Neospora caninum and neosporosis

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Neospora caninum is a protozoan cyst forming apicomplexan parasite that causes neosporosis, notably in cattle (*Bos taurus*) and domestic dogs (*Canis familiaris*) [1,2]. It is closely related to *Toxoplasma gondii* and has emerged as a major cause of reproductive failure in cattle world-wide [2]. The parasite was first described in a dog with encephalomyelitis and myositis [1] and was later described in calves with myeloencephalitis [3,4]. *Neospora caninum* has been isolated from a variety of animal host species, such as the dog (Nc1;[5] and Nc-Liverpool [6]), cattle (BPA1 and BPA2 [7]), sheep (NC-Sheep [8]) and water buffalo (NCBrbuf-1,2,3,4,5 [9]). Antibodies to *N. caninum* have also been identified in the captive sika deer (Cerus Nippon), mouflon (Ovis musimon), fallow deer (*Dama dama*), moose (*Alces alces*), European bison (*Bison bonasusbonasus* L.), wild rabbits (*Oryctolagus cuniculus*), brown hares (*Lepus europeus*) and dolphin (*Tursiops truncatus*) (reviewed in [10]). Apart from the dog (*Canis familiaris*), other canids have also been considered as potential definitive hosts of *N. caninum.* To date, coyotes [11], wolves (*Canis lupus*) [12] and dingoes [13] have also been named as definitive hosts because they have been shown to shed oocysts after being fed infected tissues, while many other domestic and wild mammal species have been identified as intermediate hosts [10,14].

Horses are an intermediate hosts of *Neospora hughesi* [15], which was identified in an aborted equine foetus [16]. The parasite was later isolated from an 11-year old horse and described as a new species, with molecular differences to *N. caninum* [15]. It is still unknown whether *N. hughesi* is the sole species of *Neospora* that infects horses or if *N. caninum* also plays a part in infection as both species can cross react serologically with each other [17].

The parasite has three asexual infectious development stages, i.e. tachyzoites, bradyzoites and sporozoites [18]. The sexual stages of *Neospora* have not been described, but it is likely that the schizont (asexual) and gamont (sexual) stages also exist in the gut epithelium of the definitive hosts as was shown for *T. gondii* when kittens were fed *Toxoplasma* cysts, since these two parasites are closed related [19]. *Neospora caninum* is regarded as one of the most important infectious causes of abortion in cattle worldwide, yet there are no definitive studies that quantify losses due to neosporosis in the cattle industry; however, losses are estimated to be in the millions of dollars per year [20,21]. The seroprevalence of *N. caninum* was found to be approximately 26% (PhD thesis, Patrick Craig) and has been estimated at approximately 12.9% in the UK [22], whereas it was as low as 2.8% in Sweden [23] and reached 55.9% in Romania [24], 14-40% in the Americas [25], 6-36% in Asia [26] and 6-21% in Oceania [27].

The two major routes of *N. caninum* transmission are horizontal, where cattle ingest sporulated *N. caninum* oocysts and vertical transmission, which includes transmission of the agent to the foetus during pregnancy, either following reactivation of bradyzoites in the infected dam or *de novo* infection of the dam during pregnancy [28]. Vertical transmission contributes significantly to the persistence of *N. caninum* in a herd by propagating the infection to successive generations. The prevalence of congenital infection varies with reported infection rates ranging from approximately 40% to 95% [29-33]. The shedding of oocysts by infected canids in cattle-feeding areas is a likely cause of horizontal transmission that could play an important role in infected herds. Cow-to-cow transmission has not been observed to date, but many authors have looked at the possibility of infection via contaminated semen from infected bulls [34-37]. However, artificial insemination of cows with semen *in vitro* contaminated with *N. caninum* tachyzoites (6.5 x 10^7 and 1.8 x 10^7 on day one and two, respectively) failed to induce infection [34]. Although one animal developed a low antibody titre of 1:80 in the direct agglutination test at day ten after insemination, it was negative after 45 days, which shows that the parasites were able to stimulate the immune system of this animal without causing infection.

