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Survey on Carrier State of Sheep in *Chlamydia pecorum* Infection

Research Article

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ABSTRACT

Over the last 40 years, evidence has accumulated to suggest the ubiquitous presence of infections with intracellular bacteria of the genus *Chlamydia* in different livestock species. Different methods to clinical specimens substantiated such widespread, but mostly clinically unapparent, presumably low-level infections. In this initial epidemiological study, we addressed the question of chlamydial infection of conjunctiva and genital tract in apparently healthy sheep. In this research, 33 sheep and 20 goats which had previously been exposed to the possibility of sexual transmission of *Chlamydia*, examined by conjunctiva (53 swabs) and vaginal swab (53 swabs). After DNA extraction by boiling method, presence of *Chlamydia pecorum* was investigated by nested PCR. *Chlamydia pecorum* strain W73 was used as positive control. In this research, 10 infected swab samples (from 106 swab samples) contain of 7 vaginal (70%) and 3 conjunctival swabs (30%) were detected. All positive vaginal swabs and 3 positive conjunctival swabs were related to aborted and adult animals, respectively. According to higher percentage of *Chlamydia pecorum* infection in apparently healthy sheep, carrier state in sheep is more probable than goats. Vaginal secretion is more important route of chlamydial infection dissemination towards conjunctival secretion. Because of high risk of chlamydial infection in cows, attention to role of sheep in disease epidemiology was recommended.

KEY WORDS *Chlamydia pecorum*, polymerase chain reaction, sheep.

INTRODUCTION

Chlamydia pecorum is the obligate intracellular bacterial pathogen which is associated with several diseases in sheep such as polyarthritis and keratoconjunctivitis around the world (Polkinghorne *et al*. 2009; Yousef Mohamad and Rodolakis, 2010). Although several cases of ovine abortion associated with this pathogen has also been reported, parasites and nutrition as co-factors could also be important in chlamydial infected small ruminant's abortions. Despite this, *C. pecorum* was demonstrated in the intestine of normal 3 months lamb when starting to graze (Rekiki *et al*. 2004) and subsequently excreted in the feces (Clarkson and Philips, 1997). Chlamydial polyarthritis is not only can be appear typically by lameness in four to eight months of age lambs, but also can be unapparent and demonstrated by lose weight and retardation growth (Watt, 2011). Concurrent with chlamydial polyarthritis, conjunctivitis in range from mild to severe is commonly observed (Walker, 2013; Watt, 2011). Study of *C. pecorum* strains in livestock with or without symptoms was done by molecular methods (Jelocnik *et al*. 2013; Kaltenboeck *et al*. 2009; Yousef Mohamad *et al*. 2008). By compared of isolated *C. pecorum* from infected Koala with and without symptoms (Kollipara *et al*. 2013; Marsh *et al*. 2011), genetically diverse of this pathogen have been reported.

It is doubtful that, the certain *C. pecorum* strains may be involved in prevalence of disease (Sait *et al*. 2014; Yousef Mohamad *et al*. 2008). Interspecies diversity in *Chlamydia* has been reported in several studies, for example, Sheehy *et al*. (1996) identified that encephalomyelitis, polyarthritis, and enteric *C. pecorum* strains has greater than 99% identity. This percentage for *C. psittaci* abortion strains with the avian strains 6BC, A2 2/M, and P94/1 greater than 95% was estimated. Different methods based on characteristics growth, inclusion morphology (Cox *et al*. 1988), and monoclonal antibodies (Andersen, 1991) are documented for this aim.

Hence, several studies focused on typing of isolated *C. pecorum* from ovine conjunctival, vaginal and rectal swabs which was genetically distinct *C. pecorum* STs. In addition, different factors such as: constant re-exposure to the pathogen, farm management and environmental factors should be noted (Entrican *et al*. 2012; Lenzko *et al*. 2011; Longbottom *et al*. 2013) and the role of adaptive and innate immune responses, which is particularly important in susceptible lambs, shouldn't be forgotten (Balamurugan *et al*. 2012; Buchanan *et al*. 2013).

Chlamydiae are very wide spread in many host organisms, but not nearly all carriers develop symptoms of disease. Recognition of carrier animals in each area is one of the most important steps to identifying chlamydial infections epidemiology, and after it, comparison of pathogenic and nonpathogenic isolated strains genetically.

