

Original Article

Infectious Causes of Bovine Abortion in Qazvin Province, Iran

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ABSTRACT

Abortion in dairy cattle is generally defined as the loss of fetus on days 47-265 of pregnancy and can significantly reduce the income and capital gain of farmers and producers. Therefore, sufficient economical support and preventive measurements are essential for this issue. In this study, genetic materials were extracted from the abomasum rennet, as well as homogenous preparations of brain, kidney, spleen, liver, and lung tissues of 128 aborted fetuses. Afterward, polymerase chain reaction (PCR) and Reverse Transcription Polymerase Chain Reaction (RT-PCR) tests were performed to identify the bovine viral diarrhea virus (BVDV), bovine herpesvirus 1 (BHV-1), *Neospora caninum* parasite, and serovars of *Leptospira* species. In the PCR test, the samples with 380 bp, 340 bp, and 173 bp bands were considered as positive for serovars of *Leptospira*, *Neospora caninum*, and BHV-1, respectively. Moreover, a 290 bp band was regarded to be BVDV in the RT-PCR. According to the findings of the current study, 39 (30.47%) of the samples were infected with *Neospora caninum*, 26 (20.31%) with BVDV, 17 (13.28%) with BHV-1, and 18 (14.06%) with serovars of *Leptospira*. In addition, multiple-agent infections were also detected in the samples. As a conclusion, the microorganism *Neospora caninum* was identified as the most prevalent infectious cause of abortion in the eight agro-industrial and livestock complexes, in Qazvin province, Iran.

Keywords: Abortion, Bovine herpes virus, Bovine viral diarrhea virus, *Leptospira*, *Neospora caninum*, PCR, RT-PCR

Causes Infectieuses d'Avortement bovine en Province Qazvin, Iran

Résumé: L'avortement chez les vaches laitières se réfère généralement à la mort du fœtus entre les jours 47 à 265 de la grossesse. Étant donné que l'avortement peut engendrer la réduction considérable des revenus et la perte de capital des producteurs, il est nécessaire de prendre des mesures de prévention et d'accorder un budget approprié à cet effet. Dans la présente étude, 128 fœtus avortés provenant de 8 fermes laitières de la province de Qazvin en Iran ont été examinés au second semestre de l'an 2015. Les échantillons de fœtus testés comprenaient un mélange homogène des tissus du cerveau, des reins, de la rate, du foie et des poumons du fœtus avorté. Après l'extraction du matériel génétique, des expériences PCR et RT-PCR ont été réalisées pour identifier BVDV, BHV-1, parasite de *Neospora caninum* et la bactérie de *Leptospira*. Finalement, en effectuant le test de PCR, les échantillons de bandes bp380 pour les bactéries leptospires, bandes bp340 pour le parasite de *Neospora caninum* et la bande bp173 pour le virus BHV-1 ont été considérés comme positifs dans l'électrophorèse sur gel. En effectuant également le test RT-PCR, le résultat de la bande bp290 pour le virus BVDV était positif. À la fin,

sur 128 fœtus avortés, 39 échantillons (30,47%) ont été infectés par *Neospora caninum*, 26 (20,31%) ont été infectés par le virus BVDV, 17 (13,28%) ont été infectés par le BHV- 1 et 18 échantillons (14,06%) ont été infectés par les bactéries de *Leptospira*.

Mots-clés: Avortement, réaction en chaîne par polymérase, *Neospora caninum*, Diarrhée virale bovine, Herpèsvirus Bovin de Type 1, *Leptospira*

INTRODUCTION

Abortion in dairy cattle is generally defined as the loss of fetus on days 47-265 of pregnancy. The infectious agents causing fetus abortion include the bacterial (45-50%), viral (15-25%), fungal (20-25%), and parasitic (10-15%) ones. In addition, some non-infectious factors can also be involved, such as the nutritional compounds, chemical agents, drugs, poisons, and hormones (Andrews et al., 2008). Infection with *Neospora caninum* can lead to fetus abortion, stillbirth, or birth of a weak calf (Almería, 2013). Moreover, the importance of leptospirosis in livestock is mostly attributed to the ensuing economic loss. This financial loss results from a rapid drop in milk production, abortion, stillbirth, birth of weak calves, agalactia, weight loss, impaired fertility, in addition to the high expenses of disease prevention, control, and treatment (Zachary and McGavin, 2013). Likewise, the significance of bovine viral diarrhea virus (BVDV) lies in its role in abortion, fetal mummification, stillbirth, teratogenesis, infertility, reduced production, and even death which could result in economic loss for the producers (Driskell and Ridpath, 2006). It should be noted that the surviving infected fetuses are protected against the virus throughout their lives (persistent infection). Therefore, identification of these carrier animals is of central role both epidemiologically and economically (Liu et al., 2009). According to the literature, bovine herpesvirus 1 (BHV-1) known as the cause of infectious bovine rhinotracheitis can account for abortion, infertility, stillbirth, reduced production, encephalitis, and gastrointestinal disorders in cattle (Hart et al., 2007). It is noteworthy that the major economic loss due to this

