A RAPID SINGLE STEP MULTIPLEX PCR ASSAY FOR THE DETECTION OF *CHLAMYDOPHILA ABORTUS, CHLAMYDOPHILA PECORUM AND COXIELLA BURNETII* FROM RUMINANTS CLINICAL SAMPLES

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Introduction

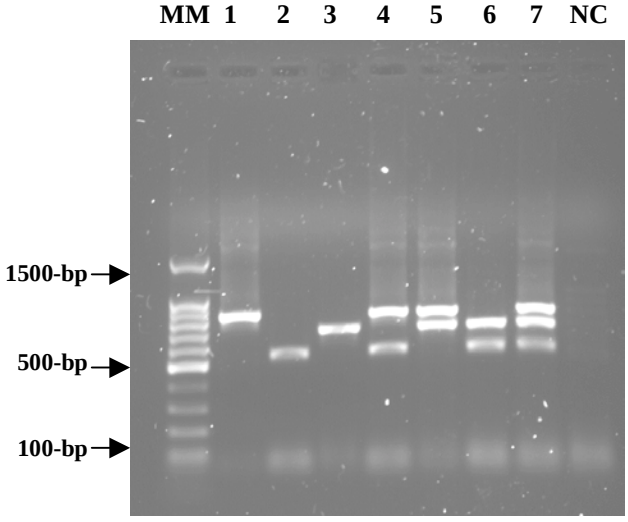
Chlamydiosis and Q fever are important causes of ruminants' abortion around the world (Rodolakis et al. 2004). They are caused respectively by intracellular and gram negative bacterium, *Chlamydophila* and *Coxiella*. Two species of the genus *Chlamydophila* cause diseases in ruminants, *C. abortus* and *C. pecorum* (Rodolakis et al. 1998). The available detection methods such bacteriological examination, culture or serology either lack both sensitivity and specificity or give only retrospective diagnosis. In order to improve *Chlamydophila* and *Coxiella* detection, specific PCR primers were designed and a sensitive multiplex PCR (m-PCR) was developed for rapid simultaneous detection and differentiation of *C. abortus*, *C. pecorum* and *C. burnetii.*

Material and methods

Three primer sets were designed and used to amplify the respective fragment of *C. abortus*, *C. pecorum* and *C. burnetii* reference strains (AB7, iB1 and Nine Mile respectively). As this test will be commercialised by a manufacturer, the sequence of the primers and the experimental protocol could not be given. This m-PCR assay was performed on 257 clinical samples taken from infected ruminant's flocks that have showed problems of abortion diseases.

Results

Fig.1 Multiplex PCR amplification of C. abortus, C. pecorum and C. burnetii reference strains individually and all possible combination. Lane MM: 100-bp ladder, 1: C. abortus AB7, 2: C. pecorum iB1, 3: C. burnetii Nine Mile, 4-6: duplex reactions, 7: triplex reaction, NC: negative control.



PCR reaction performed with the primers, designed in this study, resulted in the amplification of PCR product allowing a specific identification of *C. abortus* (821-bp), *C. pecorum* (600-bp) and *C. burnetii* (687-bp) micro-organisms (Fig.1). Multiplex as well as duplex or single PCR performed on reference strain purified DNA detect as little as 50 bacteria per PCR reaction. Amplification experiments performed with several *C. abortus*, *C. pecorum* and *C. burnetii* strains gave specific PCR product. However, no amplification was noted using DNA from other pathogens suspected to be present into tested clinical samples.

This m-PCR assay was performed on 257 clinical samples and showed that 67 samples were infected by either one of the three pathogens. Two vaginal swabs were m-PCR positive of both *C. abortus* and *C. burnetii* and none of the tested samples was shown to be infected simultaneously with the three pathogens. However *C. pecorum* strain was detected in one vaginal swab taken from aborted ewe and in epididymus of infected ram.

Discussion

Several tests that detect *Chlamydophila* and *Coxiella* antibodies made chlamydiosis and Q fever individual diagnosis tests widely available. However, these tests are not specific and poorly sensitive. Previous works have reported the use of PCR to detect individually *C. abortus* (Laroucau et al. 2001) and *C. burnetii* (Berri et al. 2000) in vaginal swab samples taken after lambing or abortion of infected ruminants. Here, we reported the successful development of multiplex PCR assay for the simultaneous detection and differentiation of *C. abortus*, *C. pecorum* and *C. burnetii*. Amplification experiments performed with both purified genomic DNA of bacteria or with spiked clinical samples showed that this assay was sensitive and specific. The performance of the m-PCR in field study showed that these two infections are widespread within the tested flocks. Two clinical samples were contaminated with both *C. abortus* and *C. burnetii* and the ability of this assay to detect dual infections was therefore known. Furthermore, *C. pecorum* was detected in vaginal swab taken from a female ewe that has aborted showing that this strain could be associated with small ruminant's abortion.

Conclusion

To conclude, we have successfully developed a multiplex PCR that can detect and differentiate three causative agents of ruminant's disease with a good sensitivity and specificity. The diagnosis of chlamydiosis and Q fever may be greatly simplified and performed at low cost. In addition, the result can be obtained rapidly which is helpful clinically if antibiotherapy has to be undertaken.

References

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