Research Article

Molecular Detection of *Chlamydophila Abortus* In Aborted Fetal Tissues by Using Polymerase Chain Reaction (PCR) In Tabriz, Northwest of Iran

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Abstract

Enzootic abortion of ewes (EAE) induced by *Chlamydophila abortus* (formerly Chlamydia psittaci serotype1) is a major cause of reproductive failures in most sheep producing countries. In this study, the abortion prevalence of *Chlamydophila abortus* (*C. abortus*) in sheep abortion cases are evaluated by polymerase chain reaction (PCR) and objectives are considered Chlamydiosis incidence of abortion in sheep in the northwest region of Iran. For this purpose, the contents of fetal tissue from 50 aborted fetuses were homogenized and DNA extracted from them and then PCR is done using specific primers for Chlamydophila (CHOMP191, CHOMP371). The study showed that about 26% of the total sample is contained Chlamydophila. This study confirms that *C. abortus* as an important cause of ovine abortion in the northwest of Iran and showed the PCR in tissue pools of aborted fetuses is a useful method for rapid detection of *Chlamydophila abortus* Ovis infections.

***Keywords*:** *Chlamydophila abortus*, Molecular detection, Polymerase Chain Reaction (PCR)

Introduction

Ovine enzootic abortion (OEA) induced by *Chlamydophila abortus* (*C. abortus*) (formerly Chlamydia psittaci serotype 1) is a major cause of reproductive failures in most sheep producing countries (Aitken et al., 1993). In an epidemic of OEA, up to 30% of ewes abort in the last three weeks of gestation or give premature birth to weak or dead lambs. After the abortion, the ewes develop a protective immunity and, in endemically infected flocks, 5-10% of the ewes abort annually (Rodlakis et al., 1998; Aitken, 2000).

Despite the economically important losses due to late-term abortions and weak lambs, the prevalence of *C. abortus* is not well known in most Asian countries, including Iran. Diagnosis of OEA, by examination of fetal tissues following postmortem examination, can be achieved by a variety of methods.

All of these methods are based on direct antigen detection and include modified Ziehl-Neelsen (MZN) staining of placental smears and direct fluorescent-antibody staining of Chlamydiae in frozen cryostat sections of the placenta or fetal tissues. Several enzyme-linked immunosorbent assays (ELISAs) have been proposed for testing for *C. abortus* antibodies in sheep (Anderson et al., 1995; Longbottom et al., 2002).

However, the majority of sheep have preexisting background levels of antibody acquired from natural widespread infection by strains of C. pecorum. PCR appears to be an ideal alternate means of Chlamydiaceae detection because it offers advantages in sensitivity and reduced processing time over the conventional serological techniques. Furthermore, PCR does not rely on the presence and maintenance of viable organisms and poses less risk to laboratory staff than culture through eggs or cells. There have been many reports of PCR for the detection of Chlamydophila nucleic acids in specific animals or from particular tissues (Hewinson et al., 1997; Laroucau et al., 2001; Messmer et al., 1997; Bomhardvon et al., 2003), but at the time of the manuscript developing there are limited publications on the impact of a pool of samples in preparation for molecular detection of *Chlamydophila abortus* in aborted fetuses of ewes.

The aim of the present study was to determine the possible role of *Chlamydophila abortus* ovis as an etiological agent of ovine abortion by PCR in a pool of samples of varying tissues of aborted fetuses.

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Materials and Methods

Sampling Methods

During breeding season of 2011-2012, 50 abomasal aborted ovine fetus samples were collected from 10 flocks in Tabriz, northwest of Iran. Placentas were collected, macroscopically assessed for EAE lesions and representative cotyledons removed for molecular analysis. Following macroscopic examination, brain, skeletal muscle, liver, spleen, and abomasum were removed from each fetus. However, the condition of some of the fetuses was such that not all tissues could be collected. Fetal tissues and placental samples were then washed with PBS containing 1000 units/ml of penicillin (Pharmacia-Upjohn) and 1000 units/ml of streptomycin sulfate (Bristol-Myers Squibb) and then different tissues from each fetus were mixed in equal proportion and pulverized under liquid nitrogen. Approximately 200 mg of the sample powder stored as pool samples at −20°C until required for DNA extraction.

