

## Molecular Study for Detection of *Chlamydia psittaci* caused Abortion in Iranian Cattle

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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 determine the abortion rate caused by *C. psittaci* in Iranian cattle using PCR method. 145 aborted bovine fetus samples from different townships in Chaharmahal Va Bakhtiari province were collected and DNA was extracted. Then, *ompA* region was amplified by PCR using specific primers. *C. psittaci* was found in 26 out of 145 samples (17.93%). The frequency of *C. psittaci* infection was 19.15%, 17.07%, 18.75% and 16% in Shahrekord, Borujen, Farsan and Kiar townships in Chaharmahal Va Bakhtiari province, respectively. Amplified fragments for *ompA* region on 1% agarose gel revealed a fragment of about 1058 bp. The results showed that the *C. psittaci* is also 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.

**Key words:** *Chlamydia psittaci*, abortion, PCR, Iranian cattle.

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*Chlamydia psittaci* (*C. psittaci*), an obligate, intracellular, gram-negative bacterium, has 7 known genotypes (A–F and E/B)<sup>1</sup>. All genotypes can be transmitted to humans and cause psittacosis or parrot fever<sup>2</sup>. Genotypes are distinguished by sequencing of the outer membrane protein A (*ompA*) gene or by a recently developed *ompA* genotype-specific real-time PCR<sup>1,3</sup>. *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<sup>4</sup>. *C. psittaci* can infect 465 avian species in 30 avian orders, with at least 153 species in the order Psittaciformes<sup>5</sup>. Chlamydia was recognized in 1999<sup>6</sup>. 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<sup>7</sup>. *C. psittaci* affects many species of animals and birds and causes a variety of disease. In ruminants, the organism infection

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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<sup>8</sup>. Up to 60% of the animals in a particular herd may shed organisms for several years, in levels that vary from minimally detectable to  $10^4$ - $10^6$  infectious units per gram of feces<sup>9</sup>. 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<sup>10,11</sup>. 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<sup>12</sup>.

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<sup>13</sup>. The PCR-based molecular techniques are quicker than microbiological susceptibility testing, and more importantly<sup>14</sup>. 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<sup>15</sup>. The aim of this study was to determine the abortion rate caused by *C. psittaci* in Iranian cattle using molecular technique.

## MATERIALS AND METHODS

### Samples 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  $-20^{\circ}\text{C}$  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<sup>16</sup>.

### 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'-ATGAAAAA CTC TTG AAA TCG G-3' (forward); C-Pesi-R: 5'-CAA GAT TTT CTA GAC TTC ATT TTG TT-3' (reverse)<sup>17</sup>. Amplification reactions were carried out in a final volume of 25  $\mu\text{l}$ , containing 100 ng of DNA, 0.5  $\mu\text{M}$  of each primer, 2.5  $\mu\text{l}$  10X PCR buffer, 1.5 mM  $\text{MgCl}_2$ , 0.2 mM dNTPs and 1 unit of *Taq* DNA polymerase. The following cycles were applied: initial denaturation step at  $95^{\circ}\text{C}$  for 5 min followed by 30 cycles: denaturation at  $94^{\circ}\text{C}$  for 1 min, primer annealing at  $58^{\circ}\text{C}$  for 1 min, PCR products synthesis at  $72^{\circ}\text{C}$  for 1 min and final synthesis step at  $72^{\circ}\text{C}$  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).

