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INFECTIOUS AGENTS OF BOVINE ABORTION - LABORATORY DIAGNOSIS

By

Valeria Moojen

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

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ABSTRACT

INFECTIOUS AGENTS OF BOVINE ABORTION -
LABORATORY DIAGNOSIS

By

Valeria Moojen

Specimens from 98 aborted bovine fetuses were collected at the Animal Health Diagnostic Laboratory of Michigan State University, between June 1977 and September 1978. The fetuses were mostly from Holstein-Friesian herds from many different areas within the State of Michigan. The primary cause of abortion was recognized in 43 (43.8%) of the aborted fetuses examined. Noninfectious factors were associated with 6.1% of the abortions and infectious agents were incriminated in 37.7%. Bacteria accounted for 22.4%, viruses for 14.3%, and fungi for 1%. Antibodies to leptospira were found in 6 fetuses, and 4 of the 50 fetal fluids examined had antibodies to parainfluenza-3. Electron microscopy was the most effective method in demonstrating viral agents in fetal tissues. The presence of herpesviruses was detected in 8.2% of the fetuses, correlating in all instances with positive fluorescent antibody (FA) results for infectious bovine rhinotracheitis (IBR) virus.

Herpesviruses were demonstrated in 3 additional fetuses that were negative for IBR by FA. Nutritional deficiencies were noted in 3 fetuses and congenital anomalies were found in 2 fetuses.

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INTRODUCTION

Bovine abortion is recognized as one of the major economic losses in dairy and beef cattle production, and infectious agents are considered to play a major role. However, a definitive diagnosis is obtained in only 25% of the total cases submitted to most laboratories. This low percentage is likely due to three factors:

1. Most abortions are not due to infectious agents.
2. Inappropriate specimens are examined (either incorrect tissues or tissues in poor condition).
3. Inappropriate tests are utilized.

The purpose of this study was to increase the effectiveness of the diagnostic laboratory in dealing with infectious agents of bovine abortion. This was accomplished by supplementing conventional tests (routine bacteriologic tests including darkfield examination of stomach contents, routine virologic procedures including fluorescent antibody examination for infectious bovine rhinotracheitis (IBR) and bovine virus diarrhea (BVD), and pathologic examination) with serologic examination of fetal fluids and electron microscopy. Serologic examination of fetal fluids takes advantage of the relative early immune competence of the fetus, and the fact that

immunoglobulins do not normally cross the placental membranes in the cow. Electron microscopy permits the detection of infectious agents not normally associated with abortion, and allows the demonstration of known infectious agents no longer viable as a result of autolytic changes of the fetus.

REVIEW OF THE LITERATURE

Factors Associated with Bovine Abortion

Factors associated with the cause of bovine abortions have been categorized mainly as genetic, environmental, and infectious, according to Hubbert et al. (1973) and Miller (1977).

Genetic Factors

Gilmore (1949) reviewed the inherited causes of reproductive failure. He discussed and characterized the lethal genes responsible for death such as those responsible for mummification and subsequent abortion, and "the Ljutikow's lethal" that results either in abortion, stillbirth or death shortly after birth.

Knudsen (1956) postulated that bulls with normal appearing semen may have abnormally arranged genes which might contribute to unbalanced genetic constitution of the embryo resulting in its death. Bishop (1964) also discussed the male influence on embryonic death. Attention was directed to the often undetected recessive lethal factors which could contribute to a large economic loss based on their wide dissemination as a result of artificial insemination.

Mylrea (1963) reported an outbreak of abortion in a Shorthorn herd in which a genetic cause was suspected. The abortions occurred in late pregnancy, and no significant organisms were detected on bacteriological examination. Also, no evidence of brucellosis, leptospirosis, vibriosis or trichomoniasis was found.

Stevens and King (1968) reported cases of midterm abortion in a Holstein-Friesian herd. By analyzing pedigrees and breeding histories and eliminating suspect animals from the mating program, they found evidence to support an autosomal recessive lethal gene involved in the abortion problem.

Wijeratne and Stewart (1971) studied the data from abortion in cattle over an 8-year period, with special reference to genetic factors. They found that rates of abortion increased with the number of inseminations, and that there was also a slight increase among twin fetuses. A significant difference was observed among the breeds Friesian, Guernsey, Jersey, Ayrshire, Dairy Shorthorn, Hereford, Aberdeen-Angus, and Charolais.

Environmental Factors

Crane (1965) provided evidence of an interrelationship between IBR related abortion and nutrition. Deficiencies such as phosphorus, vitamin A, protein, and some of the trace minerals were found in California at certain times of the year. Research with 800 cows in which supplemented feed and controlled vaccination for IBR,

BVD, leptospirosis, and brucellosis were provided, showed an increase in the weaned calf crop. Neighboring ranches with analogous vaccination programs, but without supplemented feed, did not have a reduction in their loss rate.

Andrews (1972), studying cattle from different environmental areas, found nutrition, management and breed to be important contributors to reproductive failures. For example, poorer body condition was found in nonconceiving cows compared to those that were pregnant, and it was suggested that supplementary feeding to correct the protein concentration would increase reproduction. Age of the stock was also considered to influence reproductive performance.

A study by Hignett (1959) showed that cows with high levels of phosphorus in relation to calcium in the diet had their resistance to infectious agents decreased. Woelffer (1953) reported that pregnancies were maintained in cows by the administration of progesterone. Schiefer and Moffatt (1974) described a case of bovine abortion associated with renal oxalosis in the fetus. They emphasized the need for further studies to determine the effects of oxalate on the fetus.

Infectious Agents

Viral, bacterial, mycotic and protozoan agents are well documented as causes of abortion. General articles have been published by Gilman (1939), Lawson (1963), Ashfar (1965), Dennis (1969), Woelffer (1972), Hubbert

et al. (1973), and Miller (1977). The studies by Dennis (1969) and Woelffer (1972) were also directed at the diagnosis of infectious agents.

Viral agents. Among the viruses causing bovine abortion, IBR virus is one of the more frequent. This herpesvirus, first known to cause a respiratory disease (Miller, 1955), was isolated from an aborted fetus by McKercher and Wadda in 1964. Reports by Crane et al. and Lukas et al. in the same year also incriminated IBR virus as a cause of abortion. Experimentally induced abortion in cattle with IBR virus was reported in 1964 by Chow et al., and Mitchell (1974) described epizootic abortions after inoculation with live IBR vaccine. Reports have indicated that IBR continues to be a major cause of bovine abortion (Kirkbride et al., 1973; Miller and Quinn, 1975; Kradel, 1978). IBR abortion was experimentally produced and the lesions in the fetus were described (Owen et al., 1964; Sattar et al., 1967; Kendrick and Straub, 1976). Kennedy and Richards (1964) studied the changes produced in the aborted fetuses by IBR virus infection acquired naturally, experimentally and by vaccination and concluded that they were essentially the same. Lesions consisted of foci of necrosis found consistently in the liver and irregularly in other organs. Owen et al. (1968) studied the pathogenesis of IBR virus infection in the fetus and correlated fetal and placental lesions with viral isolations.

