Specific Detection of Pasteurella multocida in Chickens with Fowl Cholera and in Pig Lung Tissues Using Fluorescent rRNA In Situ Hybridization

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Abstract

A Pasteurella multocida species-specific oligonucleotide probe, pmhyb449, targeting 16S rRNA was designed and evaluated by whole-cell hybridization against 22 selected reference strains in animal tissues. It differentiated P. multocida from other bacterial species of the families Pasteurellaceae and Enterobacteriaceae and also from divergent species of the order Cytophagales (except biovar 2 strains of Pasteurella avium and Pasteurella canis, which have high 16S rRNA similarity to P. multocida). The potential of the probe for specific identification and differentiation of P. multocida was further detected in formalin-fixed paraffin-embedded lung tissues from experimental fowl cholera in chickens and infections in pigs. In chicken lung tissues P. multocida cells were detected singly, in pairs, as microcolonies, and as massive colonies within air capillaries (septa and lumen), parabronchial septa, and blood vessels (wall and lumen). In pig lung, postmortem-injected P. multocida was detected in the alveoli (lumen and wall), and in both animals the bacterial cells were seen in the bronchi. The results showed that with the oligonucleotide probe pmhyb449, fluorescent in situ hybridization is a suitable and fast method for specific detection of P. multocida in histological formalin-fixed tissues. The test was replicable and reproducible and is recommended as a supplementary test for diagnosis and as a tool in pathogenesis studies of fowl cholera and respiratory tract infections in pigs due to P. multocida