Oocysts and high seroprevalence of *Neospora caninum* in dogs living in remote Aboriginal communities and wild dogs in Australia

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**ABSTRACT**

Canines are definitive hosts of *Neospora caninum* (Apicomplexa). For horizontal transmission from canines to occur, viable oocysts of *N. caninum* must occur in the environment of susceptible intermediate hosts. Canids in Australia include wild dogs and Aboriginal community dogs. Wild dogs are those dogs that are not dependent on humans for survival and consist of the dingo, feral domestic dog and their hybrid genotypes. Aboriginal community dogs are dependent on humans, domesticated and owned by a family, but are free-roaming and have free access throughout the community. In this study the extent of *N. caninum* infection was determined in a total of 374 dogs (75 wild dogs and 299 Aboriginal community dogs) using a combination of microscopic, molecular and serological techniques. Oocysts of *N. caninum* were observed in the faeces of two juvenile Aboriginal community dogs (2/132; 1.5%). To estimate *N. caninum* prevalence, a new optimised cut-off of 18.5% inhibition for a commercial competitive ELISA was calculated using a two-graph receiver-operating characteristic (TG-ROC) analysis and IFAT as the gold standard resulting in equal sensitivity and specificity of 67.8%. Of the 263 dog sera tested the true prevalence of *N. caninum* antibodies was 27.0% (95% confidence limit: 10.3–44.1%). The association between the competitive ELISA results in dogs less than 12 month old and older dogs was significant (P=0.042). To our knowledge this is the first large scale parasitological survey of the Aboriginal community dogs and wild dogs from Australia. The high prevalence of *N. caninum* infection in Aboriginal community dogs illustrates that horizontal transmission of *N. caninum* is occurring in Australia. These results demonstrated that *N. caninum* in dogs is widespread, including the semi-arid to arid regions of north-western New South Wales and the Northern Territory. The populations of free-ranging dogs are likely to be important contributors to the sylvatic life cycle of *N. caninum*.

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1. Introduction

Canines (domestic dog, coyote, dingo and grey wolf) are definitive hosts of *Neospora caninum* (McAllister et al., 1998; Gondim et al., 2004; King et al., 2010; Dubey et al., 2011). For horizontal transmission from canines to bovines to occur, the presence of viable oocysts of *N. caninum* must
Fig. 1. Locations of dog sampling sites in Australia. Tiwi Islands (1); Bidyadanga (2); Yuennumu (3); Ti Tree (4); Yarrabah (5); Gooloooga (6); Walgett (7); Collarenebri (8); Inglewood (9); Kempsey (10); Tuggolo National Park (11); Port Macquarie (12); Blue Mountains (13); Milton (14); Tumbarumba (15); Kosciuszko National Park (16). Black circles represent Aboriginal community dogs and white stars represent wild dogs sampling sites.

Besides wild dogs, Australia has a large population of dogs living in Aboriginal communities. Dogs in Aboriginal communities historically consisted of dingoes brought to Australia from South-East Asia 3500–5000 years ago (Corbett, 2001; Savolainen et al., 2004). However Indigenous Australians quickly adopted domestic dogs after their introduction by early European settlers and today, Aboriginal community dogs are mostly domestic dogs or domestic dog-dingo hybrids (Breckwoldt, 1988; Constable et al., 2010). Although dogs in Aboriginal communities are domesticated and owned by a family, they are mostly free-roaming as they are not confined and are allowed free access throughout the community (Brown et al., 2006). These animals are free to interact with wild dogs and cattle wherever their distributions intersect. To date, no study has investigated the presence of *N. caninum* in Aboriginal community dogs.

Given the expected interactions of Aboriginal community dogs with cattle and cattle carrion, and their common distribution with wild dogs, the aims of this study were to determine the extent of *N. caninum* infection in such a dog population using a combination of microscopic, molecular and serological techniques.

2. Material and methods

2.1. Wild dog samples

Samples from 75 wild dogs were collected from eight rural areas in New South Wales and Queensland, between 2006 and 2009 (Fig. 1). Wild dogs were captured or shot by registered trappers and hunters during their normal hunting and trapping activities under New South Wales and Queensland legislation requiring their control for livestock protection and opportunistically donated for this study (no additional wild dogs were killed for this study). Samples collected from wild dogs included blood (*n* = 4), serum (*n* = 14) and faecal samples (*n* = 66), although it was not always possible to obtain all three samples from each wild dog (Table 1). The locations where the animal or samples were collected, and the age and sex of each animal were recorded where possible. All dogs were assigned into one of two age classes, juveniles (≤12 months) or adults (>12 months). Wild dog capture, culling and sampling were carried out according to the relevant State legislation.

