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Immune and inflammatory biomarkers in cases of bovine perinatal mortality with and without infection in utero

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ABSTRACT

The objective of this study was to compare acute-phase protein [serum amyloid A (SAA) and haptoglobin (Hp)] and immunoglobulin G_1 and M concentrations in blood plasma of cases of bovine perinatal mortality due to infection in utero or traumotocia and in unexplained cases. Plasma samples were collected from 110 stillborn calves with bacterial infection (INF_B, n = 16), with viral or parasitic infection (INF_V/P, n = 31) during pregnancy, with lesions of fatal traumotocia (TRAUM, n = 22), and from unexplained deaths (UNEXPL, n = 41). Plasma immunoglobulin and SAA concentrations were measured by ELISA, and Hp concentrations were measured by the guaiacol method and ELISA. Concentrations of SAA in the INF_B group were higher than in the UNEXPL group and tended to be higher than in the INF_V/P group. A reference range (0–29 mg/L) was established for SAA in stillborn calves. Concentrations of Hp tended to be higher in the INF_B group compared with INF_V/P group. Concentrations of IgM tended to be higher in the INF_B group compared with the TRAUM and INF_V/P groups. Concentrations of IgG_1 were numerically, but not significantly, higher in the INF_V/P and INF_B groups compared with the other groups. The results demonstrate upregulation of immune and inflammatory responses in stillborn calves exposed to bacterial infection in utero. The immune-inflammatory parameters did not differ between calves with viral or parasitic infections and traumotocia. These immune-inflammatory profiles did not contribute to the diagnosis of unexplained stillbirth. This is the first report of an elevated acute phase protein response in stillborn calves. Measurement of SAA and IgM concentrations may be used in the diagnosis of bacterial infections in stillborn calves.

Key words: bovine, stillbirth, acute phase proteins, in utero infection

INTRODUCTION

Bovine perinatal mortality may be defined as calf death at full-term pregnancy (≥260 d), before, during, or within 48 h of calving (Berglund et al., 2003; Mee et al., 2014). Herd-level perinatal mortality rates vary between 0 and 30%, with median values between approximately 4 and 7% (Fourichon et al., 2001; Mee et al., 2008). In recent years, high and increasing perinatal mortality rates have been reported internationally, particularly in Holstein primiparae (Meyer et al., 2001; Berglund et al., 2003; Mee et al., 2008). The significant modifiable and nonmodifiable risk factors associated with bovine perinatal mortality/stillbirth have recently been documented (year of calving, month of calving, plurality, primiparity, previous perinatal mortality, and fetal sex; Mee et al., 2014).

Despite this knowledge about risk factors, stillborn calves remain a diagnostic challenge. The causes of death (**COD**) in bovine stillbirth vary internationally (Mee et al., 2013) but they may be classified simply as noninfectious and infectious. Multiple COD (co-mortality) and causative agents complicate diagnosis. The most common noninfectious cause of bovine stillbirth is moderate and severe calving assistance (dystocia or traumotocia; Mee and Szenci, 2012; Mee, 2013); even slight calving assistance (but not dystocia) is associated with increased risk of stillbirth (Mee et al., 2008). However, in some studies, dystocia explained only about half of the stillbirths from primiparae (Berglund et al., 2003). This suggests that other non-dystocial factors are associated with bovine stillbirths.

Numerous infectious causes of bovine stillbirth have been documented. Compared with abortion (birth of a non-viable fetus preterm), infection is a less common COD in stillborn calves. Infections account for between 3 and 12% of stillbirths diagnosed internationally in necropsy studies (Mee, 2013). Infectious agents detected in stillborn calves include *Salmonella* Dublin,

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Escherichia coli, *Aeromonas*, *Proteus*, *Streptococcus*, *Staphylococcus*, *Neisseria*, *Absidia*, *Acinetobacter* spp. (Smyth et al., 1992), *Corynobacterium pyogenes*, *Leptospira* spp. (Smyth et al., 1992, 1999), *Bacillus licheniformis*, *Mannheimia varigena* (Syrjala et al., 2007), *Listeria* spp., *Neospora caninum* (Waldner et al., 2010), *Salmonella* Stanley (Jawor et al., 2013), *Coxiella burnetii* (Waldner et al., 2010; Muskens et al., 2012), bovine viral diarrhea virus (Smyth et al., 1992), Schmallenberg virus (Bayrou et al., 2014), and bovine herpesvirus 1 and 4 (Kirkbride, 1992; Egyed et al., 2011). It is recognized that some of these isolates are probably contaminants (e.g., *Proteus*) and others are probably secondary opportunistic infections (e.g., *Streptococcus*).

