Centre for Endangered Arabian Wildlife in the United Arab Emirates and Al Wabra Wildlife Preservation, the State of Qatar

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ABSTRACT

The Sand cat (*Felis margarita*) is a small-sized felid found in sand and stone deserts ranging from the north of Africa to Asia, with the Arabian Peninsula as its centre of distribution. The Sand cat captive breeding program at the Breeding Centre for Endangered Arabian Wildlife (BCEAW), Sharjah, UAE, has experienced high newborn mortality rates, and congenital toxoplasmosis was recently recognized as one of the causes of this mortality. In the present study, one 18-month-old Sand cat (FM019) died of acute toxoplasmosis-associated hepatitis and pneumonitis acquired after birth; *Toxoplasma gondii* was demonstrated in histological sections which reacted with *T. gondii* polyclonal antibodies by immunohistochemistry (IHC). *T. gondii* DNA was found by PCR of extracted DNA from liver and lung tissues of this cat. Antibodies to *T. gondii* were found in serum examined in 1:1600 dilution in the modified agglutination test (MAT); its 2-year-old cage mate seroconverted (MAT titer 1:3200) at the same time. Another Sand cat (FM017) was euthanized because of ill health when 3 years old; its MAT titer was >1:3200, and *T. gondii* tissue cysts were found in brain, heart, ocular muscles and skeletal muscle, confirmed by IHC. Viable *T. gondii* was isolated by bioassays in mice inoculated with tissues of another chronically infected Sand cat (FM002); *T. gondii* was not found in histological sections of this cat. *T. gondii* antibodies were found in several species of animals tested, notably in 49 of 57 wild felids at BCEAW. A 7-year-old Sand cat (3657) from Al Wabra Wildlife Preservation (AWWP), Doha, State of Qatar died of acute visceral toxoplasmosis with demonstrable *T. gondii* tachyzoites by IHC, and *T. gondii* DNA by PCR, and a MAT titer of >3200. *T. gondii* antibodies were found in 21 of 27 of wild felids at AWWP. PCR-RFLP genotyping at 10 genetic loci revealed that these *T. gondii* isolates from Sand cat (FM002 and FM019) at BCEAW have an atypical genotype, which was previously reported in *T. gondii* isolates of dogs from Sri Lanka. The genotype from the cat from AWWP (3657) is a genetic Type II strain with a Type I allele at locus Apico. This is the first report of genetic characterization of *T. gondii* isolates from Middle East.

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1. Introduction

Felids are important in the epidemiology of *Toxoplasma gondii* infection because they are the only hosts that can shed environmentally resistant oocysts (Dubey, 2009). Although antibodies to *T. gondii* are widely prevalent in many species of felids, including the domestic cat, clinical toxoplasmosis is rare in wild felids (Dubey, 2009; Jones and Dubey, 2010). However, toxoplasmosis causes severe illness in Pallas cats (*Felis manul*), and the parasite can be transmitted transplacentally from asymptomatic mothers to kittens (Kenny et al., 2002; Basso et al., 2005; Dubey, 2009). Recently, a similar phenomenon was described for Sand cats (*Felis margarita*) from the United Arab Emirates (UAE) (Pas and Dubey, 2008a).

The Sand cat is a small-sized desert felid weighing 2–3 kg. It is found in sand and stone deserts ranging from the north of Africa to Asia, with the Arabian Peninsula as its centre of distribution. It is well adapted to living in arid areas and in areas where temperature changes are extreme with temperatures ranging from 0 to 58 °C. The Sand cat is mainly nocturnal, spending the days in a shallow burrow or under vegetation. Their prey consists of small rodents and occasional birds and reptiles. It may be able to exist without drinking free water (Sausman, 1997).

Sand cats are held and bred in captivity at institutes worldwide. In captivity, Sand cats are fed a variety of diets from small whole chicks and rodents to commercially prepared feline diets. Some vegetables are added to the diets and water is kept available (Sausman, 1997). At the Breeding Centre for Endangered Arabian Wildlife (BCEAW), the Sand cats are kept in large outside enclosures with an inner den. The enclosures are only accessed by the keepers and the breeding animals are not on display to the public. The cats are kept individually and only paired for part of the year to avoid having offspring during the extremely hot summer months.

We recently reported severe congenitally acquired toxoplasmosis in Sand cats at the BCEAW (Pas and Dubey, 2008a). In the present paper we report additional cases of fatal toxoplasmosis in Sand cats in UAE and the State of Qatar, and genetically characterize *T. gondii* isolates from these cats for the first time from this host. We also document prevalence of *T. gondii* antibodies in several other species of animals from the UAE and Qatar.

