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The role of the colostrum and milk in *Neospora caninum* transmission

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Summary

Neospora caninum, an apicomplexan protozoan causes economical losses due to reproductive failure associated with abortion among cattle. The transmission of *N. caninum* is possible through vertical transmission in utero, or according to the modern nomenclature endogenous and exogenous infection modes and horizontal transmission through ingestion of oocysts. Limited data is available on the vertical transmission during suckling time, *via* colostrum and milk.
In this paper the main scientific aim focused on *N. caninum* DNA detection in the milk and colostrum of seropositive cows have been reviewed. In this term, the risk of animals and humans infection has been discussed.

Key words: *Neospora caninum;* vertical transmission; milk; colostrum; DNA; PCR

Introduction

Neospora caninum is an apicomplexan parasite, closely related to *Toxoplasma gondii* (Dubey, 1999; Holmdahl *et al.,* 1994). This pathogenic intracellular parasite can infect warm-blooded vertebrates worldwide as dogs, cats, cattle, sheep, horses, rats, foxes, goats, alpacas, lamas, deer, camel, bison and other animals (Cabaj *et al.,* 2004, 2005; Dubey, 2005; Dubey & Dubey, 2003).
The infection causes important economical losses in cattle due to reproductive failure associated with abortion and mortality in congenitally infected calves (Anderson *et al*., 1995: Cabaj *et al.,* 2000; Davison *et al*., 1999; Moskwa and Cabaj, 2003; Wouda *et al.,* 1999).
Due to the specific localization of *N. caninum* in the brain, spinal cord and muscles, it is difficult to demonstrate the presence of the parasite by the direct methods (Dubey & Lindsay, 1996; Peters *et al.,* 2001). This renders the development of specific serological tests for parasite detection necessary (Björkman & Uggla, 1999; Maley *et al.,* 2001; Packman *et al.,* 1998). *Neospora caninum* infection is diagnosed in post-mortem samples by the detection of parasites, parasite antigen or parasite DNA using histopathology, immunohistochemistry, and polymerase chain reaction (Hůrková-Hofmannová *et al.,* 2006; Peters *et al.,* 2001; Yamage *et al.,* 1996). Molecular techniques such as PCR offer a highly sensitive and specific alternative to immunological methods for the diagnosis of neosporosis. Using this method the presence of *N. caninum* DNA was confirmed in different tissues such as brain, spinal cord and muscle (Guy *et al.,* 2001; Sager *et al.,* 2003; Wiśniewski *et al.,* 2002). In living animals, the presence of specific antibodies and DNA in serum, milk and semen indicates the possibility of the parasite infection (Björkman *et al*., 1997; Chanlun *et al*., 2002; Moskwa *et al.,* 2003; Ortega-Mora *et. al.,* 2003; Schares *et al.,* 2004, 2005; Serrano-Martinez *et al*., 2007; Varcasia *et al*., 2006).

Sources of transmission
The transmission of *N. caninum* is possible through vertical route in *uterus*, or according to the modern nomenclature endogenous and exogenous infection modes and horizontal infection (Trees & Williams, 2005). The probability that infected cattle will pass the infection to their offspring during gestation is very high (81 – 100 %) (Pare *et al*., 1996). The horizontal transmission occurs through ingestion of oocysts, tachyzoites or tissue cysts (Anderson *et al.,* 1997; Dijkstra *et al*., 2002; Pare *et al.,* 1996; Schares & Conraths, 2001). Consumption of placenta, material of aborted foetuses or uterine discharge in combination with defecation on the feeding alley, storage of the grass or corn silage was observed in 75 % of the post-natally infected farms (Dijkstra *et al.,* 2002). The ingestion of carcasses of infected animals with cysts in the muscle is suspected in *N. caninum* sylvatic transmission cycle (Dubey, 2003).
Limited data is available on the vertical transmission during suckling time.
The data of Uggla *et al.* (1998) have shown that oral infection with *N. caninum via* colostrum might be a possible

route of vertical transmission resulting in the infection of newborn calves within the first few hours of their life. Although a previous study done by Dubey *et al.* (1998) indicated that *N. caninum* tachyzoites may not be resistant to HCL-pepsin.
Subsequently, Dijkstra *et al.* (2001) revealed that dogs shed *N. caninum* oocysts after ingestion of naturally infected bovine placenta but not after ingestion of colostrum spiked with tachyzoites. The authors concluded that may be possible that tachyzoites incorporated in placenta passage the stomach before being destroyed by HCL-pepsin. The work of Davison *et al.* (2001) showed that calves can be infected with *N. caninum* by the oral route with experimentally inoculated colostrum or milk repleacer, even one week after birth. However, studies performed by Davison *et al.* (2001) revealed that cattle were not infected when given milk or colostrum from dams naturally infected with *N. caninum*, showing no evidence of *N. caninum* tachyzoites or parasite DNA in the tissues of experimental animals. There may be wide variation in the number of parasites present in milk both between individual cows and at different stages of lactation. The authors summarized that in some situation calves may potentially become infected *via* this route, if for example large numbers of tachyzoites were present in the colostrum or milk in an individual cow.