disease is known to be related to infertility, fetus abortion, stillbirth, and reduced production (Driskell and Ridpath, 2006). The optimal method for confirming BVDV, BHV-1, *Leptospira* species, or *Neospora caninum* as the causes of abortion is polymerase chain reaction (PCR) technique using the tissue samples collected from liver, lung, brain, and spleen. It has been demonstrated in the literature that samples from the aborted fetus or placenta are suitable for determining the cause of abortion (Liu et al., 2009). With this background in mind, in this study, we aimed to investigate the prevalence and etiology of bovine abortion by PCR and Reverse Transcription PCR (RT-PCR) in eight agro-industrial and livestock complexes of Qazvin province, Iran on April-September, 2015.

MATERIALS AND METHODS

A total of 128 aborted fetuses were collected during the second six months of 2015 from eight agro-industrial and livestock complexes in Qazvin province, Iran. Sampling was performed from the abomasum rennet content, as well as homogenous preparations of liver, lung, kidney, spleen, and brain tissues of the aborted fetuses using the sterile and disposable tools. All the samples were kept at -20 °C until they were transferred to the laboratory. Concurrent with sampling from the aborted fetuses, some essential information was collected from the agro-industrial complexes. The data entailed the cattle parity, age of the aborted fetus, type of the sperm used in artificial insemination, history of vaccination or drug injection, and changes in the diet of registered units.

DNA and RNA Extraction. In order to detect the BVDV, RNA was used as the diagnostic genetic material. In the current study, a commercial Kit

(MBST Co., Iran) was used to extract the RNA from the lung and liver tissue samples. On the other hand, DNA was used for identification of BHV-1, *Leptospira* species, and *Neospora caninum*. For this purpose, a commercial Kit (MBST Co., Iran) was used to extract DNA from the lung, liver, and brain tissue samples.

RT-PCR and Gel Electrophoresis Techniques. All the primers used for the target sequences of the intended pathogens are shown in Table 1. The RT-PCR test using a one-step RT-PCR Kit (catalogue No. 210212, QIAGEN Co., Germany) was applied to diagnose the BVDV infection in the fetuses. Finally, the products were transferred to a gel electrophoresis tank and the samples with bands of 290 bp were considered as positive for BVDV. Figure 1 indicates the final products of RT-PCR which were run on the agarose gel and visualized by ethidium bromide.

Table 1. Primers used in the PCR test

BVDV	Forward: 5 > - GCCATGCCCTTAGTAGGACTAGC >
	Reverse: 5 > - CAACTCCATGTGCCATGTACAGC >
BHV-1	Forward: 5 > - CTGCTGTTCGTAGCCCACAACG >
	Reverse: 5 > - TGTGACTTGGTGCCCATGTCGC >
Leptospira species	Forward: 5 > - TTCCCCCATTGAGCAAGATT >
	Reverse: 5 > - GGCGGCGGTCTTAAACATG >
Neospora caninum	Forward: 5 > - CCCAGTGCGTCCAATCCTGTAAC >
	Reverse: 5 > - CTCGCCAGTCAACCTACGTCTTCT >

PCR and Electrophoresis Techniques. The PCR test was used to identify the fetuses infected with BHV-1, *Leptospira* species, and *Neospora caninum*. For this purpose, Taq DNA Polymerase Master Mix (Catalogue No. 180301, Amplicon Co., Denmark) was applied to perform this test. Afterwards, the products were loaded on an electrophoresis gel, and the samples with bands of 173 bp, 380 bp, and 340 bp lengths were regarded as positive for BHV-1, serovars of *Leptospira* species, and *Neospora caninum* protozoans, respectively. Figures 2-

4 show the final PCR products which were run on agarose gel and visualized by ethidium bromide.