2. Materials and methods

2.1. Samples from BCEAW

2.1.1. Sand cats with clinical toxoplasmosis (Table 1)

2.1.1.1. FM016 and FM017. On 2 March 2006 two kittens (FM016 and FM017) were born in captivity to a breeding pair that had given birth to another *T. gondii* infected litter the previous year (Pas and Dubey, 2008a). The kittens were weighed and examined twice a week in an attempt to detect disease in the early stages. They were developing normally until day 26, when FM016 died of acute toxoplasmosis (Pas and Dubey, 2008a). The surviving kitten, FM017, had a MAT titer of >3200 and was medicated with clindamycin (Dalacin CTM, Pharmacia, Puurs, Belgium) for 15

weeks at a dosage of 20 mg/kg given orally with a syringe. Once the kitten started eating solid food the clindamycin powder was disguised in liquid fat on the food. The animal developed no clinical signs during this period but at the age of 1 year however, it was observed to walk into objects and a severe bilateral mydriasis was seen. Direct and indirect pupil reflexes were negative and ophthalmological examination revealed a partial degeneration of the retina. Blood samples excluded infections with feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) or *Bartonella* sp. Further differentials for retinal degeneration included taurine deficiency and feline infectious peritonitis (FIP) but no further indications could support these etiologies. Over several months the animal showed an increasing muscle atrophy of the hind legs and became ataxic. A general examination under anesthesia did not reveal any obvious cause for the problems seen. Liver values AST and ALT were slightly increased as well as bile acids (AST 78.9 U/l, ALT 144.5 U/l, and bile acids 35.98 µmol/l). The animal was treated several times with vitamin B complex (B.Complex-C, Bio-Pharmachemie Co. Ltd., Vietnam) and anabolica (Ilium Boldebal-H, Troy laboratories PTY Limited, Australia) injections and de-wormed regularly. At the age of 3 years, she became incontinent. Hematuria and high alkalinity was seen and many crystals were found in the urine sediment. After 2 weeks of treatment with marbofloxacin injected and later given orally at a dosage of 2 mg/kg (Marbocyl 2% and Marbocyl 20 mg tablets, Vetoquinol, B.V.'s-Hertogenbosch, The Netherlands) there was no improvement and since the ataxia and general condition of the animal had already deteriorated for such a long time, the cat was euthanized. Samples of frozen liver and lungs were sent to the Animal Parasitic Diseases Laboratory (APDL), Beltsville, MD for further evaluation.

2.1.1.2. FM019 and FM020. On 12 March 2009 two new Sand cats, a 1-year-old male FM019 and a 2-year-old female FM020, were acquired from a nearby collection where they had been born in captivity. The animals were quarantined at the BCEAW for 6 weeks during which they were observed for signs of illness and examined once under anesthesia. Hematologic and biochemical evaluations were done at that time but no abnormal blood values were seen, and viral testing for FeLV and FIV (SensPERT kits, Combined FeLV Antigen/FIV antibody test, Vet All Laboratories, Korea) were negative. They were also seronegative for *T. gondii* antibodies (see serology section) on blood samples drawn on 4 April 2009. After their quarantine period, the animals were moved to a display enclosure in the zoo. In this enclosure Gordon's wildcats of which several had antibodies to *T. gondii* (Pas and Dubey, 2008b) had previously been housed. Shortly after it was noticed that the female FM020 was not eating well, but this was attributed to the rather low temperatures in the cage, which is regulated with air-conditioning. The animal received a general examination and blood was collected under sedation with medetomidine (0.05 mg/kg, Domitor, Orion Pharma, Finland). Liver enzymes ALT and AST were slightly increased (AST 110.2 U/l and ALT 144.2 U/l) as well as urea (19 mmol/l) but with a normal creatinine (60 µmol/l). All other hematology and biochemistry val-

Table 1

Data on Sand cats with clinical toxoplasmosis.

Source (ID)	Age (years)	Date died	MAT titer ^a	Main lesions	<i>T. gondii</i> demonstration				
					Hist.	IHC	PCR	Bioassay	Strain designation
BCEAW									
FM002	15	2-15-2010	>3200	None	Neg.	Neg	+	Positive	TgSandcatUAE1
FM017	3	4-28-2009	>3200	Mild encephalitis	+	+	+	Not done	TgSandcatUAE2
FM019	2	6-15-2009	1600	Pneumonia and hepatitis	+	+	+	Negative	TgSandcatUAE3
AWWP									
3657	7	1-14-2010	>3200	Mesenteric lymph node necrosis	+	+	+	Not done	TgSandcatQA1

^a Near death or postmortem.

ues tested were in normal range. No further abnormalities could be detected during examination and her weight was as expected. Radiographs of the thorax and abdomen did not reveal any abnormalities either. Apart from some subcutaneous fluid during the procedure, no other treatment was indicated and since it was thought that the low temperature might have been the reason for the inappetence, only environmental changes were made. This cat is now apparently healthy. A blood sample was obtained from cat FM020 on 15 July 2009 for *T. gondii* antibodies.

Four weeks later her male cage mate FM019 looked depressed and ate less than normal. He was hand caught to perform an examination but during normal restraint and handling to take radiographs he developed a severe dyspnoea, collapsed and died on 15 June 2009. A necropsy was performed on the same day.

