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Enrofloxacin treatment affects the colonization stage of *Haemophilus parasuis* in weaned pigs

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Introduction: *Haemophilus parasuis* (HPS) is one of the first pathogens to colonize pigs after birth, but factors involved in systemic invasion and colonization are largely unknown. Therefore, a better understanding of HPS colonization over time is necessary for prevention and control of the disease. Antibiotics are used to prevent and control disease and enrofloxacin is considered efficacious against HPS. However, limited information is available on the effects of enrofloxacin on HPS colonization. Therefore, the objective of this study was to evaluate the effect of enrofloxacin in HPS colonization in weaned pigs.

Materials and Methods: Twenty three weaned pigs positive for HPS by PCR were selected from a conventional farm and moved to the UMN research isolation facility. On arrival, pigs were divided in two groups and blood samples and nasal and tonsil swabs collected from all pigs. Twelve pigs in the treatment group (TG) received a single dose of injectable enrofloxacin (7.5 mg/Kg Baytril, Bayer Animal Health) at 24 h post arrival. Eleven pigs in the control group (CG) received saline. Pigs were monitored for 15 days and were sampled every day by tonsillar and nasal swabs, which were tested by qPCR. At 4, 8 and 15 days post treatment (DPT), 4 pigs were euthanized and blood samples collected. At necropsy, swabs from the nasal cavity, tonsil, trachea, lung, and peritoneal and pleural serosas were collected in duplicates, one submitted for bacteriological examination and the other was tested by qPCR. ERIC-PCR genotyping[1] and PCR to detect HPS virulent gene (vtaA)[2] were applied to characterize HPS isolates obtained. Blood samples were tested for HPS antibodies using OppA-ELISA[3]. Differences between the proportion of HPS positive pigs in treated vs control groups at each sampling time point were calculated using Fisher's Exact Probability Test, with Bonferroni correction ($\alpha = 0.003$).

Results: All pigs tested positive by PCR on the day of arrival. Pigs in the CG tested positive throughout most of the study, while all pigs in the TG tested HPS negative by qPCR at 1 DPT and the treatment effect persisted partially until 12 DPT. The average of CFU/reaction/day for tonsil and nasal indicate that the daily bacterial load was less in the TG than in the CG. The proportion of positive pigs was statistically higher in the CG than in the TG on days 1, 2, 3, 4, 5, 6 and 7 DPT for nasal swabs and on days 2, 4 and 5 DPT for tonsil swabs (P-value < 0.003).
At necropsy, 9 out 11 control pigs were positive for HPS by qPCR. In contrast, only 4 out of 12 pigs in the TG tested positive at necropsy. Four HPS isolates were recovered at 15 DPT, one from serosas and 3 from nasal cavity. No clinical signs or lesions were observed during the experiment. Similar fingerprinting by ERIC-PCR demonstrated that pigs were colonized with similar HPS strains, which were also negative for the vtaA gene, which suggests that these isolates do not have this virulence factor.
Serum antibody titers, as measured by ELISA S/P at the time of arrival and at the time of necropsy for both groups were under the cut-off value of 0.2.

Discussion and Conclusion: Enrofloxacin treatment significantly reduced the number of pigs colonized with HPS and this effect was mostly seen during the first week post treatment. Enrofloxacin also reduced the presence and load of HPS on the nasal cavity and the tonsils of naturally colonized pigs, but was unable to completely eliminate the organism. Additionally, pigs did not seem to mount a humoral immune response to HPS colonization. Further research is needed to evaluate the lasting effect of enrofloxacin in HPS colonization patterns and disease dynamics.

References

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