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David Brown
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Logo Design
Ruth Cronje, and Jan Swanson;
based on the original design by Dr. Robert Dunlop

Identification of nontypable *Brachyspira* sp. by sequencing: New trends on potentially virulent species

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Introduction

The genus *Brachyspira* contains several intestinal spirochaete species which colonize the large intestine of a wide range of animals. *Brachyspira hyodysenteriae* and *Brachspira pilosicoli* are the etiologic agents of swine dysentery and intestinal spirochaetosis, respectively, and can be definitively diagnosed by PCR. Several other *Brachyspira* species are known to infect swine with varying degrees of clinical significance, yet there currently are no diagnostic tests available to identify these organisms. Additionally, these non-typable *Brachyspira* infections may be an emerging issue in swine. Here we present the development of a diagnostic test that can be used to identify *Brachyspira* species in otherwise non-typable clinical swine samples. This test involves amplifying a highly variable region of the *Brachyspira* NADH Oxidase (*nox*) gene by PCR followed by species identification by sequencing.

Materials and methods

Thirty non-typable *Brachypira* sp. isolates obtained from clinical samples submitted to the Minnesota Veterinary Diagnostic Laboratory were characterized in this study. These isolates tested negative for *B hyodysenteriae* and *B pilosicoli* by PCR. Five *Brachyspira* species commonly isolated from swine including *B pilosicoli, B hyodysenteriae, B intermedia, B murdochii*, and *B innocens* were obtained from ATCC and used as controls for the new typing system. A PCR targeting a highly discriminatory and species-specific region of the *Brachyspira nox* gene was developed. Amplicons were sequenced and results were compared with those available in GenBank for species identification. Identities of 98% or greater were considered good indicators of the *Brachyspira* species identified.

Results

The newly developed *nox* PCR followed by sequencing method correctly identified the *Brachyspira* species of all ATCC isolates. Reliable sequencing data was obtained for 29 of the 30 nontypable *Brachyspira* isolates. Of these, 15 matched *B murdochii* with 99-100% identity, 3 matched *B innocens* with 100% identity, 1 matched a *B murdochii* with 97% identity, and 10 matched an unidentified *Serpulina* sp. with 96% identity. The 10 isolates closely matching the *Serpulina sp.* sequence are strongly hemolytic and were recovered from tissues of animals with evident clinical signs.

Discussion

Non-typable *Brachyspira* species represent 68% percentage of isolates recovered from pig intestines at the Minnesota Veterinary Diagnostic Laboratory. Isolation of non-typable *Brachyspira* sp. has increased in the past years. The new method for *Brachyspira* sp. identification developed in this study allowed the classification of 19 of 30 non-typable isolates. We have apparently identified a new *Brachyspira* species that did not key in as any of the previously known swine *Brachyspira*. These 10 isolates were highly hemolytic and were recovered from affected pigs, which suggest that they are potentially virulent and associated with clinical disease in the field. We are currently performing whole 16S rRNA sequencing to better classify these isolates.

Reference

1. Rohde J, Rothkamp A, Gerlach GF Differentiation of porcine *Brachyspira* species by a novel nox PCR-based restriction fragment length polymorphism analysis. J Clin Microbiol. 2002 40(7):2598–2600.