2.1.1.3. FM002. Cat FM002 was imported from the USA in 1998 at the age of 3 years. In 2006 a blood sample was taken and the *T. gondii* antibody titer was determined with a latex agglutination test at the University of

Glasgow, Scotland, using a commercial kit (Eiken, Tokyo, Japan), which showed a positive titer of >512. Testing for FIV, FeLV and FeCoV were negative at that time. In 2007 he started to show signs of ataxia in the hind legs and a slight head tilt. A full examination under sedation revealed no obvious abnormalities but hematology results revealed a severe increase in globulins (80 g/l) and proteinuria. Because of previous problems in the Sand cats with toxoplasmosis infection, the animal was started on clindamycin (Dalacin CTM, Pharmacia, Puurs, Belgium) at 20 mg/kg twice daily for 32 days. Clinical signs improved slowly but the animal never completely recovered and remained slightly ataxic. Behavioural changes indicated that he also might have had impaired vision and a full ophthalmological examination was performed which revealed an inactive chorioretinitis with partial degeneration of the retina.

In February 2010 the condition of this cat deteriorated quickly. He was anorexic, had diarrhoea and had severe difficulties walking. A full examination revealed a severely extended bladder without obstruction and general mus-

Table 2Antibodies to *T. gondii* in sera of animals from BCEAW, United Arab Emirates.

Species	No. tested	No. positive	MAT titer (no. of animals)
Felids			
Gordon' wild cat (<i>Felis sylvestris gordonii</i>)	5	5	100, >3200 (4)
Arabian leopard (<i>Panthera pardus nimr</i>)	7	6	50 (2), 100, 200, 800 (2)
Cheetah (<i>Acinonyx jubatus soemmerringii</i>)	34	31	50, 100, 400, 800 (5), 1600 (4), >3200 (15)
Caracal (<i>Caracal caracal schmitzi</i>)	6	5	100 (2), 200 (2), 3200
African caracal (<i>Caracal caracal algira</i>)	1	1	200
Feral domestic cat (<i>Felis catus</i>)	4	1	>1600
Foxes			
Sand fox (<i>Vulpes rueppellii</i>)	3	3	800, >3200 (2)
Red fox (<i>Vulpes vulpes arabica</i>) ^a	15	9	50, 100 (3), 200 (3), 800, 3200
Blanford's fox (<i>Vulpes cana</i>) ^b	14	8	All >3200
Others			
Brandt's hedgehog (<i>Paraechinus hypomelas</i>)	2	1	3200
Long eared hedgehog (<i>Hemiechinus auritus</i>)	6	3	>3200
Ethiopian hedgehog (<i>Paraechinus aethiopicus</i>)	2	1	400
Wolf (<i>Canis lupus arabs</i>) ^c	8	5	50 (3), 200, 1600
Jackal (<i>Canis aureus</i>)	8	6	25, 50 (2), 100, 100, 200
Striped hyaena (<i>Hyaena hyaena</i>)	6	3	25, 50, 100
Small spotted genet (<i>Genetta genetta</i>)	1	1	800
White tailed mongoose (<i>Ichneumia albicauda</i>)	2	2	800 (2)
Indian grey mongoose (<i>Herpestes edwardsii</i>)	1	1	3200
Hamadryas baboon (<i>Papio hamadryas</i>)	1	1	400

^a Sampled in 2008, three of seropositive foxes were wild caught.^b Three sampled in 2008.^c One sampled in 2008.

Table 3Antibodies to *T. gondii* in sera of animals from AWWP, Qatar.

Species	No. tested	No. positive	MAT titer (no. of animals)
Arabian Sand cat (<i>Felis margarita harrisoni</i>)	20	14	200 (5), 400 (2), 800 (1), >3200 (6)
African wild cat (<i>Felis sylvestris gordonii</i>)	1	1	1600
Cheetah (<i>Acinonyx jubatus soemmerringii</i>)	5	5	50 (1), 200 (1), 400 (1), 800 (1), 3200 (2)
King cheetah (<i>Acinonyx jubatus rex</i>)	1	1	>3200

cle atrophy. A urine examination revealed proteinuria, but no bacterial growth. Blood samples revealed an increased urea (14.7 mmol/l) with a normal creatinine (95 μ mol/l). X-rays showed some consolidated areas in the frontal lung lobes, round shaped kidneys and a severe scoliosis of the thoracic vertebrae with bone demineralization. Because of the poor condition of the animal, and its old age, the cat was euthanized.

2.1.2. Serum samples from captive animals

Serum samples were obtained from Sand cats and other animals at BCEAW (Table 2).

2.2. Samples from Al Wabra Wildlife Preservation, Doha, Qatar (AWWP)

AWWP is home to 27 Sand cats and other felids. The management of this facility (Deb et al., 2009) is similar to that of BCEAW.

