Infection exposure, detection and causes of death in perinatal mortalities in Polish dairy herds

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ABSTRACT

The objective of this study was to determine the prevalence and types of infections in perinatal mortality (PM) cases from Polish dairy farms and the relevance of the presence of infection to the cause of death. This prospective longitudinal study was carried out on 121 PM and 21 control calves with a gestation of ≥260 days. Six control calves were euthanized and examined using the same protocol as for PM calves. Material was collected over a 20-month period between November 2013 and June 2015. The PM and control calves were collected from 29 to 5 herds, respectively.

Blood samples from calves were tested for antibodies to *Neospora caninum*, glycoprotein B of BoHV-1, BVDV and SBV using ELISAs and *Leptospira hardjo* and *Leptospira pomona* with the microscopic agglutination test. Brain and kidney samples from all PM and six euthanized control calves were tested using real time PCR to detect *Neospora caninum,* pathogenic *Leptospira* spp., BoHV-1 and SBV; brain was examined histopathologically for detection of *N. caninum* cysts. Samples from eight inner organs from all PM and six control calves were cultured aerobically, anaerobically and microaerobically. Ear samples from all PM and control calves were tested for BVDV using an antigen ELISA.

In total, 21.5% of PM calves were infected (antigen and/or antibody-positive) *in utero*; none of the control calves were infected. Direct evidence of infection (culture, Ag-ELISA, PCR, histopathology) was detected in 9.1% of PM calves. Gestation length in infected singletons was shorter than in uninfected singletons (274 ± 8 vs. 279 ± 7 days; P < 0.01). The odds ratio for diagnosis of infection in single pregnancies ≤275 days was 3.75 (95% CI:1.2−12.1), (*P* < 0.05). Infection was the cause of death in 10% of calves. The most common infections detected in these Polish PM calves were parasitic (11.6% of PM cases), viral (7.4%) and bacterial (5%). This study demonstrated that indirect evidence of infection is detected more frequently than direct, coinfection is rare, infection is rarely accompanied by gross lesions and is rarely a cause of death in cases of PM.

1. Introduction

Perinatal mortality (PM) has a detrimental effect on cow health, survival, reproductive performance and milk production [1,2]. Perinatal mortality may be defined as calf death at full-term (≥260 days), prior to or during, or within 12−48 h of parturition [3,4].

There is wide variation in the incidence of PM by farm [2]. In Poland the mean PM rate was 8.1 and 4.8% in first and later calvings, respectively [5]. Important risk factors for PM include age at first calving [6], breed of dam, breeding method, calving management, feto-maternal health status, length of gestation [7], gestational nutrition, calf sex and sire [4].

The causes of death in PM are multifactorial and include non-infectious and infectious causes. The major causes of bovine PM, based on necropsy studies, are dystocia and anoxia and to a lesser extent, infections and congenital defects [8]. Compared to abortion

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(birth of a non-independently viable fetus pre-term), infection is a less common cause of death in PM calves accounting for 3–12% of cases [8].

Numerous infectious agents have been detected in cases of bovine PM including; *Salmonella* Dublin, *E. coli, Aeromonas, Proteus, Streptococcus, Staphylococcus, Neisseria, Absidia, Acinetobacter* species [9], *Corynebacterium pyogenes, Leptospira* species [9,10], *Bacillus licheniformis, Mannheimia varigena* [11], *Listeria* spp., *Neospora caninum* [12], *Salmonella* Stanley [13], *Coxiella burnetii* [12,14], *Brucella abortus, Aspergillus* spp., *Campylobacter* spp., *Trichomonas foetus* [15], bovine viral diarrhea virus (BVDV) [17,18], orthobunyaviruses including the recently emerged Schmallenberg virus (SBV) [16] and bovine herpes virus 1 and 4 (BoHV-1, BoHV-4) [17,18]. It is recognized that some of these isolates are probably contaminants (e.g. *Proteus*), while others are probably secondary opportunistic infections (e.g. *Streptococcus*). Although there are many reports investigating infection in abortion cases, few focus on involvement of infectious agents in PM cases and in some cases results for aborted and PM calves are combined [18–20].

There is no information about the prevalence of infectious agents in PM calves in Poland. Therefore the objective of this study was to examine PM cases from Polish dairy farms to detect direct and indirect evidence of infections and the relevance of the presence of infection to the cause of death. A secondary objective was to compare findings in PM cases with new-born live calves.

2. Material and methods

The study design was approved by the II Local Ethics Commission in the Wroclaw University of Environmental and Life Sciences (permission no. 23/2012, 58/2014, 60/2014).

2.1. Calves

This prospective study was carried out on 121 perinatal mortality (PM) cases and 21 control (C) calves. Material was collected over a 20-month period between November 2013 and June 2015. None of the calves received colostrum.