### Statistical analysis

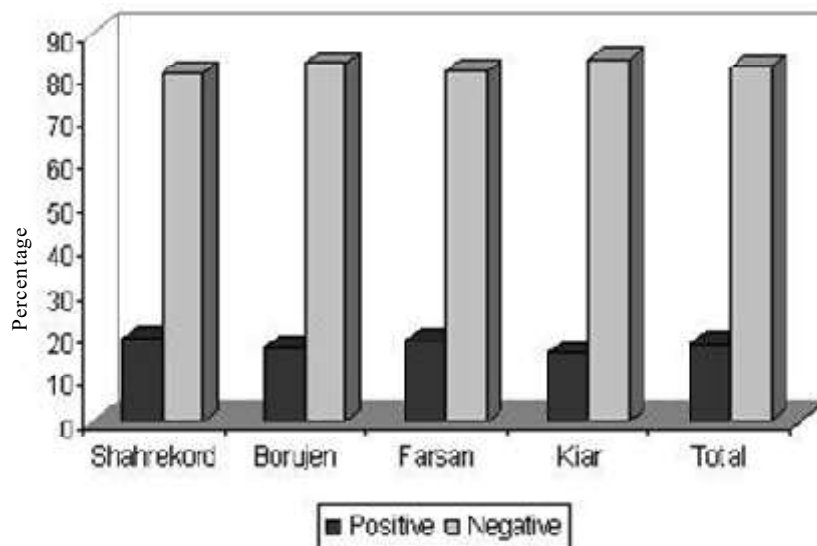
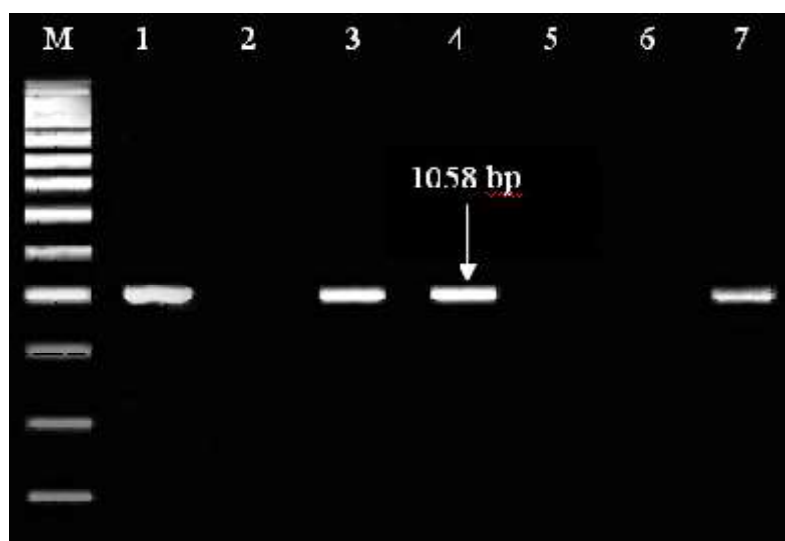
Analysis of data was performed using the SPSS version 17.0 computer software (SPSS, Chicago, IL). Also, association between *Chlamydia* infection and abortion in cattle were examined by T test statistical analysis. P values  $<0.05$  were considered significant.

## 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

**Table 1.** Frequency of *C. psittaci* at aborted bovine fetus in Chaharmahal Va Bakhtiari province located in southwest Iran

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

**Fig. 1.** Furthermore the information of *C. psittaci* percentage in different township**Fig. 2.**

run for each reaction. *C. psittaci* was isolated in 26 out of 145 cases of bovine abortion (17.93%). The positive control showed the expected 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. Agarose gel electrophoresis of PCR amplification products were showed in Fig. 2.

## DISCUSSION

*C. psittaci* is a small bacterium (0.5  $\mu\text{m}$ ) which undergoes several transformations during its life cycle<sup>18</sup>. The addition of other Chlamydia spp. has been proposed recently<sup>6</sup>. Chlamydial 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<sup>19</sup>. *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<sup>8</sup>. *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<sup>20</sup>. *C. psittaci* may be a cause of human placentitis and subsequent abortion<sup>21</sup>. The intestinal tract is the natural habitat for Chlamydia and inapparent enteric infections are common in ruminants<sup>15</sup>. 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<sup>22</sup>. 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<sup>23</sup>. 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<sup>24</sup>. 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.

Recently enzootic or sporadic abortion has become a most important disease in Europe and North America<sup>25</sup>. Chlamydial abortions have been diagnosed in cattle in California, Colorado, Idaho, Montana, Texas, Utah, and Wyoming<sup>26</sup>. In a study in Egypt, 22% of total domestic ruminant sera (3 out of 49 sheep (6.1%), 84 out of 352 cattle (23.9%), 6 out of 54 camels (11.1%), 17 out of 40 buffaloes (42.5%) showed antibodies against *C. psittaci*<sup>27</sup>. Margaret et al. in 1990 showed that the abortion rate is usually about 5%, but may be much higher in flocks exposed for the first time<sup>19</sup>. In another study in Italia, out of 671 aborted-cow sera, 290 were positive for *C. psittaci* antibody and 139 out of 600 control cows were seropositive<sup>28</sup>. Their used molecular technique for detection of *C. psittaci* infection and it was same to the method of present study and confirmed the results of our study in aborted bovine.

Travnicek et al. in 2001 reported that *C. psittaci* were detected in 272 animals, i.e. 6.37%. 6.37% sheep's was positive. Positive reaction in higher dilution of sera was recorded in 22 cases and in 9 cases (6.37%). From the total number of 837 examined sera from goats, 33 samples (3.94%) were positive<sup>29</sup>. The results of their study confirmed the findings of current research. Considering the difficulty in demonstrating *C. psittaci* infection in fetal tissue samples, the involvement of the pathogen in inducing abortion outbreaks in cattle might be underestimated<sup>28</sup>.

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.

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