Current Research in Parasitology & Vector-Borne Diseases 1 (2021) 100005

Contents lists available at ScienceDirect

Current Research in Parasitology & Vector-Borne Diseases

journal homepage: www.editorialmanager.com/crpvbd/default.aspx

Seroprevalence of *Neospora caninum* in dogs from greater Sydney, Australia unchanged from 1997 to 2019 and worldwide review of adult-onset of canine neosporosis

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ARTICLE INFO

Keywords:
Adult-onset
Dog
IFAT
Neosporosis
Seroprevalence

ABSTRACT

Neospora caninum causes disease of the muscle and nervous systems in canine pups and is known for producing disease in older dogs. We aimed to determine anti-*N. caninum* seroprevalence in healthy adult dogs from greater Sydney, New South Wales, Australia and summarise published reports of adult-onset of canine neosporosis. Indirect fluorescent antibody tests (IFAT) were used to detect anti-*N. caninum* IgG and compared to the value from an equivalent 1997 study. The *N. caninum* seroprevalence (anti-IgG, 1:50 serum dilution) was 14.1% (27/192; 95% CI: 9.1–19.0%) demonstrating no significant change compared to 1997 (12%; 18/150; 95% CI: 7.7–18.2%) ($P = 0.58$). A literature search identified 56 published cases of adult-onset canine neosporosis, most associated with neurological or myopathic signs (66.1%, 37/56) or cutaneous lesions (25.0%, 14/56). We confirm that a single IFAT *N. caninum* IgG titre has limited diagnostic value regardless of the cut-off because healthy adult dogs exhibit a range of titres.

1. Introduction

Neospora caninum is an obligate intracellular protozoan parasite causing disease in canine pups, characterised most commonly by hindlimb paralysis (Dubey et al., 1988, b; Dubey et al., 1996). It is best known for being an economically important abortion-causing parasite of cattle (Dubey et al., 2017). The parasite infects a wide variety of animals, but only undergoes sexual reproduction in canids, which are the definitive hosts (McAllister et al., 1998; Gondim et al., 2004; King et al., 2010; Dubey et al., 2011). Dogs may be infected vertically by *N. caninum* tachyzoites *in utero* or at the time of parturition, but post-natal transmission is thought to be more frequent (Barber & Trees, 1998). There is evidence of such vertical transmission in pups from Australia and New Zealand (McAllister et al., 1998; Reichel et al., 1998). The parasite forms tissue cysts, predominately in nervous tissue but may occur in muscles (Dubey et al., 1988; Peters et al., 2001). Tissue cysts, when ingested by the dog, may lead to seroconversion and oocyst shedding (Lindsay et al., 2001).

Clinical canine neosporosis occurs but is infrequently reported in adult dogs (Dubey et al., 2017) and can cause neurological, myopathic and dermatological clinical signs. The factors behind the adult-onset of clinical neosporosis are not known, but recrudescence and reactivation of *N. caninum* is likely triggered by analogous processes as recrudescence of feline toxoplasmosis (Dubey et al., 2017). While not all *Neospora*-seropositive dogs develop clinical signs, accurate information on *N. caninum* seroprevalence in adult dogs is important because it enables veterinary clinicians to make an informed decision when interpreting a positive test result for anti-*N. caninum* antibodies. Seroprevalence of anti-*N. caninum* IgG was established for domestic dogs in Melbourne, Perth and Sydney in 1997 (Barber et al., 1997). Seroprevalence in Melbourne was recently demonstrated to increase from 5% in 1997 to 34% in 2017 (Sloan et al., 2017). This rise in seroprevalence means a higher proportion of dogs at risk of developing adult-onset canine neosporosis as a consequence of recrudescence of *N. caninum*.

The aim of this study was to determine the prevalence of anti-*N. caninum* IgG antibodies in domestic dogs in the greater Sydney region and compare it to historical prevalence for Sydney (Barber et al., 1997). The context of adult-onset canine neosporosis is explained using an historical review of published data reporting cases of canine neosporosis with an adult-onset, based on a reproducible strategy.

