



## NEOSPOROSIS IN CATTLE

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### 1. INTRODUCTION

Neosporosis is caused by the protozoan parasite, *Neospora caninum*. It is essentially responsible for abortion in cattle and various neuro-muscular conditions in dogs. Until 1988, *N. caninum* was confused with *Toxoplasma gondii*, a closely related parasite (Dubey *et al.* 1988a,b). In 1989, Thilsted and Dubey reported the presence of cerebral and cardiac lesions in several aborted bovine foetuses originating from a single farm. In the following years the parasite was identified in the tissues of many bovine aborted fetuses but also of stillborn calves and, rarely, of clinically affected newborn calves (Dubey *et al.* 1989; Dubey *et al.* 1990; Anderson *et al.* 1991; Barr *et al.* 1991). Since then, *N. caninum* has been identified on all continents and is considered as a major cause of abortion in cattle (Dubey, 2005).

### 2. MORPHOLOGY, LIFE CYCLE AND TRANSMISSION

*N. caninum* has been identified and sometimes isolated from many animal species. The first clinical cases were observed in several naturally infected dogs; since then the parasite was observed in cattle, in sheep, in horses and different wild animals such as the black tail deer (*Odocoileus hemionus columbianus*) and a rhinoceros (*Ceratotherium simum*). Additionally, specific antibodies were detected in water buffaloes (*Bubalus bubalis*) in coyotes (*Canis latrans*), in red foxes (*Vulpes vulpes*) and in camels (Dubey, 2003) which suggests that these species could also act as intermediate hosts. Many species are susceptible to the parasite following the experimental inoculation of tachyzoites. The laboratory mouse is a useful model to study the parasite biology or the immune response or to screen potential chemotherapeutic agents whereas the goat and the sheep represent excellent models for the study of congenital neosporosis (Dubey & Lindsay, 1996). *N. caninum* is a coccidian parasite and three different stages have been described i.e. the oocyst, the tachyzoite and the tissue cyst containing numerous bradyzoites. The unsporulated oocyst has been found in the faeces of dogs and coyotes; thus dogs are both the intermediate and final hosts for *N. caninum* (Mac Allister *et al.* 1998; Lindsay *et al.* 1999). The oocyst measures approximately 12 microns in diameter and sporulate in the environment in 24 hours; sporulated oocysts contain 2 sporocysts containing each 4 sporozoites. Usually the total number of oocysts excreted via the dog's faeces is low. It is important to note that at present little is known regarding the frequency of

shedding of oocysts, the survival of the oocysts in the environment and whether other canids such as the fox can act as final host. After experimental infection the dogs passing oocysts in their faeces may remain seronegative (Mac Allister *et al.* 1998). The excretion of oocysts in naturally infected dogs has been reported in a very limited number of cases. The source of canine infection is not easy to identify. However, Dijkstra *et al.* (2001) reported the shedding of oocysts in dogs fed infected tissues (placenta) collected from naturally infected cows. The tachyzoite is considered as the pathogenic stage; it multiplies actively by endodyogeny in many cell types (neural cells, hepatocytes, macrophages, fibroblasts, endothelial cells, myocytes...) and measures 3-7 microns in length. The tissue cysts are round or oval in shape and can reach 100 microns and more. They are essentially found in the central nervous system although the presence of cysts in the muscles of naturally infected dogs and calves was reported (Peters *et al.* 2001). The wall of the cyst is thick (up to 4 microns) smooth and devoided of septa; it is positively stained by the PAS stain. The tissue cysts contain many bradyzoites which are morphologically similar to tachyzoites. The parasite can be transmitted horizontally (i.e. through the ingestion of oocysts shed in the environment by the final host) or vertically from an infected cow to her offspring. Vertical route is the major route of transmission in cattle and is extremely efficient as the rate of transmission is usually between 80 and 100% (Anderson *et al.* 1997; 2000). *N. caninum* DNA was found in bull semen (Ortega-Mora *et al.* 2003) but recent data indicate that venereal transmission is very unlikely to occur (Canada *et al.* 2006). Lactogenic transmission was achieved experimentally but there is no evidence that it occurs under natural conditions (Uggla *et al.* 1998; Davison *et al.* 2001; Dijkstra *et al.* 2001). Embryo transfer is recommended as a method of control as long as the status of the donor cow is checked (Baillargeon *et al.* 2001; Landmann *et al.* 2002). There is no cow-to-cow transmission of *N. caninum* (Anderson *et al.* 1997).

