

Canine neosporosis

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Figure 1 Electromicrograph of a small cluster of tachyzoites. Note the residual body still connecting tachyzoites following division by repeated endodyogeny.

KEY POINTS

- Neosporosis is a neuromuscular disease usually characterized by ascending paralysis, although a wide range of clinical signs is possible.
- The disease is most commonly reported in puppies and young dogs, but it may occur at any age.
- Neosporosis occurs worldwide, and there are no breed or sex predilections.
- Serology is the most useful diagnostic test. Immunohistochemical stains of biopsy or postmortem tissues will confirm the finding.
- Appropriate treatment procures functional recovery in many cases.
- Control/prevention is difficult since the life cycle and modes of transmission of *Neospora caninum* (the causative organism) are incompletely understood.

INTRODUCTION

Neosporosis is a relatively recently recognized disease complex, most commonly reported as an ascending paralysis of young dogs, although it can cause a wide variety of clinical signs in dogs of all ages.

A parasite similar to *Toxoplasma gondii* (*T. gondii*) causing paresis was described by Bjerkås, Mohn, and Presthus in three litters of Boxer puppies in Norway in 1984 (1). In 1988 researchers in the USA described a protozoan parasite causing a range of *Toxoplasma*-like illnesses in 10 dogs and named the causative organism *Neospora caninum* (2). The Norwegian parasite was later confirmed as being *N. caninum*. The parasite was isolated in 1988, grown in tissue culture and serological tests were developed (3). In 1989 immunohistochemical methods were described, enabling identification of the parasite in tissue sections (4). There followed many reports of neosporosis from all over the world, particularly in a neuromuscular form of the disease. Retrospective examination of

stored tissue samples revealed that neosporosis was not a new disease but occurred in dogs at least as early as the 1950s (5).

NEOSPORA CANINUM – THE CAUSATIVE ORGANISM

In addition to canine infections, *N. caninum* occurs naturally in cattle (where it is an important cause of abortion), sheep, goats, deer, and horses (causing aborted fetuses and weak foals in older mothers) (6). Experimental infections have been reported in many species (6), including rats, mice, and monkeys (rhesus macaques) (7), but no natural cases have yet been reported in cats or humans (6).

The life cycle of *N. caninum* is not yet fully understood; tachyzoites (the rapidly replicating stage) have been described in many different tissues; bradyzoites (the slowly replicating or latent stage) have been identified only in the central nervous system and the eye, in thick-walled tissue cysts. These stages cannot be differentiated from *T. gondii* by light microscopy. Electron microscopy (**Figure 1**) reveals some subtle differences between the two organisms (8), but unless many zoites are examined, this method is unreliable. *Neospora caninum* is, however, antigenically distinct from *T. gondii*, enabling immunological tests to distinguish between the two parasites. It has been assumed that, as with *T. gondii*, oocysts will be shed by an as yet unidentified definitive host, but experimental infections of cats, dogs, racoons, coyotes, a red-tailed hawk, a turkey vulture, a barn owl, and an American crow did not result in any detectable oocyst production (6).

PREVALENCE

Neosporosis occurs worldwide. Cases have been reported from Europe, USA, Canada, Australia, South Africa, Japan, and Costa Rica (6). There is also serological evidence of infection in equatorial Africa and South America (26). Seroprevalence (of infection, not disease) varies from 0.5% to 17% in Europe (27–30) and has been reported at 2% in the USA (31). Studies in the UK have shown a decline in seroprevalence over the past 10 years (25). Whether such a decline is also occurring in other countries is not known.





Figure 2
Six-week-old Labrador retriever puppy showing rigid hyperextension of the right hindlimb.



Figure 3
Fifteen-week-old Labrador retriever puppy showing paraplegia and rigid hyperextension of both hindlimbs.

