

Control options for *Neospora caninum* infections in cattle – current state of knowledge

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Abstract

This article reviews control options for *Neospora caninum* infection and abortion in cattle, drawing on published literature and the authors' own research in this field. Apart from the successful use of embryo transfer to prevent congenital infection in calves born to infected cows, there are currently no accepted control methods for the prevention of abortions in cattle. The epidemiological data at hand suggest that concomitant infections with bovine pestivirus increase the risk of abortion significantly and that these infections, for which effective vaccines exist, should therefore be controlled. While vertical transmission appears to be the major route of infection in cattle, there is also a role for postnatal transmission, involving a definitive host. Presently, the control of dogs and their access to bovine tissues, particularly potentially infected placentae and other foetal tissues, appear to be the most prudent control methods. There are some indications that vaccination against *N. caninum* may aid in the prevention of abortions. Suggestions for control options are limited by our current lack of actual experiences with control strategies. Further practical fieldwork is needed in this area.

KEY WORDS: *Neospora caninum*, abortion, cattle, epidemiology, immunology, control

Introduction

Neospora caninum is a protozoan parasite that was first described in a litter of dogs in Norway in 1984 (Bjerkås et al 1984). Today it is recognised worldwide as an infection, predominantly, of dogs and cattle (Dubey and Lindsay 1996). Other species such as sheep, goats, deer and horses (Dubey and Porterfield 1990; Dubey et al 1990b; Barr et al 1992; Woods et al 1994) have also infrequently been reported to be naturally infected. Infection has been reported from retrospective examination of stored tissues from dogs and cattle, dating back to 1957 (Dubey 1992) and 1974 (Dubey et al 1990a), respectively.

Since its recognition as a new parasite entity, *N. caninum* has emerged as a major cause of abortion in cattle. The economic impact of *N. caninum* infection in cattle has been estimated at

Key Points

- *Neospora caninum* is recognised as a major cause of abortions in (dairy) cattle around the world.
- Control options have rarely been explored and hitherto only embryo transfer has been successfully applied, resulting in uninfected calves born from infected dams.
- Treatment appears to be uneconomical in cattle, but better knowledge of the interaction between parasite and host during pregnancy might yet identify a period for strategic, preventive treatment of short duration.
- Test-and-cull, and replacement policies hinge on highly sensitive assays for which cut-off thresholds may need to be redefined.
- Infection with bovine pestivirus appears to increase the risk of *Neospora* infection threefold, hence control of pestivirus infection by means of vaccination seems indicated where cattle are at high risk from *Neospora* infection.
- Recent research indicates an association between *Neospora* abortions and dogs on farms – it seems prudent to advocate that dogs and cattle should not mix on dairy farms.

AUS\$85 million per annum for the dairy and AUS\$25 million for the beef cattle industry in Australia (Ellis 1997), and NZ\$17.8 million for the dairy industry in New Zealand (Pfeiffer et al 1998). In comparison, Dubey (1999b) summarised the economic losses in California to be US\$35 million/year. The true costs are probably higher, since these calculations only took account of losses in animal and milk production after abortion outbreaks of epidemic proportions. Sporadic and low-level endemic abortions

BALB/c	Bagg albino inbred mice
BVDV	Bovine viral diarrhoea virus
IFAT	Indirect fluorescent antibody test
IFN- γ	Interferon gamma
IgG	Immunoglobulin G
IL-4	Interleukin 4
NcSAG1	<i>N. caninum</i> surface antigen 1
NcSRS2	<i>N. caninum</i> surface antigen-1 related sequence 2
Th1	T cell helper 1

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also occur (Thornton et al 1991; Boulton et al 1995; Wouda et al 1997), but their costs and relative importance are less well defined.