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https://doi.org/10.1016/j.crpvbd.2020.100005
Received 6 December 2020; Received in revised form 13 December 2020; Accepted 17 December 2020

2. Materials and methods

2.1. Canine blood sampling

Two sets of blood samples were used in the study. All blood samples were from dogs present in the greater Sydney region, New South Wales, Australia at the time of blood sampling. (i) Excess of canine blood serum samples ($n = 100$) submitted to the Veterinary Pathology Diagnostics Services, The University of Sydney, Australia (VPDS). The VPDS samples were submitted from June 2019 to September 2019 and exclude any dogs that had neurological signs or samples that were submitted for VPDS testing for *Toxoplasma gondii* and/or *N. caninum* serology; all samples were unique dogs. Dogs with neurological signs were excluded, because their inclusion would introduce bias towards dogs being tested for *Neospora* or *Toxoplasma* serology that are submitted to VPDS for such purpose. (ii) Serum from retired racing Greyhounds ($n = 91$) and a Whippet ($n = 1$) were collected as part of an investigation into canine heartworm disease (Orr et al., 2020). The samples were collected between September 2018 and October 2019. All dogs in this cohort were clinically healthy. The samples were obtained under a University of Sydney Animal Ethics Committee-approved project. All serum samples were stored at −20°C. For each sample the following information was provided: age, sex and breed. A sample size of at least 163 was required to assess the apparent prevalence with a precision of 0.05 and confidence level of 0.95, as calculated using EpiTools epidemiological calculators (Sergeant, 2018). The estimate of true prevalence used was 12%, which is the seroprevalence figure reported from 1997 (Barber et al., 1997).

2.2. Indirect fluorescent antibody test for Neospora caninum

Indirect fluorescent antibody test for *N. caninum* (IFAT) was conducted as previously described (Dubey et al., 1988) using an initial 1:50 cut-off. Canine serum was diluted to 1:50 with serum diluting buffer (phosphate-buffered saline, 0.09% sodium azide and 10% adult bovine serum) (VMRD, Pullman, WA, USA) followed by serial double dilution (King et al., 2012; Kwok et al., 2018). Each slide included known positive and negative *N. caninum* IgG dog serum as controls. A FITC-conjugated anti-Canine IgG (VMRD) was used as a secondary antibody (used at neat concentration according to manufacturer's instructions). The slide was viewed at 20× objective using an Olympus BX60 microscope equipped for FITC fluorescence (Olympus, Australia). The serum was considered positive when the tachyzoites had a clear and complete green fluorescent outline.

2.3. Statistical analysis

Four comparisons were made in the statistical analysis: (i) a comparison between the VPDS cohort and the Greyhound cohort; (ii) a comparison between age groups (< 5, 5+ years); (iii) a comparison between males and females; (iv) a comparison between the seroprevalence estimated in 1997 (Barber et al., 1997) and the seroprevalence estimated in this study. All comparisons used the N-1 χ^2 test available on EpiTools epidemiological calculators (Sergeant, 2018). A P-value < 0.05 was considered significant.

2.4. Literature review

A literature review was conducted into adult-onset neosporosis using Scopus, Medline and Web of Science. The databases were searched for "neospor* AND adult AND (dog OR canine)". All fields were searched. Review papers, books and book chapters were excluded. The final search was conducted on 8 May 2020, yielding 675 results. Articles were excluded if the title and abstract made it clear that the article was about (i) a pathogen other than *N. caninum*, (ii) a host species other than the dog, or (iii) about a dog less than 1 year of age. The full-text of the remaining 39 articles was acquired and selected if the full-text was in English, an individual case history was included and a diagnosis of neosporosis was made. On this basis, 11 articles were excluded. The search was enriched by 12 papers referenced by Dubey et al. (2017), it is possible these 12 papers were not labelled as "adult" or "adult-onset" and consequently were not captured by the initial search.

