

Causes of Bovine Abortion in the North-Central United States: Survey of 1618 Cases (1983-2001)

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Abstract

During 1983-2001 a total of 1618 aborted fetuses from farms with multiple abortions were examined to determine the cause. A specific cause was determined in 592 (36.59%) cases. Infectious agents associated with inflammatory reaction were detected in 435 (26.88%) cases. Bacterial abortions were identified as the most common infectious cause of abortion, 265 (16.38%) cases, with *Arcanobacterium pyogenes* being the most frequently isolated bacterium. Viral causes of abortion were diagnosed in 77 (4.76%) cases, protozoal in 55 (3.39%) cases, and 38 (2.34%) of the cases were diagnosed as mycotic abortion. There were also 65 (4.01%) cases of congenital myopathy and/or cardiomyopathy, 12 (0.74%) cases of congenital renal oxalosis and 38 (2.34%) cases of multiple congenital malformations.

Lesions suggestive of infection were present in 157 (9.70%) fetuses or placentas in which no infectious organisms were identified. The cause of 873 (53.96%) abortions was not determined. Serologic examination of 590 single or paired serum samples from aborting dams was of little diagnostic value for bovine viral diarrhea (BVD), infectious bovine rhinotracheitis (IBR) and leptospiral abortions. Five serum samples from aborting dams (38 examined) had high *Neospora caninum* antibody titers (800-12,800). Aborted fetuses from these dams had encephalitis and myocarditis. Serologic examination of thoracic fluid from 80 fetuses was of little diagnostic value for IBR, BVD, *Neospora*, *Toxoplasma* and leptospiral abortions.

Résumé

Un total de 1618 fœtus avortés, provenant de fermes présentant des avortements multiples, ont été examinés pendant la période de 1983 à 2001 pour en déterminer la cause. Il a été possible de déterminer la

cause dans 592 (36.59%) de ces cas. Des agents infectieux associés avec une réaction inflammatoire ont été détectés dans 435 (26.88%) cas. Les avortements causés par des bactéries représentaient la cause infectieuse la plus commune d'avortement avec 265 cas (16.38%). La bactérie *Arcanobacterium pyogenes* était l'agent le plus fréquemment isolé dans ces cas. Les avortements étaient associés à des virus dans 77 cas (4.76%), à des protozoaires dans 38 cas (2.24%) et à une infection mycotique dans 38 cas (2.34%). Il y avait aussi 65 cas (4.01%) de myopathie congénitale et/ou de cardiomyopathie, 12 cas (0.74%) d'oxalose rénale congénitale et 38 cas (2.34%) de malformations congénitales multiples. Des lésions possiblement associées avec une infection étaient présentes dans 157 fœtus ou placentas (9.70%) bien qu'aucun agent infectieux n'ait été identifié. La cause de 873 avortements (53.96%) n'a pu être identifiée. L'examen sérologique de 590 échantillons simples ou appariés de sérum provenant de mères ayant avorté n'avait qu'une faible valeur diagnostique pour l'identification d'avortements reliés à la diarrhée virale bovine (BVD), à la rhinotrachéite infectieuse bovine (IBR) ou à la leptospirose. On a identifié la présence de titres élevés (800-12 800) d'anticorps contre *Neospora caninum* dans cinq échantillons de sérum (38 cas examinés) de mères ayant avorté. Les fœtus avortés de ces mères avaient une encéphalite et une myocardite. L'examen sérologique du fluide thoracique de 80 fœtus avait une faible valeur diagnostique pour l'identification d'avortements associés avec l'IBR, le BVD, *Neospora*, *Toxoplasma* et la leptospirose.

