10th International Symposium
MODERN TRENDS
IN LIVESTOCK PRODUCTION

PROCEEDINGS

Belgrade, Serbia, 2 - 4 October, 2013
www.istocar.bg.ac.rs
NEOSPORA CANINUM IN CATTLE: EPIZOOTIOLOGY, DIAGNOSTICS AND CONTROL MEASURES

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Invited paper

Abstract: Neospora caninum is a coccidian parasite and well established pathogen for cattle, which is responsible for numerous abortions. The organism is worldwide distributed in dairy and fattening cattle. Neosporosis can occur at sporadic, endemic, and epidemic levels in herds. Dog and other carnivores are the primary hosts for N. caninum, and the infection is disseminated via feces of infected animals excreting oocysts in the environment. Cattle get infected by ingesting the oocysts in feed and drinking water. Transplacental route (vertical transmission) is the most common transmission route. The agent is transmitted to the developing embryo or fetus, and severity and outcome of such infections are determined by the pregnancy stage. The abortions occur mainly between the 3rd and 7th month of pregnancy. Besides dogs, birds and poultry are reported as potential source of infection. The percentage of seropositive cows in the world population of dairy cattle ranges from 5-65%. In some areas, it was established that N. caninum is responsible for more than 30% of all abortions in cattle. Little is known about the risk factors for N. caninum infection; however, contact between cattle and dogs is considered highly hazardous. Some research revealed highly positive correlation between seropositivity in dogs and prevalence of N. caninum infection in cows. Laboratory diagnosis is established by agent detection by the method of immunohistochemical staining (IHC), immunofluorescence antibody test (IFAT), Western blot method (WB) and PCR or by detection of specific N. caninum antibodies applying direct agglutination test (DAT), ELISA and cELISA. ELISA test proved adequately specific and sensitive to identify the infection at herd level. This article gives and overview of importance and role of N. caninum in the pathology of dairy cattle diseases. Our aim was to outline contemporary knowledge and potential risk factors at dairy farms in Serbia, as well as the available control measures. This paper offers the analysis and comparison of pathogen prevalence in dairy herds in Serbia as well as its role in the abortions.
Key words: Neospora caninum, cows, epizootiology, diagnostics, control measures

Introduction

Neospora caninum (Apicomplexa) is a coccidian parasite and well established causative agent of the disease, primarily in dogs and cattle worldwide. The parasite was first recognized in Norway in 1984 in dogs manifesting symptoms of myositis and encephalitis. The organism is described and named Neospora caninum (Bjerkas et al., 1984). In 1988, N. caninum was recognized as a causative agent for abortions in cows (Dubey et al., 1988), and has emerged as an important pathogen causing reproductive disorders and abortions in numerous countries (Haddad et al., 2005; Vidić et al., 2011c). In cattle, the N. caninum infection often can take a subclinical course usually resulting in reduced milk yield, reduced growth in fattening herds and poor food conversion. Thus, considerable economic losses are likely in dairy cows and beef cattle. Records on prevalence of cattle neosporosis in numerous European countries strongly indicate high percentage of seroreactor animals in dairy herds (Pare and Hietala, 1995; Davison et al., 2001; Otranto et al., 2003; Schares et al., 2003). A recent research in Sweden and Holland revealed the rates of seropositive cows being 16% and 76%, respectively (Bartels et al., 2006).

Clinical neosporosis was identified in sheep, goats, deers, rhinoceroses, horses and experimental animals. Antibodies against N. caninum were detected in buffalos, foxes, coyotes and camels, indicating that wild animals are potential infection reservoirs (Godmin et al., 2004).

Etiology and epizootiology

Dogs proved to be definitive hosts for N. caninum. Dogs shed the unsporulated cysts in the feces, and sporulation occurs outside the host, in the environment (Vidić et al., 2011a). Dogs can excrete infectious oocysts during undefined time after infection onset, and data on the survival of oocysts in the environment are still lacking (Lindsay and Dubey, 2000). In bitches, the infection is mostly subclinical and transplacental transmission of the parasite results in bringing forth the infected offspring (Bjerkas et al., 1984). The most commonly observed symptom in these animals involves hind limb paresis, which progresses to paralysis. Such dogs can survive even several months. In dogs older than six months, muscular and neurological symptoms are dominant, including head tilt, swallowing difficulties, jaw paralysis, muscular pain and muscle atrophy.
Moreover, dyspnoea, pneumonia, hepatitis, ulcerous dermatitis and sometimes encephalitis can be observed (Peters et al., 2000; Cantile and Arispici, 2002). The disease occurs as localized or generalized form that may involve all organs, including skin.

