



Molecular Detection of *Brucella melitensis*, *Chlamydomphila* spp., *Campylobacter* spp., *Brucella abortus* and *Coxiella burnetii* in Genital Tracts of Goats

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ABSTRACT

Abortion is the major reproductive problem in goats faced by goat-keepers. Abortion in animals including goats is caused mainly by infectious agents. The current study was planned to assess the presence of *Brucella melitensis*, *Chlamydomphila* spp., *Campylobacter* spp., *Brucella abortus* and *Coxiella burnetii* in the genital tracts of goats and foetal stomach contents. A total of 260 biological samples (vaginal/preputial secretions and foetal stomach contents) were randomly collected from the goats. The genomic DNA extraction from the samples was done by using the commercial kit. The detection of the targeted infectious agents in the biological samples was done by species as well as genus-specific polymerase chain reactions. Standardization of PCRs was done using gradient PCR. The PCR products were run in a 1% agarose gel to determine the gene size. Out of 260 samples, the presence of *Coxiella burnetii* was not detected in any sample. *Brucella abortus* was detected in two aborted goats. Only 10 samples out of 260 (6 vaginal swabs from the aborted goats and 4 foetal stomach contents) were found positive for *Brucella melitensis*. A total of 117 samples were found positive for *Chlamydomphila* spp., whereas 84 were found positive for *Campylobacter* spp. A total of 63 samples were found positive for both *Chlamydomphila* spp. and *Campylobacter* spp. The present study concludes that *Chlamydomphila* and *Campylobacter* spp. were found as the prevalent microbial agents, which may be associated with abortion in goats in the forthcoming pregnancies in adverse conditions.

Key words: Abortion, *Brucella abortus*, *Brucella melitensis*, *Campylobacter*, *Chlamydomphila*, *Coxiella burnetii*, Goats.

The diseases are one of the major problems faced in goat farming which are responsible for significant economic losses to the farmers (Mahdi *et al.*, 2015; Doley and Nekibuddin, 2017). Amongst the diseases, genital abnormalities are the important ones that adversely affect reproductive potential of the goats (Azawi *et al.*, 2010). The important reproductive ailments in goats are abortion, retention of placenta and stillbirth (Beena *et al.*, 2017). Out of the ailments mentioned above, abortion is the commonest one, which affects productivity profoundly in goats and sheep (Menzie, 2011).

Both infectious and non-infectious agents may cause abortion in goats (Entrican *et al.*, 2009), but infectious ones are the predominant cause of caprine abortions (Smith and Sherman, 2009). Further, caprine abortion is mainly caused by the bacterial pathogens amongst the infectious agents (Smith and Sherman, 2009; Kahn and Line, 2010). There are several viruses such as Akabane, Cache Valley, Blue Tongue, Border Disease, Herpes, Nairobi Sheep Disease, PPR, Rift Valley Fever, Wesselsbron Disease, Bovine Viral Diarrhoea, etc., which are capable to cause abortions in goats (Smith and Sherman, 2009) and caprine herpes virus is the most common one in causing abortions in goats (Moeller, 2001; Kahn and Line, 2010). Under the parasitic agents, *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis*, *Anaplasma*, etc. may cause caprine abortions and the most common one is *Toxoplasma gondii*

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(Shaapan, 2016). The fungal agents associated with caprine abortions are *Aspergillus*, *Candida*, *Mucor*, *Rhizopus*, *Penicillium*, *Cladosporium*, *Rhodotorula*, *Absidia*, *Alternaria*, *Fusarium*, etc. (Pal *et al.*, 1985; Pal, 1988; Verma *et al.*, 1999). *Aspergillus* followed by *Candida* is the most prevalent fungi associated with abortion in goats (Vandyousefi and Zoghi, 1988). *Brucella*, *Chlamydia*, *Coxiella burnetii*, *Campylobacter*, *Listeria monocytogenes*, *Leptospira* (mostly interrogans, grippotyphosa and pomona), *Mycoplasma*, *Salmonella*, *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, *Streptococcus*, *Aeromonas*, *Fusobacterium*, *Trueperella pyogenes*, etc. are the main causative agents of caprine