Herpesviruses other than IBR have also been isolated from aborted fetuses. Crandell et al. (1976) reported the isolation of a virus designated BH-1247 with characteristics fitting the herpes group of viruses. This new isolate differed from IBR, DN-599 and MOVAR 33/63 viruses, and its significance as an abortifacient agent was not determined. Reed et al. (1979) reported a concurrent infection of an aborted bovine fetus with BVD virus and a herpesvirus. This latter isolate was serologically indistinguishable from DN-599 and MOVAR 33/66. Experimental studies by Kendrick et al. (1976) have suggested that this virus does not cause abortion. Malignant catarrhal fever (MCF) virus has been reported to infect wildebeest *in utero* (Plowright et al., 1960), but in cattle transplacental infection could not be demonstrated (Plowright, 1968). Even though abortion is reported in some cases of MCF, it is attributed to a lowered resistance of the animal due to the disease and not to the virus per se (Berkman and Barner, 1958). Cytomegalovirus was isolated from an aborted bovine fetus by Schiefer (1974); its role as a cause of abortion in cattle has not been established.

Bovine virus diarrhea was first described by Olafson et al. (1946), and abortion was one of the clinical signs observed. Cytopathic strains (Scott et al., 1972) as well as noncytopathic strains (Gillespie et al., 1967) capable of producing abortion have been described. The pathological changes produced by BVD virus in the bovine

fetus were characterized by Casaro et al. (1971), Braun et al. (1973) and Brown et al. (1974).

Sattar et al. (1965) first isolated bovine parainfluenza (PI-3) virus from an aborted bovine fetus. Its possible role in abortion was investigated experimentally by Sattar et al. (1967) and Swift and Kennedy (1972). Serological studies by Dunne et al. (1973) and Ruth et al. (1974) on aborted fetuses suggested the possible role of this paramyxovirus in bovine abortions.

Moll and Davis (1957) and Moll and Finlayson (1959) isolated enteric viruses (BEV) from feces of cattle and from animals having respiratory tract infection. Abortion was observed in those herds. Continuing the study, Moll (1964) experimentally exposed guinea pigs to BEV and produced abortion and stillbirths. Later, Dunne et al. (1973) reported the results of serologic examination of aborted bovine fetuses and incriminated BEV as potentially important agents in bovine abortion.

Parvoviruses are known to cause reproductive failures in other animal species (Kirkbride and McAdaragh, 1978). The possibility of this group of viruses causing abortion in cattle was investigated by Storz et al. (1978) by inoculation of the virus into pregnant cows, recovery of the virus from the aborted fetuses, and demonstration of hemagglutination inhibition antibodies in those animals.

Bluetongue (BT) virus was first isolated in the United States from cattle in 1959 (Bowne et al., 1968); Luedke et al. (1970) isolated BT virus from the spleen of

an aborted fetus. The heifer that aborted had clinical signs suggesting BT infection. Experimental studies confirmed the abortogenic nature of the BT virus in cattle (Luedke et al., 1977).

Dierks et al. (1976) isolated subgroup 2 adenoviruses from aborted bovine fetuses, and suggested that they may be involved with intrauterine infection and abortion.

A member of the Simbu group of the *Bunyaviridae* family of viruses, Akabane virus, is known to cause abortions in cattle. It has been reported in Australia (Blood, 1956), Kenya (Metselaar, 1976), and Japan (Kurogi et al., 1976), but not in the United States. Weiss et al. (1956) studied Wesselsbron virus and discussed its association with abortion in domestic animals. Their findings suggested that Wesselsbron virus infection in cattle could clinically mimic brucellosis. Of 252 clinical cases of brucellosis that were serologically negative, 39.62% had specific antibody to Wesselsbron virus.

The agent of sheep infection, Border disease, produced abortion when inoculated into pregnant heifers (Gibbons et al., 1974).

Bacterial agents. Bovine leptospirosis was clinically described first in 1935 in Russia by Michin and Azinov (1937), followed by reports in Australia (Johnson, 1943), the United States (Jungherr, 1944), and Palestine (Bernkopf et al., 1947; Freund, 1947). Leptospira were

not isolated until 1948 by Baker and Little in the United States. Stoenner et al. (1956) investigated the epizootiology of bovine leptospirosis, and serological studies on leptospiral abortion in cattle were done by Burk and Wiesmann (1963). The pathogenesis of leptospiral abortion was studied experimentally by Murphy and Jensen (1969). The difficulty in isolating the bacterium from the fetus was attributed to autolytic changes occurring in the tissues (Hanson, 1977). There are reports on instances of abortions in which significant levels of antibodies, specific for *Leptospira* species, were found in the fetus (Knott and Dadswell, 1970; Ellis et al., 1978).

Campylobacter fetus (*Vibrio fetus*) was first isolated from bovine fetuses in 1919 by Smith. Experimental studies on its etiological significance were carried out by the same worker in 1923. Based on serum-agglutination titers and isolation, Plastridge and Williams (1943) reported cases of bovine abortion associated with this bacterium. Many additional reports on isolation of the organism from aborted fetuses have been published (Apice, 1956; Mitchel, 1960; Hubbert et al., 1973). Whitford et al. (1977) reported the isolation of *Campylobacter fetus* ss. *intestinalis* and other campylobacters from aborted bovine fetuses.

Brucellosis has a worldwide distribution, but has been eradicated from Norway (1952), Sweden (1957), Finland (1960), and Japan (1974) (15). The United

States is currently conducting a program of control and eradication. Although bovine brucellosis (contagious epizootic abortion) was recognized as early as the 18th century (Jensen and Mackey, 1974), the causative agent was not isolated until 1897 by Bang in Denmark. Reports by Bolton et al. (1969), Kirkbride et al. (1973) and Ewalt and Harrington (1979) emphasized the continued importance of this disease in the United States in spite of efforts at control. The latter investigators stressed the problem of animal condemnation resulting from unofficial adult cattle vaccination.

A *Salmonella* species was reported by Gibson (1965) and Richardson and Watson (1971) to be associated with abortion in cattle. Maddox (1976) described an "abortion storm" due to *Salmonella typhimurium*, and Hall and Jones (1977) studied the pathogenesis of experimentally induced abortion in cattle due to *S. dublin*.

