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A multiplex HRM METHOD for the diagnosis of abortive diseases

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INTRODUCTION

Among ruminants, abortion is a pathological event that affects productivity and causes considerable direct and indirect economic losses to farmers because of fetal death and the diagnostic, therapeutic and prophylactic costs.
For these reasons, an accurate and fast diagnosis is always desirable to establish effective control measures. Microbial agents, such as Chlamydophila spp., Coxiella burnetii, Neospora caninum, Toxoplasma gondii, Brucella spp, Leptospira spp., Listeria monocytogenes, Anaplasma phagocytophilum, Campylobacter fetus spp. and Salmonella spp. are among the main infectious causes of abortion and require rapid and reliable diagnosis. Innovative diagnostics developed a screening qualitative assay, using HRM technology that allows, the simultaneous identification of the mentioned abortive agents. Concurrently, we have developed a software that enables the simultaneous discrimination of pathogens from their HRM profile. This innovative multiplex kit offers a fully automated analysis of pathogens.

MATERIAL & METHODS

This kit is a ready-to-use multiplex HRM assay based on the simultaneous detection of 10 pathogens by using two different reaction mixes. This also allows the real-time detection of Coxiella burnetii and Brucella spp.
It may be used for swabs (endocervical, vaginal and placenta), organs and tissues (placenta, brain, spleen or aborted heart), preputial washing and whole blood.
Results may be obtained in less than two hours (extraction in only 20 minutes, and amplification around 1 hour).

RESULTS

The kit correctly identified all reference extracts tested and did not show any cross-reactions with 60 other pathogens tested, demonstrating high inclusivity and exclusivity. The detection limit of the PCR was < 25 copies/PCR, indicating high sensitivity. The assay has a limit of detection (LOD) ranging from 2,5×102 to 2,5×104 copies/ml (depending on the pathogen) and a very good concordance with individual qPCR assays used in routine diagnostic activity.
Clinical validation is ongoing on around one thousand ruminants samples from several European veterinary diagnostics laboratories. Results will be presented during the presentation.

CONCLUSION

IDvet developed an innovative multiplex HRM qPCR assay for the diagnosis of abortive diseases. Results indicate excellent inclusivity and exclusivity, and good detection limits. The use of HRM technology for the development of this multiplex tests combined with the construction of a database allow to answer the need for screening diagnostic of abortive pathogens.
This innovative diagnostic tool represents a rapid approach to the simultaneous detection of the main and secondary abortive agents in ruminants for routine diagnostic laboratories. The method allows to make an accurate diagnosis and to set up appropriate control measures in a short period of time. It has the advantages to propose an unambiguous diagnosis with a significant reduction of response times together with an easier interpretation of results thanks to the software that comes with the kit.