3. Results

3.1. No significant difference in seroprevalence of Neospora caninum between canines visiting veterinary hospital and retired racing greyhounds

The seroprevalence of *N. caninum* (anti-IgG, 1:50 serum cut-off dilution) in greater Sydney dogs was 14.1% (27/192; 95% CI: 9.1–19.0%) (Table 1). There was no significant difference between dogs from VPDS and retired racing Greyhounds, $\chi^2_{(1,\ N=191)} = 2.96$, $P = 0.085$, where the seroprevalence was 10.0% (10/100; 95% CI: 4.1–15.9%) and 18.7% (17/91; 95% CI: 10.7–26.7%), respectively (Table 1). The Whippet was excluded in analyses involving the Greyhound group since it is not a retired racing Greyhound. The Whippet serum had no anti-*N. caninum* IgG activity at a dilution of 1:50.

The dogs from VPDS ($n = 100$) were all over 1 year of age, except one 5-month-old female Labrador (anti-*N. caninum* negative). The median age was 9.5 years, and the mean age was 8.8 years (anti-*N. caninum* positive dogs, $n = 10$, age median 9.6 and mean 9.1 years; 3 females and 7 males). There were 47 female dogs, 52 male dogs and 1 dog with no recorded sex. The retired racing Greyhounds ($n = 91$) were all more than 2 years of age, with a median age of 5 years and mean age of 5.4 years

Table 2
Number of IFAT-positive dogs at various dilutions

Dilution	Total no. of positive dogs	No. of positive dogs from VPDS	No. of positive retired racing Greyhounds
1:50	27	10	17
1:200	22	7	15
1:800	18	7	11
1:3200	9	5	4

Table 1
Summary of results

Group	Seroprevalence (%)	Numerical	95% LCL	95% UCL	χ^2 statistic	P-value[a]
1997	12.0	18/150	7.7	18.2	0.31	0.58
2019	14.1	27/192	9.1	19.0		
VPDS	10.0	10/100	4.1	15.9	2.96	0.085
Greyhounds	18.7	17/91	10.7	26.7		
Age < 5	20.6	11/53	12.0	33.5	0.78	0.38
Age 5+	14.2	16/112	0.09	0.22		
Male	18.5	15/81	0.12	0.28	0.57	0.48
Female	13.4	11/82	0.077	0.22		

[a] χ^2 test.
Abbreviations: LCL, lower confidence level; UCL, upper confidence level.