The association of bovine abortion with *Corynebacterium pyogenes* was reviewed by Hinton (1972). The organism was considered to play a minor role in abortions in the studies of Langneau (1964) and Dennis (1969). Hinton (1974) examined paired-serum samples from cases in which only *C. pyogenes* was isolated and concluded that it was the primary cause of abortions.

Epstein et al. (1972) reported an abortion associated with concurrent infection of the fetus with *Listeria monocytogenes* and IBR virus. Reports by Siddique et al.

(1976) and Miller (1977) have also incriminated *L. monocytogenes* as a cause of bovine abortion.

Although epizootic bovine abortion (EBA) has been referred to as a disease syndrome, not necessarily infectious, a chlamydial agent has been demonstrated in aborted fetuses from cows manifesting this particular syndrome (Wada et al., 1976; McKercher et al., 1976). Reed et al. (1971), in investigations of EBA in Colorado and South Dakota, suggested the possibility of other agents being responsible for this syndrome in addition to *Chlamydia*. Experimental studies on chlamydial abortion were done by Storz and McKercher (1962). They isolated EBA agent from 5 aborted bovine fetuses and successfully fulfilled Koch's postulates.

Sporadic abortions have been attributed to the following bacteria: *Haemophilus somnus* (Chladek, 1975; vanDreumel and Kierstad, 1975); *Aeromonas hydrophila* (Wohlgemuth et al., 1972); and *Pastuerella (Yersinia) pseudotuberculosis* (Langford, 1969).

Streptococcus species, *Escherichia coli*, *Staphylococcus* species, and *Pseudomonas* species have also been incriminated as occasional causes of bovine abortion, although their significance as abortifacient agents is doubted by some (Bolton et al., 1973; Dennis, 1969; Woelffer, 1972; Hubbert et al., 1973; Siddique et al., 1976).

Mycoplasma species have been isolated from aborted bovine fetuses (Langford, 1975; Ball et al., 1978), and have been shown to produce abortion experimentally by Stalheim and Proctor (1976).

Mycotic agents. Mycotic infections of the bovine fetus, placenta, and uterus were reported as early as 1920 by Smith, and experimental studies on abortion associated with *Mucor* were done by Gilman and Birch in 1925. Reports of mycotic pneumonia and associated abortion were presented by Cordes et al. (1964) and Harcourt and Thompson (1969).

Kirkbride et al. (1971), studying the diagnosis of mycotic abortion in cattle, called attention to the importance of examination of the placental tissue in diagnosing bovine abortion. In a 3-year period, 1,556 aborted bovine fetuses were examined in which 485 placentas were included. Mycotic placentitis was diagnosed in 3.85% of the cases.

Aspergillus species and *Mucor* species were reported as causes of abortion by Bolton et al. (1969), Siddique et al. (1976), and Williams et al. (1977).

Isolated cases of mycotic abortion due to other fungi have been reported: *Torulopsis glabrata* (Kirkbride et al., 1972; Knudtson et al., 1976); *Candida tropicalis* (Wohlgemuth and Knudtson, 1973); *Mortirella wolfii* (Harcourt and Thompson, 1969; Wohlgemuth and Knudtson, 1977).

Protozoan agents. An unidentified protozoan was reported by Corner et al. (1963) as the cause of abortion in cows. This report prompted Fayer et al. (1976) to study abortion in cattle experimentally inoculated with sporocysts of *Sarcocystis fusiformis*. *Trichomonas foetus*, an organism usually associated with infertility, has also been reported as a cause of abortion in cattle (Dennis, 1969; Woelffer, 1972). Abortion is described as a clinical sign of toxoplasmosis in other animal species (Hartley and Marshall, 1957), and a study by Dennis (1969) on the diagnosis of bovine abortion drew attention to the fact that toxoplasmosis may play a role in bovine abortion. Abortions due to this protozoan were reported in studies of bovine abortion (Schiefer and Moffatt, 1974; Miller and Quinn, 1975).

Laboratory Diagnosis of Infectious Agents of Bovine Abortion

The specimens most often received at the laboratory for the determination of the cause of bovine abortion are fetal tissues such as lung, liver, spleen, kidney, and stomach. Placenta is seldom submitted. The routine procedures applied to these specimens are bacterial and viral isolation attempts, darkfield microscopy (examination for *Leptospira*, *Campylobacter* and *Trichomonas*), fluorescent antibody tests for IBR and BVD, and gross and histologic examinations (Kirkbride et al., 1973; Schultz, 1973; Schiefer and Moffatt, 1974).

Reports have generally shown a figure in the range of 23% to 40% for the successful diagnosis of the cause of bovine abortion. Mitchel (1960) examined 227 cases of bovine abortion in the Ottawa (Canada) area and reported a diagnostic success of 33%, of which 8.8% was attributed to *B. abortus*, 22.5% to *L. pomona*, and 1.72% to *V. fetus*, *S. pyogenes*, and *C. pyogenes*. Sattar et al. (1965) examined 28 bovine fetuses for viruses, by virus isolation, and found IBR virus in 6 (21.4%) and PI-3 virus in 1 (3.5%).

In 1968, in a work published by Faulkner, it is stated that only 20 to 30% of cases of bovine abortion submitted to diagnostic laboratories yielded a positive diagnosis. Bolton et al. (1969) examined 1,412 aborted bovine fetuses in Vermont and reported a diagnostic success of 26.48%, of which 1.56% was due to mycotic infection and 24.92% to bacterial causes. Hubbert et al. (1973) reported that 23% of bovine abortions examined in an 11-year period from 5 Northeastern states had an infectious cause: 3.49% were viruses, 5.11% fungi, and 14.64% bacteria. The most frequently diagnosed infections were aspergillosis, IBR, streptococcosis, leptospirosis, vibriosis, and infection due to *Corynebacterium* species.

Kirkbride et al. (1971) and Kirkbride et al. (1973), examining fetal specimens and fetal placental tissue from 2,544 aborted bovine fetuses during a 4-year period in the Northern Plains States, successfully determined the etiologic agent in 35.3%. IBR virus was the most commonly

found agent (16%). Mycotic abortion was diagnosed in 3.5%, and vibriosis in 3.0%. Other bacterial agents, dystocias, and anomalies accounted for 7.4% of the abortions or stillbirths.

According to data obtained from the New Zealand Animal Disease Data Bank records (Kirkbride et al., 1977) for 1974, 34.8% of 15,741 bovine abortions examined had an etiologic diagnosis which included 9% of abortions due to brucellosis.

In a study carried out in Canada, of 1,509 abortion cases examined, 17.83% were due to viruses, 10.66% to bacteria, 3.98% to fungi, 0.07% to protozoan agents, and 7.82% to noninfectious causes (Schiefer and Moffatt, 1974).