DNA Extraction of Tissues

Total DNA was extracted from approximately 200 mg powdered pool tissues using a commercial kit (Aquaprep DNA Tissue kit Bioneer, S. Korea) according to manufacturer's instructions. DNA concentration was measured at 260 and 280 nm (Biophotometer plus, Eppendorf, Germany). Electrophoresis of each DNA sample on 0.5% agarose gel in 1X Tris/Borate/EDTA (TBA) buffer was done to check the integrity of the DNA. A 60 µl aliquot of total DNA was produced from each sample and stored at -20 ºC until required for PCR analysis.

Polymerase Chain Reaction

DNA extract (5ul) was used to amplify a 630-bp fragment of ompA gene of Chlamydophila, using primers CHOMP191 (5′-GCI YTI TGG GAR TGY GGI TGY GCI AC-3′) and CHOMP371 (5′-TTA GAA ICK GAA TTG IGC RTT IAY GTG IGC IGC-3′), as described by Kaltenbock et al. (1997) and modified by Sachse and Hotzel (2005). One µl of the amplicon was used as template for the second-round amplification, using the primer set CHOMP218 (sense) (5′-GTA ATT TCI AGC CCA GCA CAA TTY GTG-3′)/ CHOMP336 (antisense) (5′-CCR CAA GMT TTT CTR GAY TTC AWY TTG TTR AT-3′), that yields a 400 bp product for *C. abortus*. 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 95ºC for 5 minutes followed by 35 cycles: denaturation at 94ºC for 30 seconds, primer annealing at 50ºC for 30 seconds, PCR products synthesis at 72ºC for 30 seconds and final synthesis step at 72ºC for 5 minutes. PCR products were recognized by electrophoresis on 1.5% agarose gel, stained with ethidium bromide and images were obtained in UVIdoc gel documentation systems (UK). Positive controls (DNA from references strains; ATCC 25116 and ATCC 2215), samples controls (DNA from abomasal fluids and lungs without etiological diagnostic), and none template control (distilled water) were included in each PCR run. PCR products with the molecular size of 440bp were considered indicative for identification as *C. abortus.*

Results

Genomic DNA was successfully extracted from aborted ovine fetus tissues using the DNA extraction kit. The PCR products of the primers specific for ompA gene (CTU-F and CTL-R) revealed the 630bp DNA fragment. Chlamydophila was isolated in 13 out of 50 cases of ovine abortion (26%). The positive control showed the excepted amplification product specific for Chlamydophila (630 bp) and *C. abortus* (440bp). PCR products obtained from these samples and *C. abortus* reference strains with the molecular length of 440bp (according to the new taxonomy: Chlamydia and Chlamydophila), are shown in Fig 1.

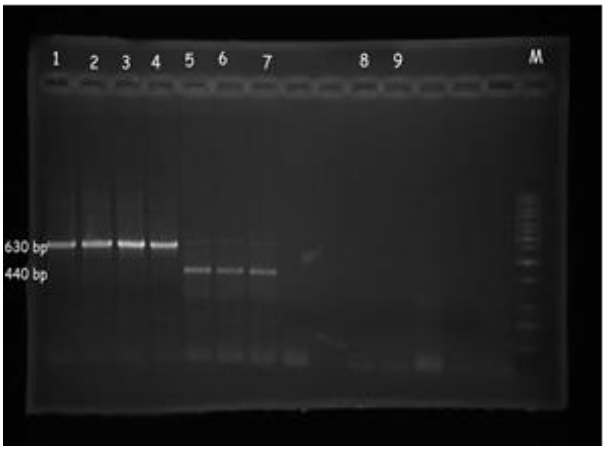

Figure 1. Ethidium bromide-stained 1.5% agarose gel electrophoresis of PCR products amplified using the mentioned primer. M, 100bp DNA ladder; lane 1, positive control sample of Chlamydophila; lane 2-4, Chlamydophila positive sample; lane 5, positive control sample of *C. abortus* ovis; lane 6-7, *C. abortus* ovis positive sample; lane 8, NTC (negative template control); lane 9, negative control (distilled water).

Discussion

OEA is one of the most important diseases affecting sheep flocks and because of abortion, weak lambs and a decrease in milk production can have negative economic effects. In previously reported serosurveys, the less-sensitive and -specific CFT had been used. Positive CFT titers can be caused by *C. abortus* as well as by C. pecorum.

According to the World Organisation for Animal Health (OIE) (2004), the most commonly used method for serodiagnosis of animal chlamydiosis the complement fixation test (CFT). However, the technique is laborious, has limited sensitivity and often impaired by cross-reactions between chlamydial species (McCauley et al., 2007). Recently developed serodiagnostic tests are mainly based on two main cross-reactive antigens present in all chlamydial species, lipopolysaccharide and the major outer membrane protein (MOMP) and thus, are not species-specific for diagnosing animals infected by OEA.

