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Markers related to the diagnosis and to the risk of abortion in bovine neosporosis

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ABSTRACT

Bovine neosporosis has emerged as a main cause of abortion in cattle worldwide. An important question to understand the disease is why not all infected cows abort. In the present review we summarize the knowledge on markers related to the diagnosis and more importantly to the risk of abortion in the infected cow. Markers considered herein include those based on specific antibodies, antibody titers and antibody subtypes, cellular immunological markers, hormones and other proteins related to gestation. The identification of parasite molecules that are specifically identified in the aborting cows might help to understand the mechanism of parasite-associated abortion and control the disease.

1. Introduction

Neospora caninum is an obligate intracellular protozoan closely related to *Toxoplasma gondii* that was first described in dogs in 1984 (Bjerkas et al., 1984) and identified since then in a wide range of warm-blooded animals (Almería, 2013; Dubey and Schares, 2011; Dubey et al., 2007). Despite a high host range, the disease is primarily of cattle (intermediate hosts) and dogs (definitive hosts). As an important cause of abortion in cattle, bovine neosporosis is today a disease of worldwide concern, causing severe economic impacts in both dairy and beef industries (Dubey and Schares, 2011; Moore et al., 2013; Reichel et al., 2013).

In the life cycle of *N. caninum*, there are three known infectious stages: tachyzoites and bradizoites in tissue cysts are found intracellular in the intermediate hosts in which asexual replication takes place, while oocysts are excreted by the definitive hosts in which sexual replication occurs. Post-natal transmission occurs by ingestion of tissues infected with tachyzoites or tissue cysts, and/or by ingestion of food or drinking water contaminated with sporulated oocysts. The ingestion of oocysts is the only demonstrated mode for horizontal transmission in herbivores. Cow-to-cow transmission of *N. caninum* has not been observed (Dubey et al., 2007). Dogs are able to excrete environmentally-resistant oocysts. Vertical transmission from an infected mother to the fetus is considered the main route of transmission in cattle. *N. caninum* is one of the most efficiently transplacentally transmitted organisms in cattle. Up to 95% of calves from infected mothers are born infected, clinically normal, but they are infected for life (Dubey and Schares, 2011). *Neospora*-infection can be maintained over several generations by vertical transmission in the herds (Pabón et al., 2007; Piergili Fioretti et al., 2003).

Abortion is the main clinical manifestation of the disease. Cows of any age may abort from 3 months of gestation to term, with most abortions occurring at 5–6 months of gestation. Fetuses may die *in utero* or be reabsorbed, mummified, autolyzed, stillborn, born alive with clinical signs or born clinically normal but persistently infected (Dubey, 2005). Abortion only occurs in a proportion of infected pregnancies, but *N. caninum* can cause repeated abortion (Anderson et al., 1995; Pabón et al., 2007; Williams et al., 2003). Two different abortion patterns associated with *Neospora*-infection have been observed in cattle herds: endemic and epidemic abortion outbreaks. The endemic pattern is characterized by an abortion problem in a herd persisting for several months or years, caused by reactivation of a chronic infection. In epidemic outbreaks, abortions are concentrated within a short period of time, most likely due to a recent point source exposure of naive cattle to *N. caninum* oocysts (Almería and López-Gatius, 2013; Basso et al., 2010).

For *Neospora*-associated abortion to occur, the fetus or placenta has to be damaged to the extent that pregnancy is no longer viable (Dubey and Schares, 2006). Several mechanisms might lead to abortion. Direct tissue damage can be caused by the multiplication of parasites in the placenta or in fetal tissues (Buxton et al., 2002; Innes et al., 2007) or due to insufficient oxygen/nutrition, secondary to

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placental damage (Dubey et al., 2006). Secondly, tissue damage can occur through the activation of the maternal immune system that elicits the production of pro-inflammatory cytokines, chemokines, nitric oxide or prostaglandins in the placenta (Buxton et al., 2002; Innes et al., 2007). In younger fetuses, neosporosis may cause death because of its uncontrolled proliferation, whereas its effect is more restricted with small focal necrosis in older fetuses that exhibit a more developed immunity (Dubey et al., 2006; Gibney et al., 2008).

No drugs exist to efficiently treat bovine neosporosis and there is not an effective vaccine for its control although many studies are focusing on the development of an efficacious vaccine. A summary of vaccine candidates and animal model tested for the prevention of the disease has been recently summarized (Monney and Hemphill, 2014; Monney et al., 2011). An important question is why only a proportion of infected pregnant cows abort. In a recent review we described epidemiological aspects of neosporosis in cattle from a clinical perspective (Almería and López-Gatius, 2013). In the present review we summarize the knowledge on markers related to the diagnosis, and more importantly, to the risk of abortion of the infected cow. Markers considered herein include those based on specific antibodies and antibody subtypes, cellular immune markers, such as gamma interferon (IFN-γ) and other cytokines, hormones and pregnancy-associated glycoproteins (PAGs). The identification of parasite molecules that are specifically induced in the aborting cows might help to understand the mechanism of parasite-associated abortions and control the disease.

