Diagnostic Survey of Bovine Abortion in Korea: With Special Emphasis on Neospora caninum


¹National Veterinary Research and Quarantine Service, Anyang and ²Departments of Pathology, ³Theriogenology, ⁴Virology, ⁵Infectious Disease and ⁶Parasitology, College of Veterinary Medicine and School of Agricultural Biotechnology, Seoul National University, Suwon, 441–744, Korea

(Received 1 February 2002/Accepted 6 September 2002)

ABSTRACT. Causes of bovine abortion were surveyed in Korea within a designated period from the cases submitted to the Department of Veterinary Pathology, College of Veterinary Medicine, Seoul National University. One hundred and eighty aborted fetuses and maternal sera were evaluated by necropsy, histopathology, bacteriology, virology, PCR, and serologic tests. The causes of abortion were identified in 108 (60%) cases, of which 38 (21.1%) were due to the infection with Neospora caninum. None of the 38 cases showed any co-infection with either virus or bacteria. Viral and bacterial causes were diagnosed in 28 (15.5%) and 13 (7.2%) aborted fetuses, respectively. Non-infectious causes such as multiple pregnancy, maternal weakness or torsion of umbilical cord were observed in 22 (12.3%) cases.

Results of the present study suggest that N. caninum is believed to be the leading cause of bovine abortion in Korea. Thus, more attention should be paid to this emerging disease in Korea. However, the causes of many aborted fetuses remain undiagnosed in this study. Therefore, this enigma should be clarified through further studies such as chromosomal analysis.

KEY WORDS: abortion, bovine, Neospora caninum, survey.


Bovine abortion is one of the most critical disease problems in the dairy cattle industry worldwide due to its negative economic impact. Surveys on the causes of bovine abortion have been reported from several countries [1, 2, 11]. Identifying the causes of abortion is important for establishing a proper rational herd management, which can protect the dairy farmers from economic losses. This type of survey is also significant for investigating whether any regional differences due to the climate and types of cattle management can affect the pattern or prevalence of abortifacients [1], which had not been previously studied in Korea.

This study reports on the results of an 18-month survey of bovine abortion in Korea. We found that a cyst-forming protozoan parasite, N. caninum, was responsible for 21.1% of bovine abortion in Korea, causing considerable economic loss in the dairy cattle industry.

MATERIALS AND METHODS

Study subjects: Over an 18-month period (between June 1999 and November 2000), a total of 180 aborted bovine fetuses at different stages of gestation and maternal blood samples were submitted to the Department of Veterinary Pathology, College of Veterinary Medicine, Seoul National University to identify the causes of abortions. The samples were from 147 farms showing approximately 4 to 16% abortion rate. Gestational age was determined based on the date of artificial insemination.

Histopathology and immunohistochemistry: After a full gross examination, brain, heart, lung, liver, spleen, kidney, spinal cord, skeletal muscle, stomach, and small and large intestines were fixed in 10% neutral phosphate-buffered formalin, routinely processed, and embedded in paraffin for light microscopic examination. Replicate sections of the brain and heart were used for immunohistochemical identification of N. caninum with commercially available hyper-immune goat serum (VMRD Inc., Pullman, WA, U.S.A.). The standard avidin-biotin-peroxidase (ABC) technique (Vector Lab, Burlingame, CA, U.S.A.) was used to demonstrate the antigen.

PCR from formalin-fixed tissues: Protozoal DNA was extracted from paraffin blocks of the brain and heart that contained lesions suspected of N. caninum infection through routine protease K digestion and phenol/chloroform extraction, and was pooled for PCR analysis. PCR was carried out as previously described using Np4 and Np7 as the primer set [5]. Brain from a mouse experimentally infected with KBA-2 [13], a histologically normal bovine fetal brain, and paraffin block only were used as positive and negative controls, respectively.

Serology: Standard tube agglutination test, microscopic agglutination test (MAT), and microplate agglutination test were used for Brucella abortus, Leptospira interrogans, and Campylobacter fetus, respectively [14]. Enzyme-linked immunosorbant assay (ELISA) for Toxoplasma gondii, serum neutralization (SN) test for Akabane and Aino viruses, and indirect fluorescent antibody (IFA) test for Chlamydia psittaci, Trichomonas fetus, and N. caninum were performed using standard methods [8, 14, 16].

