Actinobacillus sp. biochemically and phenotypically similar to *Actinobacillus pleuropneumoniae* can be differentiated by genomic fingerprinting, toxin profiling, and sequencing of the 16S rRNA gene

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Introduction. *Actinobacillus pleuropneumoniae* colonizes the upper respiratory tract of swine and causes severe fibrinous and necrohemorrhagic pleuropneumonia in susceptible animals. Naïve populations may experience high morbidity and mortality, with death occurring between 24 and 72 hours post-infection. Many swine herds in the United States have eradicated this pathogen from their populations. The negative status of these herds is monitored using serology, PCR testing, and bacterial isolation. The correct identification of suspect bacterial isolates is critical for the definition of the herd's health status, and an equivocal result may harm the ability of these herds to supply animals to negative herds. Recently, at the University of Minnesota Veterinary Diagnostic Laboratory an *Actinobacillus sp*. isolate biochemically and phenotypically similar to *A. pleuropneumonia*e was isolated from the pleura of a pig with no lesions. Initially, this isolate was identified as *A. pleuropneumoniae* based on biochemical tests and was classified as serotype 10 at the University of Montreal. However, additional testing by genotyping, toxin profiling, and sequencing of the 16S rRNA revealed that this isolate was distinct from *A. pleuropneumoniae* and more closely related to *A. minor, A. porcinus* and *A. indolicus,* early non-pathogenic colonizers of the upper respiratory tract of swine. Molecular diagnostic tools were critical in identifying the *A. pleuropneumoniae*-like isolate as a non-pathogenic *Actinobacillus sp*., and for the accurate assessment of the herd's negative status.

Materials and methods. The *A. pleuropneumoniae*-like isolate was recovered from the pleura of a 14-week-old finishing pig with no lesions. This isolate's identification as *A. pleuropneumoniae* was based on the following characteristics: V-factor requirement (+), X-factor requirement (-), catalase (-), hemolysis (+), urease (+), CAMP test (+), esculin (-), indole (-). Serotyping was performed at the University of Montreal using indirect hemagglutination as previously described.[1] Genotyping was performed by ERIC-PCR,[2] and the *A. pleuropneumoniae*-like isolate was compared with the reference strains for the 15 known serotypes of *A. pleuropneumoniae*. Toxin profiling was performed by PCR detection of the following genes: apxIA (723bp), apxIB (811bp), apxII (965bp), apxIII (396bp), and apxIV (1600, 2000, 2400, and 2800 bp) as previously described.[3] The 16S rRNA gene from this isolate was sequenced[4] and compared with sequences available at GenBank (http://www.ncbi.nlm.nih.gov/BLAST/).

Results and discussion. The isolate recovered from the pleura was biochemically identical to *A. pleuropneumoniae*. This isolate was classified as a serotype 10 by indirect hemagglutination, suggesting a cross-reaction between non-pathogenic *Actinobacillus sp*. and *A. pleuropneumoniae*. Genotyping by ERIC-PCR revealed that the *A. pleuropneumoniae*-like isolate was distinct from all *A. pleuropneumoniae* reference strains, with only 10.9% similarity with the cluster of reference strains. Toxin profiling revealed the presence of apx II and the absence of the remaining toxin genes characteristic of *A. pleuropneumoniae* (apxIA, apxIB, apxIII, and apxIV). The partial sequence of the 16S rRNA gene was 100% similar to *A. minor, A. porcinus,* and *A. indolicus.* These bacterial species, however, were negative for all toxin genes.

Conclusions/relevance. Genotyping by ERIC-PCR, toxin profiling, and sequencing of the 16S rRNA can be used to differentiate non-pathogenic *Actinobacillus sp*. that are biochemically and phenotypically similar to *Actinobacillus pleuropneumoniae*. The correct identification of these bacterial species is critical for the accurate definition of health status for swine herds.

References: 1. Vet Microbiol 1992;32:135–148. **2.** Nucleic Acids Res 1991;19:6823-6831. **3.** J Vet Diagn Invest 2005;17:359-362. **4.** LaPara TM, et al. Appl Environ Microbiol 2000;66:3951-3959.