Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 151 (2008) 110–114
veterinary parasitology
www.elsevier.com/locate/vetpar

First isolate of *Toxoplasma gondii* from arctic fox (*Vulpes lagopus*) from Svalbard

Kristin Wear Prestrud [a,*], J.P. Dubey [b], Kjetil Åsbakk [a], Eva Fuglei [c], C. Su [d]

[a] *Norwegian School of Veterinary Science (NVH), Section of Arctic Veterinary Medicine, Stakkevollveien 23, N-9010, Norway*
[b] *United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Animal Parasitic Diseases Laboratory, Building 1001, Beltsville, MD 20705-2350, USA*
[c] *Norwegian Polar Institute, Polar Environmental Centre, N-9296 Tromsø, Norway*
[d] *Department of Microbiology, The University of Tennessee, Knoxville, TN 37996-0845, USA*

Received 7 September 2007; received in revised form 6 November 2007; accepted 8 November 2007

Abstract

Cats are considered essential for the maintenance of *Toxoplasma gondii* in nature. However, *T. gondii* infection has been reported in arctic fox (*Vulpes lagopus*) from the Svalbard high arctic archipelago where felids are virtually absent. To identify the potential source of *T. gondii*, we attempted to isolate and genetically characterize the parasite from arctic foxes in Svalbard. Eleven foxes were trapped live in Grumant (78°11′N, 15°09′E), Svalbard, in September 2005 and 2006. One of the foxes was found to be seropositive to *T. gondii* by the modified agglutination test (MAT). The fox was euthanized and its heart and brain were bioassayed in mice for the isolation of *T. gondii*. All 10 mice inoculated with brain tissue and one of the five inoculated with heart developed MAT antibodies, and tissue cysts were found in the brains of seropositive mice. Two cats fed tissues from infected mice shed *T. gondii* oocysts. Genotyping using 10 PCR-RFLP markers and DNA sequencing of gene loci *BSR4*, *GRA6*, *UPRT1* and *UPRT2* determined the isolate to be Type II strain, the predominant *T. gondii* lineage in the world.

Keywords: *Alopex lagopus*; *BSR4*; Genotyping; *GRA6*; Svalbard; *Toxoplasma gondii*; *Vulpes lagopus*

1. Introduction

The coccidian parasite *Toxoplasma gondii* has a global distribution. The domestic cat and other felids are the only definitive hosts known (Frenkel et al., 1970), in which the sexual parasite cycle takes place in the intestines and oocysts are produced (Dubey et al., 1970). In intermediate hosts, which comprise virtually all warm-blooded animals, the parasite multiplies asexually in various tissues, resulting in formation of tachyzoites and tissue cysts containing bradyzoites (Dubey and Beattie, 1988). Animals and humans can become infected by intake of food or water contaminated with oocysts, by ingestion of infected tissues, or transplacentally (Dubey and Beattie, 1988). Cats are considered essential in the life cycle of *T. gondii*; infection was either absent or of very low frequency in animals on isolated islands without cats (Wallace, 1973; Munday, 1972; Dubey et al., 1997).

The high arctic archipelago of Svalbard (78°–81°N, 10°–30°E) is located midway between the Norwegian mainland and the North pole. The arctic fox (*Vulpes lagopus*, formerly *Alopex lagopus*) is a top predator feeding both from the marine and the terrestrial food chain, and an opportunistic feeder and scavenger. Major food sources are migratory birds, arriving in the spring every year, and Svalbard reindeer (*Rangifer tarandus*

* Corresponding author. Tel.: +47 77 66 54 00; fax: +47 77 69 49 11.
E-mail address: kristin.prestrud@veths.no (K.W. Prestrud).

0304-4017/$ – see front matter
doi:10.1016/j.vetpar.2007.11.011

platyrhyncus) carcasses (Prestrud, 1992; Eide et al., 2005).

Three arctic foxes found dead on Svalbard in 2000 were previously diagnosed with disseminated toxoplasmosis (Sørensen et al., 2005). Antibodies against *T. gondii* were recently reported in mammal and bird species from Svalbard, including arctic fox (Prestrud et al., 2007). Domestic cats are prohibited on Svalbard, and there are no wild felids present. However, a very limited and stationary cat population exists in the village Barentsburg (78°5′N, 14°15′E). The harsh arctic climate does not allow the cats to roam outside the village. The results in Prestrud et al. (2007) gave reason to believe that oocysts are of minimal importance for transmission of *T. gondii* in the Svalbard ecosystem, because the terrestrial herbivores studied (reindeer and sibling voles, *Microtus rossiaemeridionalis*) were all seronegative.