SIGNALMENT AND HISTORY

Age

Dogs of any age may develop neosporosis. Most reported cases have involved several littermates, with clinical signs developing at between 2 and 20 weeks of age, but confirmed cases have occurred in dogs as young as 2 days old (9) and as old as 15 years (2). Cases have occurred in isolated individuals, as well as in groups in related dogs. It is also possible that neosporosis may result in stillbirths and abortions/resorptions, but this has not been confirmed.

Breed and sex predisposition

Any breed or type of dog may be affected. Neosporosis has been confirmed in more than 30 breeds, including the Yorkshire terrier, Cavalier King Charles spaniel, West Highland White terrier, Border collie, Springer spaniel, Husky, Great Dane, Bernese mountain dog, and Irish wolfhound. Labrador retrievers and Boxers have been well-represented, but these are very popular breeds.

Cases have been reported from owners with a single pet and by large breeders with many animals, as well as in dogs from both rural and urban areas. No sex predilections have been found. Other concurrent disease, such as canine distemper, is not common in cases of neosporosis, but immunosuppression, either natural or iatrogenic, may exacerbate the disease (6).

CLINICAL SIGNS

Polyradiculoneuritis–myositis

The most commonly reported syndrome involves a hindlimb paresis, which progresses to paralysis, and forelimb weakness with cranial nerve deficits. Death results from the progressive paralysis and meningoencephalomyelitis, heart failure, pneumonia, or euthanasia. The course of the disease is variable, with peracute cases dying within 1 week of the first signs being noticed. In other cases, there is a much more chronic course, in which signs gradually progress over several weeks. Initially owners often notice a bunny-hopping type of gait, a reluctance to jump up or a splaying out of legs when squatting. The hindlimb paresis may be unilateral or bilateral. Paralysis may be flaccid or spastic; in about half of cases a rigid hyperextension of stifle and/or hock develops in one or both hindlimbs (Figures 2 and 3). Incontinence is rare initially but may develop as the disease progresses. Fever and inappetence are rare, with most dogs remaining bright and alert until the later stages (9).

Table 1
Presenting clinical signs of polyradiculoneuritis–myositis syndrome

Principal neurological presenting signs

- Altered proprioceptive and spinal reflexes – for example, placing, patellar reflexes usually weak/absent, occasionally hyperreflexia, pain perception maintained
- Muscle atrophy
- Myalgia, especially quadriceps/lumbar muscles; more rarely neck pain
- Lumbar kyphoscoliosis
- Rigid hyperextension of one or both hindlimbs

Other neurological presentations

- Forelimb paresis (unilateral or bilateral)
- Hemiparesis/quadruparesis
- Ataxia/hypermetria
- Altered behavior
- Central blindness
- Head tilt
- Head nodding/tremors
- Seizures

Table 2
Nonneurological presentations of neosporosis

| <i>Condition</i> | <i>Age of confirmed cases</i> | <i>Presenting signs</i> | <i>References</i> |
|------------------|-----------------------------------|--|-------------------|
| Myocarditis | 2 days–10 months (plus 1 'adult') | Sudden death | 2, 9–12 |
| Pneumonia | 11 years | Cough, lethargy, pyrexia | 13 |
| Dermatitis | 6–15 years | Hemorrhagic, ulcerative, necrotic, pyogranulomatous, multifocal dermatitis | 2, 14, 15 |



Figure 4 *Hemorrhagic, necrotic, pyogranulomatous dermatitis in a 6-year-old Husky.*

Physical examination in most cases reveals signs such as those listed in Table 1, but presentations may be variable and not all signs develop simultaneously. Thus, clinical signs generally progress to involve forelimb paresis/paralysis, depression, altered ocular reflexes (e.g., sluggish pupillary reflexes, anisocoria, squint, ptosis, nystagmus), the inability to open or close the jaw, difficulty in swallowing and dyspnea. At this stage most affected dogs are euthanized (6, 9).

Nonneurological presentations

Occasional cases may present with, or have signs associated with, the heart, lungs, or skin (Table 2 and Figure 4). Pancreatitis, hepatitis, or adenitis may also occur since *N. caninum* tachyzoites have produced necrosis in such organs (2), resulting in clinical signs

such as vomiting and polydipsia as complications of 'neuromuscular cases'.