### 3. NEOSPOROSIS IN CATTLE

*N. caninum* has a worldwide distribution. In many countries this organism is the main cause of abortion in cattle (Haddad *et al.* 2005). By instance, in Belgium it is estimated that 11.9% of abortions of the entire population are attributable to *N. caninum* (De Meerschman *et al.* 2000). The seroprevalence in cattle varies according to the country, the region, the serological test used, the existence or not of an abortion and to the cut-off of the selected technique. *N. caninum* causes abortion in both beef and dairy cattle. The abortion is the only clinical sign and can occur from the third month of pregnancy and onwards. Most of the abortions take place between the 5<sup>th</sup> and 6<sup>th</sup> months of pregnancy. The foetus is either resorbed, autolysed, mummified, stillborn, born alive with clinical signs, or born clinically normal but chronically infected. Abortions due to *N. caninum* can take place all year round (Anderson *et al.* 1991; Thurmond *et al.* 1995). However in the Netherlands, abortions are more frequent in summer (Wouda *et al.* 1999) whereas in California a peak is observed in autumn and winter (Thurmond *et al.* 1995). Abortions may be epidemic (abortion storms) or endemic (Thurmond *et al.* 1997). In the first case many abortions are reported over a fairly short length of time and, in some cases, over 30% of the cows may abort within a few months (Thilsted & Dubey, 1989). In the second case the abortion rate is over 5% during several years. It is also possible to observe occasionally an abortion storm in a farm confronted to endemic neosporosis (Anderson *et al.* 2000). At calving, infected calves may be clinically normal or may have neurologic signs, be underweight or unable to rise. In these animals the following clinical signs were reported: flexion or hyperextension of forelegs, hindlegs or both, ataxia, decreased patellar reflexes, loss of conscious proprioception, exophthalmia or asymmetrical appearance of the eyes (Dubey, 2005; De Meerschman *et al.* 2005). The incidence of neonatal neosporosis is low and so far has been observed in calves under 2 months of age only. As already stated the vertical mode of transmission is the most important one (Björkman *et al.* 1996; Anderson *et al.* 1997). Several studies indicated an increased risk of abortion in seropositive vs seronegative cows (Thurmond *et al.* 1997; Moen *et al.* 1998; Wouda *et al.* 1998b; Davison *et al.* 1999, De Meerschman *et al.* 2000). Additionally many observations suggest the reactivation of a latent infection during pregnancy.

However the mechanisms responsible for this reactivation are not fully understood yet (Innes *et al.* 2005). It is likely that the immunological and endocrinal modifications which take place during pregnancy are responsible for a parasitaemia and, consequently, the infection of the foetus. In this respect it has been shown that specific antibody titres are fluctuating and that a peak is reached at around 6 to 7 months of gestation. Thereafter the titres decline until 2 months after calving. In cattle the vertical transfer of the infection seems to be effective during several successive generations (Björkman *et al.* 1996; Paré *et al.* 1996; Anderson *et al.* 1997; Schares *et al.* 1998; Davison *et al.* 1999; Bergeron *et al.* 2000). The vertical transmission of the parasite cannot by itself maintain the parasite in a herd because the rate of transmission is usually below 100% (French *et al.* 1999). At the moment little is known about the pathogenicity of *N. caninum* following the oral administration of sporulated oocysts. This horizontal route of transmission induces a seroconversion after experimental infection but does not seem to induce abortion (De Marez *et al.* 1999; Trees *et al.* 2002) in contrast with the inoculation of tachyzoites (Williams *et al.* 2000; Andrianarivo *et al.* 2001, Innes *et al.* 2001). Another important factor is the gestational age and hence immunocompetence of the foetus at the time of infection (Innes *et al.* 2005). Early in gestation, *N. caninum* infection of the placenta and subsequently the foetus usually proves fatal, whereas infection occurring in mid to late pregnancy may result in the birth of a congenitally infected but otherwise healthy calf.