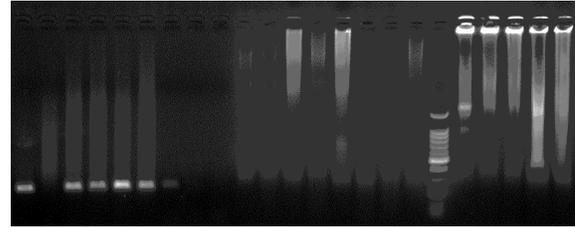


Figure 1. Agarose gel electrophoresis demonstrating the BVDV specific RT-PCR products with 290 bp length

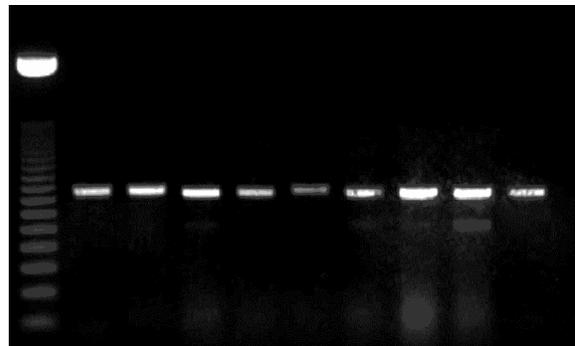


Figure 2. Agarose gel electrophoresis demonstrating the *Neospora caninum* specific RT-PCR products with 340 bp length

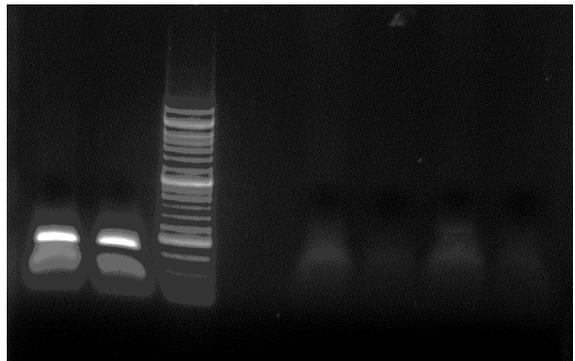


Figure 3. Agarose gel electrophoresis demonstrating the BHV-1 specific RT-PCR products with 173 bp length

RESULTS

The present study was conducted during the second six months of 2015 on aborted fetuses collected from eight agro-industrial and livestock complexes in Qazvin, Iran.

RT-PCR and Gel Electrophoresis. the primer used for the target sequences of the intended BVDV are

shown in Table 1. Afterwards, the products were loaded on an electrophoresis gel, and the samples with bands of 290 bp were considered as positive for BVDV. Figure 1 indicates the final products of RT-

PCR which were run on the agarose gel and visualized by ethidium bromide.

PCR and Electrophoresis. the primers used for the target sequences of the intended BHV-1, *Leptospira* species, and *Neospora caninum* are shown in Table 1. Afterwards, the products were loaded on an electrophoresis gel, and the samples with bands of 173 bp, 380 bp, and 340 bp lengths were regarded as positive for BHV-1, serovars of *Leptospira* species, and *Neospora caninum* protozoans, respectively. Figures 2-4 show the final PCR products which were run on agarose gel and visualized by ethidium bromide. A total of 250 fetuses were obtained, 128 of which were selected for sampling. Overall, 74 (57.8%) samples were found to be positive for at least one of the studied agents (*Neospora caninum*, *Leptospira* species, BHV-1, and BVDV). In addition, 16 (21.6%) of the aborted fetuses were diagnosed to be infected with more than one pathogens, that shown in table 2 and diagram 1.

Table 2. Frequency distribution of the studied infectious agents in the sample aborted fetuses

<i>Neospora caninum</i>	27	%21.1
BVDV	19	%14.8
BHV-1	9	%7
<i>Leptospira</i> species	13	%10.2
<i>Neospora caninum</i> + BVDV	5	%3.9
<i>Neospora caninum</i> + BHV-1	4	%3.1
<i>Neospora caninum</i> + <i>Leptospira</i> species	3	%2.3
BHV-1 + BVDV	2	%1.6
BHV-1 + <i>Leptospira</i> species	2	%1.6
Unknown	54	%42.2

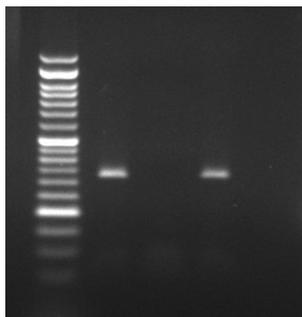


Figure 4. Agarose gel electrophoresis demonstrating the *Leptospira* spp. specific RT-PCR products with 380 bp length

