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, Chlamydia phila and Coxiella. Two species of the genus Chlamydia 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 Chlamydia phila 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 Chlamydia phila 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 chlamydioidosis 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 antibiotic therapy has to be undertaken.

References