2. Screening for humoral mechanisms

2.1. Maternal antibodies and abortion

Many studies have confirmed that *Neospora*-seropositive cows are more likely to abort than seronegative cows (Davison et al., 1999; García-Ispierto et al., 2013; Hietala and Thurmond, 1999; López-Gatius et al., 2004a; Mazuz et al., 2014; Schares et al., 2002; Williams et al., 2003). The endogenous transplacental infection is associated with an acute increase in maternal antibodies (Guy et al., 2001), and the recrudescence of latent infection during gestation is responsible for an increased abortion risk (Huang et al., 2007). In chronically infected herds, seropositive cows are three to seven times more likely to abort than uninfected cows (Innes et al., 2005). Furthermore, the risk of repetitive abortion remains in seropositive cows (Mazuz et al., 2014), increasing the abortion risk by almost twice throughout the reproductive life of cows compared to seronegative dams (Bruhn et al., 2013).

2.2. Serological tests in diagnosing bovine neosporosis

A great variety of assays are available to determine the presence of maternal *N. caninum* antibodies in cases of abortion or for the serological analyses in cattle herds. These methods include the indirect immunofluorescence test (IFAT), *Neospora*-agglutination test (NAT), enzyme-linked immunosorbent assay (ELISA), or immunoblotting, among others (Dubey and Schares, 2006; Dubey et al., 2007; Wapenaar et al., 2007). New techniques described recently include a dense granule protein (NcGRA6) incorporated in a latex beads agglutination test (LAT) (Ghalmi et al., 2014) or a disperse dye immunoassay technique for detection of antibodies against *N. caninum* (Selahi et al., 2013). Most of these techniques have been validated and have proved to be quite sensitive and specific for the detection of infection in cattle (von Blumroeder et al., 2004). However, they still have limitations. New serological tests should be designed to provide information regarding the phase of infection, the predominant route of transmission, or the abortion risk (Aguado-Martinez et al., 2005).

The ELISA tests are commonly used at the herd level which can be performed on sera, plasma or milk samples. Recently, a study compared all of the commercially available ELISAs (n = 10) and showed that most of the tests displayed high sensitivity (Se) and specificity (Sp) values when both gold standard criteria were considered. The best-adjusted ELISAs (HIPRA-CIVTEST, IDVET, BIOVET and IDEXX Rum) showed Se and Sp >95% (Alvarez-García et al., 2013). Yearly whole-herd serological screening for *N. caninum* (with the exception of calves under 6 months of age to avoid the detection of colostrum antibodies) is an effective, rapid method of detecting *N. caninum* infection and estimating the related risk of abortion in a herd. To reinforce the results of sera tests, the detection of lesions and parasite DNA of parasites in the brain (as well as heart and liver) of aborted fetuses are the best choices (Almería and López-Gatius, 2013; Ortega-Mora et al., 2006).

Many antigens have been evaluated as potential diagnosis antigens for the detection of antibodies against *N. caninum*, including tachyzoite lysates, protein fragments from tachyzoites, and recombinant proteins (Dubey and Schares, 2011). Potentially useful markers for serodiagnosis are continuously being analyzed, and some recent studies have focused on the highly antigenic region of subtilisin-like serine protease 1 (Ybañez et al., 2013), NcSRS2 surface protein (Pinheiro et al., 2013), *Neospora*-surface antigen (p40) (He et al., 2013), monoclonal antibodies against NcSAG1 (Dong et al., 2013), and anti-rNcp-43 polyclonal antibodies (Sá et al., 2014), among others.

2.3. Avidity ELISA tests: acute versus chronic infection

Avidity ELISAs have been used to distinguish acute *versus* latent infections (Aguado-Martinez et al., 2005; Basso et al., 2010; Björkman et al., 1999; Schares et al., 2002). Low avidity values are associated with acute infection (Basso et al., 2010). Serological and molecular techniques, including a p38-avidity-ELISA and typing of *N. caninum* in clinical samples by multilocus-microsatellite analysis, have been used in epidemiological studies (Basso et al., 2010). In a recent study, a *Neospora*-microneme protein (NcMIC10) was characterized and was proposed as a diagnostic marker to differentiate acute *versus* chronic infections (Yin et al., 2012).

2.4. Searching for antibody markers of abortion

To date, neither serological tests nor markers for serodiagnosis can be used to establish definitively that *N. caninum* causes an abortion in an individual cow. Huang et al. (2007) analyzed the dense-granule protein NcGRA7 as a new marker for the serodiagnosis of *Neospora*-infection in aborting cows. The results indicated that the production of the anti-NcGRA7 antibody was up-regulated in aborting cows, which could be of interest in the diagnoses of cows which will suffer abortion. In a more recent study, the use of recombinant NcGRA7 and NcSAG1 antigens as an indicator of *Neospora*-activation was reported (Hiasa et al., 2012). These results are promising for a specific serological marker linked to abortion in cattle.

2.5. Antibody titration as a marker of abortion risk

Several studies have linked high antibody titers against the parasite to an increased risk of abortion (Bech-Sabat et al., 2007; Brickell et al., 2010; Kashiwazaki et al., 2004; López-Gatius et al., 2005; Yániz et al., 2010). Therefore, it seems that cows aborting due to neosporosis have a higher *Neospora*-specific antibody response than infected but non-aborting cows. In effect, irrespective of the herd level of *Neospora*-seroprevalence, the plasma antibody titer against *N. caninum* was a good indicator of the risk of abortion (López-Gatius et al., 2004b) and *Neospora*-antibody titers were significantly