2.2.1. Clinical toxoplasmosis in a Sand cat (3657)

A 7-year-old male Sand cat died of acute illness. This cat was born at AWWP, and had been vaccinated yearly with feline panleukopaemia virus, feline rhinotracheitis virus, feline calicivirus (Felocell CVR, Pfizer GmbH, Berlin, Germany), and rabies virus (Rabdomun, Essex Tierarznei, Muenchen, Germany) (Deb et al., 2009). On 11 January 2010 this cat was examined because of anorexia and weakness. Its mucous membranes were pale and it showed labored breathing. A blood sample was obtained for biochemical and hematological tests. The cat died after 3 days of treatment with subcutaneous injections of 16.8/4.2 mg/kg amoxicillin–clavulanacid (Synulox RTU 140/30 mg/ml, Pfizer GmbH, Berlin, Germany) and 0.6 mg/kg meloxicam (Metacam 5 mg/ml, Boehringer Ingelheim, Ingelheim, Germany) and in addition 150 ml subcutaneous fluids (Electrolytloesung, Albrecht GmbH, Aulendorf, Germany). Initially formalin-fixed tissues were submitted to a commercial laboratory (Institut für Tierpathologie, Ludwig-Maximilians-Universitaet Muenchen, 80539 Muenchen, Veterinaerstr. 13, Germany) for histological examination. After the diagnosis of toxoplasmosis, formalin-fixed tissues and frozen liver and lung of the Sand cat 3657 were sent to the Animal Parasitic Diseases Laboratory (APDL), Beltsville, MD for further evaluation.

2.2.2. Serum samples from captive animals

Serum samples were obtained from Sand cats and other animals at AWWP (Table 3).

2.3. Necropsy and histological examination

Samples of all major organs were fixed in 10% buffered formalin. Paraffin-embedded tissues were sectioned at 5 μ m and examined after staining with hematoxylin and eosin (H and E).

2.4. Serological examination for *T. gondii*

Antibodies to *T. gondii* were determined using the modified agglutination test (MAT) performed at APDL as described (Dubey and Desmonts, 1987). A cut-off titer of 1:25 is considered indicative of *T. gondii* infection. Serum samples from animals at BCEAW examined for *T. gondii* antibodies are listed in Table 2. Unless indicated, the sera were obtained in 2009. Additionally, sera from 11 rodents (8 mice and 3 rats) were tested at 1:25 serum dilution. Serum samples from animals at AWWP examined for antibodies to *T. gondii* are listed in Table 3.

2.5. Attempted isolation of *T. gondii*

Unfixed liver and lungs of FM019 and FM002 were shipped by air from UAE to the APDL for *T. gondii* isolation. Seven and four days elapsed between collection of samples from cat FM019 and FM002 and their receipt at APDL, respectively.

Portions of lungs and liver from cat FM019 were homogenized in aqueous 0.85% NaCl (saline), and inoculated subcutaneously in to 8 Swiss Webster (SW) mice. The inoculated mice were observed for 81 days and examined for *T. gondii* infection (Dubey, 2009).

Tissues of cat FM002 were bioassayed in SW and gamma interferon gene knock out (KO) mice. Tissues were homogenized in saline, and the homogenate was divided into two equal portions (A and B). Portion A was digested in acid pepsin for 60 min at 37 °C, centrifuged, neutralized with sodium bicarbonate, and after adding antibiotics, inoculated subcutaneously in to five SW and two KO mice as described (Dubey, 2009). Portion B was not digested, centrifuged, and the sediment suspended in antibiotic saline containing 1000 units of penicillin and 100 μ g of streptomycin per ml of saline (Dubey, 2009). The mice inoculated with cat tissues were examined for evidence of *T. gondii* infection.

2.6. Genetic characterization of *T. gondii*

T. gondii DNA was extracted from feline tissues or infected cell culture using DNeasy kit (Qiagen) and genotyped using the genetic markers SAG1, SAG2, SAG3, BTUB,

Table 4Genetic characterization of *T. gondii* from Sand cats from UAE and Qatar.

Strain ID	Genotypes	Genetic markers										
		SAG1	(5' + 3') SAG2	alt.SAG2	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	Apico
GT	I	I	I	I	I	I	I	I	I	I	I	I
PTG	II	II	II	II	II	II	II	II	II	II	II	II
CTG	III	II or III	III	III	III	III	III	III	III	III	III	III
MAS	Atypical	u-1	I	II	III	III	III	u-1	I	I	III	I
TgCgCa1	Atypical	I	II	II	III	II	II	II	u-1	I	u-2	I
TgCtBr5	Atypical	I	III	III	III	III	III	I	I	I	u-1	I
TgCtBr64	Atypical	I	I	u-1	III	III	III	u-1	I	III	III	I
TgRsCr1	Atypical	u-1	I	II	III	I	III	u-2	I	I	III	I
Negative control	–	–	–	–	–	–	–	–	–	–	–	–
TgSandcatUAE3 (FM019)	Atypical	u-1	II	II	III	III	II	II	III	II	u-2	I
TgSandcatUAE1 (FM002)	Atypical	u-1	II	II	III	III	II	II	III	II	u-2	I
TgSandcatUAE2 (FM017)	Atypical	II or III	I	ND	ND	II	II	ND	III	II	ND	I
TgSandcatQA1 (3657)	II	II or III	II	II	II	II	II	II	II	II	II	II

ND, no data available.

GRA6, c22-8, c29-2, L358, PK1, and Apico (Su et al., 2006; Su and Dubey, 2009) (Table 4).

2.7. Immunohistochemical (IHC) examination

Immunohistochemistry was performed on paraffin-embedded sections at APDL using reagents and methods described previously (Lindsay and Dubey, 1989).