Introduction

Reproductive failure from abortion in cattle causes an estimated annual loss of 14.2 million calves in the U.S.A., and the results of extensive surveys of the causes

are not commonly reported.²⁸ Numerous bacterial, viral, fungal and protozoal organisms have been reported as the cause of infectious abortion in cattle.^{1,2,3,4,6,14,20,21,23,25,27,30}

Despite detailed efforts by many laboratories, the diagnostic success is generally low. New and improved diagnostic methods and recognition of new etiologic agents have improved the diagnostic success to some extent in several species, including the bovine.^{15,16,17,18,24}

This study is the result of 1618 cases of bovine abortion submitted to the Diagnostic Center for Population and Animal Health (DCPAH), Michigan State University, from 1983 through 2001.

Materials and Methods

A total of 1618 aborted bovine fetuses were submitted to DCPAH by cattle producers and veterinarians located in Michigan, southern Wisconsin, northern Indiana, northern Ohio and Minnesota from February 1983 through December 2001. Abortions were submitted from 350 individual farms which had experienced multiple abortions involving approximately 12,500 fetuses. Approximate gestational ages of the fetuses varied from two months to fullterm, with over 80% involving fetuses in the third trimester of pregnancy. Sources for material and data collection were the DCPAH computer database, archive and hard copies of final reports of cases generated by pathologists. Diagnostic procedures did not remain the same throughout the 18-year survey period. Nutritional and toxicologic examinations and new diagnostic assays, such as enzyme-linked immunosorbent assays (ELISA), immuno-histochemical (IHC) staining and, most recently, polymerase chain reaction (PCR) were added to the standard protocol.

Necropsy

Complete postmortem examination was performed on 1618 aborted bovine fetuses (Table 1). Fetuses were received in mild to severe postmortem autolysis. Tissues from all major organ systems, including tongue, skeletal muscle and placenta when available, were fixed in 10% formalin for histopathologic examination. Tissues were collected aseptically for microbiologic examination. Stomach contents were drawn into a sterile disposable syringe and stored in sterile clot tubes until processed.

Bacteriologic examination

Routine examination included aerobic culturing of lung, liver, stomach content and placenta. In all cases attempts were made to isolate *Ureaplasma* sp, *Mycoplasma* sp, *Haemophilus* sp, *Listeria* sp, *Campylobacter* sp and *Leptospira* sp. From 1993 through 2001 a fluorescent antibody (FA) examination using polyvalent serum was added to identify *Leptospira* sp. Anaerobic cultures were done when histologic sections from placenta

Table 1. Summary of causes of 1,618 cases of bovine abortions.

Frequency	Number	Percent
Infectious causes	592	36.55
Non-infectious causes	153	9.45
Undetermined	873	54.00
Total	1618	100.00

and lung contained gram positive or negative rods or filamentous organisms.

Virologic examination

Fluorescent antibody examination and/or virus isolation were performed on lung, liver and spleen to identify infectious bovine rhinotracheitis virus (IBRV) and bovine viral diarrhea virus (BVDV) on all cases. Attempts were made to identify Parainfluenza 3 (PI3) and bovine respiratory syncytial virus (BRSV) on all cases through 1987, and sporadically thereafter. A total of 500 (41.39%) cases were tested for PI3 and BRSV.

Examination for Chlamydia

To identify *Chlamydia* sp, impression smears of Giemsa-stained placental tissue were examined microscopically, embryonated chicken eggs were inoculated with placental tissue and fetal liver homogenate and, from 1987 through 1992, a tissue culture method was utilized using McCoy cell culture. From 1993 through 2001, ELISA was used to identify *Chlamydia* sp.

Serologic examination

When either single (acute) or paired (acute and convalescent) serum samples from aborting dams were available they were tested by the plate agglutination test for *Brucella* sp antibody, and by the micro-agglutination test for antibodies against *Leptospira* sp and *Haemophilus somnus*. Virus neutralization tests were used for IBRV, BVDV and BRSV; the hemagglutination inhibition test was used for PI3. The agar-gel immuno-diffusion (AGID) test was performed to detect *Toxoplasma* sp and *Neospora caninum* antibodies in the dam's serum or fetal thoracic fluid from selected cases based on history or histologic lesions suggestive of protozoal infection.

