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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.

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".

Criteria for dystocia included term calves with head and neck edema and hemorrhage, subcutaneous emphysema, rib fractures, intrathoracic hemorrhage, and/or ruptured liver, with lack of histologic abnormalities in any of the examined tissues. Although cases of dystocia and cow death are not "abortions" per se, these categories were summarized to determine the incidence of these diagnoses. Mineral deficiency cases were defined by subnormal mineral concentrations accompanied by histopathologic lesions (skeletal and/or cardiac myocyte necrosis); toxic causes required lesions along with chemical detection and/or known exposure of the dam to a specific toxicant. Fungal diagnoses included visualization of disseminated hyphal elements or yeast with or without positive cultures from nonplacental sites (to remove bias from possible environmental contamination).

2.4. Statistical analysis

Fetal submissions in which lung, liver, and abomasal fluid samples were cultured aerobically (and for which culture media was not overgrown by contamination [*Proteus* sp.]) were assessed for recovery of etiologic bacterial agents. The sensitivity of bacterial detection along with 95% confidence intervals was calculated for each tissue site sampled. Standard errors for sensitivity were calculated using normal approximation of standard errors for proportions. In abortions determined to be caused by a bacterial infection, the probability of agent recovery was compared among tissue sites for significant ($P < 0.05$) differences using a chi square test for independence. Two subsets of animals in this category (defined as "disseminated" with multiorgan inflammation and bacteria in examined tissues or "bronchopneumonia" with primarily diffuse neutrophilic infiltration and bacteria in the lungs) were examined separately for significant ($P < 0.05$) differences in the likelihood of bacterial detection between the three sampled sites using Fisher's exact test (because several cells of the contingency table had expected frequencies below 5; SAS version 9.4; SAS Institute, Cary, NC, USA).

3. Results

3.1. Abortion classification

Seventy-two cases were rejected for this evaluation because of missing tissue samples or indications that the calf was born alive (aerated lungs, the presence of colostrum in the abomasum). A total of 665 abortion cases (comprising 709 fetuses) were included in the study, consisting of 581 cases of one or more entire fetuses for each case and 84 cases of fetal tissues. Of the 709 individual fetuses, 303 were from dairy breeds, 284 were from beef breeds, and 122 were of nonspecified breed. Twenty cases contained twins, and 24 cases had multiple aborted fetuses (14 with two, seven with three, two with four, and one with five fetuses per case) submitted from the same farm. The summary of etiologic classes is shown in Figure 1. Over 58% (387/665) of abortions had lesions consistent with an infectious agent and 46.9% (312/665) had an infectious agent identified. Just over one fifth of the cases (138/665, 20.8%) and fetuses (144/709, 20.3%) had no detectable gross or histopathologic lesions to explain the cause of the abortion.

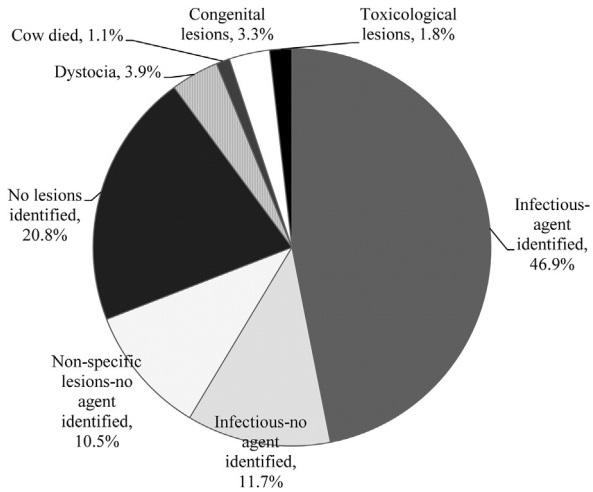

Fig. 1. Summary of etiologic classes associated with bovine abortion cases examined at California Animal Health and Food Safety Laboratory System from 2007 to 2013 (n = 665).

Among the 312 submissions for which an infectious agent was identified, the detected cause included 67.0% bacterial, 10.6% viral, 18.3% protozoal, and 4.1% fungal agents. Table 1 lists the number of fetuses diagnosed with infectious causes of abortion by trimester, with the largest proportions attributed to EBA (115/335, 34.3%), pathogenic (31/335, 9.3%) or opportunistic (73/335, 21.8%) bacterial infection, and *N. caninum* (66/335, 19.7%). Over 96% (111/115) of EBA affected fetuses demonstrated characteristic lesions consisting of lymphohistiocytic meningoencephalitis, nonsuppurative vasculitis, mononuclear lymphadenitis and thymitis, IHC demonstration of the causative bacterium in lymphoid tissue, and fetal hyperglobulinemia. The remaining cases had characteristic lesions, but IHC staining of tissues was equivocal. Similarly, *N. caninum* organisms were identified by IHC within typical lesions, such as necrotizing, nonsuppurative encephalitis, nephritis, and myositis, in 78.8% (52/66) of aborted fetuses. An additional 9.1% (6/66) of fetuses had lesions consistent with this agent and detectable fetal titers to *N. caninum* but no organisms detected by IHC. Confirmation on the remaining abortions was on the basis of presence of characteristic lesions in multiple tissues and maternal serology.

