

INVESTIGATING THE EFFICACY OF '5 IN 1' VACCINES IN MACROPODS

Claire Phillips, Damien Higgins, Cathy Herbert, Derek Spielman*

* Presenter, Faculty of Veterinary Science, University of Sydney

Introduction

Marsupials are born with an immature immune system. Most marsupials acquire immunoglobulins by passive transfer in their mother's milk. Late in gestation milk composition changes to resemble that of late lactation in eutherian mammals and the young begin to suckle intermittently. Before this pouch young generate limited immune responses but at this "switch phase" serum immunoglobulin levels reach adult levels (Adamski and Demmer, 2000).

Marsupials may have slower and reduced immune responses than eutherians (Jurd, 1994) but the success of marsupials in general indicates an immunocompetence as effective as that of eutherians. Vaccination studies show macropods produce a significant antibody response which quickly diminishes in the absence of continued challenge (Barnes *et al.*, 2009). A suggestion for the "poor humoral immune responses" seen in marsupials is that the studies used eutherian protocols which may not be appropriate for marsupials.

Orphaned and abandoned macropod joeys and juveniles are often artificially reared following the death of or separation from their mothers. Australian zoos and wildlife parks also maintain colonies of captive juvenile and adult macropods. These animals risk diseases from infectious agents, particularly clostridial and *Dichelobacter* species, *Bordetella bronchiseptica* and *Candida albicans*. Many organisations and individuals caring for these animals vaccinate them with commercial *Dichelobacter nodosus* (Footvax) and 5 in 1 clostridial vaccines (Ultravac 5 in 1) produced for livestock despite a lack of empirical or scientific evidence of their efficacy in macropod species. The macropod immune response to vaccination and thus the most appropriate vaccination protocol are poorly understood.

Administering vaccines poses risks. Foreign protein is introduced into tissues and the skin is broken allowing bacteria and other pathogens to enter which may lead to infection and abscessation or more significant disease. It is important to determine that the vaccinations are effective in adults as giving an ineffective vaccination is poor practice. It is also important to investigate the maturation of the immune system to determine an appropriate age for initial vaccination. Further, it is important to understand the immune response to vaccines to determine a regime that optimises its protective functions.

The reported incidence of tetanus in macropods varies significantly which may be due to the regional distribution of soils contaminated with the bacteria with higher incidences in areas such as northern Australia (Speare *et al.*, 1989). Infected macropods are highly susceptible (Vogelnest and Woods, 2008). Animals may display protruding third eyelids, dilated nostrils, opisthotonus when excited, hyperaesthesia, inability to masticate, respiratory problems within 48 hours and prominent tetany in the muscles of the head, neck, thorax, forelimbs and tail. The disease progresses rapidly despite intervention with antitoxin, antibiotics, sedatives and fluid therapy with death within 96 hours (Ramsay, 1960). Therefore vaccination with a tetanus toxoid is recommended for prophylaxis (Klos and Lang, 1982).

Study aims:

1. To determine whether adult macropods mount an effective immune response (defined as a fourfold rise in antibody titre) to the Ultravac '5 in 1' vaccine using the standard current vaccination schedule for eutherians.
2. To investigate the macropod immune response to vaccination to help determine the most appropriate vaccination schedule for macropods.
3. To determine whether juvenile macropods mount an effective immune response (defined above) to the standard current vaccination schedule for eutherian mammals.

To complete the first two aims 24 adult tammar wallabies were divided into three treatment groups. Two groups were administered Ultravac 5 in 1 using different schedules for boosting and the third group was administered saline as a control. Wallabies were then bled fortnightly until six months post vaccination. ELISA and flow cytometric analyses were used to examine the immune responses to vaccination. To further characterise the macropod immune response antibodies to tetanus in sera from tammars vaccinated once 2-8 years previously were also analysed. To achieve the third aim ELISA tests were run on sera from juvenile eastern grey and red kangaroos previously vaccinated with Ultravac 5 in 1 at Taronga Western Plains Zoo.