Since the disease was first registered, *N. caninum* antibodies were detected in 37.8% dogs in Argentina, 22% in New Zealand, 10% in Turkey, 6.7% in Brazil and in 6.4% dogs in Italy (Lindsay and Dubey, 2000). Neosporosis affects dogs of all age categories.

The life cycle of *N. caninum* is typified by three stages: tachyzoites, tissue cysts, and oocysts. Tachyzoites and tissue cysts are the stages observed in the intermediate hosts, and they are localized intracellularly (Haddad et al., 2005). Carnivores become infected by ingesting infected tissues containing tissue cysts with bradyzoites. Vertical (transplacental) infection is the dominant transmission route in cattle; however, horizontal infection route via feed and water contaminated with sporulated oocysts of *N. caninum* is possible (Davison et al., 2001). Neosporosis has been diagnosed in other animal species (Vidić et al., 2008). In sheep, *N. caninum* was first identified in England, in a congenitally infected lamb. Abortions associated with suspect *N. caninum* infection were reported also in dairy goats (Haddad et al., 2005).

**Bovine neosporosis**

Bovine infections associated with coccidian parasite *N. caninum* have been reported in almost all countries worldwide: Australia, New Zealand, European countries, South Africa, Korea, Japan, Thailand and America (Moore et al., 2003; Haddad et al., 2005; Vidić et al., 2011b). Quantitative studies carried out in particular geographic areas identified *N. caninum* as a major cause of abortions and neonatal deaths in dairy and beef cattle, showing the highest prevalence in the U.S.A., New Zealand, Netherlands and Germany (Rodriguez et al., 2002). Serological prevalence in cattle varies according to country region (Pare and Hietala, 1995; Packham et al., 1998), applied serology test and number of investigated animals and ranges between 87 and 90% (Davison et al., 2001; Dubey, 2003). Some 95% congenitally infected calves originating from seropositive cows are born clinically normal, healthy, although chronically infected (Moore et al., 2003; Haddad et al., 2005). The age of the cow, number of lactation cycles and abortion history do not affect the rate of congenital infection of the embryos in a subsequent pregnancy (March et al., 1998; Moore et al., 2003). Neospora abortions may occur from three months of gestation to term; however, abortions associated with *N. caninum* infection typically occur at mid gestation, i.e. between 5th and 6th gestation month. In addition to abortion, fetal deaths in utero as well as
resorption, mummification or autolysis of the fetus have been associated with Neospora outbreak. The newborn calves can manifest clinical signs of abnormalities but majority of them have no clinical signs of the disease yet being latent carriers. Seropositive cows (carriers of N. caninum antibodies) are more prone to abortions than the seronegative ones. The period from 4th to 5th month before calving is characterized by increase in antibody level, suggesting the reactivation of latent infection. Parasitemia that occurs in this period of gestation results in the infection of the fetus (Buxton et al., 2002).

To date, there is no evidence on direct transmission of N. caninum between cows; the transplacental infection is the most common infection route. So far, there is no evidence on either genital transmission or infection via the embryo transfer. Thus, embryo transfer is even recommended as the effective measure for the control and prevention of vertical transmission of the agent. Transmission of N. caninum via the milk has been experimentally confirmed; however, there is no evidence that lactogenic transmission of N. caninum occurs in nature. Dogs fed milk inoculated with N. caninum tachyzoites did not excrete oocysts in the feces. Clinical symptoms of bovine neosporosis were so far reported in animals younger than two months. Infected calves demonstrated decreased body mass, inability to rise or neurological symptoms such as ataxia, poor patellar reflex, significant loss of response to environmental stimuli, exophthalmus, hydrocephalus and spinal channel restriction. Abortions may occur as endemic or epidemic ones. Abortions occurring in 10% or more of cattle population within the time period of 6-8 weeks are considered epidemic (Haddad et al., 2005).

