Development and application of a universal hemoplasma screening assay based on the SYBR Green PCR principle

Summary / Zusammenfassung
Hemotropic mycoplasmas (also known as hemoplasmas) are the causative agents of infectious anemia in several mammalian species. Their zoonotic potential is controversial. Specific diagnostic molecular methods have been developed, but their application as screening tools is limited. The goal of the present study was to develop a universal hemoplasma screening assay based on the SYBR Green principle as a closed-tube PCR system with broad specificity, to compare the assay with hemoplasma-specific TaqMan PCR assays, and to analyze potential tick vectors and human blood samples to address zoonotic potential. The newly developed PCR assay based on the 16S rRNA gene was found to amplify feline, canine, bovine, porcine, camelid, and murine hemoplasmas, as well as M. penetrans and M. pneumoniae. The lower detection limit for feline and canine hemoplasmas was 1-10 copies/PCR reaction. Different hemoplasma species from one host species (cat, dog, cattle) could be differentiated by melting curve analysis. In a few negative controls, non-specific product formation was observed, as has been reported for other SYBR Green assays. All 1,950 Ixodes ticks analyzed were PCR-negative, suggesting that Ixodes ticks are not relevant vectors for the above-mentioned hemoplasma species in Switzerland. None of the 414 human blood samples tested revealed a clear positive result. The present study demonstrates that the developed SYBR Green PCR assay is a valuable hemoplasma screening assay and can be used as a tool for the identification of novel hemoplasma species. Positive results should be confirmed by specific TaqMan PCR or sequencing. The occurrence of hemoplasmas in humans could not yet be confirmed by molecular methods.

Publications / Publikationen

Keywords / Suchbegriffe
Haemotropic Mycoplasma, haemoplasma, real-time PCR, screening assay, Sybr green principal, prevalence, zoonosis

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