Mycologic examination

Tissue samples from placenta, lung and liver were submitted for fungal culture when gross and histologic lesions were suggestive of mycotic abortion.

Nutritional examination

Liver samples from most of the aborted fetuses were assayed for vitamin E using a high performance

liquid chromatographic (HPLC) procedure.⁵ A fluorescent spectrophotometry technique was used for selenium analysis.²⁹

Toxicologic examination

For toxicologic examination, tissue samples from fetal liver and kidney were assayed for 22 elemental concentrations (B, Ba, Ca, Cu, Co, Fe, Mg, Mn, Mo, P, Zn, Al, Sb, Cr, Cd, Hg, Pb, Se, Ti, As, Na, K) using Inductively Coupled Plasma Emission Spectroscopy (ICP) multi-elemental analysis.⁷ The elemental concentration assays were conducted by clinical toxicology to establish a database for normal values in a limited period of time (2 years). HPLC and/or calorimetric spot test was used to determine nitrite and nitrate in aqueous humor, vitreous humor or thoracic fluid, and gas chromatography/mass spectrophotometry and thin layer chromatography were used for mycotoxin (zearalenone, vomitoxin, T2 toxin, aflatoxin B1, B2, G1, G2) screening on feed from suspicious cases.

Histopathologic examination

Formalin-fixed tissues were sectioned at 6 μ , stained with hematoxylin and eosin, and examined microscopically. Special stains were used to aid in identification of microorganisms in selected cases.

Results

Results of examination of 1618 bovine aborted fetuses are given in Tables 1, 2 and 3. Infectious agents were identified as the cause of abortion in 435 (26.88%) cases, and inflammatory reactions were observed microscopically in an additional 157 (9.70%) cases with unidentified etiology (Table 2). Non-infectious and undetermined causes of abortion are reported in Table 3. There were 65 (4.02%) cases of myopathy involving either cardiac, skeletal or tongue muscle. Congenital malformations were identified in 38 (2.35%) cases, and renal oxalosis in 12 (0.74%) cases.

Vitamin E and selenium levels in fetal liver tissue varied from non-detectable to 57 μ g/g (dry weight basis), and from 0.05 to 10.69 μ g/g (dry weight basis), respectively. Elemental concentrations in all fetal liver and kidney tissue were within expected ranges, except for two cases which had decreased levels of P and Mg, and increased Ca concentration.

Macroscopically most fetuses had variable degrees of postmortem autolysis. Excessive serosanguineous fluids were present in the thoracic and abdominal cavities of nearly all fetuses. Histologic lesions consistent with inflammation were identified in 592 (36.59%) cases. Inflammatory reactions most frequently observed were necrotizing or necrosuppurative placentitis and/or bronchopneumonia, and to a lesser extent, hepatitis, myo-

Table 2. Summary of infectious causes of abortion.

Frequency of agents isolated	Number	Percent ^a
<i>Arcanobacterium pyogenes</i>	82	5.07
Protozoal	55 ^b	3.40
Infectious bovine rhinotracheitis virus	52	3.21
Mycotic	38 ^c	2.35
<i>Salmonella</i> sp	25	1.54
Bovine viral diarrhea virus	25	1.54
<i>Streptococcus</i> sp	23	1.42
<i>Mannheimia</i> sp	23	1.42
<i>Bacillus</i> sp	23	1.42
<i>Listeria monocytogenes</i>	21	1.30
<i>Escherichia coli</i>	12	0.74
<i>Ureaplasma</i>	12	0.74
<i>Staphylococcus</i> sp	9	0.56
<i>Haemophilus somnus</i>	9	0.56
<i>Klebsiella</i> sp	8	0.49
<i>Mycoplasma bovis</i>	4	0.25
<i>Campylobacter</i> sp	4	0.25
<i>Aeromonas hydrophila</i>	2	0.12
<i>Chlamydia</i> sp	2	0.12
<i>Actinobacillus</i> sp	2	0.12
<i>Leptospira</i> sp	2	0.12
<i>Pseudomonas</i> sp	1	0.06
<i>Moraxella</i>	1	0.06
Inflammation (no etiology)	157	9.70
Total	592	36.59

^aPercentage shown represents the proportion of the total of 1,618 cases.