abortions under Bacteria, Rickettsia and Chlamydia (Menzies, 2011; Elhaig *et al.*, 2018; Tejedor-Junco *et al.*, 2019) and the most common ones are *Brucella melitensis*, *Chlamydomydia*, *Coxiella burnetii*, *Campylobacter* and *Listeria monocytogenes* (Menzies, 2011; Kushwaha *et al.*, 2016; Saini *et al.*, 2017; Rossetti *et al.*, 2017; Rajagunalan *et al.*, 2019; Lakshmikanth *et al.*, 2021). These microorganisms are also capable to cause infections/diseases in human beings (Kumar *et al.*, 2010; Mändar *et al.*, 2015). The presence of the above microorganisms in the genital tract of the male goat may cause deterioration of semen quality leading to reduced sperm motility and viability (Gangwar *et al.*, 2020) and may spread the infection to female goats through natural service as well as artificial insemination (Mändar *et al.*, 2015). Likewise, the presence of the said microorganisms in the female genital tracts may cause abortion and infertility in goats and simultaneously, may infect male goats and humans as well (Gangwar *et al.*, 2020). That is why, the current study was undertaken to assess the association of *Brucella melitensis*, *Chlamydomydia* spp., *Campylobacter* spp., *Brucella abortus* and *Coxiella burnetii* with caprine abortions.

The study was conducted during the year 2020-2021 at ICAR-Central Institute for Research on Goats (ICAR-CIRG), Makhdoom, Farah, Mathura, Uttar Pradesh (India).

Sample collection

A total of 260 biological samples (number of preputial secretions=230, number of vaginal secretions=15 and foetal stomach content=15) were aseptically collected and immediately transported to the Microbiology Laboratory of ICAR-CIRG for further investigation. The samples were comprised of vaginal and preputial secretions of goats

and stomach contents from the aborted fetuses. The goats reared at ICAR-CIRG and the field goats of Districts Agra and Mathura of Uttar Pradesh and Bharatpur of Rajasthan (India) were selected for the experimentation.

Extraction of the genomic DNAs

The genomic DNA extraction from the biological samples was done by using DNASure® Tissue Mini Kit (Genetix Asia Pvt Ltd) according to the protocol of the manufacturer. The extracted DNAs were run in a 0.8% agarose gel to determine their presence and integrity.

Molecular identification of the microorganisms

The detection of the targeted infectious agents (*Brucella melitensis*, *Brucella abortus*, *Coxiella burnetii*, *Chlamydomydia* spp. and *Campylobacter* spp.) in the biological samples (vaginal/preputial secretions and foetal stomach contents) was done by specific polymerase chain reactions (PCRs; species and genus-specific). Standardization of PCRs was done using gradient PCR. Details regarding the primers, target genes and size of PCR products are given in Table 1. Likewise, the details regarding the PCR conditions are given in Table 2. The PCRs were performed by using EmeraldAmp GT PCR Master Mix (DSS Takara) following the manufacturer's instructions. The PCR products were run in a 1.5% TAE-agarose gel with a 100 bp marker (Thermo Scientific) to determine the gene size.

Out of 260 samples, the presence of *Coxiella burnetii* was not detected in any sample (Table 3; Fig 1). Thus, in our study, the role of *Coxiella burnetii* in causing an abortion in goats was not proven in the sampling area. But, Vaidya *et al.* (2010) demonstrated the prevalence of Q fever in goats as 6.1% after screening a total of 53 samples (genital swabs,

Table 1: Details regarding the target genes, the primers and size of the PCR products.

Name of organism	Name of gene	Primer sequence	Product size	Reference
<i>B. melitensis</i>	OMP-31	5'-ATGTTCCGCCACGTCCGCTATGG-3' 5'-CGGTTACGCAGCGCGCAAGT-3'	121 bp	Saini <i>et al.</i> , 2017
<i>B. abortus</i>	16SrRNA	5'-TCG AGC GCC CGC AAG GGG-3' 5'-AAC CAT AGT GTC TCC ACT AA-3'	905 bp	Marianelli <i>et al.</i> , 2008
<i>Chlamydomydia</i>	16S rRNA	5'-GATGAGGCATGCAAGTCGAACG-3' 5'-CCAGTGTGGCGGTCAATCTCTC-3'	278 bp	Borel <i>et al.</i> , 2006
<i>Campylobacter</i>	16SrRNA	5'-GGATGACACTTTTCGGAGC-3' 5'-CATTGTAGCACGTGTGTC-3'	816 bp	Inglis and Kalischuk, 2003
<i>C. burnetii</i>	IS1111	5'-TATGTATCCACCGTAGCCAGTC-3' 5'-CCCAACAACACCTCCTTATTC-3'	687 bp	Berri <i>et al.</i> , 2009

Table 2: Cycling conditions of the different primers set during the PCRs.