Perinatal mortalities were defined as calves born following a gestation of ≥260 days which died before, during or within 6 h after birth (a case definition of death within 24 h was planned but no calves died between 6 and 24 h after birth). These calves were either Holstein-Friesian (n = 113) or Holstein-Friesian crossbreds (Simmental, Jersey, Limousin, Brown Swiss sires; n = 8).

Control calf inclusion criteria were: gestation length ≥260 days, singleton calving, and Holstein-Friesian breed. All C calves (6 females and 15 males) were born after assisted calvings (6 calves without and 15 with a calving jack). From this group six male C calves (selected on the basis that owners agreed to sell the newborn male calf) were euthanized between 1.30 and 8.30 h after birth. They were premedicated with 1 ml (20 mg) xylazine (Sedazin®, Biowet Pulawy) and 1 ml (100 mg) ketamine (Bioketan®, Vetoquinol) IV and then euthanized with a mixture of pentobarbital and pentobarbital sodium, 160 mg/ml (Morbital®, Biovet Pulawy) IV at a dose of 48–96 mg/kg bw.

2.2. Herds

The study was conducted in 30 herds in the south-west of Poland. Herds were recruited as a convenience sample located within 2.5 h one-way driving distance from the University.

Each farmer was provided with a mobile phone number to call the three veterinarians who collected the material on all days and times (day and night) as soon after calf death as possible. Information on the farm management, herd records and details of the calving associated with the calf were collected at farm visits. These data included information about the herd (number of lactating cows, milk yield in the previous lactation, intercalving period, vaccination program), cow – (date of the last service), and calving (single or twin/triplets). The degree of calving assistance was recorded in seven categories (supplied to the farmers): unobserved, observed but not assisted, normal assisted calving without calving jack, normal assisted calving with calving jack, difficult calving with calving jack, difficult calving without calving jack (assistance by at least three people and/or a veterinarian) and caesarean section. The median herd size was 73 (range 1–1037 cows/herd) and the mean (SD) inter-calving period and previous lactation milk yield was 415 (38) days and 8884 (1442) kg/cow/305 DIM, respectively. Fifteen herds were vaccinated in 2013–2015; against BVD alone (n = 7), BoHV-1 alone (n = 4) or against BVD and BoHV-1 (n = 4). PM and C calves were collected from 29 to 5 herds, respectively. Between 1 and 35 cases of PM and between 2 and 7 cases of C calves were investigated per herd.

2.3. Necropsy and laboratory examinations

Necropsies were performed by the same three veterinarians, at least two of which were present at the same time in the necropsy laboratory at the University of Environmental and Life Sciences, Wroclaw. All carcasses (PM and six C calves) were subjected to systematic external and internal gross examinations and sampling according to the same project-specific protocol.

2.3.1. Samples

Samples from an ear, blood, abomasal contents and eight internal organ tissues (spleen, liver, lung, small intestine, left kidney, left adrenal gland, heart, brain) were collected from 121 PM calves. From C calves ear and blood samples were collected from 21 cases. Abomasal contents and eight internal organs (the same as in PM cases) were collected from 6 euthanized calves. For each calf a sterilized set of surgical instruments was used and during necropsy ethanol and flame sterilization was performed. Abomasal fluid was collected (by abomasocentesis) immediately after opening the abdominal cavity with a sterile needle and syringe.

2.3.1.1. Ear samples.
Ear biopsies were collected using an ear notcher and individually tested for bovine virus diarrhea virus (BVDV) using an antigen ELISA (IDEXX BVDV Ag/Serum Plus Test, Hoofddorp, The Netherlands) by the veterinary laboratory Vetlab LP, Wroclaw, Poland. The result was regarded as negative when the optical density (OD) sample minus negative (S-N) value was ≤0.2, suspect >0.2 and ≤ 0.3 and positive when OD > 0.3.

2.3.1.2. Blood samples.
Blood from a jugular vein was aseptically collected by syringe and immediately dispensed into 5 ml lithium heparinized tubes (MEUS Srl®, Piove di Sacco, Italy, 18648), centrifuged (14 min; 1860 × g), and plasma samples were aliquoted and frozen at −80 °C until analysed.

Plasma samples were tested for antibodies to *Neospora caninum*, glycoprotein B of BoHV-1 and BVDV using the IDEXX Neospora Ab Test, IDEXX IBR gB X3 Ab Test and IDEXX BVDV Total Ab Test (Hoofddorp, The Netherlands), respectively, at the veterinary diagnostic laboratory (Weterynaryjna Diagnostyka Laboratoryjna, Gietrzwald, Poland). The sample was classified as seropositive for *Neospora caninum* when the OD S/P was ≥0.5. For the BoHV-1 ELISA, the blocking % was calculated and samples with blocking values < 45% were regarded as negative; values between 45 and 55% were inconclusive and values > 55% were regarded as positive. In the BVDV Ab ELISA, samples with S/P OD values < 0.2 were classified as negative, 0.2 to <0.3 as inconclusive and ≥0.3 as