Recovered species from opportunistic bacterial dissemination cases included *Trueperella pyogenes* (n = 24), *Escherichia coli* (n = 14), *Streptococcus bovis* complex (n = 13), *Mannheimia haemolytica* (n = 9), *Pasteurella multocida* (n = 5), *Bacillus* sp. (n = 3), and a single case each of *Staphylococcus aureus*, *Nocardia* sp., *Klebsiella pneumoniae*, *Aerococcus urinae*, and *Enterobacter* sp. Among this group, 61.6% (45/73) of fetuses had evidence of bacterial dissemination, including hepatitis, conjunctivitis, nephritis, splenitis, vasculitis, fibrin thrombi, and bronchopneumonia, whereas just over 38% (28/73) were diagnosed with primary bronchopneumonia alone. Pathogenic bacterial causes consisted of *S. enterica* (n = 3), *Campylobacter jejuni* (n = 4), *Campylobacter fetus* sp., *venerealis* (n = 4),

Table 1
Numbers and percentages of examined fetuses and/or fetal tissues by trimester associated with identified infectious agents from aborted fetuses (those with an infectious cause [n = 335] and overall total [n = 709] examined at CAHFS from 2007 to 2013).

Etiologic agent	First trimester	Second trimester	Third trimester	Total	Fetuses with an infectious cause (%)	Total fetuses (%)
EBA	0	13	102	115	34.3%	16.2%
Neospora caninum	0	51	15	66	19.7%	9.3%
Total bacteria	2	15	87	104	31.0%	14.7%
Opportunistic bacteria[a]	2	13	58	73	21.8%	10.3%
Pathogenic bacteria						
Listeria monocytogenes	0	1	2	3	0.9%	0.4%
Listeria ivanovii	0	1	9	10	3.0%	1.4%
Leptospira spp.	0	0	5	5	1.5%	0.7%
C jejuni subsp. *jejuni*	0	0	4	4	1.2%	0.6%
C fetus subsp. *fetus*	0	0	1	1	0.3%	0.1%
C fetus subsp. *venerealis*	0	0	4	4	1.2%	0.6%
Salmonella enterica	0	0	3	3	0.9%	0.4%
Coxiella burnetii	0	0	1	1	0.3%	0.1%
Fungi/yeast	0	2	11	13	3.9%	1.8%
BHV-1	0	2	23	25	7.5%	3.5%
BVDV	0	0	12	12	3.6%	1.7%

Abbreviations: BVDV, Bovine Viral Diarrhea Virus; CAHFS, California Animal Health and Food Safety Laboratory System; EBA, epizootic bovine abortion; BHV-1, Bovne Herpesvirus I.

[a] Represents cases in which a bacterial etiology was identified, including *Trueperella pyogenes* (n = 24), *Escherichia coli* (n = 14), *Streptococcus bovis* complex (n = 13), *Mannheimia haemolytica* (n = 9), *Pasteurella multocida* (n = 5), *Bacillus* sp. (n = 3), and a single case each of *Staphylococcus aureus*, *Nocardia* sp., *Klebsiella pneumoniae*, *Aerococcus urinae*, and *Enterobacter* sp.

Campylobacter fetus sp., *fetus* (n = 1), *Listeria monocytogenes* (n = 3), *Listeria ivanovii* (n = 10), *Leptospira* spp. (n = 5), and *C. burnetii* (n = 1). An additional 11.7% (78/665) of cases demonstrated lesions consistent with an infectious cause (abomasitis, pleuritis, peritonitis, hepatitis, splenitis, myocarditis, encephalitis, and thymitis); however, an infectious agent was not identified. In 39 of these, bacteria could be seen histologically but either no organisms were recovered or culture plates were overgrown with nonpathogenic bacteria.

