Development of a novel high resolution melting assay for identification and differentiation of all known 19 serovars of Actinobacillus pleuropneumoniae

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Abstract

Actinobacillus pleuropneumoniae is the etiological agent of porcine pleuropneumonia, a respiratory infectious disease responsible for global economic losses in the pig industry. From a monitoring perspective as well as due to the different courses of disease associated with the various serovars, it is essential to distinguish them in different herds or countries. In this study, we developed a novel high resolution melting (HRM) assay based on reference strains for each of the 19 known serovars and additional 15 clinical A. pleuropneumoniae isolates. The novel HRM comprises the species-specific APP-HRM1 and two serovar-specific HRM assays (APP-HRM2 and APP-HRM3). APP-HRM1 allowed PCR amplification of apxIV resulting in an A. pleuropneumoniae specific melting curve, while nadV specific primers differentiated biovar 2 from biovar 1 isolates. Using APP-HRM2 and APP-HRM3, 13 A. pleuropneumoniae serovars can be determined by inspecting the assigned melting temperature. In contrast, serovar 3 and 14, serovar 9 and 11, and serovar 5 and 15 have partly overlapping melting temperatures and thus represent a challenge to accurately distinguish them. Consequently, to unambiguously ensure the correct assignment of the serovar, it is recommended to perform the serotyping HRM assay using a positive control for each serovar. This rapid and user-friendly assay showed high sensitivity with 1.25 fg - 125 pg of input DNA and a specificity of 100% to identify A. pleuropneumoniae. Characteristic melting patterns of amplicons might allow detecting new serovars. The novel HRM assay has the potential to be implemented in diagnostic laboratories for better surveillance of this pathogen.

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