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Current studies on neosporosis in Poland

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Abstract

Neospora caninum was described and named as a cause of paralysis and death in dogs, but was also recognized as a major cause of bovine abortion in many parts of the word. It is a parasite of great veterinary importance that can infect different domestic and wildlife animals species. Two transmission routes of infection in cattle have been recognized: horizontal, when cows are infected from ingestion of coccidian oocyst stages and vertical transplacental transmission.

The prevalence of *N. caninum* infection in Poland is still only partially known. The first evidence of anti-*N. caninum* antibodies in sera of dairy cows was published in 2000 (Cabaj et al. 2000). Untill now approximately 2700 cows from different districts of Poland were examined using ELISA test (IDEXX Laboratories, Inc., Westerbrook, Maine, USA). The prevalence of anti-*N. caninum* antibodies in animals sera ranged from 1.5% to 23%.

Studies ranged on vertical transmission of neosporosis have shown three generations of persistently infected animals. Clinical signs were not observed, however tested sera showed very high anti-*N. caninum* antibodies level. Serological evidence of anti-*N. caninum* antibodies was reported in more than 90% of offspring (Moskwa and Cabaj 2003).

Embryos transferred from seronegative donors to seropositive mothers resulted in seropositive offspring. Transferred embryos were imported from USA or were isolated from Polish breeds of cows (Moskwa et al. 2002).

There are no parasitological examinations useful for diagnosis of neosporosis. *In vivo* the diagnosis of neosporosis is very difficult due to the specific localization (neural tissues), the low number of parasite and non-specific clinical signs. On the other hand, molecular detection assays such as PCR are superior because of higher sensitivity and specificity in identifying *N. caninum* in animal tissues (Jenkins et al. 2002). Using conventional and nested PCR, the presence of *N. caninum* DNA in brains of calves born from seropositive mothers was confirmed (Wiśniewski et al. 2002). Additionally, PCR analysis with primers Np6 and Np21 was performed according to Yamage et al. (1996). Genomic DNA was prepared from the reference strain NC-1 and from the brains of calves born from seropositive dams. PCR amplification yielded the excepted 328 bp product thus confirming the presence of N. *caninum* DNA in all examined samples (Moskwa and Cabaj 2004).

Studies on horizontal transmission of neosporosis have shown that milk is a suitable material for the detection of *N. caninum* infection in cows through the use of both ELISA and PCR. For molecular analysis milk samples were prepared according to Chanlum et al. 2002. PCR assay using Np6 and Np21 primers yielded the expected 328 bp product and revealed for the first time the presence of *N. caninum* DNA in milk of seropositive cows. (Moskwa et al. 2003a, b, c). Additional study on colostrum collected at calving day and one day after calving revealed the 328 bp PCR product in all examined samples (Moskwa 2004, Pastusiak et al. 2004). In parallel with molecular study carried out on milk and colostrum samples, an ELISA test was also performed. To confirm serological data, Western blot analysis was used. Different patterns of protein bands were recognized by antibodies present in sera, milk and colostrum from seropositive cows (Moskwa 2004).

It has been known that both intensity of the pathology process and tissue destruction may have an influence on the enzymes activity. The estimation of some biochemical parameters was performed in sera of seropositive cows as a potential clinical signs of *N. caninum* infection (Moskwa et al. 2001). In positive sera, the highest concentrations of AST, ALT, ALP, CREA, CK, Mg and K were found. It seems that the concentrations of CK, K Mg and Fe in serum should be taken into account as a potential biochemical parametes reflecting *N. caninum* infection in cows.

Further, a preliminary study on cell proliferative response and IgG level during long lasting *N. caninum* infection in pregnant cow was done. Peripheral blood mononuclear cells were stimulated *in vitro* with Con A, LPS and *N. caninum* crude antigen. The mitogen-induced lymphocyte proliferation was assessed by the Alamar Blue method. Very slight differences were observed in the proliferative response of cells to Con A, LPS and *N. caninum* crude antigen. IgG level slightly increased and achieved the highest values after 22 weeks of pregnancy (S/P = 3.89) (unpublished data).

The existence of a sylvatic cycle of *N. caninum* was examined in European bison living in free and fenced areas in Poland (Cabaj et al. 2004a, 2005). Of the 320 examined bison, a positive antibody response was found in 23 (prevalence 7.3%). Western blot analysis confirmed the presence of antibodies to *N. caninum* tachyzoites in all 23 tested sera. It is worth to note that one cow imported from Denmark and introduced in the Bieszczady region showed a very high IgG level (Cabaj et al. 2004b).

A limited number of studies on the serological evidence of human neosporosis has been published. Based on these data and on results obtained by our team, *N. caninum* infection of humans *via* milk should be taken into account. The aim of the study was to investigate the influence of some exogenous and endogenous factors on the viability and growth of tachyzoites of the reference strain of *N. caninum* (NC-1) as well as their activity on invasion of Vero cells in an *in vitro* system (Moskwa et al. 2003c). Tachyzoites were incubated in milk from both positive and seronegative cow (at + 4˚C). Only after 1 day incubation in milk from seronegative cow tachyzoites were alive and able to invade a monolayer 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. UV treatment didn't influence on the viability of tachyzoites.

The protozoan parasite *Neospora caninum* was isolated from the brain of a 12-h-old calf, born to seropositive cow and suspected of having *Neospora* infection. The isolate has been designed as NC-PolB1 (Pastusiak 2004).

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