DIFFERENTIAL DIAGNOSIS

Many other conditions may result in similar clinical signs, including:

- Trauma – for example, could the bitch have stood on the pup or did the dog fall?
- Intervertebral disc disease, including thoracolumbar disc protrusion and caudal cervical spondylomyelopathy.
- Toxoplasmosis.
- Other infectious diseases, such as canine distemper and rabies.
- Congenital/inherited neuropathies, such as progressive axonopathy of Boxers, lysosomal storage diseases, or hypomyelination disorders.
- Granulomatous meningoencephalopathy (GME) and other inflammatory diseases of the central nervous system (CNS).
- Thromboembolic diseases.
- Neoplasia.
- Poisoning – for example, botulism.
- Various kinds of myopathies or myositides, including metabolic myopathies and muscular dystrophies.
- Other causes of myocarditis, pneumonia, etc.

DIAGNOSTIC TESTS

Radiography and **myelography** are often used to rule out differential diagnoses, as are **hematology** and **clinical biochemistry**. There are no specific changes in these tests in cases of neosporosis, although creatine kinase levels are likely to be high due to myositis, and liver enzymes are also frequently raised (9). **Electromyography/nerve conduction studies** may reveal spontaneous activity (positive sharp waves and fibrillation potentials) signifying denervation and/or myositis. **CT/MRI scans** are not yet widely enough available to be used in many cases of neosporosis, but these techniques may be able to detect some CNS changes, including large cysts and areas of inflammation, such as that occurring in toxoplasmosis in humans (16).

Analysis of cerebrospinal fluid (CSF) usually reveals nonspecific changes (elevated total protein content and pleocytosis). However, tachyzoites may be detected in sediment (17), and 'serological' tests can be carried out on CSF. Indirect fluorescent antibody test titers are lower than the corresponding serum titer, but even a titer of 1:50 in CSF is probably diagnostic (6).

Serological tests, such as the indirect fluorescent antibody test (IFAT), are usually used to measure antibodies to *N. caninum*. A titer of 1:50 or more is considered specific for *N. caninum* (6) but is evidence only of exposure, not disease. Virtually all confirmed cases of neosporosis have had high titers (1:800 or more). Although a few clinically normal dogs have had titers up to 1:12,800, a titer of 1:800 or more in a dog with clinical signs is good supportive evidence of neosporosis. Rising titers have not been demonstrated in clinical cases; indeed, most titers fall over a period of weeks following treatment. However, antibodies remain detectable for many months or even years. Test titers will depend on the antigen source, reagents, temperatures, incubation times, and other aspects of the test used, and the above information relates mainly to the IFAT used

at Liverpool University. Other laboratories may have different values for decision-making. Other serological tests, such as ELISA, have been or are being developed – for example, iscom-ELISA (18) – but most of this work relates to testing cattle sera, and many of these tests, such as the whole-tachyzoite ELISA (19), are not transferable to canine sera.

CONFIRMATION OF NEOSPOROSIS

Antemortem biopsies – for example, of muscle or skin (in dermatological cases), utilizing immunohistochemistry, may confirm the diagnosis, as would identification of the parasite within CSF samples, or lung aspirates in cases of pneumonia.

Gross postmortem findings are generally nonspecific; areas of necrosis within the CNS, granulomas in visceral tissues, yellowish-white streaking of muscle (especially diaphragm), and megaesophagus have been occasionally reported. Histopathology, utilizing ordinary stains, may reveal lesions suggestive of neosporosis, and tachyzoites or tissue cysts may be seen (**Figures 5 and 6**), but **immunohistochemistry (Figures 7–9)** is needed to differentiate from toxoplasmosis. Parasites are most likely to be found in sections of brain, spinal cord, and affected muscle but may also be seen in heart, lungs, liver, and/or kidney, especially in the peracute, generalized cases. In contrast to toxoplasmosis, parasites are only rarely identified in spleen and lymph nodes (20).