Neospora caninum DNA demonstration
The earlier results implicated to examine colostrum and the milk samples from seropositive cows to demonstrate the presence of parasite DNA. This study was done in cows with a history of abortion. Sera of these animals were tested for the presence of *N. caninum*-specific IgG using a commercially available ELISA test kit (IDEXX Laboratories, Inc., Westerbrook, Maine, USA), using an automated plate reader EL*800, Bio-tek, Instruments Inc.
Colostrum samples and the milk samples were collected from seropositive cows and prepared to the further analysis according to Chanlum *et al.* (2002), with some modifycations (Moskwa *et al.,* 2003). Colostrum samples were collected on calving day and the day after.
The skimmed colostrum and milk samples (sediment) were collected for PCR analysis (Moskwa *et al.,* 2003, 2007). Briefly, genomic DNA was isolated from tachyzoites of the reference strain NC-1, the colostrum and milk sediments using the Nucleospin® Tissue DNA extraction kit (Macherey-Nagel, Germany).
The reagents used for PCR were purchased from Fermentas (MBI Fermentas,USA).
The Nc5 region was selected as the target sequence for DNA amplification. Primers Np6 and Np21, spanning a 328 bp region, were produced according to Yamage *et al.* (1996).
Briefly, PCR was performed in a final volume of 50 µl mixture containing 5 µl of 10x PCR buffer (MBI Fermentas, USA), 0.2 mM of each dNTP, 10 pmol each of *N. caninum* primer Np6 and Np21 and 1 U Taq DNA polymerase. Amplification was carried out in a thermal cycler (Genius, Techne) using 30 cycles with denaturation (94°C, 20 sec), annealing (55°C, 30 sec) and primer extension (72°C, 25 sec). After the last cycle, additional extension was applied for 10 min at 72°C.
Two negative controls (water and DNA isolated from seronegative dams) and a positive control (DNA from the NC-1 isolate) were included in each reaction.
Amplification products were analyzed by electrophoresis through a 1 % agarose gel stained with ethidium bromide and visualised under UV light using the Kodak Electrophoresis Documentation and Analysis System (EDAS) 290.
The PCR assay yielded the expected 328 bp product in milk and colostrum samples from seropositive cows collected on calving day and one day after (Moskwa, 2004; Moskwa *et al.,* 2003, 2003a; 2007). No amplification was observed in milk and colostrum samples collected from seronegative cows.
It seems that there is possibility that *N. caninum* tachyzoites present in milk and colostrum may be transmitted to other noninfected young calves by feeding them of pooled colostrum and milk, which is a common husbandry practise in dairy farms in the UK (Davison *et al.,* 2001).

Risk for humans
Another very important consideration is whether *N. caninum* may be infective for humans. This risk seems to be higher for consumption of raw milk from an individual cows, contrary to the consumption of the bulk tank milk in which the parasites would be greatly diluted (Davison *et al.,* 2001). Several studies have not revealed the evidence of *N. caninum* infection in human (Graham *et al.,* 1999; Petersen *et al.,* 1999). But the data of Nam *et al.* (1998) revealed that twelve cases out of 172 *Toxoplasma*-positive sera cross reacted with *N. caninum* antigens, and one of 110 *Toxoplasma*-negative sera. The studies done by Tranas *et al.* (1999) revealed that 6.7 % of 1209 human blood donors were found to be seropositive. Another at-tempt to investigate the presence of human neosporosis was performed with the different groups of human popu-lation in Brazil (Lobato *et al*., 2006). Antibodies against *N. caninum* were predominantly detected in HIV-infected patients (38 %), patients with neurological disorders (18 %) and newborns (5 %).
Our earlier study was done to investigate the influence of some exogenous and endogenous factors on the viability and growth of tachyzoites of *N. caninum* as well as their virulence in an *in vitro* system. Tachyzoites were incubated in milk from both positive and seronegative cows (at + 4°C). Only after 1 day of incubation in milk from seronegative cow tachyzoites were alive and able to invade the monolayer *Vero* cells. After 21 days of incubation in PBS (at +4°C), all parasites were viable and able to grow and invade *Vero* cells. After freezing (-20°C), heating (+100°C) and sterilization, the devitalization of all tachyzoites was observed. Only UV- treated parasites retained their vitality in the cell culture (Moskwa *et al.,* 2005).
However, the limited data is still known about the epidemiology of *N. caninum* infection in humans, the risk of infection seems to be higher for consumption of the milk

straight from the cow. Results have indicated that nonhuman primates (rhesus macaque) are susceptible to *N. caninum* infection (Barr *et al.,* 1994).

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