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Diagnosis of Leptospiral Abortion in Bovine by Polymerase Chain Reaction

A. Doosti and N. Hoveizeh Tamimian

Biotechnology Research Center, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran

Abstract: Leptospirosis is an acute infectious, systemic and septisemic disease which had resent outbreaks in some parts of Iran. Infectious abortion caused by *Leptospira* is a significant cause of reproductive failure and economic losses in bovine. This disease can be serological diagnosed, but many factors may cause false positive and negative results. The purpose of present study was to determine the frequency of *Leptospira* infection in aborted bovine using Polymerase Chain Reaction technique in Chaharmahal Va Bakhtiari province located in southwest of Iran. One hundred and twenty liquid rennet samples of bovine aborted fetuses were collected and genomic DNA was extracted. PCR reaction was performed for detection of *Leptospira* DNA using specific primers for *16s rRNA* gene and PCR products were visualized in a 1% agarose gel electrophoresis. *Leptospira* DNA were positive from 17 (14.16%) of 120 aborted bovine samples. The results of current study showed that high frequency of infection with this microorganism. According to these findings examination of aborted bovine for control and prevention of leptospirosis in Chaharmahal Va Bakhtiari province it seems to be necessary.

Key words: *Leptospira* · *16s rRNA* gene PCR · Aborted bovine · Iran

INTRODUCTION

Leptospirosis is a zoonotic infection caused by spirochetes of *Leptospira* genus. Infection usually is through direct contact via injured skin mucosal membrane [1]. Leptospirosis has been recognized as an important emerging disease in the 1980s and 1990s in Andamans, Tamil Nadu and Kerala [2].

Today, it is widespread in farmland domestic animals in many parts of Iran. This disease is a zoonosis of global distribution, caused by infection with pathogenic spirochetes of the genus *Leptospira*. The disease are greatly under reported, particularly in tropical regions, but attempts at surveillance suggest that it may be the most common zoonosis that affects virtually all mammals [3].

Leptospirosis in bovine may appear as acute, sub acute or chronic forms. The present study focused on chronic form of this infection because this shape related to many abortions in bovine. Abortion or premature calving and infertility signs, can be use for diagnosis of chronic leptospirosis. Abortion may occur several weeks later, but may also occur as the only evidence of the disease in this form [4]. In some herds, abortion has occurred after leptospiral mastitis or agalactia has been observed during the previous 3 month. Abortion occurs all year round but, after correcting for any seasonal variation in calving, it is most prevalent in September and October [5].

Rapid diagnosis of leptospirosis is important for control and treatment because *Leptospira* is an important agent in animal abortion especially bovine abortion may be due to infectious, toxic, endocrine, physical or nutritional causes. Abortions have a highly negative impact on reproductive efficiency, resulting in significant economic losses for the bovine industry [6].

Diagnosis of leptospirosis is usually based on the demonstration of serum antibodies with serological tests like the microscopic agglutination test (MAT) that this method is able to detect specific antibody produced against the infecting leptospiral organism [7]. The other method is enzyme-linked immunosorbent assay (ELISA) and immuno fluorescence assay (IFA), slide agglutination test (SAT) and in addition molecular methods such as PCR used for detection of *Leptospira* [8]. MAT is based on the use of live *Leptospira* cultures *and* this method may take up to eight weeks with weekly inspection and examination. Moreover, in others method many factors may cause false positive and negative results.

Corresponding Author: Abbas Doosti, Biotechnology Research Center, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran. Postal box: 166, Tel: +98-3813-361001.

Polymerase chain reaction (PCR) has been used to detect a large number of microorganisms, including those of clinical significance and some papers described its use for the diagnosis of leptospirosis. The purpose of present study was to determine the frequency of *Leptospira* infection in aborted bovine using PCR technique in Chaharmahal Va Bakhtiari province located in southwest of Iran.

MATERIALS AND METHODS

Sampling and DNA Extraction: In present study, 120 liquid rennet samples of bovine aborted fetuses were collected from industrial livestock in Chaharmahal Va Bakhtiari province (southwest of Iran). Genomic DNA was extracted from specimens using Qiagen DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendation. The extracted DNA was quantified by spectrophotometric measurement at a wavelength of 260 nm according to the method described by Sambrook and Russell [9]. The extracted DNA of each sample was kept frozen at -20°C until used.

Gene Amplification: Species-specific oligonucleotide primers Lp-F: 5'-GCGCGTCTTAAACATGCAAG-3' and Lp-R: 5'-CTTAACTGCTGCCTCCCGTAG-3' were design from *16S ribosomal RNA* gene of *Leptospira* (accession number: JF460977.1) were used for gene amplification. PCR amplification was carried out in a total volume of 25 µl in 0.5 ml tubes containing 1µg of genomic DNA, 1µM of each primers, 2mM $Mgcl_2$, 200µM dNTP, 2.5 µl of 10X PCR buffer and 1 unit of *Taq* DNA polymerase (Roche applied science, Germany). PCR involved an initial denaturation at 94°C for 5 min; followed by 30 cycles denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min; and a final extension at 72°C for 5 min was done at the end of the amplification. The PCR amplification products (10 µl) were subjected to electrophoresis in a 1% agarose gel in 1X TBE buffer at 80V for 30 min, stained with Ethidium Bromide and images were obtained in UVIdoc gel documentation systems (UK). The PCR products were identified by 100 bp DNA size marker (Fermentas, Germany).