Over 10% (70/665) of cases had nonspecific changes in a variety of tissues which were not associated with an infectious agent, including meconium aspiration, bile canalicular stasis, equivocal to mild pulmonary neutrophilic or histiocytic infiltrate with hemorrhage, thymic necrosis, enterocyte crypt necrosis, and mild inflammation of a variety of organs (epicarditis, myositis, nephritis, and hepatitis). Identified congenital lesions (22/665, 3.3%) included vertebral column and spinal cord malformations (scoliosis, kyphosis), adrenal hypoplasia, arthrogryposis, abdominal wall malformations, hydrocephalus, cardiac abnormalities including ventricular septal defects, brachygnathism, prognathism, rectal aplasia with atresia ani, thyroid gland hyperplasia, liver shunt, and cleft palate. Mineral abnormalities or toxicological causes were present in a small number of cases (12/665, 1.8%), primarily selenium deficiency (n = 8), copper deficiency or toxicity (n = 2), and isocupressic acid metabolites implicated in pine needle abortions (n = 2). One case of suspected nitrate-associated abortion was identified with toxic levels detected in feed; however, the fetus had no gross or histologic lesions, and ocular fluid was not tested, so nitrate-associated abortion could not be confirmed.

3.2. Tissue suitability for identification of infectious agents

A total of 98 fetuses were diagnosed with bacterial abortion from culturable agents and used to assess the successful recovery of causative agents by tissue site sampled. The sensitivity (with 95% confidence intervals) for abomasal samples was 81.4% (72.3, 88.6), for lung samples was 59.8% (49.4, 69.6), and for liver samples was 35.1% (25.6, 45.4). Evaluation of a subset of these bacterial cases (n = 89) in which lung, liver, and abomasal sites were cultured demonstrated the probability of detecting the causal agent was significantly different ($P < 0.0001$) between tissues sampled. In fetuses with disseminated infections, agent recovery was greater in abomasal samples (56/61, 91.8%) than both lung (41/61, 67.2%) and liver (26/61, 42.6%) samples and differed between these sites ($P < 0.0001$). Similarly, fetuses with primary bronchopneumonia demonstrated significantly ($P < 0.0001$) different recovery rates between liver (11/28, 39.3%), lung (15/28, 53.6%), and abomasal (27/28, 96.4%) sites. *Campylobacter* spp. were rarely detected causes of abortion in this study; however, 100% of agents (9/9) were recovered from abomasal samples whereas only 44.4% (4/9) were identified from fetal liver.

Routine practice for viral agent detection included antibody-based organism staining (IHC or fluorescently labeled) on kidney and lung tissues. Although the overall prevalence was low for these agents, the probability of detection was consistent between both sites for BVDV (100%, 12/12) and for BHV-1 (96.0%, 24/25).

4. Discussion

A pathologic diagnosis was obtained for 56.9% (379/665) of abortions examined in this study, which is higher than for other published surveys that ranged from 23.3% to 47.2% [8,20–22]. One reason for this increased diagnostic rate may be due to the high incidence in our laboratory of regionally important agents including EBA and *N .caninum*. Over 31% (209/665) of abortion cases were attributed to bacterial infections compared with 14.5% to 24.4% in other

published studies [8,20–23], primarily because of the large number of EBA-associated abortions. The causative agent of EBA, currently named *P. abortibovis* [17], is a novel deltaproteobacterium, which cannot be cultured *in vitro*. This regional disease is known to occur in California, Nevada, and Oregon in the dry foothill habitat of the argasid tick vector *Ornithodoros coriaceus*. Intermittent feeding by the infected tick transmits the slow-growing bacterium to a susceptible pregnant dam in early-to-mid gestation, resulting in a chronic (3–4 months) fetal infection with late-term abortion or premature delivery [24]. Over 96% of these abortions in this study occurred in beef cattle and 86.2% occurred in third-trimester fetuses. An additional 15 cases that were not included in this review were suspicious for EBA; however, because thymus and lymph nodes were not included with submitted tissues, the diagnosis could not be confirmed.

The prevalence of *N. caninum* abortion in this study is consistent with published surveys from the western region of the U.S. over the last 20 years (10.9–11.9%) [8,20,23,25], demonstrating that although reports of abortion outbreaks may have decreased, there is still a consistent presence of the organism in cattle abortion cases. Interestingly, although *Neospora* abortions are generally regarded as a dairy cattle problem, 19.3% of these abortions occurred in beef cattle. Conversely, *Neospora* accounted for 8.4% of abortions in beef cattle and 35.2% in dairy cattle; however, establishing and comparing the actual risk (per pregnancy) for beef versus dairy cattle in the population at risk was beyond the scope of this study.

Local immune suppression in the dam at the maternal and fetal placental interface, which helps to prevent fetal rejection, may at the same time contribute to successful establishment of a fetoplacental infection [10,12]. Agents that would otherwise be rapidly cleared are able to colonize and replicate at this interface and result in a variety of damage in the fetus [12,22]. Death may be caused by agent proliferation and primary damage to the fetus or placental insufficiency causing fetal hypoxia or starvation [5,11].