Other more specific tests need to be developed and evaluated in a European context, such as those based on specific monoclonal antibodies (Salti-Montesanto et al., 1997) and recombinant protein fragments (Sheehy et al., 1996). According to antigen detection, cultivation in cell culture is still regarded as the standard. While this time-consuming method is only applicable to cultivable strains, many strains are difficult to grow and not all laboratories have the facilities and expertise to culture. The possibilities for diagnostic detection of chlamydiae have considerably improved following the introduction of DNA-based methods, particularly the PCR, which permits direct identification from clinical specimens and differentiation of species. A number of tests have been published in the literature (Anderson et al., 1996; Kaltenbock et al., 1997; Messmer et al., 1997; Longbottom et al., 2001), but none of them has been validated in veterinary laboratories so far. One of the main difficulties in evaluating new tests for detection of *C. abortus* is a lack of a completely reliable method for comparative purposes.

Isolation, which was at one time used as the standard procedure has proved to have insufficient sensitivity (Wood et al., 1992; Domeika et al., 1994). Contamination of test samples with other bacteria, inadequate transport conditions, autolysis, antibiotic treatment, and other toxic factors may all interfere with the success of isolation (Sanderson et al., 1989). A further obstacle to isolation is the possible presence of chlamydiae in samples in the form of latent infection (the so-called “cryptic” form), which is well known in infections of the human uterine tube and joints (Sachse et al., 2005).

Methods based on nucleic acid and antigen detection are recommended for diagnostic uses. Some authors have found the former (Thiele et al., 1992) and others the latter (Domeika et al., 1994; Trevejo et al., 1999), to be more sensitive in the detection of Chlamydophila. In research by Lenzko et al. (2011), PCR and DNA microarray testing revealed the presence of chlamydiae in 78% of studied flocks. In sheep and goats, when *C. abortus* is present in the fetal membranes in large numbers, the two methods probably have almost identical sensitivity. In conclusion, our results showed that *C. abortus* is one of the important factors in abortions of sheep that are unknown and the vaccine has been applied to the control of abortions by this infection. Also, this study indicates that Chlamydia infection in the abomasal is an important factor for abortion in Iranian ovine.

References

1. Aitken I. D. (2000) Chlamydial abortion, Disease of Sheep. 3rd ed. Blackwell Science, Oxford.
2. Aitken I. D., Woldehiwet Z. and Ristic M. (1993) Ovine chlamydial abortion. In Rickettsial and Chlamydial Diseases of Domestic Animals. Pergamon Press, Oxford. 349-360.
3. Anderson I. E., Baxter S. I. F., Dunbar S., Rae A. G., Philips H. L., Clarkson M. J. and Herring A. J. (1996) Analyses of the genomes of chlamydial isolates from ruminants and pigs support the adoption of the new species Chlamydia pecorum. International Journal of Systematic Bacteriology 46: 245-251.
4. Anderson I. E., Herring A. J., Jones G. E., Low J. C. and Greig A. (1995) Development and evaluation of an indirect ELISA to detect antibodies to abortion strains of Chlamydia psittaci in sheep sera. Veterinary Microbiology 43: 1–12.
5. Bomhardvon W., Polkinghorne A. L. Z. H., Vaughan L., Vögtlin A., Zimmermann D. R., Spiess B. and Pospischil A. (2003) Detection of novel chlamydiae in cats with ocular disease. American Journal of Veterinary Research 64: 1421-1428.
6. Domeika M., Ganusauskas A., Bassiri M., Froman G. and Mardh P. A. (1994) Comparison of polymerase chain reaction, direct immunofluorescence, cell culture and enzyme immunoassay for the detection of Chlamydia psittaci in bull semen. Veterinary Microbiology 42: 273-280.
7. Hewinson R. G. P., Griffiths C., Bevan B. J., Kirwan S. E. Field M. E., Woodward M. J. and Dawson M. (1997) Detection of Chlamydia psittaci DNA in avian clinical samples by

