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Brief Communication

Evidence that *Neospora Caninum* is Identical to the Toxoplasma-like Parasite of Norwegian Dogs

In 1984 *Bjerkås et al.* reported a Toxoplasma-like protozoon (TLP), associated with encephalomyelitis and myositis, in 7 Norwegian dogs. None of the dogs had antibodies against *Toxoplasma gondii.* Structurally, the TLP had more rhoptries than *T. gondii* (*Bjerkås & Presthus* 1988, 1989).

The affected dogs were born to a single dam and came from 3 succeeding litters. Six of the dogs developed ataxia and paresis at the age of 2–5 1/2 months and were subsequently euthanatized as the condition deteriorated. The 7th dog was less affected and was euthanatized at the age of approximately 2 years, after the development of serious spondylosis. The TLP was not infective to mice, but was experimentally transmitted into a blue fox (*Alopex lagopus*). Additionally, a similar parasite was found in stored histological sections from a saluki dog necropsied in 1968 (*Bjerkås & Landsverk* 1986).

Immunoperoxidase studies by means of the avidin-biotin-complex method, using rabbit antiserum raised against *T. gondii,* did not stain the parasite, while there was positive staining with serum from infected dogs (*Bjerkås & Presthus* 1988).

In a retrospective study, *Dubey et al.* (1988a) found a similar parasite in 10 out of 23 dogs with proven fatal toxoplasmosis-like illness in USA and named the parasite *Neospora caninum.* The parasite was then isolated from an infected dog and grown in cell cultures (*Dubey et al.* 1988b). Experimental studies have shown that *N. caninum* is also pathogenic for cats (*Dubey & Lindsay* 1989), mice (*Lindsay & Dubey* 1989a), rats (*Dubey* 1990) and gerbils (*Cuddon et al.* 1991). Recently, Neospora-like parasites have also been associated with neonatal mortality in cattle (*Thilsted & Dubey* 1989, *Barr et al.* 1991), sheep (*Dubey et al.* 1990) and horse (*Dubey* 1990). The tissue cyst wall in both *N. caninum* and the Neospora-like parasites (Fig. 1), including the TLP, is thicker than that of *T. gondii.*

In order to determine whether the TLP and *N. caninum* in USA are really identical the present authors exchanged sections, including some unstained paraffin sections for immunohistochemical staining. In this article we present the results of an immunohistochemical cross-testing and discuss the consistency of the morphological structures.

N. caninum in skin sections from a naturally infected dog (case 10 of *Dubey et al.* 1988a) and in liver sections from an experimentally infected dog (*Dubey et al.* 1988b) were subjected to immunohistochemical staining. The avidin-biotin-complex method was applied according to an earlier report (*Bjerkås & Presthus* 1988, 1989). Serum from a Norwegian dog naturally infected with the TLP was used as primary antibody. Brain and spinal cord sections from naturally infected Norwegian dogs (cases 3 and 5 of *Bjerkås & Presthus* 1989) were similarly stained using rabbit antiserum raised against *N. caninum* according to *Lindsay & Dubey* 1989b). Relevant controls were included. Both parasites were shown to be negative when antiserum raised against *T. gondii* was used. *N. caninum* antiserum reacted positively with both tachyzoites and bradyzoites in sections from

Figure 1. Tissue cyst in a neuron in spinal cord of the calf reported by *Barr et al.* 1991, stained with anti-*N. caninum* serum. Immunoperoxidase procedure. × 750.
Figure 2. Electron micrograph showing bradyzoites in a brain cyst of a Norwegian dog. Note the double-membraned vesicles (arrows) with a "stalk" that is directed towards the inner pellicular membrane. M, micronemes; R, rhoptries. × 26,000.
Figure 3. Electron micrograph showing part of a brain cyst of a Norwegian dog. A micropore (P) can be seen in one bradyzoite. Note numerous very electron-dense granules (A) that are judged to be amylopectin. N, nucleus; R, rhoptry; W, cyst wall. × 15,000.

the Norwegian dogs. Also, *N. caninum* in sections from a dog and a mouse reacted distinctly when serum from a Norwegian dog was applied.

The anti-*N. caninum* serum, raised in rabbits, has been found to be negative in the immunoperoxidase test with a series of protozoan parasites in tissue sections, including *T. gondii,* several *Sarcocystis spp., Besnoitia jellisoni* and *Hammondia hammondi* (*Lindsay & Dubey* 1989b).

The convalescent serum from the Norwegian dog was similarly negative when tested on *T. gondii,* a *Hammondia heydorni*-like parasite, *Sarcocystis spp.* in myocardium of sheep and *Encephalitozoon cuniculi* (unpublished).

The results of the present cross-testing indicate that we are dealing here with identical or closely related parasites. The morphological similarity is also striking (*Dubey et al.* 1988a, *Bjerkås & Presthus* 1989, *Speer & Dubey* 1989), although the ultrastructural descriptions, particularly of the bradyzoites, may indicate minor differences. The main difference may be the micropore, which has been demonstrated in bradyzoites of the TLP (Fig. 3), whereas none has been seen in *N. caninum* in USA. The amylopectin granules were more electron-dense in the TLP (*Bjerkås & Presthus* 1989) than in *N. caninum* (*Speer & Dubey* 1989), although both parasites were fixed in glutaraldehyde (Fig. 3). The difference may depend on different quality of fixation. A "washed-out" image of the amylopectin granules were, however, seen in parasites fixed in formalin (*Dubey et al.* 1988a, *Barr et al.* 1991). Numerous solid rhoptries are a common feature of the parasites considered here (Fig. 2). The numbers of rhoptries in the TLP were, apart from some very high counts in a few zoites, largely within the same range as reported in *N. caninum* in USA. Difficulties in differentiating rhoptries from dense bodies may account for some of the highest counts in the Norwegian parasite.

Barr et al. (1991) reported double-membraned vesicles, that could be continuous with the inner pellicular membrane, in bradyzoites of a *Neospora*-like protozoon in a calf, but found no such vesicles in bradyzoites of *T. gondii.* Similar structures have also been observed in bradyzoites of the TLP (Fig. 2).

Minor structural differences observed in zoites of the TLP and *N. caninum* in USA may be related to differences in fixation procedures as well as stage of parasite studied. It is likely that the discrepancies will be explained when the life cycle of *N. caninum* is discovered.

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