PCR Assay for Detection of Abortion Rate Caused By Chlamydia Psittaci in Iranian Cattle

A. Arshi, A. Doosti and A. Sharifzadeh

Abstract—Chlamydial organisms are members of an exclusive order, Chlamydiales and family Chlamydiaceae. Chlamydia psittaci is a bacterium that causes psittacosis in humans and ornithosis, pneumonitis, abortion, encephalomyelitis, and enteritis in various animals. The purpose of this study was to determination of abortion rate caused by C. psittaci in Iranian cattle using PCR method. DNA was extracted from 145 aborted bovine fetus samples of cattle. Then, ompA region of C. psittaci genome was amplified by PCR using specific primers. The primers pair CTU and CTL used for amplification of a 1058 bp fragment of the C. psittaci ompA gene. Association between Chlamydia infection and abortion in cattle were examined by T test statistical analysis. P values <0.05 were considered significant. C. psittaci was found in 14 out of 145 samples (9.65%). Amplified fragments for ompA region on 1% agarose gel revealed a fragment of about 1058 bp. The results showed that the C. psittaci is one of the major causes of infectious abortion in cattle. C. psittaci has been screening from outbreaks of epizootic cattle abortion at different stages of pregnancy. Abortion occurred mostly in middle or late pregnancy. C. psittaci is associated with significant morbidity and mortality during pregnancy, and its rarity can delay early diagnosis and treatment. Isolation of C. psittaci from aborted bovine fetus specimens indicated a wide dissemination of this pathogen among cows in Iran. The control of this microorganism is useful for prevention and reduction of the incidence of abortion and reduce the economic loses in Iran.

Keywords—Chlamydia psittaci, abortion, PCR, Iranian cattle.

I. INTRODUCTION

ChlamydoPHILA psittaci (C. psittaci), an obligate, intracellular, gram-negative bacterium, has 7 known genotypes (A–F and E/B) [1]. All genotypes can be transmitted to humans and cause psittacosis or parrot fever [2]. Genotypes are distinguished by sequencing of the outer membrane protein A (ompA) gene or by a recently developed ompA genotype-specific real-time PCR [1, 3]. OmpA is one of the most polymorphic Chlamydial genes: it encodes the major outer membrane protein (MOMP) which is the main target of the host immune response against chlamydiae [4]. C. psittaci can infect 465 avian species in 30 avian orders, with at least 153 species in the order Psittaciformes [5]. Chlamydia was recognized in 1999 [6]. There are three major types of Chlamydia: Chlamydia psittaci, Chlamydia pneumoniae, and Chlamydia trachomatis. C. psittaci is a lethal intracellular bacterial species that causes avian chlamydiosis, epizootic outbreaks in mammals and respiratory psittacosis in humans [7]. C. psittaci affects many species of animals and birds and causes a variety of disease. In ruminants, the organism infection causes enteritis, pneumonia, conjunctivitis, polyarthritis, encephalitis and enzootic abortion, depending on factors such as the virulence of the organism, the physiological state of the host, and the environmental condition. Chlamydia resembles bacteria in the composition of the cell wall, in the possession of both RNA and DNA and in multiplication by binary fission [8]. Up to 60% of the animals in a particular herd may shed organisms for several years, in levels that vary from minimally detectable to 104-106 infectious units per gram of feces [9]. The epidemiological significance of this is undetermined. Chlamydiae isolated from fecal material are capable of producing pneumonia after intratracheal inoculation and abortion after parenteral infection [10, 11]. Epizootic bovine abortion occurs suddenly in a herd. There is no clinical evidence of disease prior to abortion, usually in the seventh to ninth month of gestation. Occasionally infection results in the delivery of dead calves at term or the birth of weak calves which die later. The placenta is commonly retained and milk production drops in dairy cows but overall there is little adverse effect on the dam. Seasonal occurrences observed by some authors appear to reflect breeding practices [12].

A number of techniques, developed in the last few decades have greatly contributed to the methodology used, with the most pronounced ones, such as PCR based methods that allowed the copying of even minute amount of the sequence of interest [13]. The PCR-based molecular techniques are quicker than microbiological susceptibility testing, and more importantly [14]. Since PCR technology and ELISA are now of general use in microbiology laboratories, it can be easily implemented.

Clinical cases of chlamydiosis in cattle are very few; they may be attributable mainly to stress given by change in breeding environment, transport and delivery [15]. The aim of this study was to determine the abortion rate caused by C. psittaci in Iranian cattle using molecular technique.
II. MATERIALS AND METHODS

A. Sample collection and DNA extraction

145 aborted bovine fetus samples were collected from four townships of Chaharmahal Va Bakhtiari province located in southwest Iran. In these cattle, 47, 41, 32 and 25 specimens were obtained from Shahrekord, Borujen, Farsan, and Kiar townships, respectively.

Whole abomasal contents were stored at -20°C until required for DNA extraction. DNA was extracted using Genomic DNA Extraction Kit (QIAGEN Ltd., Crawley, UK) to obtain high molecular weight DNA for the PCR interaction for ompA gene of C. psittaci. The extracted genomic DNA was quantified by spectrophotometric measurement at a wavelength of 260 nm according to the method described by Sambrook and Russell [16].

