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NOVEL PROBE-BASED REAL-TIME PCR FOR RAPID DIAGNOSIS OF BOVINE TUBERCULOSIS IN TISSUE SAMPLES WITH SUSPECT LESIONS

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Purpose

Ongoing bTB surveillance in Germany is principally based on meat inspection at abattoirs. Suspicious organ lesions shall be confirmed by prove of the causative agent. To accelerate monitoring a novel probe based two-target real-time PCR system had to be developed for the detection of DNA of members of the *M. tuberculosis* complex (MTC), including *M. bovis* and *M. caprae*, in tissue samples from cattle.

Methods

Seven primer/probe combinations (PPC) for three different target regions (IS*6110*, IS*1081*, putative helicase [HELI]) were tested. Analytical sensitivities were determined using DNA from different MTC strains diluted in buffer and diluted in DNA extracted from bovine lymph node tissue. DNA from 35 mycobacterial species and from 9 other bovine bacterial pathogens was tested for nonspecific amplification.

Results

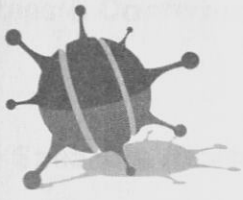
Detection of IS*6110*, the most widely used PCR target in human TB samples, was not suitable for bovine tissue samples because of low analytic sensitivity. Usage of PPC for IS*1081* and HELI, respectively, resulted in high analytical sensitivities and specificities. Based on these two PPC and β-actin as internal control, two duplex real-time PCR assays were established and combined with a modified commercial tissue DNA extraction method. The diagnostic specificity and sensitivity of the final system was 100% and 73%, respectively. Performance of this novel assay was validated in cooperation with federal state veterinary diagnostic laboratories.

Conclusions

By shortening the time for confirmation of bTB in organ samples with suspect lesions to a few days, the method is expected to improve abattoir-based bTB surveillance in Germany.

Deutsche Veterinärmedizinische Gesellschaft e.V.
German Veterinary Medical Society

16th International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD)



10th OIE Seminar

32nd Symposium of AVID

June 5 – 8, 2013
Berlin, Germany

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