Bacteriology and virology: Aerobic and anaerobic cultures were performed using fetal abomasal fluid, lung, liver,
spleen, and kidney. Isolated bacteria were identified using an automatic identification apparatus, Viteck system (VITEK Co., Hazelwood, MO, U.S.A.). Direct fluorescent antibody (FA) test for IBR and BVD were performed as previously described [14, 16]. Reverse-transcription-polymerase chain reaction (RT-PCR) was applied to detect Akabane viral antigen as described previously with minor modifications [6]. Primer pairs for BVD virus were newly designed from 5’-untranslated region of the BVD virus genome based on Hyndman and others [10]. Nucleotide sequences of the primers used to detect Akabane virus are AK4 F2, 5’-ACCAGAAGAAGGCCAGTAG-3’ , and AK4 R2, 5’-CACACGGTGCATGTCGA TAA-3’; and those for BVD virus are BVDV F1, 5’-GCTGAC- CCTATCAGGCTGT-3’. Reaction mixtures were first denatured at 94°C for 5 min, and amplification was performed for 30 cycles, at 94°C for 30 sec, 58°C for 30 sec, 72°C for 30 sec, followed by an extension for 7 min at 72°C for Akabane virus. PCR conditions for BVDV consisted of 94°C-30 sec denaturation, 52°C-30 sec annealing, and 72°C-30 sec elongation. Electrophoretic analysis of PCR products was performed after carrying out 30 cycles under the conditions. Viral isolation was also performed when necessary.

RESULTS

Results of the study are summarized in Table 1. Among the 180 fetuses examined, causes of abortion were assigned in 98 (54.5%), whereas no specific causes were found in the remaining 82 (45.5%) fetuses.

Gross pathology: At necropsy, no significant gross abnormalities were observed in most cases except for brains and skeletons. Cerebellar hypoplasia and arthrogryposis-hydranencephaly syndrome (AHS) were detected in four and nine fetuses, respectively. Numerous well-demarcated, yellowish-white foci ranging from 0.5 to 3.0 mm in diameter were observed in the skeletal muscle and myocardium of two aborted fetuses.

Histopathology and PCR from paraffin block: Among the 180 cases examined histologically, forty-five fetuses showed lesions compatible with neosporosis. The lesions were primarily focused in the brain, myocardium/epicardium, skeletal muscle, and occasionally in the liver. Mild to severe nonsuppurative necrotizing encephalomyelitis characterized by multifocal discrete foci of necrosis and gliosis and perivascular cuffing of mixed mononuclear cells (Fig. 1) was observed in the brains. Immunohistochemically, thick-walled tissue cysts containing numerous bradyzoites or tachyzoites were associated with the lesions in sixteen fetuses (Fig. 2), of which were preferentially submeningeal and periventricular in location. Multifocal-to-coalescing, mild-to-severe infiltration of mixed mononuclear cells and fragmentation and myofibrillar necrosis were noted in the heart and skeletal muscles (Fig. 3). Multifocal areas of hepatocellular necrosis and periporal infiltration of mononuclear cells were occasionally seen in the liver. Among the forty-five fetuses with the lesion, thirty-four were positive for N. caninum infection as revealed through PCR (Fig. 5), whereas the remaining eleven cases, which were all mild in lesion severity, were negative. However, among 11 fetuses which were PCR-negative but had lesions compatible with N. caninum infection, 4 cases were positive on IFA test.

Acute suppurative bronchopneumonia and purulent meningoencephalitis were found in twelve and one fetus, respectively. Focal necrotizing hepatitis and splenitis, which were negative for N. caninum by PCR and serology were observed in two fetuses. In twelve fetuses, moderate to severe nonsuppurative encephalomyelitis with neuronal loss were noted (Fig. 4). Disseminated lymphosarcoma was diagnosed in one fetus.

