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Evaluation of bovine abortion cases and tissue suitability for identification of infectious agents in California diagnostic laboratory cases from 2007 to 2012

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ABSTRACT

Establishing a definitive cause of bovine abortion is a challenging problem faced by veterinary practitioners and diagnosticians. Detection of an infectious or noninfectious source for abortion may facilitate interventions that mitigate future fetal loss in the herd. The purposes of this study were to identify the most common causes of bovine abortion in cases submitted to the California Animal Health and Food Safety Laboratory System, Davis (CAHFS) from 2007 to 2013 and to determine if detection of infectious pathogens differed with the fetal tissue evaluated. Records of 665 bovine abortion cases of 709 animals were reviewed for pathologic diagnoses, test methods used to identify causative conditions, and which tissues yielded successful identification of infectious agents associated with abortion. Over 58% of abortions were attributed to an infectious cause and 46.9% had an infectious agent identified. The most common infectious conditions were Epizootic Bovine Abortion (EBA) (16.2% of all fetuses), other fetal bacterial infections (14.7% of all fetuses), and Neospora caninum (9.3% of all fetuses.) The bacterium associated with EBA (currently named Pajaroellobacter abortibovis) was most commonly identified by immunohistochemistry (IHC) in lymphoid organs (thymus and spleen); N. caninum IHC was most frequently positive in brain, kidney, and placenta. In cases of pathogenic and opportunistic bacterial infections, abomasal samples yielded a significantly greater proportion of definitive aerobic culture results than lung or liver tissues. Direct fluorescent antibody test results for Bovine Viral Diarrhea Virus testing were identical between lung and kidney tissues and nearly identical (96.0%) for Bovine Herpesvirus I. Noninfectious abortive conditions included fetal stress (10.5%), dystocia (3.9%), congenital defects (3.3%), toxicological or mineral problems (1.8%), and death of the cow (1.1%). Just over 20% of the aborted fetuses had no gross or histopathological lesions to explain the abortion. This review highlights the need for submission of critical samples including abomasal contents, lymphoid tissues (thymus, spleen, and lymph nodes), and brain to maximize the diagnosticians' ability to identify causes of abortion.

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1. Introduction

Fetal loss is a major detriment to cattle operations that can result in large economic losses [1]. Associated impacts include costs of lost milk production associated with longer calving intervals; decreased average calf weight at weaning associated with calves born late in the calving season; loss of calf revenue from cows that abort; expenses associated with rebreeding (bull maintenance, AI); and costs of replacement for cows that have aborted and are culled [2]. Costs to the producer can be as high as \$1900 per abortion based on stage of pregnancy, cow performance, current prices, and producer decisions [3,4]. Effects on profit may be greater in







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natural-service operations because the open cow may not be identified until months after the abortion and her reproductive potential will be lost for the year, resulting in early culling and associated replacement costs [1].

Many factors influence the viability of a bovine fetus during gestation, including hormonal fluctuations, genetics, compromised blood, nutrient or oxygen supply to the fetus, and exposure to pharmacologic, environmental, toxic, or infectious agents at critical times of gestation [5–7]. Establishing a definitive cause of fetal loss can be difficult because of the absence of pathognomonic lesions, the lack of available confirmatory tests for certain conditions, and the time between insult and expulsion and/or examination of fetal tissues [8]. In addition, tissue autolysis, specific requirements for bacterial culture, and the lack of appropriate samples can interfere with recovery of causative agents [1,2,5,9–11].

Infectious agents are frequent causes of abortion in cattle; they can be associated with sporadic and epidemic abortions, often without clinical signs in the cow other than the abortion [1,2,5,10,12,13]. Placental tissues can provide useful diagnostic information but may not be available for testing or may be contaminated at the time of examination [2,10,11]. Recognition of an increased incidence of abortion is central to successful implementation of diagnostic and intervention strategies [5,14]. The purposes of the present study were to identify the most common causes of bovine abortion in cases submitted to the Davis branch of the California Animal Health and Food Safety Laboratory System (CAHFS, Davis) from 2007 to 2013 and to determine if detection of infectious pathogens differed according to the fetal tissue evaluated.