2.2. Aboriginal community dog samples

Samples from 299 free-roaming community dogs were collected from eight remote Aboriginal communities in the Northern Territory, Western Australia, north-western New South Wales and tropical north Queensland, between 2000 and 2009 (Fig. 1). All samples were collected as part of dog health programs undertaken within communities and were provided opportunistically for this study. Blood (*n* = 37), serum (*n* = 249) and faecal samples (*n* = 66) were collected from these dogs (Table 1) although it was not always possible to obtain all three samples from each dog. The location of sample collection and the age and sex of the animal were recorded. All dogs were assigned into one of the two age classes described above (see Section 2.1). Dog sampling
was approved by the University of Sydney Animal Ethics Committee.

2.3. Faecal analysis

Faecal samples (n = 132) were examined microscopically for the presence of protozoal oocysts, in particular *N. caninum*-like oocysts, by standard flotation technique as previously described (King et al., 2010). Faecal samples containing oocysts with a diameter of 11 μm (± 3 μm) and morphology similar to *Cystoisospora* spp. oocysts were considered to be *N. caninum*-like oocysts and processed for DNA extraction and PCR.

2.4. Serology for *N. caninum*

Serum samples (n = 263) were screened for *N. caninum* antibodies using a competitive ELISA (cELISA, *N. caninum* Antibody Test Kit – cELISA, VMRD, Pullman, WA, USA), that was previously validated for use in cattle and partially validated for use in dogs (Baszler et al., 2001; Capelli et al., 2006). Sera were considered positive for *N. caninum* when samples presented the manufacturer’s recommended percentage inhibition (%I) cut-off of greater than 30%.

Serum samples (n = 194) were also screened for *N. caninum* antibodies using an indirect immunofluorescence test (IFAT tachyzoite *N. caninum* 12 well slides, FITC anti-canine serum; *N. caninum* canine positive serum, *N. caninum* canine negative serum, VMRD, Pullman, WA, USA) according to the manufacturer’s instructions. Sera were tested for *N. caninum* at the dilution of 1:50 in phosphate buffered saline (pH = 7.4) and only complete peripheral tachyzoite labelling was considered as positive.

2.5. Isolation of DNA, PCR and DNA sequencing

Total DNA was extracted from canid whole blood (200 μL samples) using the Invitrogen PureLink<sup>™</sup> Genomic DNA Kit (Invitrogen, Victoria, Australia) following the manufacturer’s instructions. Extracted DNA from the blood samples was resuspended in a final volume of 200 μL of elution buffer. Dog faecal samples were concentrated prior to DNA extraction by standard salt flotation to enrich for oocysts and DNA extraction was carried out using either the glass bead and liquid nitrogen technique as previously described (Šlapeta et al., 2002a; King et al., 2010) and the Qiagen QIAamp DNA stool mini kit (Qiagen, Victoria, Australia), or using the FastDNA Soil Kit with a Fast Prep-24 Homogenisation System equipped with QuickPrep Adapter (MP Biomedicals, Australia) at a speed setting of 6.0 for 40 s. Extracted DNA from all faecal samples were resuspended in a final volume of 200 μL (Qiagen QIAamp) or 50 μL (MP – FastDNA Soil). PCR was performed on all extracted DNA samples using *N. caninum*-specific primers, Np6plus and Np21plus, that amplify the Nc5 polymorphic region (Müller et al., 1996). Dog faecal DNA samples that exhibited positive bands of the expected size for this marker gene (~330 bp) were validated by amplifying the internal transcribed spacer 1 of the rDNA (ITS1) using the primer pair JS4 and Tim 11 (Payne and Ellis, 1996; Šlapeta et al., 2002b).

2.6. Statistical analysis

Two-graph receiver-operating characteristic (TG-ROC) analysis was used to assess the diagnostic performance of the cELISA results against the IFAT results (Greiner, 1995; Reichel and Pfeiffer, 2002). An optimised %I cut-off value was estimated from the TG-ROC plot, using

**Table 1**

Summary of diagnostic test results obtained for *Neospora caninum* using the dog samples.