Serology and microbiology: No bacteria responsible for abortion were isolated from any of the samples even though thirteen aborted fetuses (12 bronchopneumonia and 1 meningoencephalitis) were suspected of bacterial abortion by histopathological examination. Furthermore, no serological evidences of bacterial abortion were found against B. abortus, L. interrogans serovar icterohemorrhagiae, pomona, canicola, hardjo, C. fetus subsp fets, C. fetus subsp venerealis, and C. psittaci. In addition, no evidences of T. gondii or T. fetus infection were found based on serology. On virology, seventeen and five fetuses were positive for BVD and IBR on IFA test, respectively. However, among the five cases positive for IBR, two were also positive for BVD. Six fetuses were found positive for Akabane virus by RT-PCR,

<table>
<thead>
<tr>
<th>Causes</th>
<th>Number of fetus</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious</td>
<td>86</td>
<td>47.7</td>
</tr>
<tr>
<td>Neospora caninum confirmed</td>
<td>45</td>
<td>25</td>
</tr>
<tr>
<td>possible</td>
<td>38</td>
<td>21.1</td>
</tr>
<tr>
<td>Bacterial infection</td>
<td>13</td>
<td>7.2</td>
</tr>
<tr>
<td>bronchopneumonia</td>
<td>12</td>
<td>6.6</td>
</tr>
<tr>
<td>meningoencephalitis</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Viral infection</td>
<td>28</td>
<td>15.5</td>
</tr>
<tr>
<td>Akabane</td>
<td>12</td>
<td>6.6</td>
</tr>
<tr>
<td>IBR</td>
<td>5</td>
<td>2.7</td>
</tr>
<tr>
<td>BVD</td>
<td>11</td>
<td>6.2</td>
</tr>
<tr>
<td>Non-infectious</td>
<td>22</td>
<td>12.3</td>
</tr>
<tr>
<td>Iatrogenic</td>
<td>2</td>
<td>1.1</td>
</tr>
<tr>
<td>Dystocia</td>
<td>2</td>
<td>1.1</td>
</tr>
<tr>
<td>Congenital lymphosarcoma</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Multiple pregnant</td>
<td>3</td>
<td>1.6</td>
</tr>
<tr>
<td>Atresia coli</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Ventricle septal defects</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Diprosopus</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Maternal weakness and toxemia</td>
<td>8</td>
<td>4.4</td>
</tr>
<tr>
<td>Environmental stress</td>
<td>2</td>
<td>1.1</td>
</tr>
<tr>
<td>Torsion of umbilical cord</td>
<td>1</td>
<td>0.6</td>
</tr>
</tbody>
</table>

a) Lesion-, PCR- and sero-positive.  
b) Lesion-positive but PCR- and sero-negative.  
c) Based on histopathology.  
d) Four and two fetuses, dual positive for IBR and bacterial infections, respectively, were excluded and assigned in the respective section.
and twelve fetuses which developed AHS syndrome with nonsuppurative encephalomyelitis and neuronal had fetal antibody titers ranging from 1: 4 to 1:256 against Akabane virus but all were negative for Aino virus on SN test.

DISCUSSION

In this study, thirty-eight of the 180 (21.1%) aborted fetuses examined were diagnosed as *N. caninum*-associated abortion by histopathology, immunohistochemistry, PCR, and serology. Our study confirms and extends the study of Baszler and others [5]. PCR-based assay can be performed practically for the routine diagnosis of *N. caninum* as well as for the analysis of strain genotypes. However, when the degree of inflammatory changes is mild, PCR assay might not be able to detect *N. caninum* DNA as revealed in this study. Baszler and others [5] suggested that at least 20 to 40 tachyzoites in 20 mg of tissue are needed to pursue PCR detection of *N. caninum* in clinical samples. In this study, even though the lesion distributions and patterns of 7 fetuses pointed towards *N. caninum* infection, a definite diagnosis remained open due to the negative PCR and serology results. In addition, these seven cases were also immunohistochemically negative to *N. caninum*. However, this still does not rule out the possibility of *N. caninum* infection since no other specific cause is identified in any of these cases.

Based on serology, all tested sera were negative for *T. gondii* and *T. fetus*, with no evidence of abortion due to *T. fetus* noted through histopathology. None of the thirty-four cases revealed co-infection with other viruses or bacteria, which is an indication of a causative relationship between *N. caninum*.
**N. caninum** infection and abortion.