Electron microscopy is generally not sufficiently sensitive to differentiate reliably between the two parasites. **Culture** of *N. caninum* from biopsy or postmortem tissues into tissue culture or mice would confirm the diagnosis, but such attempts are often unsuccessful.

DNA tests, such as polymerase chain reaction (PCR) techniques (21), are likely to be more widely available in the near future to identify parasite nucleic acid in CSF samples, biopsy material, or tissue sections.

TREATMENT

Many drugs have been tested *in vitro* or in mice, including sulfonamides, dihydrofolate reductase/thymidylate synthase inhibitors, ionophorus antibiotics (e.g., monensin, lasalocid, salinomycin), macrolides, tetracyclines, and lincosamide antibiotics. Many have been found to have some degree of activity against *N. caninum*, although metronidazole, amprolium, paromomycin, and roxarsone had little to no activity against *N. caninum* tachyzoites *in vitro* (6).

In dogs, treatment regimens have largely been extrapolated from those used to treat toxoplasmosis. The drugs and dosages used are indicated in **Table 3**. The drugs may be given singly, or two or even all three drugs may be given simultaneously. Treatment should be instituted as soon as possible when neosporosis is suspected. Since the drugs have few sideeffects and are relatively cheap, treatment may be initiated even before serological test results are available. If the dog is going to respond, there should be some improvement within a few days of commencing treatment. Treatment should continue until the dog has fully recovered or no further clinical improvement is seen. In published cases duration of therapy has varied from 2 to 9 weeks (9, 22).

Supportive treatment such as nonsteroidal antiinflammatory drugs (NSAIDs), antiinflammatory doses of corticosteroids, smooth muscle agonists, plus good nursing care (e.g., manual bladder expression) and physiotherapy are also beneficial.



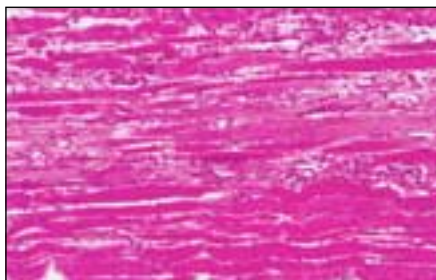


Figure 5 Far left. Myositis in a longitudinal section of quadriceps muscle from a 12-week-old Boxer; a small, elongated cluster of tachyzoites is visible adjacent to a myocyte (H&E stain $\times 100$).

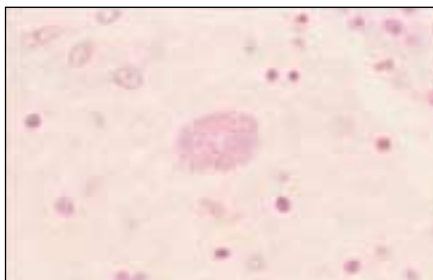


Figure 6 Left. A cluster of tachyzoites within the brain of a Boxer puppy (H&E $\times 100$).

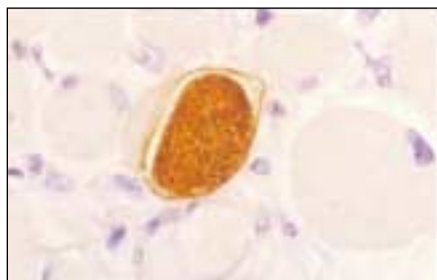


Figure 7 Transverse section of quadriceps muscle from same puppy as in **Figure 6**. The brown-stained tachyzoites are clearly visible within one of the myocytes (immunoperoxidase stain $\times 1000$).



Figure 8 Bradyzoites of *N. caninum* within a thick-walled tissue cyst in the cerebellum of a 2-year-old Rottweiler (immunoperoxidase stain $\times 1000$).