2. Materials and methods

2.1. Case selection

Diagnostic reports of bovine abortions submitted to CAHFS, Davis between 2007 and 2013 were reviewed for inclusion in this study. A case was defined as one or more fetuses or tissues submitted at the same time from a single herd. Cases consisting of intact fetuses or tissues from necropsies conducted in the field were included only if all the following fresh and/or fixed tissues were available for evaluation: brain, thymus, heart, lung, liver, kidneys, abomasum and/or abomasal contents, intestine, and fetal blood and/or fluid. Perinatal submissions with aerated lungs were excluded from the study. Cases with multiple fetuses in which any one was diagnosed with an infectious cause were included in a category of infectious agent (viral, bacterial, protozoal, and fungal). Each individual fetal result was included in the count of specific infectious agents.

2.2. Abortion diagnostics

Abortion work-ups included evaluation of clinical history, gross and histopathologic examinations, immunoglobulin G quantitation on fetal fluid and/or blood and if elevated (>20 mg/dL) specific serologic titer testing for Bovine Herpesvirus 1 (BHV-1), Bovine Viral Diarrhea Virus types 1 and 2 [BVDV-1, BVDV-2], *Neospora caninum, Brucella abortus*,

Bluetongue Virus, and Parainfluenza 3 Virus; bacteriologic cultures for Brucella spp., Campylobacter spp., and aerobic bacteria, direct fluorescent antibody testing of kidney tissue for Leptospira spp. (multivalent conjugant, rabbit origin, National Veterinary Services Laboratory, Ames, IA, USA) and on frozen sections of kidney and lung for BHV-1 and BVDV (fluorescein isothiocyanate-conjugated, monoclonal, Veterinary Medical Research and Development, Pullman, Washington). Serologic testing for BVDV-1, BVDV-2, B. abortus, BHV-1, and Leptospira spp. serovars Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, and Pomona was performed on fetal blood and dam's blood (if available). Additional testing was completed if indicated by lesions present. These included immunohistochemistry (IHC) for N. caninum [15], Coxiella burnetii, [16], and the bacterium (presently identified as Pajaroellobacter abortibovis) [17] that causes Epizootic Bovine Abortion (EBA) [18]. Fungal cultures on placenta and/or abomasal tissues were performed if indicated. A combination of histochemical staining (Gormori's methenamine silver [GMS] and periodic acid-Schiff [PAS]) and IHC diagnostics for other specific etiologic agents was performed as requested by the pathologist. Gestational age was estimated using crown-rump length measurements at the time of necropsy [19]. The diagnosis was on the basis of combination of observed characteristic gross and histopathological changes detected by the case pathologist along with results of ancillary diagnostic tests and was considered the true disease status of each fetus when evaluating the sensitivity of an individual diagnostic method or site sampled; however, because nondiseased status could not be defined, specificity was not determined in this study.

2.3. Abortion classification

Abortion diagnoses were categorized into those that had (1) pathologic changes attributable to an identified infectious agent, (2) an infectious cause with no agent identified, (3) nonspecific lesions with no agent identified, (4) no detectible gross or histopathologic abnormalities to explain fetal loss, (5) obvious congenital defects, (6) lesions associated with toxins or mineral abnormalities, (7) term calves showing evidence of death because of dystocia, and (8) cases in which the cow died. Fetuses for which an infectious cause was identified were categorized by trimester, and suitability of sampled fetal sites was evaluated for detection of abortive agents. Bacterial dissemination included cases with systemic inflammation associated with bacterial organisms, including bronchopneumonia, pleuritis, peritonitis, hepatitis, splenitis, enteritis, placentitis, nephritis, and vasculitis from which a pure or nearly pure culture of bacteria was recovered. "Opportunistic bacteria" were defined as agents other than Campylobacter spp., Leptospira spp., Listeria spp., Salmonella enterica, or C. burnetii. Etiologic agents were defined as moderate to large numbers of single bacterial organism. Mixed cultures containing low numbers of greater than three distinct bacterial colony types with no confirmed pathogenic bacteria were not considered as positive for an etiologic agent. Cases which had a bacterial agent recovered without associated gross or histologic lesions were classified "No lesions detected".

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