

One-year status report of the project “Host genotype: a new target for control of *Staphylococcus pseudintermedius*?”

Pre-phase

- In the first months of 2012, protocols for sampling and laboratory procedures were made and tested. Applications for ethical permission to sample Swedish dogs were also made - ethical approval was not needed for Danish dogs. Pilot studies were conducted on samples from (our own) dogs in Denmark to test and optimize laboratory procedures prior to arrival of the study samples.

Sampling

- The original plan was to enrol previously genotyped dogs from Denmark, Sweden and Belgium. However, due to logistical problems, dogs from Belgium were not included in the study anyway. We invited Danish and Swedish owners to participate by sending out a letter explaining the purpose of study. In total, 64 dog owners responded and agreed to participate: 34 from Denmark and 30 from Sweden. These owners were then sent (i) instructions on how to sample their dogs, (ii) sterile swabs for sampling, (iii) questionnaires to describe the clinical history of their dogs, and (iv) stamped envelopes for returning swabs and questionnaires to our laboratory at the University of Copenhagen. Samples were taken from dogs on three separate occasions within a two-month period during the summer. Two owners did not send samples as promised, and we therefore ended up with a total of 62 dogs for the study.

Laboratory work

- Due to the low response rate and exclusion of dogs from Belgium, we decided to send out twice as many swabs to the owners compared to the original plan. This allowed us to try and study colonization with *S. pseudintermedius* both qualitatively and quantitatively. We are not yet sure whether our quantification has worked, since this is a very difficult method to standardize: owners need to sample their dogs the exact same way and swabs should be processed in a special way to quantify the number of *S. pseudintermedius* in each swab.

Preliminary results

- The following table summarizes the results of our sampling by showing how many dogs were non-carriers, intermittent carriers and permanent carriers of *S. pseudintermedius*:

<i>S. pseudintermedius</i> carrier status	Swedish dogs	Danish dogs	Total
Non-carriers (negative in all samplings)	3	3	6
Intermittent carriers (1 or 2 of the three samples positive)	5	15	20
Permanent carriers (all 3 samples positive)	22	14	36
Total	30	32	62

Results are in conjunction with previous research from our department showing that 50-60% of dogs are permanent carriers, 30-40% are intermittent carriers, and 10-20% are never colonized with *S. pseudintermedius* (Guardabassi et al., 2011).

Isolates obtained from each culture-positive sample are currently being verified to the species level and are also investigated by PCR to screen for methicillin-resistant *S. pseudintermedius* (MRSP).

Upcoming work

- The next step will be to analyse the genetic data previously obtained from the dogs to see if there are any genetic traits associated with carriage or non-carriage of *S. pseudintermedius*.

We will also study adherence of staphylococci to skin of participating dogs by an *in vitro* corneocyte assay used previously by our research group: corneocytes taken from the skin surface of participating dogs by a non-invasive method will be transferred to glass slides followed by inoculation of *S. pseudintermedius* strains onto the slides. Adhesion of bacteria to the corneocytes will then be measured by microscopy – we hypothesize that *S. pseudintermedius* will adhere poorly to corneocytes of non-carriers, whereas a stronger adhesion is expected for permanent carriers. The plan was originally to use the assay for 10 permanent carriers and 10 non-carriers. However, we identified only 6 non-carriers so the numbers will be lower. Taking corneocytes from dogs is not painful for the dogs but is technically demanding. We will therefore visit dog owners in both Denmark and Sweden to obtain such samples from their dogs.

Other tasks for the upcoming year will be to analyse quantitative data from the laboratory procedures and to analyse questionnaire data from dog owners to relate the clinical status of dogs (pyoderma / no pyoderma) to the *S. pseudintermedius* carrier status of dogs.

Revised budget

- The following table describes the revised budget. A detailed description of reasons for any changes has been provided separately through the online application website.

	Year 1 (planned)	Year 1 (actual)	Year 2 (planned)
Diagnostic processing	350.000 SEK	290.500 SEK*	-
Materials for sampling and shipment of samples	20.000 SEK	16.600 SEK*	-
Corneocyte experiment (travel to ~15 dog owners in Denmark and Sweden, consumables for the analysis)	-	-	40.000 SEK
SNP data analysis by Merete Fredholm	-	-	30.000 SEK

* Adjusted to 83% of the planned budget due to receipt of 83% of the expected number of samples.

Based on the actual budget for the first year and the planned budget for the second year, the following calculation has been made:

Total budget, year 1+2 incl. 25% administration:.....	471.375 SEK
Received by Agria in 2011:.....	200.000 SEK
Total sum applied for in 2012:.....	<u>271.375 SEK</u>

Reference

Guardabassi, L. 2011. Is MRSP ST71 just a *Staphylococcus pseudintermedius* strain containing *mecA*? Abstract presented at the 2nd ASM-ESCMID conference on methicillin-resistant staphylococci in animals: veterinary and public health implications. Washington DC, USA.