Infection is thought to reduce milk yield in dairy cows through its effects on fertility as cows that have aborted often have a lower milk yield and are more likely to suffer retained fetal membranes. Neospora infections in young calves have been reported to cause neuromuscular disease and may affect growth rate of finishing animals (Innes et al., 2002). Direct and indirect economic losses associated with this coccidium implicate the price of the lost calf, expenses of the expertise and diagnostics, costs of individual animals until returns to service, decreased milk production as well as costs for the replacement of culled animals.

Diagnosis

Bovine neosporosis is manifested by early embryonic death, abortion, stillborn or non-vital calf as well as by delivering normal calf without apparent clinical manifestations (Innes et al., 2002). Clinical manifestations in cows infected with N. caninum are associated with two major factors, i.e. whether the animal is pregnant at the moment of infection and at which gestation stadium (early, mid or late gestation period). Non-pregnant cows typically do not manifest any clinical
symptoms. Seroconversion, i.e. presence of specific *N. caninum* antibodies in blood or milk serum is the only indicator of the infection.

If the dam gets infected at the beginning of gestation period (2-3 months), early embryonic death is likely to occur. In *Neospora* infections acquired from 3\textsuperscript{rd} to 7\textsuperscript{th} gestation month, abortions, delivery of non-vital calves or calves with diverse disorders may occur, depending on the gestation month (*Buxton et al., 2002*). In infections acquired by the end of gestation period, weakly vital or normal calves without symptoms yet seropositive to *N. caninum* are delivered. During this gestation stadium, the immune system of the calf better responds to infection, which results in only limited or not at all consequences for the newborn animal (*Innes et al., 2001*).

Since the first identification of *N. caninum*, a range of diagnostic tests have been developed and assessed. The most widely used methods include immunohistochemical staining, indirect immunofluorescence, ELISA, direct agglutination, Western blot analysis and PCR (*Lindsay and Dubey, 1989; Pare and Hietala 1995; Packham et al., 1998; Romand et al., 1998*). The only indicative diagnostic procedure for identification of *N. caninum* is examination of the serum of aborted cows. Confirmation of the diagnosis of neosporosis requires histopathological examination of aborted fetuses. Most commonly, samples of the brain, heart, liver, placenta and tissue liquids are submitted for examination. If possible, blood serum should be examined, too. Although characteristic changes are observed in multiple organs of the fetus, major changes are most clearly seen in the brain. Since most aborted fetuses are autolized, partly digested brain samples should be submitted for histopathology. Mostly small amount of *Neospora* organisms is present in fetal brain, thus immunohistochemical examination of tissue portions is required. Histopathological changes mostly implicate focal encephalomyelitis (associated with necrosis and non-suppurative inflammation), hepatitis (occurring more often in epizootic abortions than in sporadic ones) and placental changes. It is to be emphasized that detection of this coccidium in the placenta is quite complex and intricate process.

Although immunohistochemical identification of *N. caninum* offers the best evidence in the etiology of abortion, this method is nowadays considered highly non-specific. Another diagnostic possibility is PCR detection of *N. caninum* DNA in brain preparations of the aborted calf. The efficacy of PCR in the diagnostics of this disease is closely associated with the laboratory itself, sampling procedure and degree of fetal autolysis (*Lally at al., 1996*).

Detection of *N. caninum* antibodies by serodiagnosis includes specifically modified ELISA (enables differentiation between acute and chronic infections), indirect fluorescent antibody (IFA) test, direct agglutination test (enables detection of IgG class) and immunoblotting assay (*Harkins et al., 1998; Sondgen et al., 2001*);
Although a valid serological diagnosis of neosporosis can be established using the blood serum or any fetal body fluid, sampling of fetal peritoneal fluid is highly recommended. For a serodiagnosis of congenital infection, calf serum should be collected immediately before obtaining of colostrum. Detection of *N. caninum* antibodies in calves’ sera indicates the existence of this coccidial infection. Negative result does not necessarily exclude the infection considering that synthesis of fetal antibodies is strongly dependent on gestation stage, exposure level and the time period between infection onset and abortion. Antibody titer of 1:25 can be considered specific for *N. caninum*, especially in the fetus.