Cattle with serum antibody responses to *N. caninum* are thought to be chronically infected. A study from the United States of America reported a decrease in milk yield in seropositive cattle (Thurmond and Hietala 1997). Recently, Barling et al (2000a) demonstrated that seropositivity was associated with significant reductions in average daily weight gain and liveweight at slaughter. Serologically positive animals also had higher veterinary treatment costs and showed significantly lower economic returns (Barling et al 2000a). Trees et al (1999), in their review, discussed additional costs such as early foetal death, stillbirth, neonatal death, and reduced breeding value. Early culling would further increase the cost of *N. caninum* infection to primary producers (Thurmond and Hietala 1996). Because of our poor knowledge of their relative contribution, the total cost of these factors remains to be quantified.

More than 10 years have passed since the realisation that *N. caninum* is a major cause of abortion in cattle and, less frequently, of disease in dogs. The general structure of the life cycle was elucidated recently and some of the modes of transmission clarified. Dogs were demonstrated to be a definitive host of *N. caninum*. Oocysts of *N. caninum* were excreted by experimentally infected dogs, if only in low numbers (McAllister et al 1998; Lindsay et al 1999a). Vertical transmission of infection occurs both in dogs and cattle (Paré et al 1994; Barber et al 1998). In the latter species it appears to be the main pathway for infection from one cow to the next (Paré et al 1996; Schares et al 1998). However, epidemiological investigations point to postnatal infections with *N. caninum* as the cause of abortion storms (McAllister et al 1996). Antony and Williamson (2001) and other authors (Thornton et al 1994; Schares et al 1999) have suggested that postnatal infection occurred more often in New Zealand than is reported from other parts of the world.

Diagnosis and diagnostic techniques have been improved and optimised (Atkinson et al 2000). Control of *Neospora* infection or prevention of abortions is the critical issue for primary producers. Control or treatment options have been discussed in the literature, but rarely tried in the field. This paper discusses possible strategies for the control of *N. caninum* infection and/or abortions in cattle in the context of the current state of knowledge about the parasite, its life cycle and epidemiology, and makes recommendations for its control.

Control of infection

Treatment options

Presently, only dogs with clinical signs of neosporosis receive treatment. Barber and Trees (1996) reviewed 27 clinical cases and their treatment. In vitro data suggest that a number of chemicals might be effective (Lindsay and Dubey 1989b; Lindsay et al 1994, 1996, 1997), and in dogs the drugs of choice appear to be clindamycin (Dubey et al 1995) and potentiated sulphonamides.

In vivo data for cattle are few. Gottstein et al (2001) recently presented data that suggest that the severity of clinical outcome of experimental infection with 2×10^8 tachyzoites of *N. caninum* in calves could be modulated by treatment with toltrazuril and

ponazuril at 20 mg/kg/day. However, the economics of treating cattle are questionable. The fact that treatment could only be used as a preventive measure and hence be long-term, might do little to curb an ongoing abortion epidemic. Additionally, it would result in considerable and likely unacceptable milk or meat residues or withdrawal periods, which would be further restrictive. Also, the treatment was directed at a tachyzoite challenge, whereas it is doubtful that encysted bradyzoites are as susceptible to chemical treatment.

Guy et al (2001) reported a rise in antibody response in pregnant cows, and thought this was due to recrudescence of the parasite resulting in congenital infection of the calves. Innes et al (2001b) demonstrated a transient immunosuppression during gestation. Thus, chemical treatment of very limited duration during pregnancy might be efficacious, assisting the dam's immune system during this period of immunosuppression. The aim of such treatment would be to prevent recrudescence of the parasite. However, just when in relation to stage of pregnancy such a treatment would need to be administered to be effective is uncertain and more research is needed before the practicalities of this option can be evaluated.