Results:

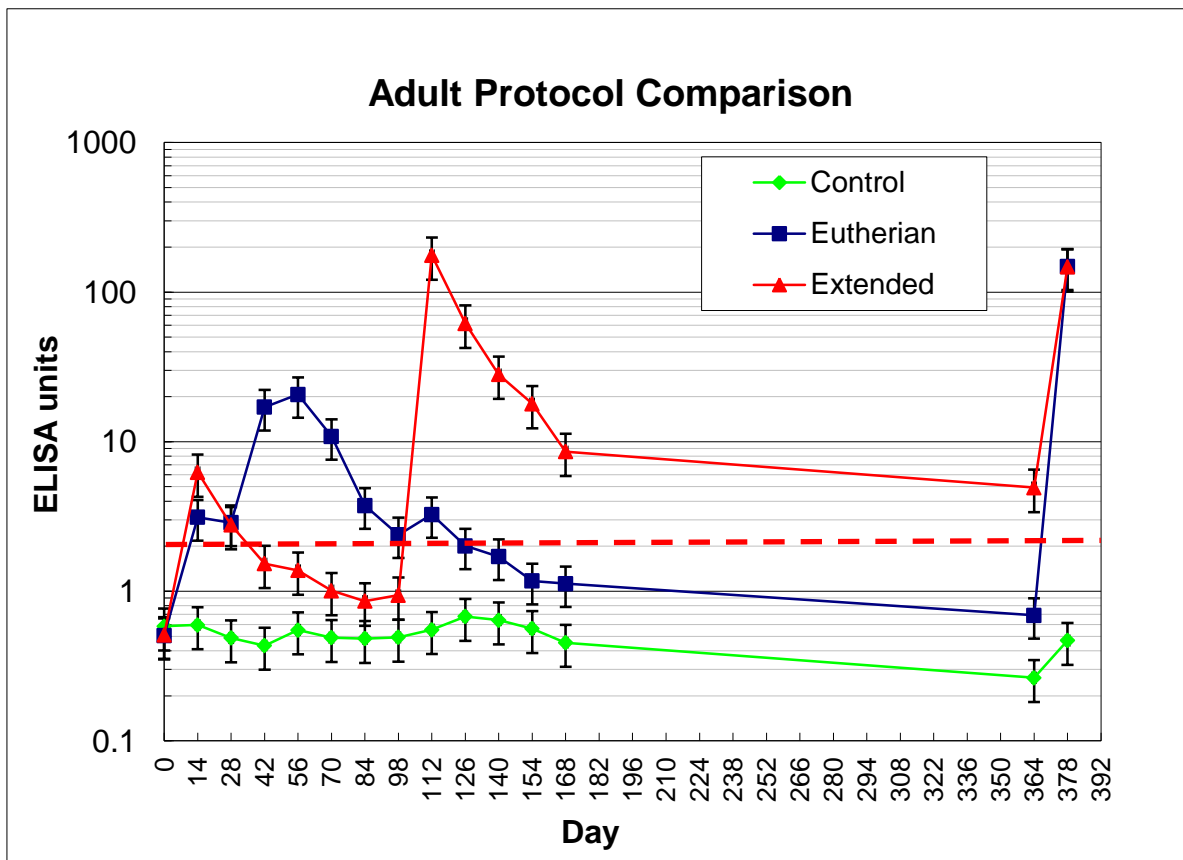


Figure 1. Temporal variation in antibody titres for all three treatment protocols

The ELISA results show the tammar wallabies mounted strong immune responses to the initial tetanus antigen and strong secondary immune responses to booster vaccinations at four weeks. However, titres decreased within six weeks after the first booster injections, as shown in other studies in macropods. Interestingly while titres in the extended group declined to levels similar to control animals and below the

“protective” level, the rapid response following the day 98 booster injection to a much higher level clearly shows persisting immune memory. If the response to protective levels is very rapid this could provide strong and effective immunological protection to repeated challenge. The extended group maintained protective titres until the 12 month booster vaccinations which stimulated equal to stronger responses in both groups.