B. Gene amplification

The primers used for amplification of a 1058 bp fragment of the ompA gene were those described by Yang et al. (2007), with the following nucleotide sequence: C-Pesi-F: 5’-ATG AAA AAA CTC TTG AAA TCG G-3’ (forward); C-Pesi-R: 5’-CAA GAT TTT CTA GAC TTC ATT TTG TT-3’ (reverse)[17]. Amplification reactions were carried out in a final volume of 25 μl, containing 100 ng of DNA, 0.5 μM of each primer, 2.5 μl 10X PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTPs and 1 unit of Taq DNA polymerase. The following cycles were applied: initial denaturation step at 95°C for 5 min followed by 30 cycles: denaturation at 94°C for 1 min, primer annealing at 58°C for 1 min, PCR products synthesis at 72°C for 1 min and final synthesis step at 72°C for 5 min. PCR products were recognized by electrophoresis on 1% agarose gel (0.20 g agarose was dissolved in 25 ml TBE 1X buffer), stained with Ethidium Bromide and images were obtained in UVIdoc gel documentation systems (UK).

C. Statistical analysis

Analysis of data was performed using the SPSS version 17.0 computer software (SPSS, Chicago, IL). Also, association between Chlamydia infection and abortion in cattle were examined by T test statistical analysis. P values <0.05 were considered significant.

III. RESULTS

Genomic DNA was successfully extracted from aborted bovine fetus samples using the DNA extraction kit. The PCR products of the primer specific for ompA gene (CTU-F and CTL-R) revealed the 1058 bp DNA fragment. Positive and negative controls of known sequence were also run for each reaction. C. psittaci was isolated in 26 out of 145 cases of bovine abortion (17.93%). The positive control showed the excpected amplification product specific for C. psittaci (1058 bp). In Shahrekord, 9 aborted bovine fetuses were found positive out of 47 fetuses giving an apparent frequency rate of 19.15%. In Borujen, 7 fetuses out of 41 samples were found to have C. psittaci infection. The apparent prevalence rate of C. psittaci was 6 out of 32 in Farsan (18.75%), and only 4 fetuses were found out of 25 samples in Kiar Township (16%). These results were shown in Table 1. The results demonstrate the association between Chlamydia infection and abortion in Iranian cattle by using T test statistical analysis (P<0.05). These findings showed a wide occurrence of Chlamydia infections in Iranian cattle.

<table>
<thead>
<tr>
<th>Township</th>
<th>Number of samples</th>
<th>C. psittaci – negative, number (%)</th>
<th>C. psittaci – positive, number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shahrekord</td>
<td>47</td>
<td>38 (80.85%)</td>
<td>9 (19.15%)</td>
</tr>
<tr>
<td>Borujen</td>
<td>41</td>
<td>34 (82.93%)</td>
<td>7 (17.07%)</td>
</tr>
<tr>
<td>Farsan</td>
<td>32</td>
<td>26 (81.25%)</td>
<td>6 (18.75%)</td>
</tr>
<tr>
<td>Kiar</td>
<td>25</td>
<td>21 (84%)</td>
<td>4 (16%)</td>
</tr>
<tr>
<td>Total</td>
<td>145</td>
<td>119 (82.07%)</td>
<td>26 (17.93%)</td>
</tr>
</tbody>
</table>

IV. DISCUSSION

C. psittaci is a small bacterium (0.5 μm) which undergoes several transformations during its life cycle [18]. The addition of other Chlamydia spp. has been proposed recently [6]. Chlamydia infections in cattle have been described worldwide and cause disease syndromes such as pneumonia, enteritis, conjunctivitis, polyarthritis, encephalitis, mastitis, abortion and other urogenital tract infections as well as subclinical infections [19]. C. psittaci is identified by the formation of dispersed microcolonies, which do not stain with iodine and resist inhibition by sodium sulfadiazine. It can infect most domestic animals, many wild mammals and more than 100 species of wild and domestic birds are also susceptible [8]. C. psittaci was considered the cause for fetal death when Chlamydial isolation was associated with placentitis or inflammation of other fetal tissues and when other abortifacient agents were not detected [20]. C. psittaci may be a cause of human placentitis and subsequent abortion [21]. The intestinal tract is the natural habitat for Chlamydia and inapparent enteric infections are common in ruminants [15]. Ovine and bovine C. psittaci strains were divided into two distinct serotypes: type 1 isolated from abortion, pneumonia or enteric infection and type 2 associated with polyarthritis, encephalitis or conjunctivitis [22]. These two groups do not cross react with each other or with avian strains by a plaque reduction test. Within each type, isolates from sheep and cattle are antigenic ally alike [23]. Since the affected cows did not show any evidence of viral or bacterial infections such as infectious bovine rhinotracheitis virus and...
bovine respiratory syncytial virus infection, they were suspected of Chlamydia infection [24]. In present study PCR technique was used to detection of abortion rate in Iranian cattle caused by C. psittaci infection. The result was showed 26 out of 145 samples (17.93%) are positive for C. psittaci infection. The frequency of this microorganism was 19.15% in Shahrekord, 17.07% in Borujen, 18.75% in Farsan and 16% in Kiar Townships. Agarose gel electrophoresis of PCR amplification products were showed in figure 1.

In conclusion our results showed that C. psittaci is one of important factors in abortions of ruminants that unknown and vaccine has been applied to control abortions by this infection. Also, this study indicates that Chlamydia infection in the abomasal as an important factor for abortion in Iranian cattle.

ACKNOWLEDGMENT

The authors would like to thank all the staff of the Biotechnology Research Center of Islamic Azad University of Shahrekord Branch located in southwest Iran for their sincere support.

REFERENCES