Neosporosis, caused by the protozoan parasite Neospora caninum, is a frequent cause of bovine abortion worldwide (Dubey, 2003). Dogs are a definitive host of the parasite (McAllister et al., 1998). When identifying definitive hosts of N. caninum, it is essential to avoid confusion with the closely related organism Hammondia heydorni (Ellis et al., 1999). Besides dogs, other canids have been considered to be potential definitive hosts of N. caninum (McAllister, 1999). Antibodies to N. caninum have been found in North American coyotes (Canis latrans), British red foxes (Vulpes vulpes), and Australian dingoes (Canis familiaris dingo; Lindsay et al., 1996; Barber et al., 1997; Buxton et al., 1997). In Texas, Barling et al. (2000) performed a spatial analysis study, and found statistical associations among the density of farmed cattle, seropositivity for N. caninum, and abundance of coyotes and grey foxes (Urocyon cinereoargenteus).

The aim of our study was to determine if coyotes are a definitive host of N. caninum, by feeding them infected bovine tissues and then examining their faeces.

Three newborn dairy bull calves (1, 2, and 3) seronegative for N. caninum at 1:25 dilution by indirect fluorescent antibody test (IFAT), were acquired from the University of Illinois Dairy. The calves were kept indoors and were exclusively fed milk replacer without antibiotics. The three calves were infected during the first week of life with N. caninum. Calf 1 was injected intravenously with 5 × 10⁵ tachyzoites (NC-Illinois strain; Gondim et al., 2002), calf 2 was injected intravenously with a mixture of 1 × 10⁴ tachyzoites of NC-beef strain (McAllister et al., 1998, 2000) and NC-Illinois strain (5 × 10⁵ tachyzoites from each strain), and calf 3 was orally administered 29,000 oocysts of the NC-beef strain via an oesophageal feeder. Calves were euthanised 6–12 weeks after infection. At the time of euthanasia the three calves had N. caninum antibody titres ≥ 1:800 by IFAT. The entire brain and spinal cord, as well as a portion of the heart, tongue, diaphragm, other skeletal muscles and kidney, were cut into pieces of approximately 3 cm³, mixed, and shipped on ice to the Logan Field Station of the National Wildlife Research Center, in Logan, UT.

Four female coyote puppies were used in the experiment. Two littermates (A and B) were infected when they were 8 weeks old, and the other two pups (C and D), from different litters, were infected when they were 12 weeks old. They were born and raised in captivity at the Logan Field Station. The puppies were housed in outdoor pens with their littermates and mothers, prior to study initiation, and were fed a commercially prepared carnivore diet (Fur Breeders Agriculture Cooperative, Sandy, UT). The pups were negative for antibodies against N. caninum at a 1:25 dilution, as determined using an IFAT (Dubey et al., 1988). During the study, animals were housed individually; coyotes A and B...
in indoor kennels, and C and D in outdoor pens. Pups A and B each consumed 1 kg of tissue from calf 1, within a 2-day period (time of first exposure, day 0). Pups C and D refused to eat the infected tissue and were difficult to handle; therefore it was necessary to induce general anaesthesia (using sodium thiopental) on two successive days, in order to administer blended tissues mixed with water, via an oesophageal tube. Pup C was administered a total of 1 kg of mixed tissues from calf 3, and pup D was administered a total of 0.8 kg of mixed tissues from calf 2.

Faecal samples were collected for 4 days before the coyotes were infected with calf tissues, and for 28 days after infection. Faeces were examined by a standard sucrose flotation technique as described earlier (Gondim et al., 2002). No N. caninum-like oocysts were observed in any specimens prior to consuming the infected tissues, although Isospora spp. oocysts were observed in the faeces of 2/4 coyotes before and after infection. Isospora spp. have a direct faecal-oral life cycle (Baek et al., 1993) and were presumed to have been transmitted from the pups’ mothers. One of the four coyotes (C) shed approximately 500 Neospora-like oocysts between 8 and 10 days after infection (Fig. 1A). The unsporulated oocysts were spherical to subspherical and measured 10 \( \mu \text{m} \) in diameter (n = 10). They were aerated in 2% \( \text{H}_2\text{SO}_4 \) as previously described (Gondim et al., 2002). After aeration, sporulated oocysts contained two sporocysts, each with sporozoites (Fig. 1B).

Approximately 150 oocysts were concentrated by sucrose, washed thrice in water, and a final volume of 200 \( \mu \text{l} \) of sediment containing oocysts was obtained. The sediment was suspended with 500 \( \mu \text{l} \) of PBS and ground for 10 min by vortexing in a 1.5 ml tube with 500 \( \mu \text{m} \) glass beads (500 \( \mu \text{m} \) in diameter). The solution was transferred to a new tube, mixed with 600 \( \mu \text{l} \) of a digestion buffer [100 mM NaCl, 10 mM Tris–HCl (pH 8.0), 25 mM ethylenediaminetetraacetic acid, 0.5% sodium dodecyl sulphate], 5 \( \mu \text{l} \) of proteinase K (20 mg/ml), and incubated at 65 °C for 2 h. DNA was extracted by standard phenol–chloroform techniques. This DNA was used to infect cattle, although only slight shedding of oocysts is typical of coyotes, then they could not be an efficient definitive host of this organism. However, the first experimental protocols in

![Fig. 1. Neospora caninum oocysts shed by a coyote (Canis latrans). Bars 10 \( \mu \text{m} \). (A) Unsporulated oocyst. (B) Sporulated oocyst containing two sporocysts.](image1)

![Fig. 2. PCR for Neospora caninum and Hammondia heydorni using DNA extracted from oocysts shed by a coyote (Canis latrans). (1) Vero cell DNA using specific primers to N. caninum (negative control), (2) N. caninum DNA (positive control) showing prominent band at approximately 328 bp, (3) DNA extracted from the coyote oocysts using specific primers to N. caninum, (4) Vero cell DNA using specific primers to H. heydorni (negative control), (5) H. heydorni DNA (positive control) showing prominent band at approximately 270 bp, (6) DNA extracted from the coyote oocysts using specific primers to H. heydorni, (7) \( \Phi \times 174 \) DNA marker (Invitrogen).](image2)
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