Miller and Quinn (1975) compared data from aborted and nonaborted bovine fetuses in Ontario, using pathological, microbiological, and immunological criteria. They considered a positive finding in at least one of the three categories as indicative of a positive etiological diagnosis. This analysis resulted in a positive diagnostic rate of 48%.

Kradel (1978), in a study of the abortion problem in bovine herds, stated that etiological agents are determined in less than 25% of the cases submitted to most diagnostic laboratories, but that this figure could likely be increased to 34-50% with an improvement in diagnostic methods.

Serology

Serological studies currently being done on bovine fetuses are contributing to an increase in the number of positive diagnoses of abortions. The placental transfer of maternal immunoglobulins (Ig) to the fetus does not normally occur in the bovine (Brambell, 1958), and early studies by Fennestad and Borg-Petersen (1958) provided evidence of the bovine fetus' capability to respond immunologically. Later, in 1962, the same workers emphasized that this capability to respond immunologically probably depended upon the animal species, period of gestation when infected, and the antigen. In this study, bovine fetuses were infected with *Leptospira saxkoebing* and specific antibody was detected as early as the 132nd day of fetal life.

Valuable studies have been carried out on the bovine immune system. Buttler (1969) studied the different bovine immunoglobulins, and a symposium was conducted in 1970 on the bovine immune system (Buttler et al., 1971). Schultz et al. (1973) provided important information concerning the ontogeny of the immune system in the bovine fetus. In their work, 106 fetuses of various ages were studied. The development of the bovine immune response and the human immune response was compared. Although fetuses were not inoculated with infectious agents, the results suggested the need of antigenic stimulation for the immune system to show morphological and functional activities. A review by Schultz published in the same

year (1973) stated that periods in which IgM and IgG producing cells were recognized in the spleen were found to be 56 and 145 days, respectively. The appearance of immunologic competence of specific antigens occurred chronologically, and was approximately 90 to 120 days for BVD, IBR, and PI-3.

In view of the aforementioned considerations, the presence of significant levels of Ig in fetal serum and body fluids is indicative of intrauterine antigenic stimulation (Tyzard, 1977). The applicability of the results of these studies may be of value in the laboratory diagnosis of bovine abortion. For instance, Kahrs et al. (1971) concluded that serologic tests could be a valuable source of information in the diagnosis of bovine abortion and congenital defects. They worked with BVD and IBR viruses, and 11 aborted fetuses. Sawyer et al. (1973) reported the use of the radialimmunodiffusion (RID) test for detection of congenital infections in the bovine fetus, and Dunne et al. (1973), based on results of serologic examination of fetal fluids, presented evidence that viruses other than IBR and BVD were associated with bovine abortion. The serologic tests were done specifically for IBR, BVD, BEV, and PI-3 viruses. The number of positive diagnoses was increased by 58% compared to standard diagnostic procedures. Ruth et al. (1974) also showed serologic tests of fetal fluids to be useful in the diagnosis of bovine abortion. The presence of IgG was determined by RID, and it was often associated with

fetuses aborted due to infectious agents. The fetal fluids were specifically tested for antibodies to IBR, BT, BVD, PI-3, parvoviruses, and *Leptospira*. Kirkbride et al. (1977) compared levels of Ig (IgG and IgM) in aborted and abattoir fetuses, and correlated the presence of Ig to lesions in these fetuses. They found a significant difference in the level in aborted fetuses as compared to fetuses from the abattoir. According to the results obtained, the measurement of fetal Ig by RID may be of value as a first step in the diagnosis of infectious abortion.

Virus Concentration

Various techniques have been described and applied in an attempt to concentrate different viruses. Among the methods utilized was the alcohol precipitation of proteins from solutions containing influenza virus (Cox et al., 1947). He and co-workers found methanol to be superior to ethanol, and concluded that if precipitation was conducted under controlled conditions, such as temperature, methanol concentration and pH, the technique was applicable to a wide range of viruses.

Later, in 1949, Pollard et al., following essentially the procedure of Cox et al. (1947), studied the effects of methanol on 5 viruses from 4 different groups. In this study, the optimal pH for elution was given, and it was observed that the nitrogen content of the virus suspension decreased after precipitation. Goodheart et

al. (1974), working with oncogenic herpesviruses concentrated by methanol precipitation, called attention to the usefulness of this technique in virus concentration trials. Verrilli (1978) successfully used the methanol precipitation procedure in his electron microscopy study of representative viruses of veterinary significance.

Electron Microscopy

Morphology is an important feature of viruses that can be determined only by electron microscopy (EM) and which obviously is useful in viral identification.

Spradbrow and Francis (1969), using virus infected cell culture lysates, emphasized the possibility of EM playing an important role in diagnosis of viral infections, and Gibbs and Johnson (1970), working with udder and teat lesions, stated that the examination of clinical specimens by EM provides a satisfactory method for a rapid and accurate differential diagnosis of cowpox, pseudocowpox, and bovine herpes mammilitis.

Marsolais et al. (1971) reported the use of EM for the rapid diagnosis of avian coronavirus infection, and McFerran et al. (1971) discussed the application of negative contrast EM to routine virus diagnosis. They worked with different animal viruses obtained directly from clinical material or isolated in eggs or cell cultures.

England et al. (1976) reported the greater efficiency of EM for demonstration of viruses associated with neonatal calf diarrhea as compared to viral isolation and

the fluorescent antibody techniques, but Draayer and Kirkbride (1977) doubted the need for the routine examination of fetal tissue by EM. Verrilli (1978), working on negative contrast EM of viruses of veterinary significance, stressed the usefulness of its application in clinical virology.

Viral Interference

The usefulness of viral interference to detect noncytopathogenic viruses was emphasized by Gillespie et al. (1967) in their report on the isolation of noncytopathogenic BVD virus from 2 aborted fetuses. Dellers (1977), in his evaluation of the diagnostic capability of veterinary virology, drew attention to the importance of using the interference test to detect noncytopathogenic viruses.

Nuttall et al. (1977) used the interference test in the examination of the bovine fetal serum employed in cell cultures for possible virus contaminants. Results indicated that the test was less sensitive than the quantitative plaque technique, and probably would not detect low levels of interference.

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ARTICLE

INFECTIOUS AGENTS OF BOVINE ABORTION - LABORATORY DIAGNOSIS

By

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SUMMARY

Specimens from 98 aborted fetuses were collected at the Animal Health Diagnostic Laboratory of Michigan State University between June 1977 and September 1978. The fetuses examined were mostly from Holstein-Friesian herds coming from many different areas within the State of Michigan. The primary cause of abortion was recognized in 43 (43.8%) of the 98 aborted fetuses examined. Non-infectious factors were associated with 6.1% of the abortions and infectious agents were incriminated in 37.7%. Bacteria accounted for 22.4%, viruses for 14.3%, and fungi for 15%. Fluids from 50 fetuses were tested and antibodies to leptospira were found in 6 fetuses, and 4 had antibodies to PI-3. Electron microscopy was the most effective method in demonstrating viral agents in fetal tissues. The presence of herpesviruses was detected in 8.2% of the fetuses, correlating in all instances with positive fluorescent antibody (FA) results for infectious bovine rhinotracheitis (IBR) virus. Herpesviruses were demonstrated in 3 additional fetuses that were negative for IBR by FA. Nutritional deficiencies were noted in 3 fetuses and congenital anomalies were found in 2 fetuses.