RESULTS

In present study, 120 liquid rennet samples of bovine aborted fetuses were examined for presence of Leptospiral DNA. Analysis of PCR products for presence of *16S rRNA* gene of *Leptospira* on 1% agarose gel revealed a 306 bp fragment (Figure 1). The existence of Leptospiral DNA was detected in the positive control also the positive control showed a target sequence on agarose gel electrophoresis but no product observed for negative control.

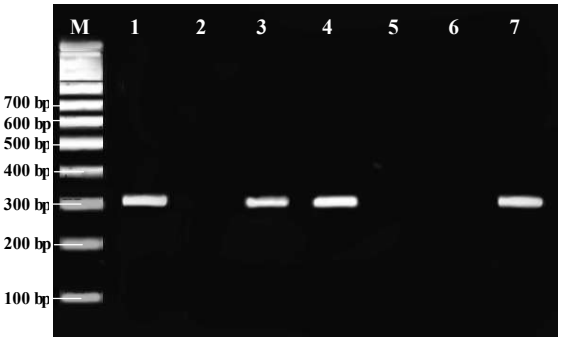

Fig. 1: Gel electrophoresis for identification of leptospiral infection in aborted bovine samples (Line M is 100 bp DNA ladder (Fermentas, Germany), line 1 is positive control, line 2 is negative control, lines 3, 4 and 7 are positive samples and lines 5 and 6 are negative samples).

The frequency of Leptospiral DNA in liquid rennet samples of aborted bovine was 17 (14.16%) of 120. The results showed a high frequency of *Leptospira* infection in aborted bovine in Chaharmahal Va Bakhtiari province. These findings indicated that examination of aborted bovine for control and prevention of leptospirosis in this region located in southwest Iran it seems to be necessary.

DISCUSSION

Some factors are influential on bovine abortion such as open days, age, previous abortion, seasonal effects and contamination with different organisms for example bacteria, viruses and protozoa that between them the bacterial cause have the most frequently. Different bacteria cause abortion such as *actinomyces pyogenes*, *Leptospira* spp., *streptococcus* spp., salmonella spp., etc [10].

Leptospirosis is an important zoonotic disease of bovine caused by parasitic spirochetes classified within the genus *Leptospira* [8]. Its distribution is worldwide and bovine can be infected with a number of serigroups of which have specific effects upon the genital system causing fetal death with abortion, stillbirth and wakly live calves [5]. Because of the wide diversity of clinical signs, diagnosis of leptospirosis is difficult and depends

upon a variety of laboratory assays such MAT, IHA and ELISA [4]. This methods are time consuming and difficult. In addition, detection of bovine abortion cause is very important and it is better to use the molecular methods such as PCR technique. Sensitivity of PCR is so high, that other methods could not compete with this method anymore. In current study the frequency of *Leptospira* infection determined in aborted bovine using PCR technique and *16S rRNA* gene of this microorganism were amplified by species-specific oligonucleotide primers. Leptospiral DNA was diagnosed in 17 abortions (%14.16) from 120 samples. The results of present study showed a high frequency of this microorganism in aborted bovine in southwest of Iran.

Some studies were performed about *Leptospira* infection and its correlation with bovine abortion. The rate of leptospiral bovine abortion in different studies is between 4 to 40% in the world wild. Some agents are effective on leptospiral infection for example seasonal effects, kind of rodents, the animals which are presence in the ranch such as cat or dog, also the type of ranch (industrial or non industrial). Naturally, leptospiral infection has a direct association with abortion in animals, especially in the chronic form of disease. Different studies were reported about this problem. In 1981, 265 bovine abortion tested by serological method for presence of *Leptospira* infection and 10 (3.9%) of 265 samples were positive for *Leptospira* spp [10]. Another study in 1984 showed that 8 (3.1%) of 265aborted bovine fetus had *Leptospira* [11]. In 1988 Prescott, *et al*. [12] in Ontario by immunofluorescence techniques understood that 34 (6.1%) of 553 aborted bovine fetuses had leptospires. 65% of these fetuses were from submissions made between November and January, 1988 [12]. The study of Anderson in 1990, showed that survey of causes of bovine abortion occurring in the California consideration and leptospirosis were relatively uncommon and leptospirosis was diagnosed in nine (1.9%) abortions from 468 samples by serological test, which occurred in all season [13]. Incidence of leptospiral abortion in Brazilian dairy cattle was studied in 1999, in total, 72 (60%) of 120 aborted fetuses had evidence of leptospiral infection [14].

As we said, the leptospires persistence in different region is related to high humidity, temperature and the seasonal effect. In the wet season, *Leptospira* survival was expected to be higher and in the dry season, *Leptospira* survival is likely to be poor. In the present study, the frequency of leptospiral infection in aborted bovine was 14% and samples were collected in the winter months, this fact suggests that the frequency of bovine abortion in each region, related to the climate conditions. The result of present study was showed the importance of leptospiral infection in bovine abortion in Chaharmahal Va Bakhtiari province and according to high frequency of abortion caused by *Leptospira* in this region, prevention and control of leptospirosis is so important. Prevention of leptospirosis may be achieved by avoidance of high-risk exposures and immunization. Leptospiral abortion can be controlled by vaccination and biosecurity to the point that no serious economic effect should occur in a dairy herd. Recent findings suggest that for maximum protection particularly in herds with *Leptospira* hardjo-bovis, it is necessary to use a vaccine that is specifically designed to provide protection against this *Leptospira*. In addition, rapid detection can help to effective and time treatment. Therefore, using PCR method is effective to increase the abortion rate in Chaharmahal Va Bakhtiari province.

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