polymerase chain reaction. Veterinary Microbiology 54: 155-66.
8. Kaltenbock B., Heard D., De Graves F. J. and Schmeer N. (1997) Use of synthetic antigens improves detection by enzyme-linked immunosorbent assay of antibodies against abortigenic Chlamydia psittaci in ruminants. Journal of Clinical Microbiology 35: 2293-2298.
9. Kunimoto D. and Burnham R. C. (1985) Human immune response and Chlamydia trachomatis infection. Reviews of Infectious Diseases 7: 665–673.
10. Laroucau K., Souriau A. and Rodolakis A. (2001) Improved sensitivity of PCR for Chlamydophila using pmp genes. Veterinary Microbiology 82: 155-164.
11. Lenzko H., Moog U., Klaus H., Lederbach R., Diller R., Menge C., Sachse K. and Sprague L. D. (2011) High frequency of chlamydial co-infections in clinically healthy sheep flocks. BMC Veterinary Research 7: 29.
12. Livingstone M., Entrican G. and Wattegedera S. (2005) Antibody responses to recombinant protein fragments of the major outer membrane protein and polymorphic outer membrane protein POMP90 in Chlamydia abortus-infected pregnant sheep. Clinical and Diagnostic Laboratory Immunology 12: 770–777.
13. Longbottom D., Fairley S. and Chapman S. (2002) Serological diagnosis of ovine enzootic abortion by enzyme-linked immunosorbent assay with a recombinant protein fragment of the polymorphic outer membrane protein POMP90 of *Chlamydophila abortus*. Journal of Clinical Microbiology 40: 4235–4243.
14. Longbottom D., Psarrou E., Livingstone M. and Vretou E. (2001) Diagnosis of ovine enzootic abortion using an indirect ELISA (roMP91B iELISA) based on a recombinant protein fragment of the polymorphic outer membrane protein POMP91B of *Chlamydophila abortus*. FEMS Microbiology Letters 195: 157-161.
15. Messmer T. O., Skelton S. K., Moroney J. F., Daugharty H. and Fields B. S. (1997) Application of a nested, multiplex PCR to psittacosis outbreaks. Journal of Clinical Microbiology 35: 2043-2046.
16. McCauley L. M. E., Lancaster M. J. Young P., Butler K. L. and Ainsworth C. G. V. (2007) Comparison of ELISA and CFT assays for *Chlamydophila abortus* antibodies in ovine sera. Australian Veterinary Journal 85: 325-328.
17. Rodlakis A., Salinas J. and Papp J. (1998) Recent advances on ovine chlamydial abortion. Veterinary Research 29: 275-288.
18. Sachse K., Hotzel H., Slickers P., Ellinger T. and Ehricht R. (2005). DNA microarray-based detection and identification of Chlamydia and Chlamydophila spp. Molecular and Cellular Probes 19: 41–50.
19. Salti-Montesanto V., Tsoli E. and Papavassiliou P. (1997) Diagnosis of ovine enzootic abortion, using a competitive ELISA based on monoclonal antibodies against variable segments 1 and 2 of the major outer membrane protein of Chlamydia psittaci serotype 1. American Journal of Veterinary Research 58: 228–235.
20. Sanderson T. P. and Andersen A. A. (1989) Evaluation of an enzyme immunoassay for detection of Chlamydia psittaci in vaginal secretions, placentas, and fetal tissues from aborting ewes. Journal of Veterinary Diagnostic Investigation 1: 309-315.
21. Sheehy N., Markey B., Gleeson M. and Quinn P. J. (1996) Differentiation of Chlamydia psittaci and C. pecorum strains by species-specific PCR. Journal of Clinical Microbiology 34: 3175-3179.
22. Stamp J. T., McEwen A. D., Watt J. A. A. and Nisbet D. I. (1950) Enzootic abortion in ewes. 1. Transmission of the disease. Veterinary Record 62: 251–254.
23. Thiele D., Wittenbrink M. M., Fisher D. and Krauss H. (1992) Evaluation of the polymerase chain reaction (PCR) for detection of Chlamydia psittaci in abortion material from ewes. Zbl. Bakteriol. International Journal of Medical Microbiology 277: 446-453.
24. Trevejo R. T., Chome B. B. and Kass P. H. (1999) Evaluation of the polymerase chain reaction in comparison with other diagnostic methods for the detection of Chlamydia psittaci. Journal of Veterinary Diagnostic Investigation 11: 491-496.
25. Wood M. M. and Timms P. (1992) Comparison of nine antigen detection kits for diagnosis of urogenital infections due to Chlamydia psittaci in koalas. Journal of Clinical Microbiology 30: 3200-3205.