Table 3
Summary of published adult-onset neosporosis cases

Breed	Age (years)	Sex	Form	Diagnostic method	Outcome	Reference
Labrador Retriever	11	FS	Neurological/myopathic	IFAT titre: 1:3,200 IgG, falling to 1:800 IgG in eight weeks. Polymerase chain reaction (PCR) on cerebrospinal fluid (CSF)	Resolved	Didiano et al. (2020)
Saluki	2	MN	Neurological/myopathic	IFAT titre: 1:800 IgG, falling to 1:400 IgG in eight weeks. PCR on CSF	Partially resolved	Didiano et al. (2020)
Lurcher	10	FS	Neurological/myopathic	IFAT titre: 1:800 IgG. PCR on CSF	Euthanised	Didiano et al. (2020)
English Springer Spaniel	1.6	F	Neurological/myopathic	IFAT titre: 1:800. PCR on CSF	Resolved	Didiano et al. (2020)
Labrador Retriever	2	MN	Neurological/myopathic	IFAT titre: 1:800. PCR on CSF	Partially resolved	Didiano et al. (2020)
Greyhound	4.9	MN	Neurological/myopathic	IFAT titre: 1:800, rising to 1:1600 in eight weeks, falling to 1:100 after six months. PCR on CSF	Resolved	Didiano et al. (2020)
Boxer[b]	7	FS	Neurological/myopathic + cutaneous + generalised	IHC on nervous tissue. Direct microscopy on cutaneous lesion sample. PCR on blood, CSF and skin	Euthanised	Decôme et al. (2019)
German Shepherd[a]	1	MN	Generalised	IFAT titre: 1:400, rising to 1:6,400 in four weeks	Resolved	Newton & Manens (2018)
Labrador Retriever[b]	7	MN	Neurological/myopathic	Protozoa in muscle biopsies. IFAT titre: 1:800	Resolved	Serrano & Freeman (2017)
Cocker Spaniel[a]	10	FS	Cutaneous	IFAT titre: 1:6,400. PCR on skin sample	Died	Mann et al. (2016)
Golden Retriever[a]	10	FS	Cutaneous	IFAT titre: 1:800. PCR on blood. Immunohistochemistry (IHC) on smears from lesions	Resolved	Legnani et al. (2016)
Pointer	3	F	Cutaneous	IHC of punch biopsies. IFAT titre: 1:50	Resolved	Karademir et al. (2016)
American Staffordshire Terrier	12	FS	Myocarditis	Titre 1:20 IgM, 1:80 IgG, changing to 1:10 IgM, 1:40 IgG in two weeks (serological test unknown)	Resolved	Agudelo et al. (2016)
Toy Poodle[a]	4	M	Generalised	IHC on nervous tissue and organs	Euthanised	Magaña et al. (2015)
Greyhound	9.5	FN	Neurological/myopathic	Titre: >1:1,600 (serological test unknown). PCR on CSF	Partially resolved	Parzefall et al. (2014)
Labrador Retriever	2.6	IM	Neurological/myopathic	PCR on CSF. Titre: >1:1,600 (serological test unknown)	Partially resolved	Parzefall et al. (2014)
Cavalier King Charles Spaniel[b]	3	MN	Neurological/myopathic	PCR on CSF. Titre: >1:800 (serological test unknown)	Resolved	Parzefall et al. (2014)
Border Collie[a]	4	FS	Generalised	IHC on liver	Euthanised	Hoon-Hanks et al. (2013)
Greyhound[b]	12	FS	Cutaneous + neurological/myopathic	IFAT titre: 1:80. IHC on skin lesions	Resolved	De Schuyter et al. (2013)
Maltese[a]	6	FS	Cutaneous	IHC and PCR on swabs of cutaneous lesions. IFAT titre: 1:1,600	Euthanised	Gupta et al. (2011)
Rottweiler[a]	2	F	Neurological/myopathic + gastric nodulation	IFAT titre: 1:400. PCR and IHC on muscle biopsy	Died	Gomez et al. (2011)
Shetland Sheepdog[b]	9	FS	Neurological/myopathic	IFAT titre: 1:50. IHC on nervous tissue	Died	Galgut et al. (2010)
West Highland White Terrier	5	MN	Neurological/myopathic	PCR on CSF. IFAT titre ≥1:800	Partially resolved	Garosi et al. (2010)
West Highland White Terrier	9	FS	Neurological/myopathic	IFAT titre ≥1:800	Euthanised	Garosi et al. (2010)
Greyhound	7	FS	Neurological/myopathic	PCR on CSF. IFAT titre ≥1:800	Euthanised	Garosi et al. (2010)
Dachshund	10	MN	Neurological/myopathic	PCR on CSF. IFAT titre ≥1:800	Partially resolved	Garosi et al. (2010)
Labrador Retriever	4	FN	Neurological/myopathic	PCR on CSF. IFAT titre ≥1:800	Resolved	Garosi et al. (2010)
Labrador Retriever	9	MN	Neurological/myopathic	IFAT titre ≥1:800	Partially resolved	Garosi et al. (2010)
Labrador Retriever[b]	1.5	F	Neurological/myopathic	IHC on brain	Euthanised	Garosi et al. (2010)
Standard Poodle[a]	4	FS	Generalised	PCR on liver	Died	Fry et al. (2009)
Rhodesian Ridgeback	7	MN	Generalised	IFAT titre: 1:20,480, rising to 1:81,920 in four weeks	Resolved	Holmberg et al. (2006)
West Highland White Terrier	1	F	Cutaneous + neurological/myopathic	IFAT titre: 1:400,000, falling to 1:20,000 after 6 months. PCR on blood, rectal scrapings and cutaneous nodules	Resolved	McInnes et al. (2006)
Labrador Retriever[b]	4	F	Neurological/myopathic	Tachyzoites in CSF. IHC on brain	Died	Gaitero et al. (2006)
Springer Spaniel[b]	9	IM	Neurological/myopathic	Direct microscopy on lung and liver	Euthanised	Dubey et al. (2006)
Basset Hound	2	M	Neurological/myopathic	Direct microscopy on lung and brain	Died	Dubey et al. (2006)
Basset Hound	5	F	Neurological/myopathic	IFAT titre: 1:800. IHC on nervous tissue	Died	Dubey et al. (2006)
Alaskan Malamute[b]	4	M	Neurological/myopathic	IFAT titre: 1:1,000 IgG. IHC on nervous tissue	Euthanised	Lorenzo et al. (2002)
Labrador Retriever	14	IM	Neurological/myopathic	IHC on nervous tissue	Euthanised	Cantile & Arispici (2002)
Mastiff	3.5	MN	Myocarditis	Direct microscopy, IHC and PCR on myocardium	Died	Meseck et al. (2005)
Rottweiler[a]	4	IM	Cutaneous	IHC on skin biopsies. Agglutination test titre: 1:1,600	Resolved	Ordeix et al. (2002)
Italian Greyhound[a]	9	MN	Cutaneous	IHC on skin lesion biopsy	Resolved	La Perle et al. (2001)
Labrador Retriever[a]	6	FS	Cutaneous + neurological/myopathic	IHC on skin lesion biopsy	Euthanised	La Perle et al. (2001)
Collie[b]	7	M	Neurological/myopathic	Direct microscopy on brain. IHC on nervous tissue. IFAT titre: 1:1,600 IgG	Died	Gondim et al. (2001)
Collie	7	EF	Neurological/myopathic	Titre: 1:800 (serological test unknown).	Resolved	Boydell & Brogan (2000)
Boxer	11	IM	Cutaneous	IHC on skin lesions	Euthanised	Perl et al. (1998)
Rottweiler[b]	3	IM	Neurological/myopathic	IHC on muscle biopsy	Resolved	Thate & Laanen (1998)
Bernese Cattle Dog	5	M	Cutaneous	IHC and electron microscopy on skin biopsy. IFAT titre: 1:640	Resolved	Poli et al. (1998)

