VACCINATION AGAINST *CHRYSOMYA BEZZIANA*: A SUMMARY OF CURRENT KNOWLEDGE

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ABSTRAK

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Metode pembiakan lalat *the Old World Screwworm Chrysomya bezziana* dalam laboratorium berskala kecil, produksi berbagai stadium lalat berikut jaringan yang dihasilkan yang dapat digunakan untuk studi vaksin dan teknik evaluasi efikasi vaksin secara *in vitro* dan *in vivo*, telah dikembangkan. Metode-metode tersebut akan dapat diterapkan untuk mempelajari lebih lanjut pengendalian lalat *screwworm* dengan menggunakan berbagai teknologi alternatif. Hasil selama ini menunjukkan adanya kemungkinan untuk mengintroduksi efek imunologik secara dramatik lewat pemberian makan larva, mengisolasi dan mengkarakterisasi antigen tunggal. Namun, vaksin rekombinan yang diharapkan masih belum dapat dihasilkan. Pemahaman tentang efek vaksinasi didiskusikan dan berbagai saran dibuat untuk memberi arah penelitian selanjutnya.

Kata kunci; *Chrysomya bezziana*, *Old World Screwworm fly*, vaksin, antigen rekombinan, domba

ABSTRACT

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Methods for the culture of the *Old World Screwworm Fly Chrysomya bezziana* on a small laboratory scale, the production of life stages and tissues suitable for vaccine study, and the *in vitro* and *in vivo* assessment of vaccine effects have all been developed. These methods would be applicable to further studies on screwworm control by many alternative technologies. It has been shown to be possible to induce dramatic immunological effects on feeding larvae and to isolate and characterise individual antigens. However, a recombinant vaccine remains elusive. Current understanding of the effects of vaccination is discussed and suggestions made for possible future research directions.

Key words: *Chrysomya bezziana*, Old World screwworm fly, vaccine, recombinant antigen, sheep

The previous papers in this special edition summarise knowledge gained through a four-year project investigating the feasibility of vaccinating against the screwworm fly, *C. bezziana*.

The field of vaccination against ectoparasites is in its infancy (WILLADSEN and BILLINGSLEY, 1996; WILLADSEN, 1999). Therefore, before such a project can be carried out, it is essential to establish and validate a range of experimental procedures as necessary background for further research. For *C. bezziana*, these procedures included the maintenance of a culture of *C. bezziana* in the laboratory, the production of material which acts as a source of antigens for the development of prototype vaccines and the development bioassays for potential vaccination effects. Success in each of these has been reported in preceding papers. The culture *C. bezziana* has been established and its viability ensured by the routine infusion of wild type flies into the colony. Sources of antigenic material, as described in the preceding papers, have included first and third instar larvae, larval secretory/excretory material, larval cardia and larval peritrophic membrane. Two assays for vaccine effects have been developed. The first measures the direct effect of serum on the growth and viability of larvae in an *in vitro* system. The second is an *in vivo* assay of larval growth and survival which exposes the larvae to the full immune response of the host as well as the physiological differences of individual sheep in an outbred population. These techniques are of generic applicability, not only to the quantitation of immunological effects on screwworm but also for the assessment of alternative control technologies.

With these techniques established, it was possible to examine the feasibility of vaccination. It is worthwhile briefly summarising the results described in detail in the preceding papers. The most effective vaccinations were with native whole peritrophic membrane. Here, *in vitro* results showed, on average, a 73% decrease in larval weights and a 32% decrease in larval survival, an overall decrease in the weight of larvae recovered of 82%. *In vivo*, the results were less dramatic but still striking: a 41% decrease in larval weights and a 13% decrease in larval survival (SUKARSIH *et al.,* 2000). The efficacy on some individual sheep was much greater, and no attempt has been made at any stage to optimise

the immunological responses. The effects after vaccination with cardia were less impressive as measured *in vitro*, though the *in vivo* results were comparable with those of the peritrophic membrane vaccinates. Extracts of whole first and third instar larvae were assessed only *in vitro* and, though they gave some inhibition of larval growth, these immunogens were not studied further. Vaccination with serine proteases from secretory/excretory material showed no effect *in vitro* but a significant reduction in growth coupled with an increased survival *in vivo*. Vaccinations with peritrophic membrane fractions were, in general, less effective than the full membrane, while vaccination with recombinant versions of individual peritrophic membrane proteins either showed no effect or a slight exacerbation of the infestation.

The challenge is to draw these observations together into a pattern that might lead to productive future research.

The first lesson is that both *in vitro* and *in vivo* assay systems have a role to play in any future work. The fact that the results of the two assays are, in many instances, not directly comparable demonstrates that important biological information can be obtained from each of them. For example, in two experiments, accurate weights for larvae feeding *in vitro* and *in vivo* on individual sheep vaccinated with whole peritrophic membrane were obtained. These weights showed no significant rank correlation, possibly due to differences in feeding physiology and immunological reactions in the two systems. In the final analysis, it will always be the results *in vivo* which are critical for the development of a vaccine.