Besides *N. caninum*, *T. gondii* is another potential causative agent of bovine abortions of protozoan etiology. Because of morphological similarities of *N. caninum* and *T. gondii* etiological diagnosis should be obtained by an immunohistochemical examination and PCR detection of the parasitic DNA. The attempts to isolate active forms of *N. caninum* have mostly failed and antigenic properties of diverse *N. caninum* isolates are still unclear, especially when speaking of healthy animals. Isolation of *N. caninum* form the fetus was unsuccessful, since the pathogen population dies inside the host. This coccidium can be more readily isolated from neural tissue of congenitally infected calves, as neural tissue contains the tissue cysts, which are much more resistant to autolysis than the *N. caninum* tachyzoites (Lindsay and Dubey, 2000; Dubey, 2003).

**Control measures**

Neosporosis has proved to be highly challenging disease in a view of treatment and control (Kljajić and Vidić, 2010). However, some antimicrobials applied in the therapy of toxoplasmosis (sulfadiazine, clindamycin) proved applicable. Potential prevention measures include timely detection of seroreactor animals, separation of the offspring from infected mothers, disease monitoring in low-prevalence herds, prevent dogs to access aborted fetuses, placenta or meat of dead cattle as well as prevent fecal contamination of feed and drinking water in the herd (Haddad et al., 2005). So far, reliable and effective vaccine that might prevent abortions is not available. Inactivated vaccine was applied in experimentally infected cattle, resulting in prevention of transplacental transmission of the parasite (Innes et al., 2002). In this way, effective protection from abortion was provided only in case that immunity had been developed prior to the exposure to primary infection.
Acknowledgment

This research is part of the Project EVB: 31084 financial supported by Ministry of Education, Science and Technological Development of the Republic of Serbia

*Neospora caninum* kod goveda: epizootiologija, dijagnostika i mere kontrole

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Rezime

*Neospora caninum* je protozoa, kokcidija, poznata kao uzročnik pobačaja kod krava. Raspredeljen je širom sveta kod mlečnih i tovnih grla. Neosporoza se javlja sporadično, endemic i epidemijski kod krava. Domaćin za *N. caninum* je pas i drugi karnivori, koji šire infekciju preko fecesa kada luči oociste u spoljašnju sredinu. Goveda se inficiraju ingestijom oocista hranom i vodom. Uzročnik se transplacentarno, vertikalna transmisija, prenosi na plod i posledice takve infekcije su različite i zavise od faze graviditeta. Abortusi najčešće nastaju između 3-7 meseca graviditeta. Pored pasa rezervoari infekcije mogu biti i ptice i živina.

Procenat seropozitivnih krava u svetu kreće se od 5-65% kod mlečnih krava. U nekim područjima utvrđeno je da je *N. caninum* odgovorna za više od 30% svih pobačaja kod krava. Faktori rizika za infekciju nisu još dovoljno proučeni, ali kontakt krava i pasa se smatra visoko rizičnim. Ispitivanja koja su vršena pokazala su da je seropozitivnost pasa sa farmi u visokoj korelaciji sa prevalencijom infekcije kod krava. Laboratorijska dijagnostika vrši se direktnim dokazivanjem uzročnika imunohistohemijskim bojenjem (IHC), metodom imunofluorescencije (IFAT), Western blot metodom (WB) i PCR, ili dokazivanjem specifičnih antitela za *N. caninum* metodom aglutinaicje (DA), ELISA i cELISA. ELISA test se pokazao dovoljno specifičan i osetljiv za ispitivanje prisustva infekcije na nivou zapata.

U radu su prikazana saznanja o značaju i ulozi *N. caninum* u patologiji muznih krava. Cilj rada je da se prikaže pregled sadašnjih saznanja i potencijalne faktore rizika za farme krava u Srbiji i mere kontrole koje su na raspolaganju. U radu će biti analiziran i upoređen stepen prevalencije patogena u zapatima mlečnih krava u Srbiji i uloga patogena u pobačajima.
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