Control of concomitant infections

It has been reported that *N. caninum* infection on its own increases the risk of abortion in cattle by a factor of at least three (Moen et al 1998; Wouda et al 1998). In addition, several reports suggest that concomitant infection of *N. caninum*-infected cows with bovine pestivirus increases the risk of *Neospora* abortions a further threefold (Pfeiffer et al 2000). A Swedish study (Björkman et al 2000) has also reported a significant association between bovine viral diarrhoea virus (BVDV)-infection in cattle, *Neospora*, and abortion risk. Conversely, a study from Spain could not find any increased risk of abortion in dairy cows that were infected with both BVDV and *N. caninum* (Mainar-Jaime et al 2001). It is hypothesised that infection with BVDV or other potentially immunosuppressive agents such as mycotoxins might lead to or facilitate a recrudescence of a chronic *N. caninum* infection. Recrudescence could result in parasitaemia, and then transplacental infection of the foetus, foetopathy, and ultimately abortion. Depending on the timing of the parasitaemia (Guy et al 2001), it may also result in congenitally-infected asymptomatic calves.

Effective vaccines for the control of bovine pestivirus exist (Brownlie et al 1995). However, there are currently no reports of the effect of controlling pestivirus-infection, whether by vaccination or other means of control (Mainar-Jaime et al 2001), on the incidence of *N. caninum* infections/abortions in cattle. Such an approach might decrease the risk of abortion due to *N. caninum*, and further research is clearly warranted in this area.

Control of the route of transmission

Vertical transmission

There is a large body of evidence suggesting the predominant route of infection with *N. caninum* is via vertical transmission, namely from dam to daughter in utero. Studies in several countries have provided strong evidence that this mode of transmission may be very efficient (Björkman et al 1996; Paré et al 1996; Schares et al 1998; Davison et al 1999), resulting in infection of more than 80%, and up to 95%, of offspring of seropositive dams. Some

more recent studies have reported that the efficiency of vertical transmission may be lower than 50% (Bergeron et al 2000; Dyer et al 2000).

Test and cull

It has been proposed that infection and hence abortion could be controlled by testing cows and culling those that are seropositive. Culling infected cattle and replacing them with uninfected animals hinges on the ability of serological tests to accurately identify animals according to their true infection status. Several serological tests have been validated over recent years and these have been recently reviewed by Atkinson et al (2000). Care must be taken to select the performance characteristics of any test for maximum sensitivity, in order that all infected individuals are removed from the herd and only non-infected replacements bought in. Serological responses are highest at the time of abortion and then decline (Conrad et al 1993; Cox et al 1998). Commercial tests are optimised for the diagnosis of *N. caninum* abortions, and tend to use more conservative (higher), highly specific threshold values. For a test-and-cull-policy these thresholds would have to be adjusted downwards to maximise sensitivity, so that all infected cattle in a herd could be reliably identified (Reichel and Pfeiffer 2002).

Testing and culling, as proposed and modelled by Thurmond and Hietala (1995), and French et al (1999), may be an option for herds that have a high seroprevalence of infection, if the predominant mode of transmission is vertical. Even in herds where the seroprevalence exceeds 20%, as is frequently reported (Jensen et al 1999), it should be possible to replace culled animals with non-infected animals over a number of years, provided there is no postnatal transmission.

The general consensus is that repeat abortions are a rare event in cattle and that <5% of cows that have aborted once abort again (Innes et al 2000). There is also experimental evidence suggesting that previous infection with *N. caninum* may protect foetuses against an otherwise lethal challenge with *N. caninum* tachyzoites (Williams et al 2000; Innes et al 2001b). It is unlikely that chronically infected dams that have previously aborted will abort again. Thus, culling may eliminate those animals from the herd that have established a strong immunity. If postnatal transmission is thought to present a high risk in the herd, it may be advisable to keep such cows in the herd, as they provide the strongest protection against subsequent challenge from the parasite. The benefits of retaining those animals in the herd in terms of protecting against abortions, however, need to be carefully balanced against the known lower production (e.g. milk) of infected cows (Thurmond and Hietala 1997; Barling et al 2000a).