Aim of the present study was to isolate *T. gondii* from this virtually cat free, high arctic area. The arctic fox population on Svalbard is closely monitored by the authorities (http://mosj.npolar.no/; Fuglei et al., 2003), and obtaining fresh tissues from a seropositive fox was a great challenge. For the present study, permission from the Governor of Svalbard and the Norwegian Animal Research Authority was sought to study *T. gondii* infection in arctic foxes.

2. Materials and methods

2.1. Foxes and assaying for T. gondii

A total of 11 wild arctic foxes were trapped live (Tomahawk Live Trap, 100 cm × 30 cm × 30 cm) in September 2005 ($n = 5$) and 2006 ($n = 6$) in Grumant (78°11′N, 15°09′E), Svalbard. All were less than 6 months old. A blood sample (0.5 ml) was obtained from the cephalic vein. The sample was allowed to clot, and serum was collected and assayed for antibodies (IgG) against *T. gondii* at twofold dilutions of 1:40 to 1:1280 using the direct agglutination test (DAT; Toxoscreen DA kit, bioMerieux S.A., Marcy-l′Etoile, France), performed in a heated tent (12–15 °C). The foxes were caged individually (60 cm × 46.5 cm × 54 cm metal wire dog cage) for 6–8 h during the analysis.

One of the 11 foxes, a female weighing 2 kg, had a positive DAT at serum dilutions up to 1:1280. The fox was anesthetized by intramuscular injection of a mixture of 0.5 ml (12.5 mg/kg body mass) ketamin (Ketalar® 50 mg/ml, Pfizer Inc., NY) and 0.5 ml (0.25 mg/kg) medetomidine (Domitor® 1 mg/ml, Orion Corp., Turku, Finland). This is five times the recommended dose for immobilization/anaesthesia of arctic fox (Fuglei et al., 2002; Kreeger et al., 2002). Surgical anaesthesia was induced within seconds. Blood was collected by cardiac puncture, the fox was killed by cervical dislocation, and the brain, heart and serum were kept cool but unfrozen during transportation via Norway to the Animal Parasitic Diseases Laboratory (APDL), U.S. Department of Agriculture (USDA), Beltsville, MD. Total transportation time was 16 days. The serum was retested for *T. gondii* antibodies by the modified agglutination test (MAT) as described previously (Dubey and Desmonts, 1987). The fox had a positive MAT in 1:1600 dilution; the MAT and DAT are essentially similar.

2.2. Bioassay in mice

The brain and heart were separately homogenized in a Waring® blender. The heart homogenate and half of the brain homogenate were separately digested with pepsin (Dubey, 1998), the rest of the brain homogenate was kept undigested and these three volumes of processed tissue were divided into 1 ml dosages and injected subcutaneously into three groups of five Swiss Webster (SW) outbred albino mice per group (Taconic Farms, German Town, NY).

On day 29 post-inoculation (p.i.), a blood sample was taken from each mouse and the sera were assayed by MAT at 1:25 dilution. The mice were killed by atlanto-axial dislocation after 32–60 days p.i. To verify *T. gondii* infection, brain smears were made from each mouse and examined for tissue cysts by microscopy (Dubey and Beattie, 1988).

2.3. Bioassay in cats

Skinned and eviscerated carcasses from *T. gondii* infected mice were minced and fed to two *T. gondii* seronegative cats (Dubey, 1995). The mouse brains were homogenized separately with mortar and pestle and mixed with 0.5 ml of saline per brain, and then fed to the cats using a 5 ml syringe. Faecal samples from both cats from days 3–5 were pooled prior to oocyst examination, as were samples from days 6–9. The pooled samples were examined for presence of *T. gondii* oocysts by sucrose flotation and microscopy from days 2–9 after feeding, and when oocysts appeared, they were collected by sucrose flotation and water sedimentation, and sporulated in 2% sulphuric acid (Dubey, 2006).