When the project began, the only myiasis fly that had been investigated in any detail was *L. cuprina*. Superficially, the feeding behaviour of *L. cuprina* and *C. bezziana* is similar. It is important therefore to recognise that the current research has shown the dangers of extrapolating from one parasite to the other. With *L. cuprina*, *in vitro* and *in vivo* assays are generally correlated. As above, with *C. bezziana,* this is not the case. With *L. cuprina*, the effects of vaccinating with proteases have been equivocal. With *C. bezziana*, the results are more significant, even if they appear at first sight to be contradictory, a mixture of effects which simultaneously inhibit growth but slightly enhance survival. With both parasites the peritrophic membrane is the most effective source of protective antigens. In both parasites, there is a correlation between larval weights and larval survival in the *in vitro* assays, suggesting similar effects might be occurring. In *C. bezziana*, this correlation is not found *in vivo*. In *L. cuprina*, the effect appears to be due to blockage of the peritrophic membrane by the binding of antibody. Whether this is the case with *C. bezziana* is unknown.

The interaction between any pathogenic organism and the immune system is enormously complex. It is well recognised that this interaction can lead to both protection and the exacerbation of disease or pathology. Much attention has focused on the various arms of the immunological response, as exemplified by Th1 and Th2 paradigm in rodents and humans. In working with complex parasites and parasite extracts, as with *C. bezziana*, there is an added complexity in that the host is exposed to a very large number of immunogenic molecules during vaccination, some of which are likely to be appropriate targets for a protective response, while others might, through the interaction with the immune system, stimulate pathology or facilitate parasite development.

The current work provides evidence for both protection and exacerbation of disease. With the limited evidence currently available, the factors which facilitate larval survival and those which inhibit larval growth and decrease survival are equally a matter of speculation. Nevertheless, such speculation is important since it may suggest directions for further research.

There are two lines of evidence suggesting that exacerbation of the infection can occur or, at least, can be an environment advantageous to the larvae generated through the induction of an immunological response. Firstly, there is a positive correlation between antibody titre to the SDS soluble immunogens from the peritrophic membrane and the weight of larvae recovered *in vitro* (SUKARSIH *et al*., 2000). Secondly, an increase in larval recovery *in vivo* occurs following vaccination with proteases and recombinant PM 48 expressed in *Pichia*. It is important that these effects, though scientifically interesting, appear to also be quantitatively small.

Consider the increased larval survival from sheep vaccinated with some antigens. One explanation might be an increased inflammatory response in vaccinated sheep. Feeding larvae of *C. bezziana* cause wound inflammation, probably made worse by the concomitant bacterial infections. If an immunological response adds to the inflammation, this may be to the advantage of the parasite. The peritrophic membrane is excreted continuously by larvae and so must be present in the wound. Alternatively, some of the proteins from the membrane may also exist in soluble form, even though they were obtained experimentally by extraction with detergents or denaturants. The PM95 antigen from *L. cuprina,* which experimentally is only solubilized in 8M urea, is also found in a soluble form in excretory/ secretory material (TELLAM *et al*., 2000). Nevertheless, current evidence is that Cb42, Cb48 and Cb15 all fail to stimulate a significant immunological response in sheep exposed to *C. bezziana* infections under natural conditions.

It is also difficult to understand the results from vaccination with proteases. One can imagine that antibody to proteases may inhibit growth either directly or through inhibiting wound development but it is harder to see why this inhibition of growth is accompanied by higher larval recovery. Again, it must be remembered that the increases in larval recovery, though statistically significant, are relatively small.

Far more importantly, immunological inhibition of larval growth and survival was the aim of this project. This has been found. Protection is seen in the vaccination with peritrophic membrane, inhibition of growth by vaccination with proteases, vaccination with cardia, and, to a lesser extent, through the *in vitro* results of vaccinating with fractions derived from the peritrophic membrane. The results still fall short of an effective vaccine, though it must be remembered that even the *in vivo* assay system is still only a model for a true, natural infection, severely limited in duration and effect on the host on ethical grounds and for reasons of experimental reproducibility. It is possible that a longer exposure to the host's immune system may also lead to greater larval mortality, while limitation of wound development by vaccination may limit the successive cycles of egg laying and larval infection that typify a field infestation. This is currently a matter for speculation.

It would be desirable to clarify the mechanism of those effects that have been observed. Research with *L. cuprina* suggested a simple mechanism, blockage of the peritrophic membrane by antibody (CASU *et al*., 1997; TELLAM and EISEMANN, 1998) which may well not obtain with *C. bezziana*. The evidence against it is tentative. With *L. cuprina*, this mechanism leads to clear effects *in vitro,* since it depends solely on antibody. For *C. bezziana,* although the whole peritrophic membrane has been found to be an effective immunogen, the results of vaccination with recombinant versions some of the major peritrophic membrane proteins have been negative although the antibodies bind to the peritrophic membrane. It may be, for example, that the size of pores in the peritrophic membrane of *C. bezziana* is such that blockage is more difficult to achieve. These factors can be addressed experimentally and to do so would be illuminating.

If simple blockage of the peritrophic membrane is not the mechanism of immune protection, then the protection that has been observed with some fractions may be due to more specific, more functional interaction between the immune system and the parasite. This is an exciting possibility, since it suggests that better immunological protection might be achieved if such functionally important targets could be identified.

The experimental difficulties in doing so are formidable. The target antigens may be minor rather than major components of the immunogenic extracts use to date; it would be necessary to disentangle the protective responses from those that enhance survival and much of the experimentation would rely on of the use of *in vivo* assays. Nevertheless, could this be achieved, the results would be of great scientific interest and considerable practical value in animal production systems in large areas of the tropical and sub-tropical world.

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