<table>
<thead>
<tr>
<th></th>
<th>cELISA &gt; 18.5% cut-off</th>
<th>cELISA &gt; 30% cut-off</th>
<th>Nc5 PCR on blood</th>
<th><em>Neospora</em>-like oocysts in faeces</th>
<th>Nc5 PCR on oocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Tiwi Islands, Northern Territory</td>
<td>16/27 (59%)</td>
<td>7/27 (36%)</td>
<td>3/10 (30%)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>2) Bidadanga, Western Australia</td>
<td>7/13 (54%)</td>
<td>3/13 (23%)</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>3) Yuenemu, Northern Territory</td>
<td>41/151 (27%)</td>
<td>22/151 (15%)</td>
<td>n.a.</td>
<td>1/26 (4%)</td>
<td>1/1 (100%)</td>
</tr>
<tr>
<td>4) Ti Tree, Northern Territory</td>
<td>8/9 (89%)</td>
<td>6/9 (67%)</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>5) Yarrabah, Queensland</td>
<td>9/13 (69%)</td>
<td>3/13 (23%)</td>
<td>0/2 (0%)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>6) Goodooga, New South Wales</td>
<td>10/16 (63%)</td>
<td>8/16 (50%)</td>
<td>0/11 (0%)</td>
<td>0/13 (0%)</td>
<td>n.a.</td>
</tr>
<tr>
<td>7) Walgett, New South Wales</td>
<td>11/13 (85%)</td>
<td>11/13 (85%)</td>
<td>0/12 (0%)</td>
<td>1/16 (6%)</td>
<td>1/3 (33%)</td>
</tr>
<tr>
<td>8) Collarenebri, New South Wales</td>
<td>5/7 (71%)</td>
<td>2/7 (29%)</td>
<td>0/2 (0%)</td>
<td>0/11 (0%)</td>
<td>0/6 (0%)</td>
</tr>
<tr>
<td>9) Inglewood, Queensland</td>
<td>0/7 (0%)</td>
<td>0/7 (0%)</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>10) Kempsey, New South Wales</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>0/1 (0%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>11) Tuggolo, New South Wales</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>2/15 (13%)</td>
<td>0/3 (0%)</td>
</tr>
<tr>
<td>12) Port Macquarie, New South Wales</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>0/2 (0%)</td>
<td>0/2 (0%)</td>
</tr>
<tr>
<td>13) Blue Mountains, New South Wales</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>1/42 (2%)</td>
<td>0/2 (0%)</td>
</tr>
<tr>
<td>14) Milton, New South Wales</td>
<td>1/2 (50%)</td>
<td>0/2 (0%)</td>
<td>0/2 (0%)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>15) Tumbarumba, New South Wales</td>
<td>1/3 (33%)</td>
<td>0/3 (0%)</td>
<td>n.a.</td>
<td>0/4 (0%)</td>
<td>n.a.</td>
</tr>
<tr>
<td>16) Kosciuszko National Park, New South Wales</td>
<td>1/2 (50%)</td>
<td>1/2 (50%)</td>
<td>n.a.</td>
<td>0/2 (0%)</td>
<td>n.a.</td>
</tr>
<tr>
<td>Total (%)</td>
<td>110/263 (42%)</td>
<td>63/263 (24%)</td>
<td>3/41 (7%)</td>
<td>5/132 (4%)</td>
<td>2/18 (11%)</td>
</tr>
</tbody>
</table>

95% confidence interval<sup>a</sup> 36.0–47.9% 19.2–29.5% 1.8–20.1% 1.4–8.8% 1.9–34.1%

n.a. – not available.
<sup>a</sup> Modified Wald method.
the non-parametric method, where sensitivity and specificity were equal ($\theta_0$). The 90% intermediate range was calculated which defines % values outside this range as being at least 90% accurate. The cut-off value that minimises misclassification costs (MCT) was estimated using the IFAT prevalence in the sample population (Greiner, 1996). The TG-ROC and MCT were analysed in the computer software ‘Computational Methods for Diagnostic Tests’ (CMDT, FU Berlin, Germany). True prevalence from apparent prevalence was calculated with Blaker’s 95% confidence interval (Reiczigel et al., 2010). Modified Wald method was used to calculate 95% confidence intervals for proportions (95% CI). The cELISA outcome and parasitaemia for N. caninum were analysed in relation to animal gender and age using a two-sided $\chi^2$ test. Values of $P \leq 0.05$ were considered significant.