3. Results

3.1. Samples from BCEAW

3.1.1. Sand cats with clinical toxoplasmosis

3.1.1.1. FM017. The lungs were edematous, and had multiple small nodules. The liver was congested. Microscopically, numerous tissue cysts were found in sections of brain (Fig. 1). There was a single focus of perivascular infiltration of mononuclear cells and a focus of gliosis. Large sized *T. gondii* tissue cysts were seen in sections of heart, skeletal muscle, and ocular muscle but lesions were not seen (Fig. 2). A single focus of vasculitis and mononuclear cell infiltration was seen in the retina. Lesions or *T. gondii* were not found in sections of kidneys, adrenal, lung, liver, stomach, small intestine, and urinary bladder.

DNA from tissues of this cat was typed at several of the 10 genetic loci and partial data was obtained. The data indicates this strain maybe of an atypical genotype (Table 4).

3.1.1.2. FM019. Grossly, the lungs had edema, focal consolidation, and multiple small white nodules were observed on the lung surface. There was serohemorrhagic fluid in the thorax and the liver was jaundiced. Tachyzoites were seen in impression smears of the lungs (Fig. 3). Bacteria were not isolated from culture of lung, liver and thorax fluid on standard culturing.

Histologically, this cat had severe multifocal hepatitis, and interstitial pneumonitis associated with numerous tachyzoites (Fig. 4). Focal myocarditis and nephritis were associated with few tachyzoites. Tissue cysts were not seen. The diagnosis was confirmed by IHC.

Antibodies to *T. gondii* were not detected in 1:25 serum dilution of blood sample collected on 9 April 2009 but blood

sample collected directly from the heart at postmortem had a MAT titer of ≥ 1600 .

The mice inoculated with tissues of cat FM019 remained seronegative and tissue cysts were not found in their brains when killed 81 days post-inoculation.

DNA from tissues of this cat was typed at the 10 genetic loci and was found to be an atypical strain (Table 4). This genotype was previously reported in *T. gondii* isolates of dogs from Sri Lanka (Dubey et al., 2007).

3.1.1.3. FM020. The cause of illness in cat FM020 was not determined. The cat seroconverted at the same time as FM019; the MAT titer on 9 April was <1:25 but it had a high MAT titer (1:3200) on 15 July 2009.

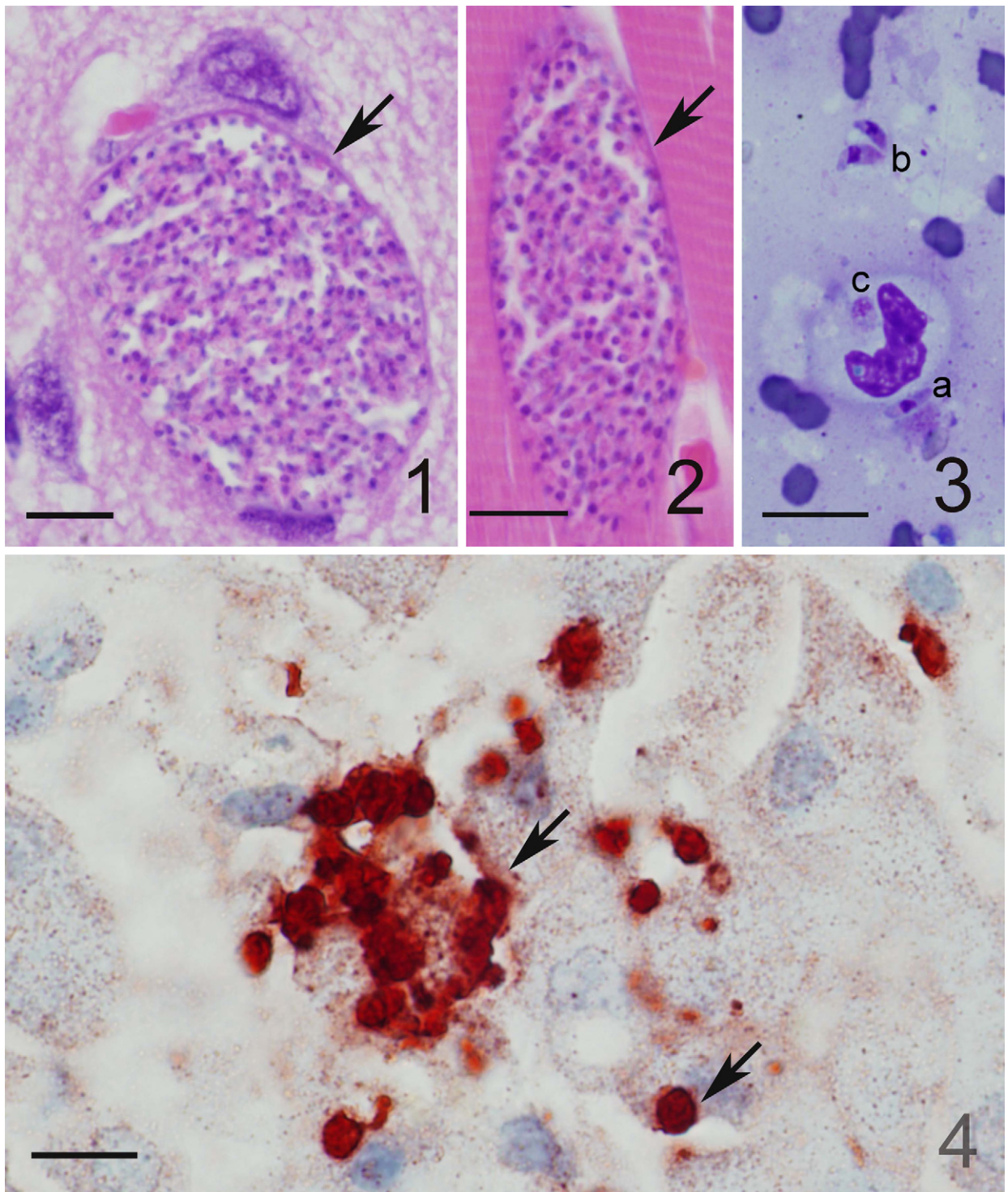
3.1.1.4. FM002. On postmortem several large cysts were observed in both kidneys and multiple pinpoint white nodules were seen. Roundworms were found in the duodenum. Immunohistochemically, lesions and protozoa were not demonstrable in histological sections (Table 1).