INTRODUCTION

Bovine abortion is recognized as one of the major economic losses in dairy and beef cattle production, and infectious agents are considered to play a major role. However, a definitive diagnosis is obtained in only 25%

of the total cases submitted to most laboratories (5,21, 36,37,41). This low percentage is likely due to three factors:

1. Most abortions are not due to infectious agents.
2. Inappropriate specimens are examined (either incorrect tissue or tissues in poor condition).
3. Inappropriate tests are utilized.

The purpose of this study was to increase the effectiveness of the diagnostic laboratory in dealing with infectious agents of bovine abortion. This was accomplished by supplementing conventional tests (routine bacteriologic tests including darkfield examination of stomach contents, routine virologic procedures including fluorescent antibody examination for IBR and BVD, and pathologic examinations) with serologic examination of fetal fluids and electron microscopy. Serologic examination of fetal fluids takes advantage of the relative early immune competence of the fetus, and the fact that immunoglobulins do not normally cross the placental membranes in the cow (6). Examination of fetal fluid previously showed an increase in the number of positive diagnoses of abortion (13,42). Electron microscopy has played an important role in diagnosis of viral infections (16,35,51,54) and should permit the detection of infectious agents not normally associated with abortion as well as allowing the demonstration of known infectious agents no longer viable as a result of autolytic changes of the fetus.

MATERIAL AND METHODS

COLLECTION OF SPECIMENS

Specimens from 98 aborted bovine fetuses were collected at the Animal Health Diagnostic Laboratory of Michigan State University between June 1977 and September 1978. The gestational age of the fetuses ranged from 2 to 8 months.

Necropsy was performed on the fetuses and gross pathologic changes were recorded. Portions of lung, liver, kidney and abomasal contents were collected aseptically for microbiological examination and serosanguineous fluid was collected for serologic tests. Samples were processed immediately or frozen at -20 C for subsequent testing. Portions of selected tissues were fixed in 10% buffered formalin for histopathological examination.

BACTERIOLOGIC EXAMINATION

Lung, liver, kidney and abomasal contents were cultured for bacteria using standard laboratory procedures, and darkfield examination was performed on abomasal contents for the detection of *Leptospira* species and *Campylobacter* species (28).

VIROLOGIC EXAMINATION

PREPARATION OF SPECIMENS

A 10 to 20% tissue suspension of pooled lung, liver and kidney was made by grinding the tissues with a mortar and pestle, and sterile sand, using sterile distilled

water as a diluent. Two milliliters of the tissue suspension was immediately added to an equal volume of 2X concentrated Eagle's minimal essential medium with Earle's base supplemented with 0.5% lactalbumin hydrolysate, sodium pyruvate and non-essential amino acids (EMEM). Also contained in the medium was a high antibiotic concentration (1,000 mg/ml streptomycin, 1,000 units/ml penicillin G, and 500 mg polymyxin B sulfate). The remainder of the sample was used for electron microscopy. Samples were examined immediately or frozen at -20 C.

VIRUS ISOLATION

The tissue suspension was centrifuged at 2,000 rpm for 10 minutes, in a clinical centrifuge, and the supernatant fluid was used as inoculum. Amounts of 0.1 and 0.3 ml were inoculated into each of 2 tubes containing early passages of bovine fetal kidney (BFK) cells or bovine fetal peritoneal (BFP) cells. The maintenance medium was EMEM with 2% bovine fetal serum. The inoculated tubes were incubated at 37 C and observed for cytopathic effect (CPE) daily for 7 days and then frozen. Second and third blind passages were made by following the same procedure as above. In the third passage, coverslips were included in the tubes for hematoxylin and eosin staining (41). Third passage samples (at 8 days post-inoculation) were also inoculated with 0.2 ml of the NADL strain of BVD virus containing 100 $TCID_{50}$ to test

for the presence of noncytopathogenic BVD virus. The cultures were examined daily for CPE for 7 days (18).

ELECTRON MICROSCOPY

Virus Concentration

Samples from the 10 to 20% tissue suspensions in distilled water were used. After these lysates were thawed and frozen twice, dry KCL was added to achieve a final concentration of 1 M (54). This suspension was mixed for 5 to 10 minutes at room temperature, and then centrifuged at 10,000 g for 10 minutes at 0 C. The supernatant fluid was collected and precipitated with methanol, essentially as described by Goodheart et al. (1974).

Briefly, one-half volume of chilled methanol (-65 C) was added slowly to one volume of magnetically stirred tissue suspension in an ice bath (2 C). The temperature of the mixture was not allowed to rise above 3 C. The tissue suspension-methanol mixture was allowed to stir at 2 C in an ice bath for 3 hours, followed by centrifugation at 10,000 g for 20 minutes at 0 C. The pellets obtained were collected in small volumes of phosphate buffered saline (PBS) (0.2 M, pH 7.4) to achieve an approximately final volume concentration of 20 to 30 fold. Elution was allowed to proceed at room temperature for 10 minutes, followed by centrifugation at 10,000 g in an Eppendorf microcentrifuge at 2 C for 30 minutes.

The supernatant fluid, if clear, was collected, placed into small glass vials, and stored at -70 C for EM examination. The non-clear supernatants were recentrifuged for an additional 10 minutes.

Electron Microscopic Examination

The methanol precipitated samples as well as unprecipitated samples were negatively stained as described by Verrilli (1978). Two grids were used for each sample: one grid for the methanol and another for the direct preparation. A Philips 300 electron microscope operated at 80KV was used for the examination; grids were scanned initially at instrumental magnification of 20,000X and 60,000X to determine the presence of virus. Fifteen minutes were allowed for each preparation, as suggested by McFerran et al. (1971). A second grid was prepared if the first preparation was unsatisfactory.

FLUORESCENT ANTIBODY TECHNIQUE

The fluorescent antibody (FA) tissue section technique was done essentially as described by Reed et al. (1971) and recommended by Carbrey et al. (1971) for diagnosis of IBR and BVD. Briefly, frozen sections 8 μ thick were made from lung, liver and kidney. The sections were placed on slides, fixed in acetone for 10 to 20 minutes at room temperature, and overlaid with fluorescent conjugates to IBR and BVD viruses. The tissue sections were allowed to stain for 30 minutes in a humid chamber at 37 C followed

by 10 minutes wash in 0.85% saline. Slides were then mounted with a coverslip using a drop of equal parts of saline and glycerol.