Polymerase chain reaction (PCR) is the most modern practical technology in diagnosing infectious diseases and compared with classical techniques, it has been shown to be more rapid, with results obtained in a few hours, and also more reliable. Moreover, PCR allows a faster bacterial identification directly from clinical samples. Genotyping, which is based on a more stable marker, DNA, is not dependent on gene expression. Another advantage of genotyping methods is that the discriminatory power of DNA-based methods is generally superior to that of phenotypic methods. The ability to distinguish between genomes is important to several disciplines of microbiological research, for example in studies on population genetics and microbial epidemiology (Zandi *et al*. 2014). Of great importance when choosing a method for genotyping are the typing ability, reproducibility, discriminatory power and also the ease and cost of performing the analysis. With PCR, selected segments of any DNA molecule can be amplified exponentially. Goat products have a favorable image in the world; thus, goat farming is practiced worldwide. The goat population has increased globally despite major changes in the agriculture due to industrial mergers, globalization, and technological advances in developed countries. The goat production is one of the key elements contributing to the economy of farmers living in the arid and semi-arid regions including most areas of Iran (Askari *et al*. 2011). There are also more than 50 million heads of sheep in Iran, of 27 breeds and ecotypes (Zamani *et al*. 2015), some from which breed in Khuzestan province. Because of importance of these animals, several studies regarding to *Chlamydia aborus* infection in sheep and goat by serology (Ghorbanpoor *et al*. 2007), and *Chlamydia psittaci* infection in pigeons (Ghorbanpoor *et al*. 2015) by PCR is documented. Because of suspected *Chlamydia pecorum* infection symptoms observation in sheep and goats, and no report about this in Iran, the primary aim of this research is the study on presence of *C. pecorum* in apparently healthy sheep and goats, through vaginal and conjunctival swab exam in some regions of Khuzestan.

MATERIALS AND METHODS

In the present study, *Chlamydia pecorum* infection of 33 sheep and 20 goats from Baghmalek and Shushtar cities was evaluated by vaginal and conjunctival swab. One vaginal and one conjunctival swab was collected with at least environmental infections from each animal and transferred to the microbiology lab in sterile 1.5 mL micro tubes and stored at -20 ˚C. Sterile cotton swabs with wooden handle were used for sampling.

Boiling method was used for DNA extraction. For this aim, vaginal and conjunctival swabs material were homogenized in 0.5 ml TE buffer (10 m*M* Tris-Hcl, 1 m*M* ethylenediaminetetraacetic acid (EDTA), pH 8) supplemented with 2% w/w dithiothreithol and boiled for 10 minutes. After centrifugation with 13000 rpm for 2 minutes, the supernatant was collected as DNA stock in new sterile micro tube (Hewinson *et al*. 1991). The extracted DNA of each sample was kept frozen at -20 ˚C until PCR assay.

In this research, Nested PCR assay was used for detection of *momp* gene in samples extracted DNA. Designed primers by Kaltenbock *et al*. (1997), were used for this aim (Table 1). Two fragments 576-597 bp and 426-441 bp of *momp* gene were amplified at the first and second steps of PCR, respectively.

Final volume of each reaction was 25 µL and was containing: 12.5 µL 2x master mix (SinaClon, Iran), 1 µL (10 pmol/µL) of each primer, 3 µL of extracted DNA (step one) and PCR product (step two) and 7.5 µL DEPC treated sterile water.

Assigned temperature program to the thermal cycler device (Eppendorph, Germany) in two steps, was according to Kaltenbock and colleagues study (Kaltenbock *et al*. 1997). PCR results became visible with run of 8 µl of each reaction in stained 1.5% agarose gel (KBC, Spain) with safe stain (SinaGen, Iran).

Table 1 Primer sequence and the aim pieces of *momp* gene in nested PCR

Gene	Sequences	Segment (bp)	Ref.
C. pecorum (*momp*)	F:5-GCICTITGGGAATGCGGITGCGCIAC-3 R:5-TTAGAAICGGAATTGIGCATTIACGTGIGCICG-3 F: 5-CCAATACGCACAATCGAAACCTCGC-3 R:5-CCACAAAATTTTCTAGACTTCAACTTGTTAAT-3	576-597 426-441	Kaltenbock *et al.* (1997)

Chlamydia pecorum W73 strain and DEPC treated sterile water, were used as positive and negative control in PCR assay, respectively.

RESULTS AND DISCUSSION

By genomic analysis and electrophoresis of PCR products, from 106 swab samples of 53 apparently healthy animals (33 sheep and 20 goats), 426-441 bp segment was detected in 10 swab samples (9.44%) preparation of 10 animals (18.87%) (Figure 1).