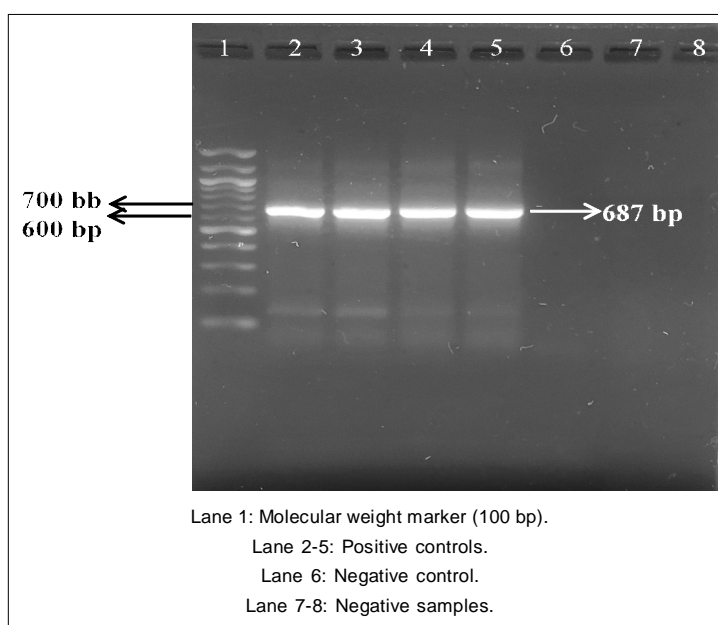
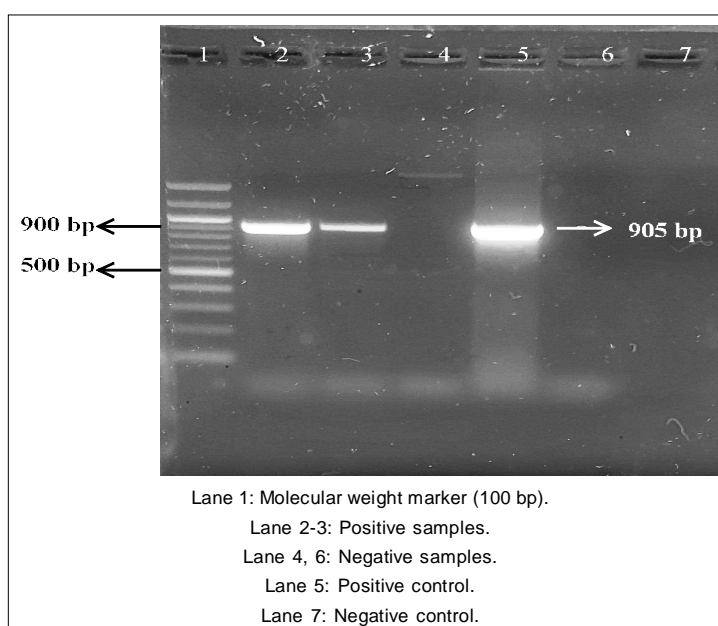
Name of microorganism	Initial denaturation	Secondary denaturation	Annealing temperature	Extension	Final extension	Cycles
<i>B. melitensis</i>	98°C/2 min	98°C/0.5 min	60°C/0.5 min	72°C/0.5 min	72/5 min	35
<i>B. abortus</i>	95°C/2 min	95°C/0.5 min	54°C/0.5 min	72°C/1.5 min	72/10 min	35
<i>Chlamydomydia</i>	98°C/2 min	98°C/0.5 min	56°C/0.5 min	72°C/0.5 min	72/5 min	35
<i>Campylobacter</i>	98°C/2 min	98°C/0.5 min	52°C/0.5 min	72°C/0.5 min	72/5 min	35
<i>C. burnetii</i>	98°C/2 min	98°C/0.5 min	56°C/0.5 min	72°C/0.5 min	72/5 min	35

Table 3: Percent positivity of the microorganisms in the genital tracts of the goats.