The slides were observed under a Zeiss fluorescence microscope with a halogen lamp source using a Schott OG5 barrier filter. The presence of IBR or BVD virus was indicated by the presence of bright focal areas of fluorescence or by the characteristic staining of individual cells.

SEROLOGIC TESTS

Serologic tests were done on serosanguineous fluid or pooled fluids extracted from lung, liver and kidney of aborted fetuses. Fluid samples were obtained from 50 fetuses.

Microtiter serum neutralization (SN) tests were performed according to Carbrey et al. (1971) to determine antibodies specific for IBR, BVD (NADL), and BEV types 2, 4, 5, and 6. The screening dilutions were 1:8 and 1:16 (final).

Hemagglutination inhibition (HI) tests were used to detect antibodies to PI-3 and bovine parvovirus (HADEN), at the screening dilutions of 1:8 and 1:16. The technique employed was based on that described by Carbrey et al. (1971), except a microtiter system was used. A complement fixation (CF) test was performed for antibodies to *Chlamydia* according to the technique used for the viruses of the encephalitides as described by Senne and Pearson

(1978). A standard serum agglutination procedure similar to that employed for brucellosis diagnosis was used to detect antibodies to *Campylobacter fetus* (2), and antibodies to the following serogroups of *Leptospira* were determined by the microscopic agglutination test (11): *pomona, hardjo, grippotyphosa, icterohaemorrhagiae* and *canicola*. The standard plate agglutination test was employed to examine for antibodies to *Brucella abortus* (2). After an initial screening test, positive samples were retested at higher dilutions. Negative and positive controls were periodically included in all serologic tests.

HISTOLOGIC EXAMINATION

Specimens were fixed in 10% buffered formalin and sectioned at 6 μ, stained with hematoxylin and eosin, and examined under the microscope. Histologic examinations were not carried out on fetuses that were too decomposed.

RESULTS

The fetuses examined were mostly from Holstein-Friesian herds coming from many different areas within the State of Michigan. Histories indicated that 15.3% of these herds were vaccinated for IBR, 11.2% for BVD, 10.2% for leptospirosis, 6.1% for brucellosis, 5.1% for PI-3, and 3.1% for vibriosis. The majority of abortions occurred between 6 and 7 months of gestation.

Infectious causes of abortion were recognized in 37 (37.7%) of 98 aborted fetuses examined (Table 1). In addition, noninfectious causes were associated with 6 (6.1%) aborted bovine fetuses. Frequently the fetuses had no gross changes with the exception of autolysis. Infectious conditions in the aborted bovine fetuses were detected by the tests indicated in Tables 2 and 4.

BACTERIOLOGY

The bacteriologic results are summarized in Table 2. Darkfield examination of the fetal abomasal contents demonstrated the presence of vibrio and leptospiral organisms in 5 and 3 fetuses, respectively, and *C. pyogenes* was isolated in pure culture or as a predominant organism from 6 fetuses. Bacteria isolated but not generally known to induce abortion are listed in Table 3. Abortion was attributed to these agents only when they were isolated in pure culture or as the predominant organism from abomasal contents and/or other fetal tissues, when inflammatory lesions were observed in the fetal tissues, and in the absence of other known causes of abortion.

Antibodies to leptospira were found in 6 fetuses, of which 4 reacted to *L. hardjo* and 4 to *L. pomona* and, among them, 2 fetuses reacted to both serotypes, with titers ranging from 10 (+) to 40 (++). In one of the reactors, enterovirus-like particles were found in the

Table 1. Infectious agents isolated or demonstrated in studies of 98 bovine abortions in Michigan, 1977-1978

Factors	Number	Percentage
INFECTIOUS:		
Herpesvirus		(8.2)
IBR virus	3	
IBR virus + *L. monocytogenes*	1	
IBR virus + *K. pneumoniae*	1	
Others	3	
Togavirus		(3.1)
BVD virus	3	
Picornavirus		(2.0)
Enterovirus	2	
Parvovirus	1	(1.0)
Paramyxovirus		(4.1)[a]
PI-3 virus	4	
C. pyogenes		(6.1)
C. pyogenes	5	
C. pyogenes + Parvovirus	1	
Campylobacter species		(5.1)
Campylobacter species	4	
Campylobacter species + Enterovirus	1	
Leptospira species	9	(7.1)[b]
E. coli	1	(1.0)
Aspergillus species	1	(1.0)
Miscellaneous	3	(3.1)
TOTAL	43	(37.7)

[a]Percentage not reflected in the total because of overlap with other significant findings.

[b]Percentage calculated on the basis of 7 fetuses; the other 2 were not included because of overlap with other significant findings.

Table 2. Results of microbiological, pathological and serological[a] examination of 98 bovine aborted fetuses, Michigan, 1977-1978

Bacteria & Fungi	Total	DF[b]	ISOL[c]	AGG[d]	PREC[e]	PATHOL[f]
Campylobacter species	5	5	ND[h]	-	NA	+
Leptospira species	3	3	ND	6	NA	+
C. pyogenes	6	NA[g]	6	NA	NA	+
E. coli	1	NA	1	NA	NA	+
Aspergillus species	1	NA	1	NA	-	+
Miscellaneous	4	NA	4	NA	NA	+

[a]Only 50 fetal sera were tested.

[b]Darkfield examination

[c]Isolation of the microorganism

[d]Agglutination test

[e]Precipitation test

[f]Pathology: lesions on Table 5

[g]Not applicable

[h]Not done

Table 3. Results of bacteriological examination of 98 aborted bovine fetuses in Michigan, 1977-1978

Isolates	Number of Specimens
E. coli	27
Streptococcus species	17
Acinetobacter species	11
Bacillus species	9
Aeromonas species	8
Enterobacter species	8
Proteus species	8
Moraxella species	7
C. pyogenes	6
Klebsiella species	5
Pseudomonas species	3
Staphylococcus species	2
Pasteurella species	1
L. monocytogenes	1
Haemophilus species	1

tissues by EM and, in another, lesions of arthrogryposis and cleft palate were observed.

VIROLOGY

The results of virological examinations are summarized in Table 4. Electron microscopy (EM) was the most effective method utilized, demonstrating viruses in 15 of the 85 fetuses. Eight were herpesviruses, 2 were toga-like viruses, 3 were entero-like viruses, and 2 were parvo-like viruses. Of the 8 herpesviruses, 5 were presumed to be IBR on the basis of positive FA tests on original tissues. The FA test did not detect any IBR virus that was not demonstrated by EM. One of two fetuses positive for toga-like virus by EM was also positive for BVD by FA, and FA revealed BVD virus in 1 fetus that was negative for toga-like virus by EM. Methanol precipitated material was generally more suitable for EM examination, being somewhat cleaner, but variability in concentration was occasionally noticed.