The pathogenesis of *N. caninum* induced abortion in cattle is yet to be fully elucidated. *Neospora caninum* infection in cows is mainly manifested in the placenta and foetal tissues following a maternal parasitaemia. Experimental studies have shown that infection of pregnant cows in early gestation leads to foetal death, which could be due to both extensive placental necrosis and necrosis in foetal tissues, such as the liver and brain [38-44]. Changes observed in first trimester foetuses include necrosis and apoptosis in the CNS (brain and spinal cord), liver, lung, kidney, spleen and thymus. The parasite seems to replicate in the heart without causing cell death (studied extensively in PhD thesis, Chapter 3). In foetuses following infection of the dams in mid to late gestation, mild to moderate histological changes are detected in foetal tissues and are restricted mainly to the CNS [38].

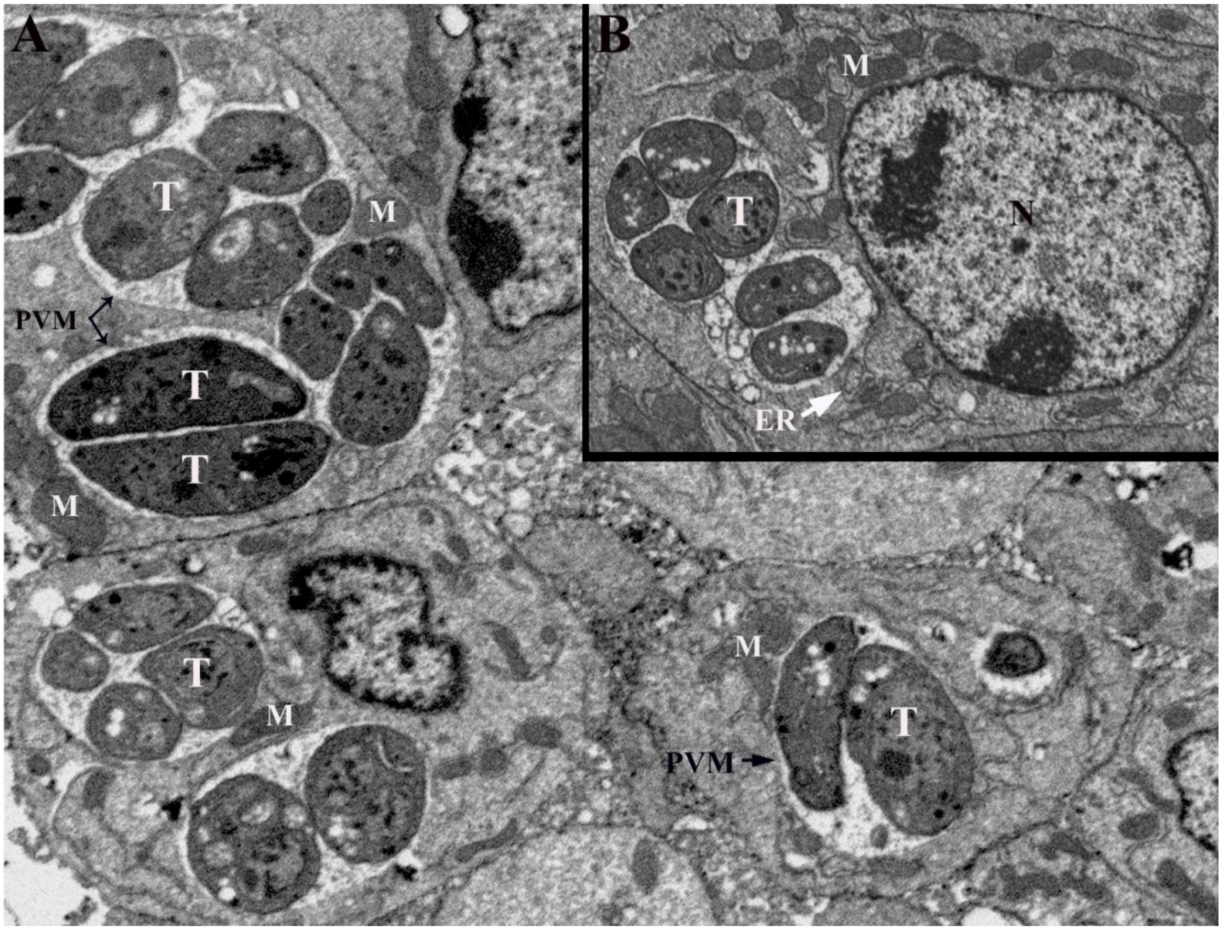
The host immune response towards *N. caninum* is of paramount importance in controlling parasite multiplication. *Neospora caninum* is an obligate intracellular pathogen, which means that cell mediated immune responses are likely to play a pivotal role in protective immunity, by reducing the multiplication of parasites within the host, hence reducing parasitaemia [45,46]. Protective immunity to intracellular pathogens is associated with type 1 T helper cells (Th1 cells), which secrete pro-inflammatory cytokines, such as interferon gamma (IFN-γ), tumour necrosis factor alpha (TNF-α) and interleukin (IL)12 [47]. The Th2 type anti-inflammatory cytokines, namely IL-4, and regulatory cytokines which includes IL-10 and transforming growth factor beta (TGF-β) are produced at the foeto-maternal interface in the placenta and can counteract the effect of the pro-inflammatory cytokines [48]. The Th2 cytokines are associated with implantation of the foetus and maintenance of early pregnancy by suppression of local inflammatory responses [49,50].