Bacteria crossing the placentome and reaching the fetus thorough the umbilical veins result in disseminated infection, hepatitis, and circulatory damage; this pattern of fetal "septicemia" is characteristic of known bacterial pathogens including *Listeria* spp., *S. enterica*, and *Campylobacter* spp. [11,12,14,26,27]. Opportunistic agents more commonly cause a primary placentitis and reach the fetus via ingestion and/or inhalation of contaminated amniotic fluid. Bronchopneumonia, gastroenteritis, and dermatitis are typical lesions resulting from these infections because these organs would be in contact with compromised amniotic fluid [11,12,14]. In the present study, infections with opportunistic agents were more commonly associated with lesions of septicemia than lesions of primary bronchopneumonia. It is difficult to determine if the initial damage occurred in fetal lungs and the dissemination was secondary or if the tissue damage was concurrent; however, abomasal samples had a greater likelihood of bacterial agent recovery than lung tissues, even in cases with primary lung pathology. Fluid present in the abomasum may be more resistant to effects of postmortem autolysis and permit longer bacterial pathogen survival than lung or liver tissues.

Cases that are classified with histologic evidence of response to an infectious cause but no agent identified (11.7%) in this survey represent a large gap in diagnostic methodologies. More traditional methods were used in these cases because they were consistently applied throughout the study period; however, the use of molecular diagnostics can dramatically improve etiologic agent detection, especially in compromised samples. Utilization of more sensitive DNA or RNA recovery methods may improve agent detection; however, a thorough examination of fetal tissues remains crucial when evaluating abortions to verify the significance of ancillary diagnostic results by demonstrating compatible lesions indicative of disease. Novel techniques such as fluorescent *in situ* hybridization (FISH) can strengthen the causative attribution of potential pathogens and may help to enhance diagnoses in the future [28].

Several limitations were identified in this review. Cases presented to a diagnostic facility are by definition a convenience sample, and the present summary is not a true representation of bovine fetal loss. Placental tissues were only included in 83 of 665 cases (12.5%), which represents a large deficiency. Agents for which the placenta is the target organ, particularly nonculturable bacteria such as *Chlamydia* spp. and *C. burnetii*, would not be identified in these cases and could contribute to missed diagnoses in this study. Abomasal samples provide the greatest likelihood of bacterial and fungal detection in infectious abortions but may not be included from field necropsies or compromised when expelled fetuses have been scavenged.

Maternal serology is often used as a component of abortion diagnostics; however, results must be taken in context with the management of the herd [2,8]. A positive titer to an infectious agent indicates that the dam has been exposed to a specific antigen that may or may not be associated with the abortion. Vaccination status of the cow and her herd mates is important when interpreting titer response. Paired serum samples taken 10 to 14 days apart demonstrating a rising titer to an abortive agent provide stronger evidence of association; however, maternal seroconversion may have occurred before the abortion and may not be observed in follow-up testing [2,5,8]. Paired serologic results are most useful when blood is collected from nonvaccinated animals and from multiple animals in a herd [2,5,8]. In this study, results on maternal serum were only available for 8.6% (57/665) of the cases examined, and information on paired samples was not identified so these data were not included.

A substantial number (144/709, 20.3%) of fetuses had no detectable lesions to explain gestational loss (Fig. 1). Hormonal, nutritional, environmental, and toxic stressors can affect the ability of the cow to sustain the pregnancy and the viability of the fetus and often result in no definitive fetal lesions [2,6]. Nearly 75% of these cases without lesions occurred during the third trimester of gestation when fetal demands on the dam are greatest. Confirmation of mineral imbalances is severely limited by the lack of references on expected fetal concentrations of minerals, whereas toxic compounds responsible for fetal loss may not be present once tissues are examined [8]. Enhanced diagnostic

methods for such conditions would increase the likelihood of obtaining a diagnosis in these cases.

Etiologic identification in bovine abortion cases is a difficult undertaking for veterinary practitioners and diagnosticians [9]. An integrated approach is critical to the successful diagnosis of fertility and/or abortion problems in a herd [6,14]. Although the present study used cases submitted to a single diagnostic laboratory system and is susceptible to submission bias, important trends in causes of bovine abortion can be ascertained through these types of reviews [29]. Providing a detailed history, sufficient quantities of appropriate samples, and ancillary materials such as feed and water samples allow diagnosticians' access to the most valuable information available and will maximize the likelihood of determining a cause [14]. Agent identification may be missed if specific samples, particularly abomasal content, lymphoid organs, and placenta are not included with the submission or if special techniques needed to identify causes are not available. Nevertheless, there is benefit to producers and practitioners making herd health management decisions in ruling out abortive agents even in cases in which no definitive diagnosis is made, and enhanced diagnostic techniques will continue to provide useful investigative information [8,14].

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