Name of microorganisms	Number of samples tested	Number of positive samples	% Positivity
<i>Coxiella burnetii</i>	260	0	0.0
<i>Brucella abortus</i>	260	2	0.76
<i>Brucella melitensis</i>	260	10	3.80
<i>Campylobacter</i> spp.	260	84	32.30
<i>Chlamydophila</i> spp.	260	117	45.0

milk, fecal swabs, urine and serum). Rajagunalan *et al.*, 2019 reported the first case of abortion induced by *Coxiella burnetii* in goats in India.

Brucella abortus was detected in the vaginal secretions of two aborted goats, whereas presence of *Brucella melitensis*, *Campylobacter* spp., *Chlamydophila* spp. and *Coxiella burnetii* in these goats was not confirmed, which indicates the potential of *Brucella abortus* to cause abortion in goats too (Table 3; Fig 2). This is the unique finding of this study. However, Moeller (2001) also reported that caprine abortion occurred due to *Brucella abortus*.

**Fig 1:** Detection of *C. burnetii* by PCR.**Fig 2:** Detection of *B. abortus* by PCR.

Only 10 samples out of 260 (6 vaginal swabs from the aborted goats and 4 fetal stomach contents) were found positive for *Brucella melitensis* (Table 3; Fig 3), whereas *Brucella melitensis*, *Brucella abortus*, *Campylobacter* spp., *Chlamydophila* spp. and *Coxiella burnetii* were not detected in these goats. However, many other workers reported a higher prevalence of *B. melitensis* in genital tracts of goats as well as the main cause of caprine abortions (Smith and Sherman, 2009; Kahn and Line, 2010; Samadi *et al.*, 2010; Sadhu *et al.*, 2015). Hence, further study is required to evaluate the current finding about role of *Brucella melitensis* in caprine abortion.

A total of 117 samples were found positive for *Chlamydophila* spp. (110 preputial secretions and 7 stomach contents) (Table 3; Fig 4), whereas 84 were found positive for *Campylobacter* spp. (all preputial secretions) (Table 3; Fig 5). A total of 63 samples were found positive for both *Chlamydophila* spp. and *Campylobacter* spp. These two microorganisms, being commensals, can act as opportunistic pathogens and their pathological significance needs to be associated with the gross and histopathological changes in the affected animals. Gangwar *et al.* (2020) found 53.13% of samples positive for *Chlamydophila* spp.

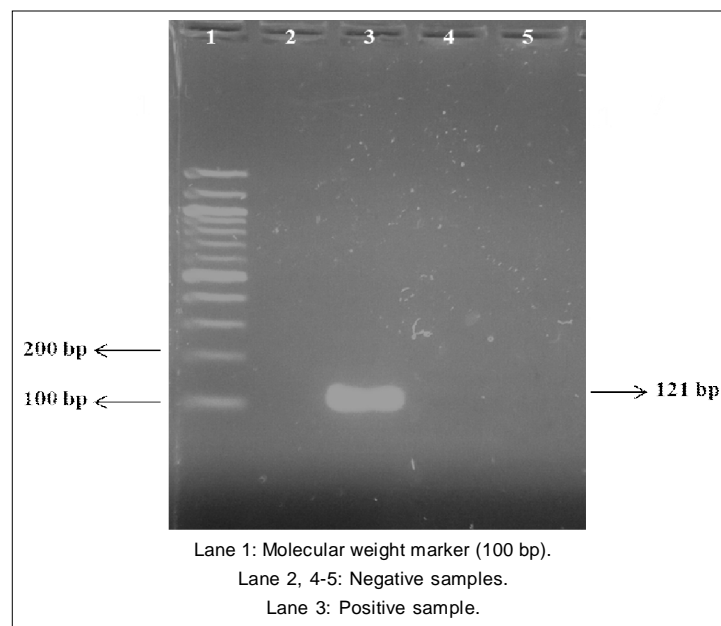


Fig 3: Detection of *B. melitensis* by PCR.

