RESEARCHABSTRACT

SWINE HEALTH

Title: Application of an Epidemiologic Survey Tool for *Lawsonia intracellularis* – **NPB #07-053**

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Date submitted: November 3, 2008

Scientific Abstract:

The whole genome of *Lawsonia intracellularis* (strain PHE/MN-00) was analyzed for the presence of tandem repeats and this analysis identified four genomic regions containing putative variable number tandem repeat (VNTR) sequences. These regions consisted of two sequences of ATA_n and two sequences of CA_n nucleic acid repeats. Specific primer sequences were then designed, using Primer 3 software program, upstream and downstream of these four respective regions and used to generate VNTR profiles for isolates of *L. intracellularis* from diverse sources.

L. intracellularis isolates of geographic and temporal diversity, including the type strains PHE/MN1-00, VPB4, 15540D, and 963/93, were used to determine if there was variability of VNTRs among isolates of *L. intracellularis*. The results of our analysis show that each available pure culture *L. intracellularis* isolate examined herein had a unique VNTR profile and these data demonstrate that there are genetic differences between *L. intracellularis* isolates as reflected by VNTR typing.

To assess if the VNTR profiles were conserved and stable in a specific isolate, we tested an isolate prior to cultivating in cell culture, after low- and high- passage cell culture, and after serial passage through a pig. The results also showed that the VNTR profiles of a sample obtained directly from a diseased pig intestine was identical to that obtained after purification and inoculation into cell culture, after low passage, and after serial passage through a pig. These results suggest that the VNTR profiles are relatively stable during laboratory culture and over short periods of time and produce repeatable, unique profiles.

In this project we further used the VNTR method of typing *L. intracellularis* to analyze the genetic relatedness of field outbreaks obtained from pigs with various forms of proliferative enteropathy (PE; ileitis), from different geographical locations, and from various animal species.

During evaluation of a larger number of samples, patterns emerged that provide some insight into the sources and phylogenetic relatedness of field *L. intracellularis* isolates.

We have now extended our database of *L. intracellularis* VNTR types to include isolates from 58 proliferative enteropathy outbreaks occurring in pigs, 20 horses, and 7 other animal species which include ostrich, spider monkey, ferret, hamster, skunk, and rabbit. For this effort, we have performed numerous Lawsonia serologies and fecal PCRs on horses and other species submitted to the University of Minnesota Veterinary Diagnostic Laboratory for the purpose of identifying these outbreaks.

We continue to find that VNTR types obtained from pigs with proliferative enteropathy are very different from those obtained from horses or other non-pig species. From the data analyzed so far, little or no genetic variation (using a 70% cut-off value) was found within clinical types, including proliferative hemorrhagic enteropathy (PHE), porcine intestinal adenomatosis (PIA) and subclinical forms of proliferative enteropathy within a pig barn. Slight variation between isolates from different geographic locations was found, but this difference was no more pronounced between Lawsonia isolates from Minnesota herds than between Minnesota herds and European herds. Marked variation in VNTR types were found between isolates from pig sources and those obtained from non-pig sources.