Embryo transfer

Congenitally infected calves appear to become infected late rather than early in gestation. Recrudescence of *N. caninum* infection leading to infected calves also occurred late in gestation in some of the experiments reported by Guy et al (2001). Protecting calves from congenital infection by embryo transfer into uninfected recipient cows has been tried successfully. This resulted in seronegative offspring born to seropositive, infected dams (Baillargeon et al 2001). As this is an expensive way of breaking the life cycle of *N. caninum*, it will most likely be reserved for valuable dams and their embryos.

Postnatal transmission

Control of dogs and their access to bovine tissues

While the main route of bovine infection appears to be vertical,

transmission between cattle and their offspring by means of foetal fluids, and from cattle to dogs via ingestion of infected tissues, has been suggested. There is epidemiological evidence linking the presence of dogs to abortion epidemics in cattle.

McAllister et al (1998) demonstrated that dogs are a definitive host for *N. caninum*. While many cattle appear to be born congenitally infected, and hence may not get infected by oocysts produced by a definitive host, there is evidence that point-source infections may have caused some of the abortion epidemics that have been recorded (Thornton et al 1994; McAllister et al 1996). Mathematical modelling also supports the notion that vertical transmission alone is not sufficient to maintain infection in a population (French et al 1999). Investigations in North America and Europe suggest an association between serological status or infection of cattle and the presence and density of domestic and wild canids (Paré et al 1998; Wouda et al 1999; Barling et al 2000b). It thus seems prudent to restrict numbers of dogs and their association with cattle and access to bovine tissues, especially foetal, placental and aborted tissues. Dijkstra et al (2001) demonstrated that dogs shed *N. caninum* oocysts after they had been fed placentae from cattle naturally infected with *N. caninum*. However, others (Bergeron et al 2001) have argued that the presence of *N. caninum* in the bovine placenta is rare. The dogs in the study by Dijkstra et al (2001), and some dogs in the earlier transmission experiments conducted by McAllister et al (1998) and Lindsay et al (1999a), did not seroconvert, and hence were impossible to identify as a potential source of infection for cattle. This is not dissimilar to the situation with *Toxoplasma gondii*, in which not all infected cats seroconvert (Dubey et al 1977; Ruiz and Frenkel 1980). This suggests that all dogs must be considered a potential source of *N. caninum* infection for cattle, whether or not they are serologically positive.

The role of colostrum as a potential source of infection for cattle and dogs is equivocal. Infection in calves was based on demonstration of the parasite (Uggla et al 1998), or on the production of antibodies (Davison et al 2001), whereas other authors (Dijkstra et al 2001) provided no evidence that dogs may be infected in this way.

Dijkstra et al (2002ab) have reported epidemiological associations between abortions and postnatal infection, and the presence/arrival of dogs on farms. In controlled epidemiological studies, access by dogs to foetal fluids and placental tissue appeared to be linked to postnatal transmission in cattle. However, little direct evidence exists that cows may be infected in any way other than by the well-documented vertical route of transmission. Trees et al (2001) reported experiments in which three cows were each fed approximately 600 *N. caninum* oocysts but apparently did not become infected. The number of oocysts shed by experimentally infected dogs always remained very low (McAllister et al 1998; Lindsay et al 1999a) and it is difficult to understand how such low faecal shedding of oocysts could cause the abortion outbreaks observed in cattle.

Vaccination approaches

A vaccine that is claimed to aid in the control of abortions associated with *N. caninum* in cattle (Bovilis Neoguard, Intervet, Auckland, New Zealand) has recently been released and is currently the subject of field trials in New Zealand. However, to date there are no published data on which its efficacy can be assessed.