(*continued on next page*)

Table 3 (*continued*)

Breed	Age (years)	Sex	Form	Diagnostic method	Outcome	Reference
Siberian Husky[b]	6	F	Cutaneous	IFAT titre: 1:12,800 IgG. Direct microscopy on swab from cutaneous lesion	Euthanised	Fritz et al. (1997)
Labrador Retriever	2.5	M	Neurological/myopathic	IHC on nervous tissue. IFAT 1:51,200	Euthanised	Barber et al. (1996)
Rottweiler[a]	1.3	M	Neurological/myopathic	Titre 1:800 (serological test unknown). Direct microscopy of muscle and nervous tissue	Euthanised	Little (1996)
Rottweiler	2	F	Blindness	IHC on nervous tissue. IFAT 1:12,800	Euthanised	Van Ham et al. (1996)
Golden Retriever	12	FS	Cutaneous	IHC on skin lesions. IFAT titre: 1:3,200	Resolved	Dubey et al. (1995)
Boxer	1.5	F	Neurological/myopathic	IFAT titre: 1:12,800	Euthanised	Knowler & Wheeler (1995)
Cocker Spaniel	2	F	Neurological/myopathic	Direct microscopy and IHC on nervous tissue	Euthanised	Morales et al. (1995)
West Highland White Terrier	11	FS	Pneumonia	IHC on lung sections	Euthanised	Greig et al. (1995)
Basset Hound[b]	10	MN	Neurological/myopathic + generalised	IHC on myocardium	Euthanised	Hoskins et al. (1991)

[a] On immunosuppressive drugs prior to presentation with clinical signs consistent with neosporosis.
[b] Treated with immunosuppressive drugs for clinical signs consistent with neosporosis.
Abbreviations: MN, male, neutered; FS, female, spayed; IM, intact male; EF, entire female; M/F, male/female; neutering not reported.

