Final Report: Investigations on *Arcanobacterium phocae* in “Foot Pad Necrosis” of Mink

Patrick Boerlin, Department of Pathobiology, Ontario Veterinary College, Guelph
July 15th 2014

Background

“Foot Pad Necrosis” (FPN) has been associated in the past with feeding seal meat to minks, and suspected to be caused by seal caliciviruses. However, the virus could be neither grown nor detected in foot pad lesions. Lesions have been induced by experimental injection of seal caliciviruses in minks, but not through feeding of infected seal meat. It is unclear if these lesions were really FPN. The disease occurs mainly during the breeding season in ‘hyperactive’ heavy males, but it has apparently behaved like a transmissible disease, even in the absence of seal meat feeding. Previous information suggested that *Staphylococcus pseudintermedius* and *Streptococcus canis* were regularly recovered from FPN lesions and could potentially be involved in its pathogenesis. These organisms are part of the normal flora of mink and the apparent transmissible nature of FPN suggested that specific strains of these two bacterial species, more able to cause the disease than others, could have emerged and spread across mink populations. To test this hypothesis, and with the support of the Canada Mink Breeder Association, we recently examined the diversity of coagulase-positive staphylococci and *S. canis* in minks with and without feet lesions. Our results first showed that *Staphylococcus delphini* rather than *S. pseudintermedius* was associated with FPN. Second, our molecular typing of *S. delphini* and *S. canis* strains from mink feet lesions and from healthy minks showed that no specific strain could be associated with the disease. This supports the hypothesis that any strain of these two organisms behaves in minks as opportunistic pathogens causing secondary infections on primary lesions of another etiology. However, during the course of this study, our collaborators at the Animal Health Laboratory isolated *Arcanobacterium phocae* from mink feet lesions at several occasions. Interestingly, *A. phocae* is known to occur regularly in sea mammals, including seals (hence the name “phocae”), which could suggest some links with the feeding of seal meat to minks. This organism is relatively fastidious and slow growing on bacterial culture media. Consequently, it gets overgrown and probably often overseen in the middle of all the bacteria from the normal flora of foot pads growing on such media. We strongly suspected that *A. phocae* is more frequent than it appears with classical bacteriological diagnostic methods. Therefore, we developed a sensitive real-time PCR for the specific detection of *A. phocae*. In laboratory experiments, this PCR was extremely sensitive. However, it needed to be validated on real world materials, including materials from mink feet. This PCR was subsequently used for comparison of samples from minks with feet lesions and healthy minks. The results of these studies (the previous ones on *S. delphini* and *S. canis* and those from this study on *A. phocae*) are described in detail in a manuscript which has now been accepted for publication in the Canadian Journal of Veterinary Research (accepted manuscript attached to this report).
Objectives and results summary

Objective 1: Assess the exact detection limit of our new *A. phocae* PCR.
The sensitivity of the PCR for *A. phocae* developed here was very high. The detection limit of the PCR using broth cultures and spiked feet tissue extracts was of approximately five colony forming units (CFU or individual live bacteria) per mL. Spiking of feet sample extracts with positive controls did not detect the presence of significant PCR inhibitors either. The specificity of positive results was also excellent, since small amounts of non-specific amplification products were detected only with high concentrations of bacteria closely related to *A. phocae* and not with other less related bacteria. These non-specific products could also easily be differentiated from those specific for *A. phocae* by their different melting peak temperatures.

Objective 2: Collect feet samples from cases of FPN and from healthy minks from the same farms, as well as from farms without a history of FPN problems.
One major issue we encountered during the study was that most feet with lesions we received did not present the extensive acute necrotizing lesions described in the literature for FPN, but were rather more chronic pododermatitis lesions (see figure 1 in the attached manuscript). We therefore used the general definition of pododermatitis (inflammation of the skin of the foot) to differentiate cases from healthy control animals for our study. Feet from mink were collected on 14 farms in Central and Eastern Canada. Three farms had not experienced pododermatitis problems, while the eleven others had regular pododermatitis problems. Feet from healthy control animals were collected in the three farms without pododermatitis (n=24) and in nine of the farms with pododermatitis problems (n=54). Feet with lesions were collected in all 11 farms with pododermatitis problems (n=60).

Objective 3: Test these samples with our *A. phocae* PCR in order to assess if the presence of this organism can be associated with FPN.
*A. phocae* was not detected in any of the feet samples from healthy control mink of farms without pododermatitis problems. However, it was detected in 39% of the feet from healthy mink in farms with pododermatitis problems and in 62% of pododermatitis lesions in these problem farms. Significant statistical associations were found between both the presence and the quantity of *A. phocae* in feet samples and pododermatitis lesions. Thus, there is a definite association between *A. phocae* and pododermatitis lesions, but this is not an absolute one. Despite the very high sensitivity of the new PCR developed here, the pathogen cannot be detected in a significant proportion of pododermatitis lesions.

Conclusions and recommendations
A highly sensitive and specific detection tool was developed for *A. phocae* in the course of this project and a clear association of *A. phocae* with pododermatitis was demonstrated. However, despite this association, it appears that the organism is not present systematically in all pododermatitis lesions. This suggests that, similarly to other bacteria detected in mink pododermatitis cases such as *S. delphini* and *S. canis*, it may contribute significantly to the development of maintenance of lesions as a non-obligate pathogen only. Despite its previously described widespread presence in sea mammals (i.e. a possible origin in seals and association with feeding seal meat to mink), it is likely that this bacterium is only an opportunist causing secondary infections and worsening lesions caused by one or several other primary causes. These other primary causes remain to be identified. However, before this can be done properly, a clear evaluation and description of the current situation regarding the type of pododermatitis lesions encountered in Canadian mink, their distribution, and their exact relation with FPN as it has been historically described in mink should be conducted.