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Detection of *Francisella tularensis* in the European hare by real-time TaqMan PCR

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Tularemia in lagomorphs (European brown hare [*Lepus europaeus*] and European wild rabbit [*Oryctolagus cuniculus*]), particularly due to *Francisella tularensis* subsp. *holarctica*, is historically enzootic in continental Europe. In France, the surveillance of tularemia is carried out almost exclusively on lagomorphs found dead or dying within the National Surveillance Network of Game Death Causes (SAGIR).

Bacterium sensitivity to temperature variations, as well as frequent putrefaction of the dead bodies, hamper diagnosis of tularemia in lagomorphs' tissue samples. A multitarget Real time TaqMan PCR assay, with primers and probes targeting the *tul4*, *fopA* and *ISFtu2* genes, has been developed in this laboratory, for *F. tularensis* detection and for isolate identification. In this study, conventional bacteriology, nested-PCR and real-time PCR were compared for their reliability in the detection of *F. tularensis*. DNA was extracted using a commercial kit. Analytical specificity was first confirmed on 30 other bacterial strains, frequently isolated from wild animals, particularly from hares. Moreover, the diagnostic specificity was 100%, when evaluated on 635 spleen samples of hunted wild-boars, apparently healthy.

Diagnostic sensitivity was assessed on 298 hare tissue samples (spleen or liver) collected in France from 2004 to 2007. Our results showed that real-time PCR is significantly more sensitive than conventional bacteriology and nested-PCR (positive samples: 52% vs. 9% and 25% respectively). Real-time PCR detected all culture-positive samples and all nested-PCR positive samples. Moreover, 27% of the samples including especially those putrefied or stored for long periods of time, were detected only by real-time-PCR.

Sensitivity, fastness, simplicity and reliability of this multitarget real-time TaqMan PCR assay make it a useful tool for tularemia routine diagnosis, which appears to be more appropriate than conventional bacteriology and PCR tools for an adequate surveillance of tularemia in wildlife.