One of the two KO mice inoculated with tissues of FM002 died of acute toxoplasmosis on day 18 post-inoculation (p.i.) and numerous tachyzoites were found in smears of its lungs. *T. gondii* was successfully cultivated in CV1 cells inoculated with homogenate of lung of the infected KO mouse. This isolate was designated TgSandcat-UAE1. Neither antibodies to *T. gondii* nor tissue cysts were found in the other KO mouse killed 47 days p.i.

The SW mice inoculated with pepsin digest of tissues of FM002 remained asymptomatic. Antibodies to *T. gondii* (MAT > 1:200) were found in three of the five mice but tissue cysts were not found in their brains 47 days p.i.; however, all four KO mice inoculated with pooled brains from seropositive SW mice died of acute toxoplasmosis on days 14–19.

All SW mice inoculated with undigested tissues of the Sand cat died of bacterial infection 3 days p.i. and were discarded.

DNA from tissues of this cat was typed at the 10 genetic loci and was found to be an atypical strain, identical to FM019 (Table 4).



Figs. 1–4. Lesions and *T. gondii* in tissues of Sand cats. (1) Intracellular tissue cyst (arrow) in section of the brain of cat FM017. H and E stain. (2) Tissue cyst in section of the myocardium of cat FM017. H and E stain. (3) Tachyzoites in impression smear of lung of cat FM019. Giemsa stain. Note one tachyzoite (a) with a nucleus, two tachyzoites with nucleus dividing into two (b), and an intracellular tachyzoite (c). (4) Tachyzoites (arrows, all red areas) causing necrosis of hepatocytes in the liver of cat FM019. Section stained with *T. gondii* polyclonal rabbit antibody. Scale bar = 10 μ m.

3.1.2. Serological prevalence

T. gondii antibodies were found in several species of animals tested, notably in 49 of 57 felids (Table 1).

None of the 11 rodent (8 mice and 3 rats) sera from the breeding colony kept for feeding carnivores at the BCEAW had antibodies to *T. gondii*.

3.2. Samples from AWWP

3.2.1. Clinical toxoplasmosis in Sand cat 3657

Blood collected on the first day of illness indicated highly increased liver values AST (1000 U/l) and total bilirubin (44 μ mol/l). Postmortem gross lesions were seen in the lungs which were edematous with multiple small white nodules in all lung lobes. Similar small white nodules were found in the pancreas and omentum. The mesenteric lymph nodes were enlarged and a tapeworm was found in the small intestine. Histologically, there were multifocal area of necrosis and inflammation in adrenal glands, subacute interstitial pancreatitis, interstitial pneumonia, abdominal adipose tissue necrosis, hepatitis, and mild myocarditis. There was gastritis characterized by multifocal necrosis of glands, mixed cellular infiltration of the lamina propria, cellular debris within gland lumina. Many tachyzoites were seen in the lamina propria and in glandular epithelial cells (Fig. 5). Two loops of the colon showed mild enteritis associated with tachyzoites. Sections of small intestine and brain were not fixed for histology. There was almost total necrosis of mesenteric lymph nodes with enormous numbers of tachyzoites. *T. gondii* tissue cysts were not identified in any organ. All specimens were negative for feline coronavirus clone FIPV3-70 tested at the laboratory in Germany.

DNA from this cat was typed at the 10 genetic loci and was found to be a Type II strain with a Type I allele at locus Apico (Table 4). This Type II strain is highly prevalent worldwide.

3.2.2. Serological prevalence

T. gondii antibodies were found in 14 of 20 Sand cats, and 7 of 7 other wild felids (Table 3).

4. Discussion

In the present study, two Sand cats (FM019 and 3657) died of acute toxoplasmosis at separate establishments. Cat FM019, born in captivity at BCEAW, and appeared clinically normal until 18 months of age. Its MAT titer was 1:1600 at the time of death, but was seronegative to *T. gondii* 2 months before its demise.