(anti-*N. caninum* positive dogs, $n = 17$, age median 4.9 and mean 5 years; 13 females and 4 males). There were 51 female dogs and 40 male dogs. The Whippet ($n = 1$) was a 7.8-year-old male.

Results were analysed on the basis of age (< 5, 5+ years) and sex using the *N*-1 χ^2 test. There was no significant association between seropositivity and sex, $\chi^2_{(1,\ N=189)} = 0.57$, $P = 0.45$. There was no significant association between seropositivity and age, $\chi^2_{(1,\ N=192)} = 0.78$, $P = 0.38$.

An equivalent and only study into the *N. caninum* seroprevalence in Sydney is from 1997 (Barber et al., 1997). The *N. caninum* seroprevalence of 12.0% (18/150, 95% CI: 6.8–17.2%) from 1997 is not significantly different to the seroprevalence of the present study ($\chi^2_{(1,\ N=342)} = 0.31$, $P = 0.58$).

3.2. Dogs with no neurological signs had an IFAT anti-N. caninum IgG titres ≥ 1:200

Canine sera that tested positive at 1:50 for anti-*N. caninum* IgG ($n = 27$) were end-titrated. The number of positive animals declined as the samples became more dilute (end-titre range 1:50 to 1:6,400). At a dilution of 1:800, two-thirds of 1:50-positive dogs remained positive (18/27). At a dilution of 1:3,200, one-third of 1:50-positive dogs remained positive (9/27) (Table 2).

3.3. Evidence for adult-onset of clinical neosporosis in published literature

The literature search identified 56 published cases reporting adult-onset of canine neosporosis in 40 publications (Table 3). Cases were classified as neurological/myopathic, cutaneous or generalised. As infectious myositis requires muscle biopsies to definitively diagnose (Evans et al., 2004), it was impossible to reliably distinguish neurogenic signs from myogenic signs retrospectively. On this basis, myopathic and neurological cases were grouped together. Infrequently, cases with unusual clinical signs arose, such as blindness, gastric nodules, pneumonia and myocarditis. Cases with these signs were assigned their own groups.

Neurological or myopathic disease was overrepresented (37/56) in the published cases, followed by cutaneous disease (14/56) (Tables 3 and 4). Cutaneous lesions were typically 0.5–5.0 cm in diameter, multifocal, ulcerative and exudative (Dubey et al., 1995; Perl et al., 1998; La Perle et al., 2001; Mann et al., 2016). Forms of immunosuppression were reported in 44.6% (25/56) of the cases of adult-onset neosporosis, with the remainder having no identified cause of immunosuppression (Tables 3 and 4). Corticosteroids administered therapeutically was reported and associated with the clinical cases of adult-onset of neosporosis in 23.2% (13/56) of dogs (Table 3); such drug administration invariably led to worsening of the clinical signs. A further 12 dogs (21.4%) were on immunosuppressive drugs prior to developing clinical signs of neosporosis (Table 3). Serology was used to aid diagnosis in the majority of cases (39/56). The IFAT for *N. caninum* antibodies was used in 82.1% (32/39) of cases where serology was employed.

Table 4
Frequency of published forms of adult-onset neosporosis

Form	Frequency (%)	Frequency (%) (died/euthanised)
Neurological/myopathic	66.1 (37/56)	56.8 (21/37)
Cutaneous	25.0 (14/56)	42.9 (6/14)
Generalised	12.5 (7/56)	71.4 (5/7)
Myocarditis	3.6 (2/56)	50.0 (1/2)
Pneumonia	1.8 (1/56)	100 (1/1)
Blindness	1.8 (1/56)	100 (1/1)
Gastric nodulation	1.8 (1/56)	100 (1/1)

Note: Some animals exhibited multiple forms of the disease.