3. Results

3.1. Detection of N. caninum oocysts in Aboriginal community dogs

A total of 374 dogs (299 Aboriginal community dogs and 75 wild dogs) were examined for the presence of N. caninum. Dog samples from 16 rural regions of Australia were examined, with numbers of animals per region ranging from 1 to 177 (Fig. 1, Table 1). In total, 132 dog faecal samples were examined microscopically (Fig. 2). Oocysts matching the morphological characteristics of N. caninum were observed in the faeces of two juvenile (1.5%) Aboriginal community dogs (2/132; 95% CI 0.1–5.7%). Low numbers of unsporulated oocysts (~11 μm) were observed in the faeces of a dog from Walgett, New South Wales. Moderate numbers of unsporulated and sporulated N. caninum-like oocysts were identified in the faeces of an Aboriginal community dog from Yuendumu, Northern Territory (Table 1, Fig. 3). These oocysts measured approximately 10–11.5 μm in diameter.

Of these 132 faecal samples examined microscopically, N. caninum specific PCR was run on 18 samples that contained either N. caninum-like oocysts ($n = 2$) or a random selection of faeces with no N. caninum-like oocysts...
observed (n = 16). Both microscopically positive faecal samples for *N. caninum*-like oocysts were confirmed to contain *N. caninum* DNA using Nc5 and ITS1 rDNA. None of the randomly selected faecal samples returned positive amplification.

3.2. Optimisation of cut-off for the cELISA using an indirect immunofluorescence antibody test (IFAT) to detect *N. caninum* in dogs

Sera from Aboriginal community dogs were tested using an IFAT to provide the “gold standard” *N. caninum* infection status of the dogs (Table 4). Of the 194 dog sera tested, *N. caninum* antibodies were determined in 20.6% (n = 40) dogs using a 1:50 dilution in the IFAT (40/194; 95% CI 15.5–26.9%). The same 194 dog sera were assayed using cELISA (VMRD) for the detection of anti-*N. caninum* antibodies and %I was calculated. Using the cELISA, 23.2% (n = 45) tested positive for *N. caninum* using a %I cut-off greater than 30% (45/194; 95% CI 17.8–29.7%). Among the 40 IFAT-positive dogs only 18 were positive by cELISA with 22 dogs presenting as false negatives. Among the 154 IFAT-negative dog sera 27 tested positive using cELISA. Given the low sensitivity and moderate specificity of the cELISA, if dogs testing positive using IFAT are assumed to detect true positives, we attempted to optimise the %I cut-off using this dataset.

The results of the cELISA and IFAT were analysed using the two-graph receiver–operating characteristic (TG-ROC) analysis to optimise the cut-off point and compare it to the kit cut-off (i.e. 30%) suggested by the manufacturer of the cELISA diagnostic assays (Fig. 4A). Non-parametric analyses of the data suggested a cut-off %I of 18.5% for the cELISA, resulting in equal ($\theta_0$) sensitivity and specificity of 67.8%. The 90% intermediate range was −9.5 to 37.7%I (Fig. 4A). The frequency distribution of the %I values showed poor discrimination around the cut-off threshold (Fig. 4B). Using the minimum misclassification cost (MCT), an optimal cut-off with %I of 17.8% in the cELISA was suggested by the data. At this cut-off the sensitivity is 70.0% and specificity is 68.2% (Fig. 4C).

Using the optimised cut-offs the apparent prevalence of *N. caninum* in the dog sera was 39.2% (76/194; 95% CI 32.6–46.2%) and 39.7% (77/194; 95% CI 33.1–46.7%) applying %I of greater than 18.5% and 17.9%, respectively. Therefore, the true prevalence was 19.7% and 20.7% using %I of greater than 18.5% and 17.9%, respectively.

3.3. Seroprevalence of *N. caninum* in Aboriginal community dogs

A comparison of the number of positive and negative samples when using the new optimised cut-off (%I > 18.5%) and the kit cut-off (%I > 30%) of cELISA is presented in Table 1. Of the 263 dog sera sampled, *N. caninum* antibodies were apparently present in 41.8% (110/263; 95% CI 36.0–47.9%) and 24.0% (63/263; 95% CI 19.2–29.5%) dogs using the cELISA with the new optimised and kit %I cut-off respectively (Table 1). The true prevalence of *N. caninum* in the dog sera was 27.0% (Blaker’s 95% confidence limit 10.3–44.1%) and 24.8% (Blaker’s 95% confidence limit 7.5–44.5%) based on the optimised 18.5% and 30% cut-off respectively.