Sand cat 3657 was also born in captivity at AWWP. The diagnosis of toxoplasmosis was confirmed immunohistochemically, by serology and by PCR. The acute nature of lesions indicated that toxoplasmosis was acquired recently, and only tachyzoites (not tissue cysts) were seen in lesions. In Sand cat 3657, lesions in the mesenteric lymph nodes and stomach are noteworthy. The finding of gastritis was unusual as this is rarely seen in domestic cats (*Felis domesticus*) naturally infected with *T. gondii* (Dubey, 2009) and has not been reported in wild felids.

Why some animals develop clinical toxoplasmosis whereas most remain subclinical is unknown. Recently,

attention has been focused on the genetic makeup of the strains in pathogenesis of clinical toxoplasmosis and atypical strains were found to cause clinical toxoplasmosis in immunocompetent humans (Demar et al., 2007; Elbez-Rubinstein et al., 2009; see Dubey, 2009). Currently there is no information on the genetic characterization of *T. gondii* isolates from the Middle East or the Sand cat. In the present study, the strain of *T. gondii* from UAE was found to be atypical. This genotype has been reported in dogs from Sri Lanka (Dubey et al., 2007). The isolate from Qatar is a common Type II strain that is frequently identified worldwide.

The cause of ataxia and retinal degeneration in FM017 was not determined. The presence of numerous tissue cysts in the brain and few lesions might have contributed to illness. It is known that clindamycin acts on tachyzoites but has no effect on tissue cysts (Dubey, 2009).

Prevention of *T. gondii* infection in zoo animals is a major problem, especially in highly susceptible species, such as Pallas and Sand cats, New World primates, and marsupials. Cats, like other hosts of *T. gondii* can become infected transplacentally, by ingesting food and water contaminated with oocysts, and by the ingestion of infected tissues (Dubey, 2009). In domestic cats the ingestion of infected tissues is more efficient because even a few bradyzoites are infectious for cats whereas few oocysts are not, and transplacental transmission is considered infrequent (Dubey, 2009). However, Pallas and Sand cats are different because unlike humans, domestic cats and sheep, chronically infected asymptomatic dams transmit infection to their offspring (Dubey, 2009).

The diet of all small carnivores at the BCEAW previously consisted of imported frozen buffalo and chicken meat, locally bought fresh camel and donkey meat, frozen day old chicks, and white mice and rats, pigeons, guinea fowl and quail that have been bred at the BCEAW. After the neonatal deaths in Sand cats in 2006 were confirmed as being caused by toxoplasmosis (Pas and Dubey, 2008a), the Sand cat diet was adapted to include only meat that has been frozen at -20°C for at least 1 week or fresh mice and rats bred at the BCEAW. Limited sampling of rats and mice sera fed to cats at BCEAW did not have any evidence of *T. gondii* infection. Although a serious effort is made to keep the enclosures pest free, wild rodents, birds and reptiles can still gain access and feral cats are sometimes found roaming within the BCEAW.

The diet of the Sand cats in Qatar consists of white mice and rats bred at AWWP. In addition they are fed with wild pigeons which have been caught and slaughtered on the premises and then frozen for different periods (ranging from 1 day to 1 month). Pest control programs are limiting the number of wild mice and rats in the area of the enclosures, but are not eradicating all.

Although no method can be regarded as 100% effective, most *T. gondii* tissue cysts will be destroyed when meat is frozen and thawed as well as by heating up to 67°C or processing the meat (see Dubey, 2009). Heating or processing the meat is not considered as an option at the BCEAW for nutritional and behavioural enrichment reasons. Results of the present study indicate that several other species of animals at BCEAW were exposed to *T. gondii*. However, clinical toxoplasmosis at the BCEAW has been documented only in