Virus isolation attempts were least effective. Of 97 fetuses examined, only 1 yielded a positive isolation, viz., a noncytopathic BVD virus. Although the FA test on original tissue was negative for BVD, EM examination revealed the presence of a toga-like virus.

Serological results were negative for all viruses tested except for PI-3 virus, to which there were 4 reactors. These 4 fetuses corresponded to 4 in which other causative agents of abortion were demonstrated.

Table 4. Results of the virological examination of aborted bovine fetuses

Virus	Total	ISOL[a]	EM[b]	FA[c]	SN[d]	HI[e]	PATHOL[f]
Entero	3	-	3	NA	-	NA	+
BVD	3	1	2	2	-	NA	+
IBR	5	-	5	5	-	NA	+
Other Herpes	3	-	3	NA	-	NA	+
Parvo	2	-	2	NA	NA	-	-
PI-3	4	-	-	NA	NA	4	-
No. of fetuses examined		97	85	98	50	50	76

[a]Isolation

[b]Electron microscopy

[c]Fluorescent antibody test

[d]Serum neutralization test

[e]Hemagglutination-inhibition test

[f]Pathology: lesions on Table 5

PATHOLOGY

Some of the lesions found in the different organs examined are tabulated in Table 5 with the corresponding microbiological findings. Four of eight fetuses in which herpesvirus was demonstrated had areas of necrosis in the liver. One of these four fetuses also had similar lesions in the lung, while another had such lesions in the spleen. The remaining 4 fetuses had no significant lesions (NSL) or were too decomposed to be examined.

Hydrocephalus and severe malacia of the cerebrum in areas adjacent to the lateral ventricles were found in the fetus whose tissue preparations yielded a positive isolation of a noncytopathic strain of BVD virus. Two other cases of BVD showed NSL.

Pathologic changes found in 2 fetuses in which EM examination of the tissue preparations revealed presence of enterovirus-like particles consisted of slight inflammatory reactions in the liver, kidney and lung with focal hemorrhages in 1 fetus and the presence of thrombi in the blood vessels of the brain, lung, myocardium, spleen and kidney in the other. Significant lesions were not found in the examination of the fetus whose tissues contained only particles resembling parvovirus.

From the 6 *Corynebacterium*-associated abortions, 1 fetus had small areas of necrosis in the liver, 3 had neutrophilic infiltration in the lung, and 1 of these also had bacterial colonies in the blood vessels and areas

Table 5. Histopathological lesions associated with infectious agents of aborted bovine fetuses

Histopathology	IBR + Other Herpes	IBR + *L. monocytogenes*	IBR + *K. pneumoniae*	*C. pyogenes*
Areas of necrosis:				
Lung	+			+
Liver	+	+	+	+
Spleen			+	+
Kidney				+

	C. pyogenes	*C. pyogenes* + Parvo	*E. coli*	Mixed culture bacteria/fungi
Neutrophilic infiltration:				
Lung	+	+	+	
Liver	+			+
Blood vessels				+

	Campylobacter sp.
Lymphocytic infiltration:	
Liver	+

	C. pyogenes
Bacterial colonies:	
Blood vessels	+

	Enterovirus	Mixed culture of bacteria
Thrombi:		
Brain	+	+
Myocardium	+	
Lung	+	
Spleen	+	
Kidney	+	

	Mixed culture of bacteria	*Aspergillus* sp.
Necrotic dermatitis:		
Generalized		+
Eyelid	+	

of necrosis in the spleen and kidney. Collections of lymphocytes in the liver were found in 1 of the 5 fetuses aborted due to *Campylobacter* species; the other 4 fetuses had NSL. In a case in which *L. monocytogenes* was isolated from fetal tissue and the FA for IBR was positive, the histopathologic findings consisted of foci of necrosis in the liver. Similar findings in the liver and spleen were found in a fetus which had a positive FA reaction for IBR virus and presence of *Klebsiella pneumoniae*. Tissues from 3 fetuses yielded the following mixed culture of bacteria and fungi: a) *E. coli* and *P. mirabilis*; b) *E. coli, Bacillus* sp. and *Rhizopus* sp.; c) *Klebsiella* sp. and alpha hemolytic *Streptococcus* sp. The lesions were bile duct hyperplasia and dermatitis of the eyelid, neutrophilic infiltration of the blood vessels, and foci of inflammatory cells in the liver and thrombi in the brain, respectively.

Necrotic dermatitis with presence of fungal hyphae was found in the tissues from the fetus in which *Aspergillus* sp. was isolated.

Abortions associated with noninfectious causes were identified by pathological findings in 6 fetuses. Histopathologic examination of 2 fetuses demonstrated increased cellularity of the skeletal muscles, and fragmentation and loss of striation of individual muscle fibers. One of these also had signs of interstitial pneumonia. The placenta contained areas of neutrophilic accumulation and 1 to 2 areas with degenerative changes. These conditions

were identified as nutritional muscular dystrophy (NMD). Iodine deficiency goiter was diagnosed on 1 fetus on the basis of hyperplasia of the thyroid follicles which had a columnar epithelium and scant colloid. Histopathologic examination of another aborted fetus revealed oxalate-like crystals in the kidneys, and inflammatory changes in the lung. Developmental anomalies were present in 2 of the fetuses. One had an interventricular septal defect and severe chronic passive congestion of the liver, degenerative changes of the myocardium consisting of atrophy and hyalinization of muscle fibers, and severe and extensive encephalomalacia. No other cause of abortion was found. The anomalies found in the other fetus consisted grossly of cleft palate and arthrogryposis; microscopic examination demonstrated increased connective tissue in the liver. This particular fetus was serologically positive for *L. pomona* (1:40).

DISCUSSION

In the present study, noninfectious factors were associated with 6.1% of the abortions and infectious agents were incriminated in 37.7%. Of these 37.7%, bacteria accounted for 22.4%, viruses 14.3%, and fungi 1%. The number and types of infectious agents obtained varied considerably when compared with earlier studies (5,36,37,43) and was likely due to variability of specimens, different herd health programs, and epizootiological factors as well as the availability of diagnostic methods.