A number of adult tammar originally vaccinated when in the CSIRO colony in Canberra were sampled years later for another study. Their sera showed presumably protective titres (>2 ELISA units) two years post vaccination and one animal had elevated titres (>0.5 ELISA units) up to 7 years post vaccination.

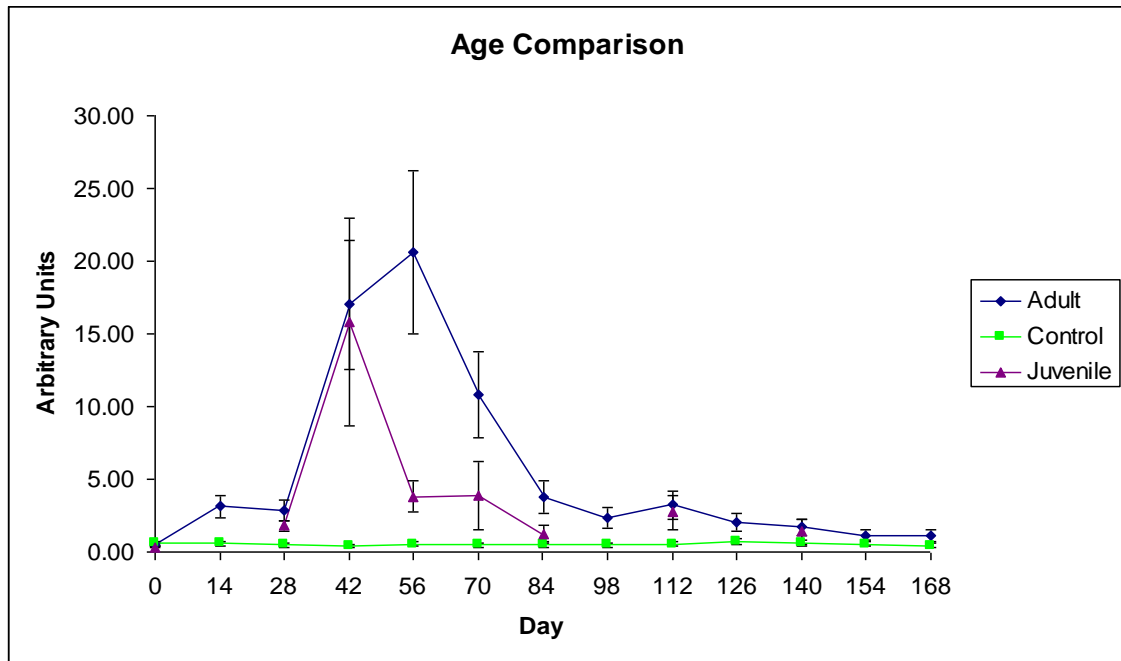


Figure 2. Temporal variation of antibody titres for adult and juvenile eastern grey and red kangaroos vaccinated using the eutherian protocol plus adult controls

Discussion/Conclusion

Juvenile eastern grey and red kangaroos mounted higher immune responses than the adult tammar controls and resemble the immune responses of the vaccinated adults with which they are not significantly different.

This study shows that adult tammar wallabies and juvenile eastern grey and red kangaroos as young as 200-300 days mount measurable immune responses to the tetanus component of the Ultravac 5 in 1 vaccine. The combined results suggest that revaccination or re-exposure can result in a rapid dramatic increase in antibody titres to a protective level and some tammar wallabies retained elevated antibody levels up to 7 years post vaccination. Our limited results indicate an extended protocol where macropods receive a booster vaccination after about 98 days instead of 28 days may produce higher antibody levels. As a “protective” level is unknown in macropods further studies are required to allow more definite recommendations about the value of vaccinating macropods against tetanus and which regime is most effective.

References

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