^b*Neospora* = 2, *Toxoplasma* = 1, protozoal = 52

^c*Aspergillus*, *Mucor*, *Candida*

Table 3. Summary of non-infectious/undetermined causes of abortion.

Frequency of cases	Number	Percent ^a
Myopathy (skeletal, cardiac, tongue)	65	4.02
Congenital malformation	38	2.35
Oxalosis renal	12	0.74
Mummification	11	0.68
Dystocia	8	0.49
Twinning	8	0.49
Anasarca	5	0.31
Congenital leukosis	4	0.25
Hydatiform mole	1	0.06
Hydrops allantois	1	0.06
Undetermined	873	53.96
Total	1026	63.41

^aPercentage shown represents the proportion of the total of 1,618 cases.

carditis, nephritis, meningoencephalitis and myositis. There were also three cases of cuffing lymphocytic pneumonia associated with *Ureaplasma* sp, three cases of lymphocytic nephritis, one of which was associated with *Leptospira* sp, (special stains), 46 cases of myositis and/or myocarditis, and 31 with encephalitis.

Results of serologic examination of 590 single and paired serum samples from the aborting dams are shown in Table 4. Thoracic fluid from 80 fetuses was examined for the presence of antibodies against common abortifacient agents. Results were negative for BVDV (n=62), IBRV (n=62), *Leptospira* sp (n=62), and *Toxoplasma* (n=32); one sample had a titer of 200 for *Neospora* antibodies, and 69 were negative.

Discussion

A review of the literature showed results of surveys of causes of bovine abortions are not frequently reported.^{1,9,10,12,22,27} Researchers at Iowa State University reported results of a one-year survey of 227 bovine abortions in 1978-1979.⁹ IBRV was isolated from 14.9% of cases, while bacterial and fungal causes accounted for 12.9% of abortions. *Arcanobacterium pyogenes* was the most common bacterial agent isolated (3.6% of cases).

In 1990 and 1996, the California Veterinary Diagnostic Laboratory reported on surveys of cattle abortions.^{3,11} In the 1990 report, infectious causes of abortion in cattle were bacterial (16.2%), viral (5.6%), protozoal (3.2%) and fungal (1.1%). In the 1996 report, infectious causes of abortion in cattle were bacterial (17.98%), protozoal (14.62%), viral (3.19%) and fungal (1.34%).

Results of a 10-year study of bovine abortion were published by South Dakota researchers in 1992¹⁷ and 1993¹⁸. Bacterial abortions were most common (16.90%), with *A. pyogenes* most frequently isolated. Viral and fungal causes were found in 10.57 and 5.23% of cases, respectively. The proportion of viral and fungal causes of abortion in South Dakota and the proportion of viral abor-

tions in Iowa were notably higher than reported in California and Michigan surveys. No protozoal causes of abortion were reported in the South Dakota and Iowa surveys.

In our survey, bacterial agents (265 cases, 16.38% of total) were the most frequently isolated organisms associated with abortion, with *A. pyogenes* most commonly isolated (Table 2). Major viruses isolated were IBRV (52 cases, 3.21%) and BVD (25 cases, 1.54%). There were also two fluorescent antibody (FA)-positive BRSV cases; this finding is of questionable significance. Viral abortions were much less common than bacterial abortions in our survey (Table 2), and significantly lower than in the Iowa and South Dakota surveys. Differences in occurrence are unclear, but could be the result of improved vaccination programs in recent years.

There was a positive relationship between IBRV FA-positive cases and the presence of microscopic liver necrosis and herpesvirus intranuclear inclusion bodies. Both PI3 and BRSV have been previously incriminated as causes of abortion in cattle;^{13,25} two fetuses in this survey were FA-positive for BRSV, but attempts to identify PI3 virus by FA or virus isolation were unsuccessful.

