NRCPD-OUAVM Joint Research Report

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Project Title
Development of a detection system for avian coccidiosis based on the Loop-Mediated Isothermal Amplification (LAMP) assay

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Research Period
Research period: 2011/04/01 - 2012/03/31
Total number of years: One year

Research Summary
Avian coccidiosis is an economically important enteric disease that is known to be caused by seven *Eimeria* species. Identification of *Eimeria* species that are present in chicken flocks will aid farmers and relevant authorities in the management of this disease, and contribute towards the development of more efficient control methods.

The main objective of this study was to establish a system to detect *Eimeria* species that infect chickens based on the Loop-Mediated Isothermal Amplification (LAMP) assay. In order to achieve this, the Houghton strain of the seven avian eimerian species, namely *E. tenella*, *E. maxima*, *E. acervulina*, *E. necatrix*, *E. brunetti*, *E. mitis* and *E. praecox* were used as controls. Genomic DNA was extracted from each of the species and used in LAMP assays containing species-specific primer sets as previously described (Barkway et al. 2011. BMC Vet. Res. 7:67). Results showed that the LAMP assays were capable of specifically detecting all the seven *Eimeria* species.

To further test their usefulness, the LAMP assays were tested against 18 *Eimeria* samples collected from local poultry farms. The presence of *Eimeria* oocysts in each of the samples was determined using morphological observation. In addition, a PCR-based method using previously described real-time quantitative PCR primers (Vrba et al. 2010. Vet. Parasitol. 174:183-190) was used to detect the presence of *Eimeria* species in the samples. Results of the PCR assays showed that *Eimeria* parasites were identified in 16 out of the 18 samples, with the majority of them showing the presence of multiple species. LAMP assays against the 18 samples showed comparable results. However, a few species that were detected by the PCR assays were not detected by the LAMP assays. This may be due to sequence divergence between strains and imply that further optimisation is required to improve the sensitivity of the LAMP assays in detecting local *Eimeria* populations.

In conclusion, LAMP assays capable of distinguishing all the seven *Eimeria* species infecting chickens were established in this study. Results of this study also provided a toolkit for the development of similar assays for the diagnosis of local *Eimeria* species and strains that are responsible for avian coccidiosis. The availability of such simple and specific detection tools will be valuable in the prevention, surveillance and control of the disease.