Histopathologically, similar to the results of previous studies, lesions were most commonly observed in the brain, heart, skeletal muscle, and occasionally in the liver. In the brain, multifocal areas of necrosis and gliosis with no inflammatory reaction were the main changes observed in fetuses less than 5 months of gestation (8 cases); whereas, in fetuses over 7 months of gestation (37 cases), perivascular infiltration of mixed mononuclear cells was more prominent than necrosis. These results were consistent with those of Ogino and others [18]. This remarkable association between inflammatory response and gestational age probably reflects the progressive development of the immune system in bovine fetuses. Presence of tachyzoites and associated hepatocellular necrosis suggest an acute infection with a high dose of parasites, which indicates a primary infection of the dams [22]. Hepatic lesion associated with **N. caninum** infection was not commonly noted in this survey.

Repeated abortions associated with **N. caninum** infection were noted in only one farm where seven cows aborted eight fetuses over a 2-year period. Although the prevalence of repeated abortion in bovine following **N. caninum** infection is unknown, repeated abortion associated with **N. caninum** is reported to be about 4 to 5% [17, 21].

According to a previous nationwide sero-survey, 49% of the sera collected from herds with a higher (7 to 17%) abortion rates than the average (3%) were positive for **N. caninum** [9]. The mean seropositive rate of these herds was 2.5 times higher than that of herds with no abortion problem, which suggests a correlation between **N. caninum** seropositivity and the abortion rate. When cows became infected with **N. caninum**, a 3- to 50-fold increase in the risk of endemic or epidemic abortion was observed [19, 20].

**N. caninum** is a cyst-forming protozoan parasites affecting various animal species including ruminants [7]. In cattle, **N. caninum** infections have been associated with neonatal paresis as well as abortion [4, 7]. In California, economic loss due to **N. caninum** abortion was estimated at $35 million annually [3, 7]. **N. caninum**-induced bovine abortion was first described in 1997 in Korea [12]. Overall, **N. caninum** was demonstrated to be one of the leading causes of bovine abortion in Korea based on the previous sero-survey and the results of this study.

Lesions such as suppurative bronchopneumonia and meningoencephalitis, which are suggestive of bacterial infections, were found in twelve and one fetus, respectively but bacterial cultures did not yield any specific causes in these cases. Furthermore, all sera tested were serologically negative for *B. abortus, L. interrogans* and *C. fetus*. Although placentas are very important for diagnosing bacterial abortion, they are not routinely evaluated in this survey because farmers, instead of submitting them for diagnoses, customarily fed them to the co-raising animals in farms. Therefore, even when evidences of bacterial infections are found in other organs histologically, due to unavailability of placentas, diagnosis of bacterial abortion is difficult in Korea. Four fetuses afflicted with bronchopneumonia were coinfected by BVD virus, suggesting that bacterial pneumonia might occurred secondary to the immunosuppression of the dams following BVD virus infection. However, dual infections may simply reflect the prevalence of each infection, and further studies are needed to draw definite conclusions.

Encephalomyelitis associated with Akabane virus infection was noted in adult cows between August and September 2000 [15]. While the outbreak subsided by the end of October 2000, a marked increase in the incidences of bovine abortion due to Akabane virus infection was observed during the fall of 2000 and early spring of 2001, which indicates a hematogenous spreading of virus from the infected dam to the fetuses. We speculate that foot and mouth disease outbreak in the spring of 2000 in Korea kept the farmers off the scheduled vaccinations including Akabane virus, consequently resulting in the sudden increases of abortion and AHS due to Akabane virus last fall. Aside from Akabane virus, five and seventeen fetuses were aborted due to IBR and BVD viruses based on gross findings, histopathology, and virology.

In conclusion, **N. caninum** is believed to account for...
BOVINE ABORTION IN KOREA

21.1% of bovine abortion causing considerable economic loss to the dairy cattle industry in Korea. Thus, more attention should be paid to this newly emerging disease in Korea. However, because the causes of many aborted fetuses could not be diagnosed in this study, similar to other studies, this enigma should be clarified through further studies such as chromosomal analysis.

ACKNOWLEDGMENT. This study was supported by grants-in-aid from the Ministry of Agriculture and Forestry (399002-3) and the Brain Korea 21 project. We also thank all the practitioners who submitted the cases for this study. Appreciation is extended to Drs. T. Umemura, I. Yamane, and Doo-Youn Cho for their valuable comments.

REFERENCES