Bovine viral diarrhea virus can cross the placental barrier, and the principal determinant of fetal response to infection is age of the fetus. Possible outcomes of fetal infection include fetal resorption, abortion, congenital malformation, mummification, and birth of normal, weak or persistently infected (PI) calves.^{13,15,16} In this study, BVDV was isolated from 14 aborted fetuses. Placentitis was evident in three cases, while hydrocephalus and cerebellar hypoplasia were associated with two cases. Gross or microscopic lesions were not seen in the fetal and placental tissues of the other 20 BVD-associated cases.

There were two additional cases of cerebellar hypoplasia, three with a cleft palate and 11 mummified fetuses. These could be the result of an *in utero* infection of the fetus by BVDV, however, attempts to identify BVDV by laboratory procedures from placenta and fetal tissues

Table 4. Antibody titers to various infectious agents from 590 serum samples from aborting dams.

Infectious agent	Number examined	Antibody titers
Infectious bovine rhinotracheitis virus	590	<4 - >4096
Bovine viral diarrhea virus	590	<4 - >4096
Parainfluenza 3 virus	245	<4 - 128
Bovine respiratory syncytial virus	245	16 - 64
<i>Haemophilus somnus</i>	150	32 - 4096
<i>Leptospira</i> sp	300	<50 - 800
<i>Brucella</i> sp	100	<25 ^a
<i>Neospora caninum</i>	40	<200 ^a - 12800 ^b
<i>Toxoplasma</i>	13	<20 ^a (n=12) - 2560 ^b (n=1)

^anegative interpretation

^bpositive interpretation

in these cases were unsuccessful, and further proof for presence of BVDV in the herd was lacking.

Chlamydia sp, responsible for up to 25% of bovine abortions in some reports,^{13,14} were identified in only two cases and therefore were not considered a significant cause of abortion in our survey.

Bacterial organisms were most commonly associated with abortions in our survey. *Arcanobacter pyogenes* (82 cases, 5.06%), *Salmonella* sp (25 cases, 1.54%), *Streptococcus* sp (23 cases, 1.42%), *Mannheimia* sp (23 cases, 1.42%), *Bacillus* sp (23 cases, 1.42%) and *Listeria monocytogenes* (21 cases, 1.30%) were the most common bacterial organisms isolated (Table 2). Incriminating a bacterial organism as a cause of abortion is difficult at best. Bacterial abortion was diagnosed on the basis of isolating of an organism with well-established abortifacient capability, or finding bacteria in pure or nearly pure culture from several fetal tissues, including placenta, liver and lung, in association with an inflammatory reaction. Mycotic organisms were the fourth most common agents associated with infectious abortion in our survey. There were 38 (2.34%) cases of mycotic abortion with diffuse severe necrotizing placentitis: 26 cases of *Aspergillus* sp; six cases of *Candida parapsilosis*; five cases of *Mucor*; and one case of *Torulopsis globrata*. Most of the bacterial and mycotic abortions were considered sporadic with no apparent seasonal occurrence. Leptospirosis and Brucellosis did not appear to be significant causes of abortion in our survey.

One abortion was due to toxoplasmosis. The diagnosis was based on necrogranulomatous inflammation in the brain, and non-suppurative myocarditis and placentitis. Paired serum samples from the dam had antibody titers of 320 (acute) and 2,560 (convalescent) for *Toxoplasma* sp. One protozoal abortion was diagnosed based on the presence of protozoal cysts in the placenta and the associated inflammatory reaction. Two abortions were due to *Neospora caninum* infection. The diagnosis was based on necrogranulomatous encephalitis, myocarditis, positive immunocytochemical staining and high serum antibody titers in the dam's serum for *Neospora*. Fifty-two additional protozoal abortions were suspected based on presence of histologic lesions (necrogranulomatous encephalitis and/or non-suppurative myocarditis and myositis). However, protozoal organisms suggestive of *N. caninum* and *Toxoplasma* infection could not be demonstrated histologically (Table 2).