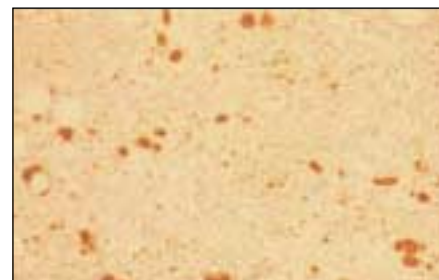


Figure 9 Many small clusters of *N. caninum* tachyzoites within section from a skin biopsy from the Husky in **Figure 4** (immunoperoxidase stain $\times 160$).

PROGNOSIS

About half of appropriately treated dogs might be expected to make a full or functional recovery, although many are left with an odd gait, muscle wastage, or roached back (**Figure 10**). Rigid hyperextension is the sign least likely to be reversed. If this hyperextension is unilateral, amputation of the affected limb may improve the dog's locomotor ability. Peracute and extremely chronic cases are the least likely to respond.

There is anecdotal evidence that relapses may occur, but these generally respond well to a further short course of treatment. There is also evidence that some, generally more mildly affected, dogs make a spontaneous recovery.

There are only limited data available on the **preventative treatment** of seropositive littermates or of bitches during pregnancy to prevent prenatal infection of pups, but such treatments have generally been unsuccessful (23).

CONTROL AND PREVENTION

Vertical transmission of the parasite from a seropositive, but clinically normal, bitch to her puppies was for a long time the only confirmed route of infection in dogs. However, the number of puppies infected in each litter varies from none to all of the litter, with overall only about 20% pups seropositive (24). Fewer than half of these infected pups are ever likely to develop clinical signs of neosporosis (24). Transmission can occur repeatedly over several consecutive litters.

Bitches with an IFAT titer of 1:50 have produced affected puppies, but infections and disease are more likely in pups born to bitches with high titers. Care should be taken in interpreting IFAT results and in the subsequent advice given to breeders. There are legal implications of advising not to breed from a valuable animal when the sensitivity and specificity of the tests used, especially when positive at very low titers, are not known exactly and the risk of producing an affected puppy is actually quite low. Similarly, there may be legal implications if a seropositive bitch is bred from. For example whether resulting puppies should be tested before sale, and whether any results should be disclosed to purchasers when puppies are apparently healthy at the time of the sale. Such matters need

Table 3

Recommended treatment for canine neosporosis

| Drug | Dose |
|--------------------------|------------------------|
| Clindamycin | 11–22 mg/kg bid or tid |
| Potentiated sulfonamides | 15 mg/kg bid |
| Pyrimethamine | 1 mg/kg daily |



Figure 10 The same Labrador retriever as in **Figure 3**, now 11 months old, taken 6 months after completion of a 6-week course of clindamycin. Despite severe muscle wastage and joint deformities, the dog could walk short distances.

careful discussion with the breeders, after which they must decide whether they want to test, breed, or disclose results to anyone else.

Vertical transmission of *N. caninum* also has implications in rare or unusual breeds, including the conservation of wild canids, where the gene pool is small, since one or two seropositive dams could have a major effect on the incidence of the disease in that group of animals. Since congenital infection is far less than 100% efficient, and experimental studies produced infections in cats and mice after oral infection (6), **postnatal infection** was also suspected to occur, and there is now epidemiological evidence that this is so (25).

The route of infection is thought to be through the ingestion of raw meat (especially beef), so it is sensible to advise dog owners to cook meat thoroughly before feeding (including scraped beef used

for weaning puppies). It is also likely that freezing would destroy the parasite, although there is a report in the literature of a successful isolation of *N. caninum* into culture following prolonged freezing of tissues to -52°C (6). It is possible that infection could follow ingestion of oocysts from environmental contamination by a definitive host, but since the identity of such a host is not known, no control measures for this possible method of transmission can be instituted.

Finally, although there is evidence that postnatal infection does occur, it is still not known whether disease in adult dogs is due to a relapse of congenital infection or follows from a recent primary infection.

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