Previous attempts to formulate a vaccine against *N. caninum* have met with limited success (Dubey 1999a). It is generally considered important in the control of protozoal infections to elicit an effective T cell helper-1 (Th1) -type response, which is evident in experimental *N. caninum* infections in cattle (Williams et al 2000). However, a very strong Th1-type response might be incompatible with a successful outcome for pregnancy (Raghupathy 1997), as it may lead to rejection of the developing foetus. Thus, the generation of a strong and successful Th1-response by vaccination needs to be compatible with the successful continuation and completion of gestation in the vaccinated dam. Successful vaccines have been introduced for some coccidian infections, such as the live attenuated vaccine Toxovax™ for *T. gondii* and the protective immunity-inducing antigens in the case of *Eimeria* (Wallach 1997). These might be examples of the direction to take in the development of a potential vaccine for *N. caninum*.

Several experimental studies (Williams et al 2000; Innes et al 2001b) and reports from the field (McAllister et al 2000) support the view that protective immunity develops in chronically infected dams. This is further supported by observations that repeat abortions due to *N. caninum* in cattle are rare (Anderson et al 1995). It has also been demonstrated that the time of challenge with *N. caninum* in relation to stage of gestation is an important determinant of the outcome of infection. Infection at 70 days gestation in naïve cows reliably resulted in foetopathy, whereas infection at 30 weeks resulted in congenitally infected, clinically asymptomatic calves (Williams et al 2000; Innes et al 2001b). Cows chronically infected with *N. caninum* and challenged at 10 weeks gestation did not abort (Guy et al 2001). One might therefore expect that vaccination could be successful in either preventing abortions or infection, or both.

Most research to date has been conducted using animal models such as dogs, mice, sheep and cows, and as some of those models, especially those involving non-target species, might not reflect the natural situation, results should be treated with caution.

Liddell et al (1999) vaccinated female BALB/c mice with a crude *N. caninum* tachyzoite lysate preparation co-administered with ImmuMAXSR™ adjuvant. The mice were subsequently mated, and pregnant dams were challenged with *N. caninum* tachyzoites at 10–12 days gestation. Results demonstrated that this single inoculation appeared to confer complete protection against vertical transmission of infection to the offspring. All pups in the experimental group were free from parasitic infection. No results have yet been reported on the efficacy of this vaccine formulation in cattle. Baszler et al (2000) examined the possibility of vaccination of BALB/c mice with a soluble *N. caninum* antigen either trapped in nonionic surfactant vesicles or formulated with Freund's Complete Adjuvant. This approach, however, resulted in exacerbation of encephalitis and neurological disease in the mice. Observations were characterised by increased antigen specific interleukin-4 (IL-4) secretion and increased IgG1:IgG2a ratios in vivo, possibly suggesting an inappropriate immune response was generated in that experiment.

Andrianarivo et al (1999) tested four different adjuvants and a killed whole *N. caninum* tachyzoite preparation for immunogenicity in cattle, and compared these with responses to experimentally-induced infection with culture-derived tachyzoites. The humoral immune responses, determined by indirect fluorescent antibody test (IFAT) titres, were always

significantly lower in immunised cattle than the experimentally infected cattle. Cell-mediated responses were studied in two adjuvant groups immunised with the tachyzoite preparation; POLYGEN™-adjuvant-immunised cattle produced interferon (IFN) levels similar to those of infected animals.

Andrianarivo et al (2000) also studied the effect of a killed *N. caninum* tachyzoite preparation in pregnant cattle using a POLYGEN™ adjuvant. Heifers were immunised at 35 and 65 days gestation, and then 4 weeks later challenged with an intravenous or intramuscular inoculation of tachyzoites. Immunised heifers developed both humoral and cell-mediated immune responses, characterised by an increase in production of IgG1 and IFN-γ, respectively. Following a challenge with *N. caninum* tachyzoites, there was no significant cell-mediated immune response. Since all foetuses from control and experimental cattle developed lesions characteristic of *N. caninum* infection, it was concluded that this formulation did not prevent foetal infection in pregnant cattle.