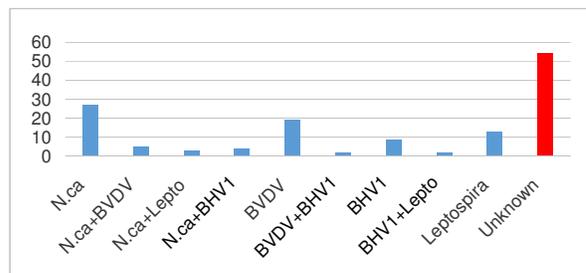


Diagram 1. Frequency of the studied infectious agents in the samples

DISCUSSION

The infectious cause of fetus abortion can be determined through collecting samples from the aborted fetus or placenta. Accordingly, sampling from the organs should rapidly be performed for efficient detection of the causative agent (Zachary and McGavin, 2013). With this background in mind, in this study, we used PCR and RT-PCR methods for detecting BVDV, BHV-1, *Leptospira* species, and *Neospora caninum* in the abomasum rennet content, as well as homogenous preparations of liver, lung, kidney, spleen, and brain tissue samples of the aborted fetuses. In the study performed by Njiro et al. in 2010, some of the infectious causes of reproductive problems were studied in cattle belonging to underprivileged farmers in South Africa. In the mentioned study, 239 blood samples of cattle from 21 regions of the country were investigated using the PCR method. According to the results of their study, the prevalence of BHV-1 and BVDV infections was 75% and 50%, respectively (Njiro et al., 2011). In the current study, rate of infection with BHV-1 and BVDV was reported as 17% and 13% in the eight studied agro-industrial and livestock complexes of Qazvin province, respectively. Although the present research showed an overall lower rate of viral infections, compared to the study by Njiro et al., the frequency of BHV-1 infection was significantly higher than BVDV. Moreover, a difference was observed between the findings of the present research and the one performed by Njiro and colleagues regarding the rate of *Neospora caninum* infection. The frequency of this infection was revealed

to be 37% in the aborted fetuses, which is four times higher than the reports of Njiro and colleagues. Furthermore, another study evaluated the dairy cattle in Querétaro region of Mexico (2008) in order to find the reasons of abortion in this area. The authors simultaneously conducted two detection techniques, including the enzyme-linked immunosorbent assay and histopathological evaluation of the lesions in the samples obtained from the aborted fetuses. Their findings indicated that 62.5% of the aborted fetuses had histopathological lesions in their lung, heart, spleen, and kidney tissues. In 70% of the cases, the observed lesions were compatible with the pathological characteristics of brucellosis, leptospirosis, and neosporosis. Additionally, vague lesions were observed in the remaining 30% of the fetuses (Escamilla et al., 2007). Both the current study and the one performed by Patricia Escamilla revealed similar results concerning fetus abortion due to BVDV and reported an infection rate of 13%. Abortion resulting from BHV-1 was shown in 17% of the fetuses of the present study, while Patricia et al. found only 6% of the aborted fetuses to be infected with this virus. A similar difference was observed regarding the rate of fetus abortion caused by neosporosis. In the present study, 37% of the aborted fetuses were infected with *Neospora caninum*, which is much higher than that of the herds in Querétaro, Mexico (14%). The major discrepancy between these two studies might be related to the serovars of *Leptospira* bacteria, which were the main cause of cattle abortion in the study completed by Escamilla and colleagues (frequency of 53%). However, no infections with this pathogen were reported in the aborted fetuses studied in the current research. One of the most important factors playing role in fetus abortion due to *Neospora caninum* is the relationship between parity and the frequency of fetus abortion caused by this agent. In a study aimed to investigate the serological status against this parasite, no significant difference was observed between the different age groups (i.e., <2, 2-4 years, and >4 years) (Sadrebazzaz et al., 2007). A

recently proposed hypothesis speculating no relationship between *Neospora caninum* infection and parity has been challenged in the literature. Fuji et al. (2001) in a study on the seroprevalence of this protozoan reported a significant relationship between infection and parity in the south western regions of Brazil (Fuji et al., 2001). Likewise, a study on the frequency of infection with *Neospora caninum* in Mexican cattle (2010) revealed a positive and significant relationship between the extent of infection and age of the cattle. The latter study reported the five-year-old cows to have the highest rate of infection (Garcia-Vazquez et al., 2009). Furthermore, in the present study, assessment of the pregnant cattle age showed a significant relationship between the parity and abortion due to *Neospora caninum* infection.

According to the results of present study, more than half of the abortions in agro-industrial and livestock complexes of Qazvin province could be attributed to contamination with the infectious agents, such as *Neospora caninum* and BVDV. Considering the vertical transmission of these agents to the progeny, infections can lead in extreme economic loss in case of neglecting the preventive and treatment measures.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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