In our survey, degenerative myopathy was associated with abortion in 65 (4.02%) cases. Degenerative myopathy lesions, involving either skeletal, cardiac or tongue muscle, were associated with low fetal liver vitamin E and/or selenium values (Table 3). The association between fetal congenital myopathy and cardiomyopathy and low vitamin E and/or selenium deficiency has been previously reported.^{31,32} Renal oxalosis with tubular neph-

rosis was identified in 12 (0.74%) of the abortion cases. A diagnosis of renal oxalosis was determined histologically based on the presence of birefringent crystals under polarized light with morphology suggestive of calcium oxalate. A disorder involving abnormal glycine metabolism has been proposed as the cause of renal oxalosis in the bovine fetus.^{8,26} Whether renal oxalosis was the primary cause of abortion in this study was not definitively determined, however, no other reason for abortion was found in these cases.

In four (0.25%) of the abortion cases, multicentric congenital leukosis was found involving major organs, including lymph nodes and spleen (Table 3). Agar gel immunodiffusion (AGID) testing of acute and convalescent serum of one of the dams for bovine leukemia virus was positive. Because bovine leukemia virus does not cause the calf form of leukosis, the significance of the positive bovine leukemia virus test result on the dam's serum is unclear.

Fetuses are received in various states of autolysis, which makes diagnosis of abortions a challenge. Despite this reality, characteristic lesions of IBR (liver), *Neospora* (brain) and other infectious agents (placenta and lung) may be found when autolyzed tissue is examined histologically. In our experience, the success rate for identifying an infectious cause of abortion is highest when the placenta is available for both histologic and microbiologic examination.

In 157 (9.70%) abortion cases, inflammation of placental and several major organ tissues was noted without being able to identify any etiologic agents (Table 2). Thirty-one of these cases also had non-suppurative inflammatory reactions in skeletal muscle, tongue and cardiac muscle, suggestive of protozoal abortion. We are uncertain whether failure to identify the causative organisms was due to inadequate laboratory procedure or environmental factors.

Serologic tests were performed on 590 single and paired serum samples from dams when submitted with aborted fetuses (Table 4). All herds had a history of vaccination with various vaccines and bacterins. We were unable to correlate antibody titers with any possible cause of abortion. The titers most likely reflected the dams' response to vaccination or to previous exposure to the pathogen. Cases with very high serum titers (IBRV, BVDV > 4096) suggest post-vaccinal exposure to the organism without the actual presence of active disease. One case of leptospiral abortion was diagnosed histologically after a history of previous leptospiral abortions in the herd. Serum from the aborting dam had no antibody titer for six *Leptospira* serotypes. One dam that aborted had an eight-fold increase in the serum antibody titer (from 320 [acute] to 2560 [convalescent]) to *Toxoplasma* sp. Five serum samples from cows that aborted had high titers (800-12,800) to *Neospora*

caninum (n=40); their aborted fetuses had encephalitis and myocarditis lesions. It is our experience that serologic testing of single or paired serum samples from an aborted dam may not be an effective method for diagnosing abortion in vaccinated herds.

Serologic tests were performed on thoracic fluid from 80 fetuses. Not all 80 samples were tested for the same antibodies. Results were negative for IBRV (n=62), BVDV (n=62), *Leptospira* sp (n=62) and *Toxoplasma* (n=32). One sample was positive for *Neospora caninum* antibodies (titer=200 with mild brain lesions) and 68 were negative. In our experience, fetal thoracic fluid was of little diagnostic value for identifying the cause of abortion.

Conclusions

In conclusion, most perinatal deaths are not due to infections but rather non-infectious causes such as genetic disorders, teratogens, nutritional disorders, toxic plants, hormonal asynchrony, corticosteroids, physical insults and toxins.¹⁹ A good history and physical examination of the aborted cow are important parts of the diagnostic investigation. In most cases, diagnosis is unlikely to be determined by laboratory examination alone.

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