Choromanski and Block (2000) formulated vaccine preparations from *N. caninum* tachyzoites using Havlogren and Bay R1005 adjuvants. Initial inoculations were followed by booster vaccinations 2 weeks later. Experimental heifers had greatly increased IFAT antibody titres, but were not challenged with *N. caninum*. There is some anecdotal evidence of efficacy of this tachyzoite formulation after a series of three inoculations. However, unvaccinated controls were not kept in the herd, making it difficult to draw conclusions from the study (Wren 2000).

Nishikawa et al (2001) used live vaccinia virus vectors to deliver NcSRS2 and NcSAG1 *N. caninum* antigens to pregnant mice. Vertical transmission of the parasite to foetuses was prevented. These workers also demonstrated the immunogenicity of their construct in dogs (Nishikawa et al 2000). Many advantages exist for live antigen delivery systems, such as ease and relatively low cost of production. Little debate has occurred, however, over the suitability of live vectors for the delivery of *N. caninum* antigens to cattle. No doubt this will occur in coming years.

Different isolates of *N. caninum* appear to have different biological characteristics, including differences in their ability to cause pathological change. Prior infection with a less virulent strain protected mice against an otherwise lethal challenge with NC-Liverpool (Atkinson et al 1999). Temperature-sensitive mutants of *N. caninum* (Lindsay et al 1999b) were able to protect mice from a potentially lethal challenge with tachyzoites. Following data presented by Williams et al (2000) and Innes et al (2001b), in which prior natural chronic infection with *N. caninum* protected foetuses against a lethal challenge, one could envisage the use of a live attenuated vaccine to prevent abortion, such as is the case with *T. gondii* and vaccination with Toxovax™. However, “vaccination” with Toxovax™ did not protect sheep foetuses from a lethal challenge of *N. caninum* (Innes et al 2001a). In contrast, “vaccination” with *N. caninum* provided protection from a lethal challenge with *T. gondii* when ewes were challenged with a moderately virulent strain but not when challenged with a highly virulent strain (Lindsay et al 1998).

The majority of vaccine preparations used in immunogenicity trials thus far have been based on whole-cell or cell-lysate formulations. It is questionable whether vaccines of this type are economical and effective to produce. In vitro culture of *N. caninum* tachyzoites is a laborious and expensive process (Lindsay

and Dubey 1989a). Economic factors may prevent the large-scale development of whole-cell or cell-lysate vaccines, especially in a global context.

Recombinant antigens of *N. caninum* have been produced, but have mainly been used in the serological diagnosis of infection (Lally et al 1996; Dubey et al 1997; Jenkins et al 1997). Future work might be directed at establishing their utility as vaccine candidates.

Conclusions

Although knowledge on the biology and epidemiology of *N. caninum*, and recognition of it as an abortifacient, has grown over the last 10 years, there is still a paucity of practical field experience with methods for prevention of infection and foetal loss. The only proven method for prevention of vertical transmission is via embryo transfer, which would likely be reserved for a small number of highly valuable *N. caninum*-infected cows.

A small number of epidemiological investigations suggest that bovine pestivirus infections significantly increase the risk of abortion in *N. caninum*-infected cattle. Efficacious vaccines for the control of pestivirus infection exist and their use is advocated in situations where there is a high risk of both bovine pestivirus and *N. caninum* infection.

Some epidemiological investigations of *N. caninum* abortions point to postnatal infections as the cause of abortion storms. Antony and Williamson (2001), Schares et al (1999) and Thornton et al (1994) suggest that this is more often the case in New Zealand than is reported from other parts of the world. Testing for *N. caninum* infection and culling of infected animals may be less successful in New Zealand than in parts of the world where vertical transmission predominates.

In order to control postnatal transmission, the interaction of dogs with pregnant cattle, and their access to bovine placentae and foetal tissues should be controlled. All dogs should be presumed a potential source of infection for cattle, as serodiagnosis of the infection status of individual dogs is fraught with difficulty. As a considerable number of dogs experimentally infected with *N. caninum* did not seroconvert, it may be prudent to eliminate, as far as is practicable, all dogs from the vicinity of cattle that may be at risk.

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