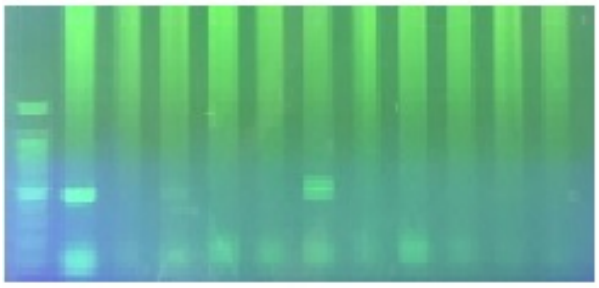

Figure 1 Results of swab samples nested PCR: left to right: 100 bp ladder; positive control (426-441 bp); negative control; positive sample; 2 negative samples; positive sample and 5 negative samples

Ten swabs were included of 7 vaginal and 3 conjunctival swabs. Nine of 10 (90%) and 1 of 10 (10%) detected infection animals were sheep and goat, respectively. Base on results, chlamydial infection rate of goats (1/20) was lesser than sheep (9/33), and none of the positive animals have an infection in vagina and conjunctiva, simultaneously.

In review of studied animal's history, 2 positive samples (20%) were from non pregnant sheep at the time of sampling (adult), 2 samples were from sheep that had not delivered until now (20%), and 6 samples were collected from aborted sheep and goats (60%). It is noteworthy that, all the positive vaginal swabs (7 of 10 positive swabs) were collected from aborted or adult animals, while 3 positive conjunctival swabs were related to animals without delivery and adult, respectively (Table 2).

During forty years ago, the presence of intracellular bacterial agents as *Chlamydia* and *Chlamydia* has reported in different species of livestock. From 1980 until now, by using the advanced diagnostic techniques, no changes were made in our knowledge related to pathogenetic and prevalence of these microorganisms.

Chlamydia pecorum because of create a long-lasting and unclear infection in vagina and intestine of ruminants, the extinction of some animal species as koalas because of infertility and abortion, and possibility distinguish of virulence in unclear and clear infection agents by differences review in some genes CTR (coding tandem repeats) of two type strains which is important in biology and their pathogenesis, Should be considered (Yusef Mohamad and Rodolakis, 2010).

Table 2 Positive result of study base on host and sample type

Sample	No.	Host	
		Goat	Sheep
Vagina	7	1	6
Conjunctiva	3	0	3
Total	10	1	9

Recently, several reports of this ubiquitous intracellular bacterial species presentation and its negative impact on growth performance and fertility in the cattle has been published (Kaltenboeck *et al.* 2005). DeGraves and colleagues in 2003, to investigate the prevalence of low-level *C. pecorum* and *C. psittaci* infections in virgin heifers using vaginal cytobrush. Fifty three percent infection was reported by them and possibility of extra genital transmission and production of anti-*Chlamydia* antibodies by stimulation of immune system during chlamydial infections is suspected (DeGraves *et al.* 2003).

During the study of *C. pecorum* infected calves within the first two months by Jee and colleagues, 51% infection was reported (Jee *et al.* 2004) but *C. pecorum* infection rate in healthy bulls preputial washing samples and semen, was low (Kauffold *et al.* 2007). The seroprevalence of chlamydial infection in healthy and infertile Swedish cattle was 28 percent, so in this research, the relation of chlamydial infection with reproduction disorder is suspected (Godin *et al.* 2008). There are Reports of *C. pecorum* infection in cattle chronic respiratory inflammation (Jaeger *et al.* 2007), co-infection with *C. abortus* in aborted buffalo (Greco *et al.* 2008), and in healthy pigeon's fecal samples (Tanaka *et al.* 2005).

Jelocnik and colleagues in 2013 by multilocus sequence analysis (MLSA) of seven housekeeping gene in different *C. pecorum* isolates (sheep, cattle and koala), revealed that Limited clustering demonstrated between *C. pecorum* isolates in sheep and koala, also they identified 10 new MLSA sequence types (STs) and at least one ST (ST2013Aa) is

interplay between sheep and koalas isolates. Sequence types 23 (ST23), which is common between sheep and cattle isolates, is associated with multiple hosts and disease states. Also, presence of ST differentiation of intestinal and conjunctiva isolates in one host, is possible (Jelocnik *et al.* 2013).

CONCLUSION

In present study, most of the positive samples (70%) were collected from ruminant's vagina that have mating, therefore the physical transfer of agents in addition to transmission through semen, is suspected and need to further studies. Also, molecular evaluation and comparison of isolated *C. pecorum* in one or different hosts, is essential.

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