Title

"Understanding population, genetic and antigenic diversity of the poultry red mite to improve prospects for successful vaccine development."

A PhD Project completed by Dr Eleanor Karp-Tatham at the Royal Veterinary College between October 2016 and December 2020, sponsored by the BEMB Research and Education Trust. Eleanor was supervised by Professor Damer Blake BSc, MSc, PhD, PGCVetEd, FHEA, Professor of Parasite Genetics.

Summary of final thesis

The poultry red mite (PRM; *Dermanyssus gallinae*), an obligatory blood feeding parasite, is primarily associated with poultry where it is predicted to incur losses of ~€230 million per annum from European farmers. Current control strategies include the use of chemicals and desiccant dusts but are frequently reported to be ineffective with resistance commonplace. Alternative methods of control are urgently required for PRM, including the production of novel drugs and development of a suitable vaccine. Understanding the genetic diversity of PRM populations can help to answer fundamental questions relating to their ability to adapt (i.e. developing resistance to anti-mite products) and provide crucial information to support development of novel and effective control measures.

The main aim of the thesis was to improve understanding of poultry red mite population structure, and to assess diversity in mite genomes and proteins with relevance to the development of, and likely response to, novel control measures. In order to achieve this, five main objectives were set out: (1) collection and processing of mites from field locations; (2) identification and validation of genetic markers from these mites; followed by application of these markers to assess: (3) population structure and regional variation; (4) the occurrence of genetic types that have been associated with resistance to anti-mite products and (5) variation in anti-mite vaccine candidates.

Sampling of *D. gallinae* was conducted across the UK and other countries in Europe. From the UK, a total of 24 farms were sampled, covering all four countries and 18 counties. Three UK farms were sampled several times, providing an opportunity to assess variation over time. From the rest of Europe, samples were received from 16 countries, covering 82 individual farms.

Work using the 'bar-coding gene' (cytochrome oxidase subunit 1; COI) as a genetic marker for *D. gallinae* was completed. Analysis revealed variations both between and within countries, suggesting extensive travel for some individual mites within mite populations. Focusing on the UK, the results have also identified variations within some single farms. Through computational analysis, 100 markers (sites known to vary amongst individuals) were selected and tested on 75 samples (each representing an individual farm), including markers relating to anti-PRM vaccine candidate and drug resistance to anti-PRM products. Analysis revealed high levels of genetic diversity, suggesting that mites from each farm were different from all other farms. These results can potentially be attributed to a sequence of events that are common in modern poultry production. First, a rapid reduction in PRM population size occurs when poultry houses are cleaned between flocks and most mites die, followed by

rapid expansion (due to the fast lifecycle of PRM) which results in changes to the occurrence and balance of genetic diversity within the population. Regular and unintentional mixing of mites can increase genetic diversity, especially following periods of population reduction. Mixing of mites can result from the introduction of new birds or equipment contaminated with PRM, or carriage by humans visiting farms. Analysis suggested that mixing occurs both within and between farms, in the UK and across Europe, suggesting that trade of poultry could be one source contributing to high genetic diversity. Production system type did not appear to impact the level of genetic diversity observed, with similar levels detected in both free-range and intensive style farms.

Research focusing on the occurrence of genetic mutations associated with drug resistance in PRM were found to be widespread across European mite populations, with the UK showing the highest presence of mutations. Patterns of these mutations were individual to countries, likely due to differences in legislation and differing practices of control against PRM. These results provide further evidence of the requirement for new control measures against PRM and the value of increased knowledge of genetic diversity. Focusing on vaccine targets, analysis of three candidates that were identified during review of the published literature showed low levels of diversity. The results encourage ongoing development of these vaccine candidates for use in a vaccine against *D. gallinae,* suggesting efficacy against mites from a broad geographic range, although further research is essential to fully understand the diversity present and how this diversity would impact on vaccination efficacy.