#### 4. DIAGNOSIS IN CATTLE

The diagnosis of bovine neosporosis is based on the examination of maternal and foetal sera ideally combined with the examination of foetal tissues. Ideally the whole foetus should be provided. When this is not possible the head should be collected and forwarded to the laboratory. The examination of the foetus's tissues is necessary. The brain, the heart, the liver and the placenta are the prelection organs. The histopathological examination of these tissues reveals a multifocal non suppurative encephalomyelitis and sub acute myocarditis and hepatitis (Anderson *et al.* 1991; Barr *et al.* 1991). As the tachyzoites are few and difficult to observe with classical stains (Shivaprasad *et al.* 1989; Bergeron *et al.* 2001) it is recommended to use immunohistochemistry especially on the sections presenting compatible lesions (Boger & Hattel, 2003). The detection of specific antibodies in the cow's serum is only indicative of exposure to *N. caninum* and is not diagnostic by itself (Dubey, 2005). Maternal serology is also used to study the epidemiology of the disease in the herds. Several ELISA's, indirect immunofluorescence techniques and an agglutination test were developed (Dubey, 2005). There are several modifications of the ELISA test used to detect antibodies to *N. caninum* in sera or milk, using whole parasite lysate, purified proteins, recombinant proteins and tachyzoite proteins absorbed on immunostimulating complex (ISCOM) particles. Recently some of these tests were compared in a multicentric study in different places in Europe (von Blumröder *et al.* 2004). Additionally an avidity ELISA which allows to discriminate between recent and chronic infections was developed. In this test sera are treated with urea to release low-avidity antibodies and values obtained before and after treatment with urea are used to estimate recency of infection (Björkman *et al.* 1999; Jenkins *et al.* 2002; Schares *et al.* 2002). Immunoblots are useful in detecting *N. caninum*-specific antibodies (Schares *et al.* 1999; Söndgen *et al.* 2001). The presence of specific antibodies in the foetal serum is very useful; however the absence of specific antibodies does not exclude the possible implication of *N. caninum*. Indeed the foetus might be too young or the infection might be too recent (Barr *et al.* 1995; Otter *et al.* 1997; Wouda *et al.* 1997; Slotved *et al.* 1999). A titre equal or over 1:25 can be considered as indicative of foetal neosporosis (Slotved *et al.* 1999). The use of peritoneal liquid seems preferable to the use of other foetal fluids (Söndgen *et al.* 2001). Usually the titres observed in aborting cows are higher than those in non aborting cows. However the titres are fluctuating and sometimes below the cut off even during the pregnancy. Although closely related to *N. caninum*, *Toxoplasma gondii* and *Sarcocystis spp* do not cross react with the parasite (Dubey *et al.* 1996; Williams *et al.* 1997; Wouda *et al.* 1998a; Schares *et al.* 1999). The efficiency of the diagnosis by polymerase chain reaction (PCR) is dependent on the laboratory,

stage of the autolysis of the foetus and sampling procedure (Bazler *et al.* 1999). This technique is highly sensitive and very specific. However it remains expensive and requires sophisticated equipment and an highly qualified technical staff. The differential diagnosis includes toxoplasmosis and sarcosporidiosis (Dubey, 2005). Abortions due to *Sarcocystis* spp are rare in cattle and the schizonts are observed at the level of the endothelial cells of the blood vessels. *T. gondii* is not recognized as an abortifacient in cattle. If necessary immunohistochemistry and PCR should be performed in order to identify the different agents.

## 5. CONTROL

At the moment very few control measures are available. As the vertical transmission of the disease represents the major source of infection in cattle it is advisable not to breed seropositive heifers born to seropositive mothers. This is only feasible if the initial seroprevalence in the herd is not too high. This could be assessed through bulk-milk testing (Björkman *et al.* 1997). If bulk-milk testing is positive additional studies on dam-heifer sera and on animals of different ages could provide valuable data on the transmission of *N. caninum* in a given herd (vertical vs horizontal transmissions). In herds with a high level of vertical transmission the seroprevalence does not increase significantly with the age of animals. In this case seropositive animals should be culled. Embryo transfer from seropositive valuable cows to seronegative cows is an option under some circumstances (Baillargeon *et al.* 2001; Landmann *et al.* 2002). To prevent horizontal transmission it is important to prevent the contamination of feed and water via the shedding of oocysts by dogs and possibly other canids like the fox. These animals should not have access to animal premises although this might be difficult to achieve. A known source of infection for the final hosts is the placenta. Many drugs with an *in vitro* activity on the tachyzoites of *N. caninum* have been tested either in a murine model or in cattle. So far there is no known drugs that will prevent transmission from the mother to the offspring although further work is being done with the triazine derivatives (Gottstein *et al.* 2001; Kritzner *et al.* 2002; Haerdi *et al.* 2006).