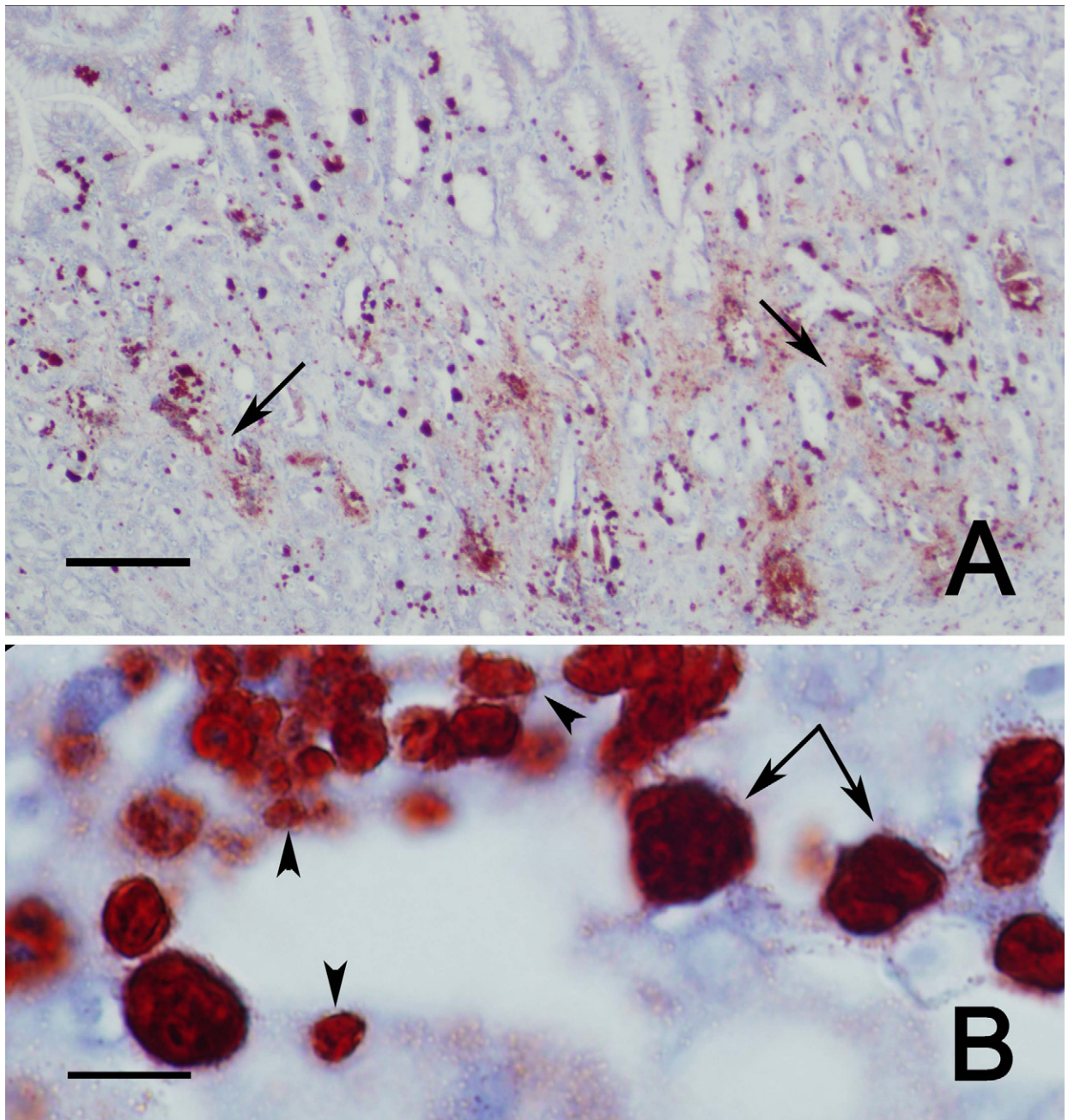


Fig. 5. Gastritis in Sand cat 3657 from AWWP. Immunohistochemical staining with *T. gondii* rabbit polyclonal antibodies. (A) Note foci of necrosis (arrows) and tachyzoites (all red bodies) in gastric glands and the lamina propria. Scale bar = 100 μ m. (B) Several groups (arrows) and individual (arrowheads) tachyzoites in epithelial cells of a gastric gland. Scale bar = 10 μ m.

Sand cats (Pas and Dubey, 2008a, and present study), in a Blanford's fox (Dubey and Pas, 2008) and in a Sandfox (Pas and Dubey, 2008c) and now in Sand cat at AWWP.

In the present study, *T. gondii* antibodies were found in several species of carnivores in addition to Sand cats, Sand foxes and Blanford's foxes previously reported (Dubey and Pas, 2008; Pas and Dubey, 2008c). We are not aware of previous reports of serological prevalence in the long eared hedgehog (*Hemiechinus auritus*), white tailed mongoose (*Ichneumia albicauda*), Indian grey mongoose (*Herpestes*

edwardsii), Hamadryas baboon (*Papio hamadryas*) and the Arabian wolf (*Canis lupus arabs*). Sobrino et al. (2008) reported *T. gondii* antibodies in 59.1% of 22 Egyptian mongoose (*Herpestis ichneumon*) from Spain. Pas and Dubey (2008b) found MAT antibodies in 86.1% of 36 Gordon's wild cat from the BCEAW. In the present study, *T. gondii* antibodies were found in 5 of 5 Gordon's wild cat. Whether Sand cat, Gordon's wild cat, caracal, or leopard can excrete *T. gondii* oocysts is unknown (Dubey, 2009), whereas it is known that cheetahs can excrete *T. gondii* oocysts

(Marchiondo et al., 1976; Polomoshnov, 1979). There are several Gordon's wild cats resident at the BCEAW. They were housed in the same pen 2 months before the Sand cats FM019 and FM020 were introduced in the enclosure. Oocysts excreted by these cats might have been present in the pen where the Sand cats were housed and could be the source of infection for Sand cats at BCEAW.

It is of interest to see that the *T. gondii* strain identified in Sand cats from UAE has an atypical genotype that is different from the clonal Type I, II and III strains. This genotype was previously reported in *T. gondii* isolates of dogs from Sri Lanka (Dubey et al., 2007), indicating it is widely spread in Asia and the Arabian Peninsula. In addition, the strain identified from Sand cat from Qatar belongs to the Type II lineage that is also widely spread at the global scale. The high seropositivity of *T. gondii* in captive wild animals tested in this study, in addition with the lack of epidemiological studies on this parasite in the Persian Gulf region, makes it necessary to carry out further studies to investigate the diversity and transmission of *T. gondii* in animal and populations in the region.

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