4. Discussion

Timely *N. caninum* seroprevalence updates enable clinicians to assess the risk of dogs developing adult-onset neosporosis and make informed judgements when presented with suspected cases. Our Australian study shows there has been no significant change in *N. caninum* seroprevalence in Sydney between our study and the study conducted in 1997 by Barber et al. (1997). We could not confirm the hypothesised increase in the *N. caninum* seroprevalence in Sydney, New South Wales, since 1997, based on a six-fold increase in *N. caninum* seroprevalence recently demonstrated for dogs in Melbourne, Victoria (Sloan et al., 2017). The knowledge of the seroprevalence is of equal value when treating animals with immunosuppressive drugs which is common for a wide variety of non-neosporosis related canine diseases. Seropositive dogs (in our case 14%) carry viable *N. caninum*, thus possess the risk to develop active neosporosis infection as a consequence of reactivation of the parasite.

Veterinary textbooks and published recommendations suggest that a presumptive diagnosis of neosporosis can be made by combining clinical signs with a single positive *N. caninum* IFAT titre of 1:200 or greater (Sherding, 2006; Dubey et al., 2009; Lyon, 2010). The finding that ~80% (22/27; 95% CI: 63.3–91.8%) of 1:50-positive dogs (with no clinical signs of neosporosis) will test positive for *N. caninum* at 1:200 demonstrates that a single titre should be regarded with caution in the diagnosis of clinical neosporosis. In one study it was recommended that 1:800 is a more appropriate titre to use as "*sub-clinically infected dogs are rarely [positive at titres above] 1:800*" (Barber et al., 1997). However, two-thirds (18/27, 95% CI: 47.8–81.4%) of 1:50-positive dogs in our study tested positive for *N. caninum* at 1:800, while healthy animals have tested positive at 1:12,800 in another study (Barber & Trees, 1998). Furthermore, six of the dogs that developed adult-onset neosporosis had IFAT titres less than 1:800 (Poli et al., 1998; Galgut et al., 2010; Gomez et al., 2011; De Schuyter et al., 2013; Karademir et al., 2016; Newton & Manens, 2018), although only Newton & Manens (2018) specified that an end-titration was performed and followed-up with sequential serology.

In analogy with toxoplasmosis caused by *T. gondii* in cats, a rising sequential *N. caninum* IgG titre should be considered to confirm a diagnosis of adult-onset neosporosis (Coelho et al., 2019). Most adult dogs suffering from neosporosis present with neurological signs, while cutaneous and generalised forms of the disease are "less common" (Sherding, 2006; Dubey et al., 2009; Lyon, 2010). Using the published record, cutaneous manifestations represented 25.0% of cases (Table 3), implying that the advice for clinicians on adult-onset neosporosis should include the notion of frequent cutaneous manifestation. Cutaneous manifestation, however, can just be more likely reported, because it is more easily diagnosed (skin biopsy) compared to disease in CNS.

In conclusion, positive anti-*N. caninum* IgG IFAT results occur over a range of titres in the general population of dogs in Sydney, New South Wales, without evidence of clinical neosporosis. A single IFAT *N. caninum* IgG titre cannot be used in isolation to definitively confirm a diagnosis of canine neosporosis and follow-up *N. caninum* IgG titre testing to demonstrate rising titres to *N. caninum* is recommended in a clinical setting. In dogs with clinical signs associated with presumptive neosporosis, rapid and aggressive treatment is imperative regardless of the serology result.

Funding

This research was supported in part by the 2019 Honours Funds, Faculty of Science, University of Sydney.

CRediT author statement

Andrew Barker: Methodology, Formal analysis, Investigation, Writing-Original Draft, Writing-Review & Editing. Denise Wigney: Investigation, Resources, Writing-Review & Editing. Georgina Child: Investigation, Writing-Review & Editing. Jan Šlapeta: Conceptualization, Methodology, Resources, Investigation, Writing-Review & Editing, Supervision, Project administration, Funding acquisition. All authors read and approved the final manuscript.

Data availability

IFAT *N. caninum* IgG data tables are available at LabArchives: https://doi.org/10.25833/88tb-bb87.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Damien Higgins for discussions; and Laura Woolfenden and Katrina Gilchrist, who assisted in laboratory procedures.

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