There is evidence that cattle can develop a protective immunity to subsequent neosporosis abortion. In fact less than 5% of the cows are aborting twice in an infected population. This protective immunity seems to be more efficient following infection through oocysts when compared to the endogenous reactivation of infection (Trees & Williams, 2003). Vaccination with killed tachyzoites is unable to prevent the vertical transmission of the parasite (Andrianarivo *et al.* 2005). A killed vaccine is currently available in different countries (Neo Guard ® Intervet) but there are no convincing data about the efficiency of the vaccine to prevent *N. caninum*-associated abortion in cattle (Dubey, 2005).

## 6. CONCLUSION

Many data were produced on *N. caninum* and neosporosis since the first description of the parasite and the disease it induces. Neosporosis is now considered as a major cause of abortion in cattle worldwide. Many reliable diagnostic tests are now commercially available. The epidemiology and pathogenesis of the affection are complex and still not fully known. In particular the mechanisms responsible for the reactivation of a latent infection in a chronically infected cow and the subsequent infection of her offspring deserve additional studies. Currently very few control strategies are available. The selective culling of infected animals and hygienic measures represent the only practical measures. In future chemotherapy and/or vaccination might become realistic options.

## 7. SUMMARY

*N. caninum* was described for the first time in 1988. Since this date neosporosis has been shown to be a major source of abortion in cattle worldwide. The life cycle of the parasite is complex and comprised three main stages i.e. the oocyst (shed by a carnivorous final host) the tachyzoite (which multiplies in the intermediate host and is the pathogenic stage) and the tissue cyst (which contains many bradyzoites and represents a latent form found mainly in the central nervous system of the final host). The reactivation of a chronic infection during pregnancy can lead to the infection of the foetus and, depending on different factors (age of the foetus, immune status of the mother...), to abortion or the birth of a chronically infected calf. The congenital infection is the main route of transmission and represents a challenge for immunologists, parasitologists and veterinarians. The horizontal transmission is due to the ingestion of sporulated oocysts through the contamination of feed and water by the faeces of the final host. Well established procedures are now available to establish a diagnosis of neosporosis. However there are no drugs or vaccines available yet. Culling and hygienic measures represent at the moment the only practical realistic control strategies.

## 8. KEY WORDS

*Neospora caninum*, neosporosis, cattle, abortion.

## 9. RESUME

*N. caninum* fut décrit pour la première fois en 1988. Depuis cette date, la néosporose est apparue comme une maladie de distribution mondiale et l'une des principales étiologies responsables d'avortements chez les bovins. Le cycle vital du parasite est complexe et comprend trois stades principaux: l'oocyste (éliminé via les selles d'un carnivore hôte final), le tachyzoïte (qui se multiplie au sein des tissus de l'hôte intermédiaire et qui est le stade pathogène) et le kyste tissulaire (qui contient de nombreux bradyzoïtes et représente une forme de latence retrouvée essentiellement dans le système nerveux central de l'hôte intermédiaire). La réactivation d'une infection chronique chez la vache durant la gestation peut mener à l'infection du fœtus et en fonction de différents facteurs (âge du fœtus, immunité de la mère...) à un avortement ou à la naissance d'un veau infecté de manière chronique. Cette voie congénitale est la principale source de contamination des bovins et représente un véritable défi pour les immunologistes, pour les parasitologistes et pour les vétérinaires. La voie horizontale est liée à l'ingestion d'oocystes sporulés via la contamination de l'eau et de la nourriture par les excréments de l'hôte final. Des procédures bien établies existent en ce qui concerne le diagnostic de la néosporose. Cependant, en l'absence de vaccins et de chimioprophylaxie, les mesures hygiéniques et la réforme sélective des animaux infectés représentent les seules options disponibles pour le moment.

## 10. MOTS CLES

*Neospora caninum*, néosporose, bovins, avortement.

## 11. REFERENCES

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