REPORT: Experimental studies in Nile crocodiles

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Funds obtained through the CSG Student Research Assistance Scheme were used to cover implementation and running costs of two experimental studies.

The first study was conducted in collaboration with the Community Reference Laboratory in Rome, Italy. The aim of the study was to detect the humoral immune response in crocodiles experimentally infected with *Trichinella zimbabwensis.* An abstract of the article in press is shown below.

Development of an ELISA to detect the humoral immune response to *Trichinella zimbabwensis* in Nile crocodiles (*Crocodylus niloticus*)

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Abstract: Crocodiles are known reservoir hosts of *Trichinella papuae* and *Trichinella zimbabwensis*, two zoonotic parasites that also infect mammals. Since commercial crocodile farming represents a key source of income in several countries, it is important to monitor this nematode infection in both farmed crocodiles and in breeding stocks which are frequently introduced from the wild. For this purpose, an indirect ELISA was developed to detect the anti-*Trichinella* immune response in crocodile sera. New Zealand rabbits were immunized with pooled sera from non-infected farmed crocodiles in the presence of Freund's complete adjuvant. The anti-crocodile serum was then conjugated with horseradish peroxidase. Serum samples from four Nile crocodiles (*Crocodylus niloticus*) experimentally-infected with *T. zimbabwensis* and from four uninfected crocodiles were used to set up the ELISA. The larval burden per gram of muscle tissue was determined by muscle biopsy. Preliminary tests were performed on serum samples from an additional 15 experimentally-infected crocodiles as well as eight wild Nile crocodiles. Among the 19 experimentally-infected crocodiles, seroconversion was observed in 11 animals. The highest antibody response was observed six weeks post infection (p.i.), but in most of these animals, antibodies were not detectable after six weeks p.i. even though live larvae were present in the muscles up to six months p.i.

The second study formed part of a MSc study and had two major goals. In this study, 15 crocodiles were randomly divided into three groups of five animals each to represent high, medium and low infection groups. Crocodiles representing each group were experimentally infected with 10000, 5000 and 2000 larvae/animal respectively. The first goal was to determine the muscle predilection and distribution of *T. zimbabwensis* larvae and to ascertain whether different infection levels had any significant impact on predilection. The results from this study were also compared to those reported in naturally infected crocodiles (La Grange *et al*. 2012). The second aim was to assess the influence of infection intensity on specific biochemical parameters. Deviations from normal parameters of serum glucose, CPK, LDH, AST and ALT were observed and compared to observations in mammalian studies. ELISA

assays were also conducted and the results compared with those reported on in the previous experimental trial (Ludovisi *et al.* 2012).

Results of the first study did confirm that serodiagnostic tests, specifically ELISA is not useful to detect *Trichinella* infections in sylvatic animals since antibody titres can only be detected for a limited period of time (Ludovisi *et al*. 2012). The results of the MSc study are still being collated but it does appear that predilection muscles in crocodiles differ from those in other sylvatic carnivores. Results also showed that the biochemical response of crocodiles to *Trichinella* infection is different to that of mammals. I hope to finish the dissertation and publish the final results by the end of this year.

I wish to take this opportunity to thank the CSG for their generous and kind support without which the projects would not have been possible and will gladly forward copies of the published results from both experimental studies to the CSG as soon as it is complete.