For the diagnosis of bovine neosporosis, clinical history, epidemiological data, information about the abortion pattern and foetal age are important factors that should be considered [51]. The definitive diagnosis of neosporosis can be very difficult, because infection does not always result in abortion and even demonstration of *N. caninum* infection, both histologically and immunohistologically, does not give conclusive evidence that the parasite is the cause of the abortion [52]. With regards to post-mortem diagnosis in aborted foetuses, the ideal diagnostic samples include both the aborted foetus and the placenta, together with sera from the dam. If this is not feasible, samples from brain, heart and liver of the foetus should be submitted [51]. The brain is the most reliable tissue for the diagnosis, but the probability of diagnosing the infection increases when other tissues, such as heart and liver, are analysed [51]. Histology has been the most commonly used method for parasite detection initially, but immunohistological detection of parasites in foetal brain, lung, liver and heart has been used to confirm the presence of the agent [53,54]. The PCR technique also plays an important role in the diagnosis of *N. caninum* infection when used in aborted foetal tissues [55-58] and other samples such as amniotic fluid [59] and cerebrospinal fluid [60,61]. The advantage of the PCR is its high specificity and high sensitivity and therefore the ability to detect small amounts of *N. caninum* DNA in a large quantity of tissue; PCR also works well when foetal tissues are autolysed, which is often the case with *Neospora* abortions [51]. Serological tests have the advantage that they can be applied *intra* *vitam* and are suitable techniques for processing large numbers of samples [62]. There is a variety of serological assays available for the detection of *N. caninum* antibodies in cattle. The immunofluorescent antibody test (IFAT) has been widely used to detect *N. caninum* specific antibody in maternal serum or foetal fluids. In addition to the IFAT, a number of *N. caninum* specific enzyme-linked immunosorbent assays (ELISAs) have been described, which utilise either whole fixed *N. caninum* tachyzoites, aqueous or detergent-soluble tachyzoite extracts, tachyzoite antigens incorporated into immunostimulating complexes (iscoms) or recombinant tachyzoite antigens [63-67].

Seropositivity to *Neospora caninum* in cattle of different age groups from three different locations in Jamaica (sampled in 2012)

Age groups	Number of animals	Positives (%)	Negatives	Pregnant (%)[a]
0-2	16	5 (31.3)	11	4 (25.0)
3-5	229	53 (23.1)	176	43 (18.8)
6-8	151	33 (21.9)	118	26 (17.2)
9-11	66	22 (33.3)	44	10 (15.1)
>12	30	13 (43.3)	17	4 (13.3)
unknown	7	2 (28.6)	5	0 (0.0)

[a] overall number of pregnant cows sampled



Ultrastructural features of *Neospora caninum*-infected human HepG2 cells cultured in vitro with 95% O_2 and 5% CO_2. **A**. *Neospora caninum* tachyzoites (T) are present in hepatocytes with numerous mitochondria (M) that are predominantly located in close contact with the parasitophorus vacuole membrane. **B**. Hepatocyte with two parasitophorus vacuoles filled with tachyzoites (T). Numerous mitochondria (M) are located in the perinuclear region and in association with the PVM. Lead citrate/uranyl acetate staining, FIB-SEM Nanotomography. (This photomicrograph is the sole property of Patrick Craig and the University of Liverpool).

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