In approximately one-third of perinatal mortality cases, no cause of death can be detected (Berglund et al., 2003; Khodakaram-Tafti and Ikede, 2005). The "unexplained stillbirth" is an additional problem for veterinary practitioners and for laboratory diagnosticians. The high rate of unexplained stillbirths in calves may be due in part to the traditionally narrow diagnostic testing menu used in veterinary laboratories. Hence, various physiological markers have been investigated to see whether it is possible to predict stillbirth even if the COD cannot be diagnosed. These included parameters in dams (Kindahl et al., 2002; Kornmatitsuk et al., 2004) and in calves (Kornmatitsuk et al., 2004) to monitor fetal well-being and predict stillbirth. However, these studies had a small number of dams and stillborn animals, limiting their ability to define useful parameters. In addition, although these studies tried to predict the risk of stillbirth, they could not determine the COD as being infectious or noninfectious.

An alternative approach to investigating unexplained stillbirths is to monitor markers of infection as possible predictors of stillbirth. Both circulating neutrophils and immunoglobulins have been studied. High maternal prepartum neutrophil counts predicted the risk of stillbirth in heifers (Chassagne et al., 1999). In cattle, immunoglobulins from the dam do not cross the placenta but the fetus has the ability to produce IgM and IgG after 90 and 111 d of pregnancy, respectively (Ellis et al., 1978). The older the fetus, the higher the concentrations of IgM and IgG detectable in serum (Sawyer et al., 1973). High serum immunoglobulin concentrations are strong indicators of fetal infection (Ohmann, 1981). For example, serum samples from bovine fetuses experimentally infected with chlamydia, *Campylobacter fetus*, *C. burnetii*, bluetongue virus, bovine virus diarrhea virus (**BVDV**), *Anaplasma marginale*, and the agents of epizootic bovine abortion had higher IgM and IgG concentrations compared with those of precolostral newborn control calves (Sawyer et al., 1973). Calves vaccinated prenatally with *E. coli* from 9 to 102 d before birth took 10 to 14 d to respond to bacterial antigen administered orally (Conner et al., 1977). In addition to a humoral immune response, intrauterine infection can lead to fetal infection and inflammation. Microorganisms may gain access to the amniotic cavity and fetus by ascending from the vagina and cervix or via hematogenous dissemination through the placenta (transplacental infection). The most common pathway of intrauterine infection is the ascending route (Romero et al., 2003). Microorganisms or their products can stimulate the fetus to produce cytokines and a systemic inflammatory response. In human medicine, this is described as the fetal inflammatory response syndrome (**FIRS**) and is diagnosed by elevated IL-6 concentration in fetal blood; FIRS is typically diagnosed in preterm fetuses but can also occur in term fetuses (Chaiworapongsa et al., 2002; Bashiri et al., 2006). Human fetuses with FIRS have a higher rate of neonatal complications and are frequently born to mothers with subclinical microbial invasion of the amniotic cavity (Bashiri et al., 2006).

In contrast to human fetuses, little is known about the immuno-inflammatory responses in bovine fetuses. To date, there have been no studies on the acute-phase response in bovine stillborn calves. The acute-phase response is nonspecific and it develops after any disturbance of homeostasis, but particularly inflammation. The body mounts a multifactorial response to remove and replace damaged tissue. One of the mechanisms involved is the production and secretion of acute-phase proteins (**APP**) by the liver (Eckersall and Conner, 1988). In ruminants, testing for APP allows early and precise detection of inflammation. The most common APP measured in cattle are haptoglobin (**Hp**) and serum amyloid A (**SAA**) (Eckersall and Bell, 2010). The APP have been widely used during monitoring of treatment (Smith et al., 1998; Jawor et al., 2008), as an objective parameter for detecting sick animals (Gruys et al., 1993; Deignan et al., 2000), monitoring of calf group health (Gånheim et al., 2007; Furman-Fratczak et al., 2011), or as a tool to estimate the safety of repeated liver biopsy (Vels et al., 2009; Jawor et al., 2016). To date, there are no published studies about APP in stillborn calves.

The hypothesis tested in this study was that in cases of perinatal bovine mortality, where infection is present, there will be increased concentrations of fetal circulatory immunoglobulins and APP. Two sub-hypotheses were tested: (1) that the magnitude of the fetal immuno-inflammatory response may differ depending on the type (bacterial, viral, parasitic) of infection, and (2)