The Aboriginal community dog from Walgett that was PCR positive for *N. caninum* specific DNA in her faeces contained *N. caninum* specific antibodies in her serum. Her female sibling tested during the same sampling period was also seropositive for *N. caninum*. Their dam was tested seven months later and was found to be seropositive for *N. caninum*. However, neither the sibling nor the dam displayed *N. caninum*-like oocysts in their faeces.

There was no significant association between the gender of the animal and the *N. caninum* cELISA assay ($\chi^2$ 1.00, $P \geq 0.05$), with 46.7% (63/135) and 40.4% (46/114) *N.
caninum cELISA positive males and females, respectively. However, the association of the N. caninum cELISA result with the age of the dogs was significant ($\chi^2 4.13, P = 0.042$), with 47.0% (86/183) of adult dogs and 32.9% (23/70) juvenile dogs tested positive in the N. caninum cELISA assay.

3.4. Detection of N. caninum DNA in blood of clinically healthy Aboriginal community dogs

Of the 41 dogs sampled (34 adult and 6 juvenile dogs), only three adult dogs had detectable N. caninum DNA in their blood (Table 1). All three dogs were from an Aboriginal community in the Northern Territory (Tiwi Islands) and one of these dogs had detectable N. caninum DNA in its blood and antibodies in her serum. There was no significant correlation between the gender of the dogs and presence of N. caninum DNA in blood ($\chi^2 0.36, P > 0.05$) nor the age of the dogs and presence of N. caninum DNA in blood ($\chi^2 0.57, P > 0.05$). One out of 20 (5%) dogs with detectable N. caninum DNA in their blood was a female and 2/20 (10%) were males.

4. Discussion

In this study, the relatively high seroprevalence of N. caninum detected in the dogs and the finding that significantly more adult dogs than juvenile dogs were infected with the parasite, indicate that post-natal exposure of this parasite is most likely occurring. The increase in prevalence of N. caninum in dogs with age was recognised in other studies (Wouda et al., 1999; Basso et al., 2001; Wanha et al., 2005) and suggests the dogs become infected through either food or environmental sources. The increase in prevalence of N. caninum in dogs with age (Wouda et al., 1999; Basso et al., 2001; Wanha et al., 2005; this study) suggests that the dogs become infected through their food.

Surprisingly, these results demonstrated that N. caninum in dogs is widespread throughout Australia, including in the semi-arid to arid regions of north-western New South Wales and the Northern Territory. Currently no information exists about the extent of bovine neosporosis in these semi-arid to arid regions (King et al., 2011a). In these areas, cattle populations are almost exclusively beef, stocking rates are commonly low and farm sizes are considerably larger in comparison to the mostly coastal areas of New South Wales and Queensland where N. caninum infection has been commonly recorded (Boulton et al., 1995; Green, 2001; Australian Bureau of Statistics, 2006; Hall et al., 2006; Landmann et al., 2011). The cELISA used in this study was partially validated for dogs, however only moderate agreement of the competitive ELISA with IFAT was demonstrated with 72% sensitivity and 89.3% specificity (Capelli et al., 2006). The IFAT is generally considered 100% sensitive in dog (Dubey et al., 1988). The TG-ROC analysis suggested a cut-off (18.5%) that was lower than the one suggested by the manufacturer (30%), but it was within the 90% TG-ROC confidence interval. The low specificity using the kit cut-off was lower than the one reported by Capelli et al. (2006). The poor agreement between the cELISA and IFAT can be explained by the different antigens these tests recognise. However further investigation is required to understand the biological relevance of the outcomes these tests give.