The number of mycotic abortions diagnosed (1%) agrees with the results of earlier studies (5,13), but is considerably lower when compared to other reports (21,30,36). This low percentage was anticipated as only a small number of placental tissues (4%) were submitted. It has been demonstrated (30,57,58) that abortions due to fungi are usually a result of placentitis and that the fetus is often not infected or does not show specific lesions. In view of the fact that placental tissue is usually not submitted, it was thought that the demonstration of antibody to fungal antigen might be useful in reaching a diagnosis of mycotic abortion. However, of 50 fluids examined, none showed evidence of antibody to *Aspergillus* sp. To determine whether or not our results indicated the ineffectiveness of fetal serology for the diagnosis of fungal abortions will require further study using larger sample size, and the inclusion of additional fungal antigens.

Similarly disappointing results were obtained in the serologic examination of fetal fluids for the detection of antibodies to various viruses (IBR, BVD, PI-3, BEV, Parvo), and to *Chlamydia*, *Campylobacter* and *Brucella*. There were no antibodies detected to any of these with the exception of PI-3 virus, to which there were 4 reactors. These results differed markedly from earlier reports. In 1971, Kahrs et al. reported serological evidence to BVD and IBR in 18.8% and 25%, respectively, of aborted fetuses examined. Dunne et al., in 1973,

examined 100 aborted fetuses and demonstrated that 10% had antibodies to IBR, 3% to BVD, 56% to PI-3 and 41% to BEV. Much lower frequencies were found in work by Ruth et al. in 1974. In analyzing the data of these earlier reports, considerable variation was found in what was considered a positive antibody response (e.g., serum dilutions from 1:2 to greater than 1:40). In our study, all fetal fluids were screened at dilutions of 1:8 and 1:16. This was necessary as most of the fluids were in poor condition and could not be tested at lower dilutions. Thus, the poor condition of the samples may have contributed to our lower success rate.

More rewarding results were obtained when fetal fluids were examined for antibodies to *Leptospira*. Six reactors were found distributed within the serotypes *L. pomona* and *L. hardjo*. None of these reactions correlated with results of darkfield examination. Whether or not this was related to the acuteness of the infection and consequent lack of antibody or to membranous materials that were confused for *Leptospira* is unknown. These results compared favorably with those obtained in a recent report (15).

Electron microscopy was the most effective method in demonstrating viral agents in fetal tissues. The presence of herpesviruses was detected in 8.2% of the fetuses, correlating in all instances with positive FA results for IBR and demonstrating herpesviruses in 3 additional fetuses that were negative for IBR by FA.

Considering the 91.5% reported accuracy of the FA test in the diagnosis of IBR abortion (29), these 3 herpesviruses may represent other bovine herpesviruses (12,40) whose role in abortion has not been elucidated. The inconsistency of methanol precipitation as a concentration technique was thought to be a result of "virus trapping" due to the large amount of tissue proteins that were precipitated. The use of ultrasonic vibration and absorption procedures is presently being attempted to rectify this problem.

Contrary to results obtained by others (29,43), viral isolation attempts were practically useless as a diagnostic aid. Only one isolate (a noncytopathogenic BVD virus) was obtained, and this virus was demonstrated directly in tissue emulsions by EM. The failure to isolate other viruses was attributed to lack of preservation and autolytic changes of fetal tissues examined. Failures in isolation were not due to the lack of susceptibility of the cell cultures employed, as these cells were previously shown to support the growth of viruses such as IBR, BVD, PI-3 and BEV. Experimentally induced viral abortions (7,10,26,38,44,45,52,53) revealed that recovery of virus from the fetus was inversely related to the length of time between infection and collection of specimens, and that fetuses with marked autolysis yielded few isolates.

Autolysis was less a factor in histopathological examination. Consistent with observations of others

(26,27,44), focal areas of necrosis were consistently found in the liver and irregularly in lung and spleen of fetuses that were shown to be infected with herpesviruses. There were no distinguishable differences in the fetuses in which IBR was confirmed and others in which herpesvirus was demonstrated but were negative for IBR virus by FA.

Histopathological changes associated with other viruses were nil or inconsistent. A correlation between BEV and degeneration of arterial walls has been suggested (13), and in the present study 1 of the 3 fetuses in which enterovirus-like particles were demonstrated had lesions consisting of thrombi in small vessels of various tissues. No lesions were observed in the other 2 fetuses or in the fetus with parvovirus-like particles. Hydrocephalus was found in a fetus whose tissues contained togavirus-like particles as seen by EM. A noncytopathic BVD virus was also isolated. Although studies have established BVD virus as a teratogenic agent (8,10,25,55), and hydrocephalus has been associated with this virus in sheep (56), hydrocephalus produced by BVD virus has not been demonstrated experimentally in cattle.

Alveolar infiltration of neutrophils and mononuclear cells, and the presence of bacteria, were observed in the fetus infected with *C. pyogenes*. Hepatic necrosis was evident in the fetus whose tissues yielded *L. monocytogenes*. These lesions are consistent with those described by other investigators (1,17,49,50). However, the fetus infected with *L. monocytogenes* was also infected

with IBR virus, which may have been responsible for the lesions observed. No specific lesions were observed in abortion associated with other bacteria.

Nutritional factors were associated with 3 abortions. Congenital nutritional muscular dystrophy has been reported in association with abortion (23,36), and 2 fetuses which we examined had lesions consistent with this myopathy. Iodine deficiency goiter was identified in an additional fetus, but its association with abortion is unknown.

Although renal oxalosis has not been established as a cause of abortion, it has been observed in aborted bovine fetuses (4,46) and in 1 fetus examined in this study.

The significance of arthrogryposis and cleft palate in 1 fetus was difficult to assess. These congenital anomalies have been associated with genetic (22,32), nutritional (14,34,48), and infectious factors including Akabane virus (31) and BVD virus (20,33). This particular fetus had serological evidence of leptospiral infection and was from a herd with a history of an abortion epidemic. An interventricular septal defect was found in 1 fetus examined. This genetic anomaly has been reported in young calves, but no causal relation to abortion was suggested (3).

In conclusion, the capability of the diagnostic laboratory in dealing with bovine abortion was enhanced by the implementation of additional aids. Our results clearly indicate the value of electron microscopy in

detecting viruses, and it is anticipated that more effective concentration and purification methods will increase the effectiveness of this tool. Such procedures should be relatively inexpensive, simple, and not unduly time-consuming. Ultracentrifugation does not appear to be the answer, as a considerable amount of extraneous proteins are pelleted, viruses are occasionally distorted, and excessive use of the centrifuge leads to frequent servicing.

Although our results were not as impressive as those of previous studies (13,42), serologic examinations of fetal fluids appears to be a useful adjunct to routine procedures. The value of serologic tests using fetal fluids will probably be more fully realized as additional antigens are screened.

Finally, the effectiveness of the diagnostic laboratory would be increased if placental membranes and fetuses in good condition were obtained. Thus, an effort should be made to communicate this requirement to practicing veterinarians through extension media. To facilitate more satisfactory specimens, diagnostic laboratories should probably provide suitable shipping containers.

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