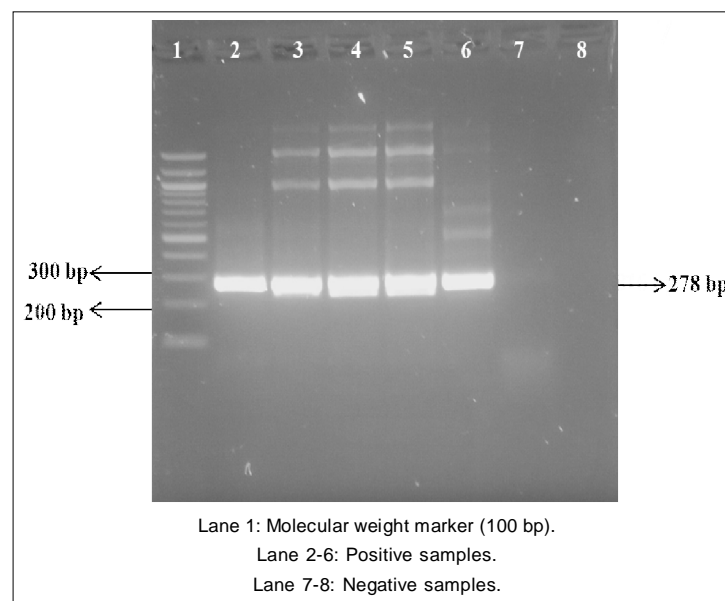


Fig 4: Detection of *Chlamydophila* spp. by PCR.

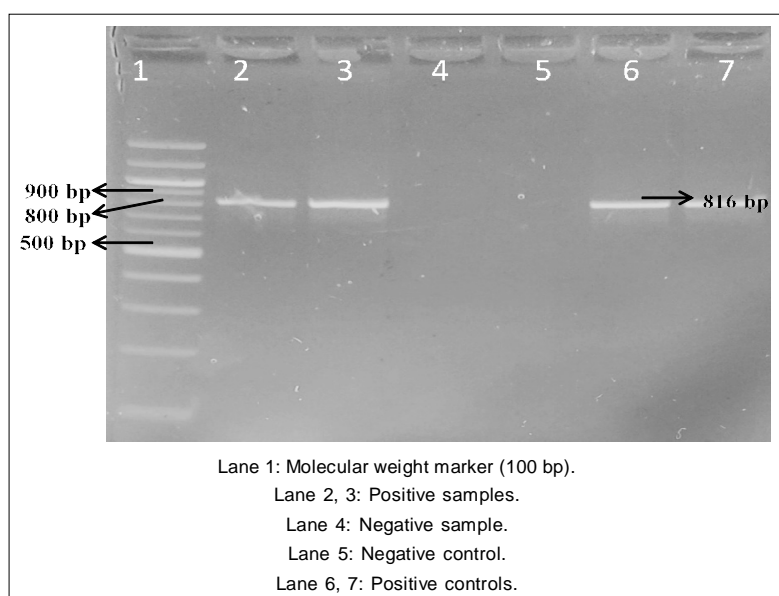


Fig 5: Detection of *Campylobacter* spp. by PCR.

and Sharma *et al.* (2008) also found *Chlamydophila* spp. as the most common cause of abortions in goats and sheep. However, contrary to our finding, in a study conducted by Beena *et al.* (2017), 5.30% positivity of *Campylobacter* spp. was reported in the genital samples from goats, whereas in another study, campylobacteriosis was reported only rarely as a cause of abortion in goats (Moeller, 2001).

Thus, the present study concludes that *Chlamydophila* spp. and *Campylobacter* spp. were found as the prevalent microbial agents, which may be associated with abortion in goats in adverse conditions during the forthcoming pregnancies.

CONCLUSION

Chlamydophila spp. followed by *Campylobacter* spp. were found as the most prevalent microorganisms in the genital tracts of goats. *Brucella abortus* was also detected in the aborted goats indicating its potential to cause abortion in goats too. The presence of *Coxiella burnetii* was not detected in a single sample and *Brucella melitensis* was found in a few samples only. This finding is not in concurrence with the fact that *Brucella melitensis* and *Coxiella burnetii* are the main cause of caprine abortions. Hence, extensive investigation with a structured sampling plan is required to establish the association of the targeted microorganisms (*Brucella melitensis*, *Chlamydophila* spp., *Campylobacter* spp., *Brucella abortus* and *Coxiella burnetii*) with caprine abortions and their epidemiological significance.

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Ethical statement

The sample collection from the animals was done after taking approval from Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Conflict of interest

All authors declare that they have no conflict of interest.

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