The presence of N. caninum oocysts in the faeces of Aboriginal community dogs from semi-arid to arid regions indicates that N. caninum is likely to be present in their food supply. Although Aboriginal community dogs are generally owned by a family or considered a family member, they are not confined or tethered and commonly have access to uncooked meat and other food waste, either provided by their owners or obtained through hunting or scavenging (Brown et al., 2006). The presence of Sarcocystis spp. sporocysts in many of the dog faeces examined, including the two dogs that shed N. caninum oocysts, supports our conclusion that these dogs had consumed uncooked meat of some kind, as muscle Sarcocystis spp. cysts are ubiquitous in cows, sheep and macropods in Australia (Munday et al., 1975, 1978; Savini et al., 1994). Scat analysis of community dogs from Yuendumu, Northern Territory indicates that a common dietary component for these dogs is meat from cattle and, to a lesser extent, macropods (C. Brown, personal observation). Our study provides evidence that Aboriginal community dogs may be involved in both the domestic transmission route of N. caninum in Australia (livestock to/from dog), and a sylvatic transmission route (dog to/from marsupial) as previously proposed and experimentally demonstrated using Australian native carnivorous marsupial species (King et al., 2011a,b). Further evidence for the presence of a sylvatic transmission route in Australia is the observation of seropositivity to N. caninum of the wild dog from the Kosciuszko National Park in south-eastern New South Wales. Kosciuszko National Park is a major wildlife refuge where nearby cattle properties are scarce. Antibodies detected in this adult wild dog, suggest that exposure to N. caninum likely resulted from the consumption of natural prey within the area. The prey for this population of wild dogs is more likely to be native or feral animals such as marsupials, rabbits or rodents that are common in the area, rather than meat from cattle (Jenkins and Morris, 2003; Claridge et al., 2009).

Oocysts of N. caninum identified in the faeces of the community dog from Yuendumu, Northern Territory contained six different MS10 genotypes of N. caninum and were able to infect Australian native carnivorous marsupial (King et al., 2011b). Previously, only single MS10 genotypes had been identified in N. caninum oocysts isolated from naturally infected dogs from Germany and Portugal (Basso et al., 2009). Identification of multiple N. caninum genotypes in the oocysts of the Aboriginal community dog faeces from Yuendumu suggests that sexual reproduction within the definitive host has the potential to produce isolates of different pathogenicity to cattle and other animals (Atkinson et al., 1999; Miller et al., 2002; Williams et al., 2007; Al-Qassab et al., 2010). Alternatively the dogs consumed multiple N. caninum genotypes via different animal prey. Mixed genotypes in Toxoplasma gondii were identified in wildlife intermediate hosts that may be involved in a sylvatic transmission cycle of toxoplasmosis (Dubey et al., 2008; Gibson et al., 2011; Wendtea et al., 2011). If this also occurs with N. caninum, then the sylvatic cycle of the parasite may be an important contributor to the genetic diversity and, thus also, the virulence of N. caninum.
To our knowledge this is the first large scale parasitological survey of wild dogs and Aboriginal community dogs from Australia (Jenkins and Andrew, 1993; Thompson et al., 1993; Brown and Copeman, 2003; Palmer et al., 2008). In addition to species of coccidia, intestinal parasites were also prevalent in the examined wild and Aboriginal community dogs, dominated by the presence of hookworm (Ancylostomatidae), Toxocara canis (dog roundworm), Spirometra erinacei (zipper tapeworm) and Taeniidae (tapeworm) eggs. A recent survey of intestinal helminths in Australian pet and refuge dogs from both urban and rural locations demonstrated a low prevalence (9.6%, n = 1,400), with hookworm eggs (Ancylostomatidae) present in 6.7%, eggs of Toxocara canis in 1.1% and Spirometra erinacei in 0.1% of faecal samples (Palmer et al., 2008). We suspect that this difference in parasite prevalence between these two studies is, in part, due to access to unprocessed food and the hunting and scavenging nature of both wild dogs and Aboriginal community dogs, because the life cycles of both hookworms and Toxocara canis can be sustained by paratelic hosts and Spirometra erinacei and taenid cestode life cycles require appropriate intermediate hosts. This implies that for dogs with a free-ranging lifestyle, a diet commonly including these paratelic/intermediate hosts has a major impact on the range and prevalence of parasites they harbour.

In conclusion, our results indicate that N. caninum is prevalent in dogs of eastern Australia where bovine neosporosis is endemic and in the Aboriginal community dogs that were investigated in other parts of Australia. N. caninum is probably similarly distributed elsewhere throughout the rest of Australia. These free-ranging dogs may be important contributors to the sylvatic life cycle of N. caninum as suggested in King et al. (2011a). To effectively determine the risk that wild dogs and other dogs may present to livestock industries through horizontal transmission of N. caninum, a more detailed study focussing on dogs, herbivorous marsupials and cattle in areas where they co-exist is necessary. The prevalence of N. caninum infection in Aboriginal community dogs illustrates that horizontal transmission of N. caninum is occurring in Australia. These dogs may have a role in maintaining both a domestic route of transmission of infection, where the Aboriginal community dog is the source of horizontal infection to livestock, and a sylvatic route of infection, with infected dogs being a potentially important source of N. caninum infection of varying pathogenicity to Australian wildlife and livestock.

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