

AN INVESTIGATION OF THE FEASIBILITY OF VACCINATING AGAINST THE OLD WORLD SCREWORM FLY, *CHRYSOMYA BEZZIANA*

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ABSTRAK

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Masalah lalat *the Old World Screwworm Chrysomya bezziana* dan keterbatasan metode pengendaliannya diuraikan secara singkat. Setiap usaha untuk meneliti kelayakan vaksinasi terhadap lalat, merupakan rujukan teknologi pengendalian, yang menuntut penguasaan teknik pembiakan lalat sekaligus produksi sumber antigen dan pengembangan teknik pengujian yang cocok bagi penilaian terhadap efek vaksinasi. Ini semua harus dirangkaikan untuk menyusun suatu strategi pengembangan vaksin myiasis. Strategi ini diuraikan sebagai pendahuluan dari suatu seri makalah tentang evaluasi kelayakan vaksinasi secara lebih rinci.

Kata kunci: *Chrysomya bezziana*, lalat *Screwworm*, vaksin

ABSTRACT

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The problem of the Old World Screwworm fly *Chrysomya bezziana* and the limitations of current methods of control are discussed briefly. Any attempt to investigate the feasibility of vaccinating against the fly, as a novel control technology, demands the establishment of methods for fly culture, the production of sources of antigens and the development of assay techniques suitable for the assessment of vaccination effects. This must be coupled to a strategy for vaccine development. This strategy is described, as a prelude to a series of papers evaluating the feasibility of vaccination in detail.

Key words: *Chrysomya bezziana*, screwworm fly, vaccine

The Old World Screwworm fly *Chrysomya bezziana*, an obligatory parasite of warm-blooded animals, was first recognized in Indonesia in 1938 during an investigation of hoof myiasis of cattle in North Sulawesi (KRANEVELD and PETTINGA, 1948). This fly can be found in tropical and sub-tropical South East Asia, Africa, the Indian subcontinent and throughout New Guinea (NORRIS and MURRAY, 1964). Observation by MUCHLIS and PARTOUTOMO (1973) in North Sulawesi estimated about 20% of cattle were suffering from myiasis, and in South Sulawesi and Sumba showed that roughly 10% of the cattle raised on ranches were infected by *C. bezziana* (SIGIT, 1978). Incidences of myiasis caused by *C. bezziana* were also reported in pigs, horses and goats in West Java, West Timor and Sumba (SUKARSIH *et al.*, 1989). The current problems caused by *C. bezziana* and case histories of its control have been described recently (REICHARD, 1999).

Two technologies are currently available for the control of both Old World and New World Screwworm

fly. The first is the use of pesticides, the second sterile male release. The use of pesticides has been frequently documented, though not in a particularly systematic fashion. Any spray-on pesticide is potentially beneficial, though penetration of pesticides into deep wounds is a serious limitation in many cases. The issues with the use of pesticides are now becoming widely recognised. Many of the older chemicals have unacceptable human toxicity, or environmental consequences. The ability of insects and other arthropod parasites to become resistant to pesticides has been extremely well documented, although no clear evidence for such resistance has been reported for *C. bezziana* in the scientific literature as yet. This may reflect the absence of research in this area rather than the intrinsic inability of *C. bezziana* to develop resistance. Systemic pesticides like the macrocyclic lactones can be very effective against the larvae of *Chrysomya*, though it would seem that relatively little use has as yet been made of this class of chemicals. In large measure, this

may be because they remain relatively expensive. With the expiry of patent coverage on some of these pesticides, the price of generic products has declined dramatically and they may become more generally available. Nevertheless, these chemicals may not be free from environmental effects. The importance of pesticide residues in dung for the ivermectins has been much discussed. In the absence of access to higher priced or toxic chemical pesticides, a variety of home remedies have been used at the village level for the control of *C. bezziana*.

The second control technology is use of sterile male release. This was first postulated by KNIPLING (1955). This has been a major success with the New World Screwworm fly (*Cochliomyia hominivorax*) in southern United States and Mexico (KRAFSUR *et al.*, 1987) and subsequently in Libya (LINDQUIST *et al.*, 1992). The technology is being adapted for *C. bezziana* at a joint Australian Malaysian research facility in Malaysia. The use of sterile male release in eradicating a potential incursion of *C. bezziana* into Australia has been examined in a sophisticated econometric and biological model (ATZENI *et al.*, 1994). Practice in the United States and Libya, and theory in case of the screwworm model for Australia, all indicate that even where sterile male release is used as the dominant control or eradication technology, intensive use of pesticides also plays a part in the control of regional outbreaks and in the suppression of the population of wild type flies (LINDQUIST *et al.*, 1992; ATZENI *et al.*, 1994; REICHARD, 1999). In the case of the model for Australia for example, intensive use of pesticides is a background assumption in the development of the model. Removal of this assumption, *i.e.* a sterile male release in the absence of pesticide application, escalates the difficulty and cost of eradication enormously.

Against this background, the collaboration between CSIRO, Balitvet, Inter-University Centre on Biotechnology, Institute of Technology Bandung, with the support of ACIAR has looked at the feasibility of vaccinating against *C. bezziana* as an alternative control technology.

Aim of vaccination

Vaccines, particularly recombinant vaccines, have a number of theoretical advantages: low cost of manufacture, stability, ease of distribution, freedom from environmental contamination and specificity for the target pest. They are easy to apply and arguably less likely to be misapplied or abused than are chemical pesticides. The great problem is that there is currently only one recombinant vaccine against an ectoparasite world wide, that against the tick *Boophilus microplus* (WILLADSEN *et al.*, 1995; WILLADSEN, 1999) and attempts to vaccinate against a variety of insect species

have been discouraging. The real reasons for poor or variable results with a number of species are very difficult to determine. The amount of research done in this field, as opposed to the total research done in pesticide development, is very small indeed.

Strategy of vaccine development

Given that the total resources which could be put into this project were restricted, the strategy for examining the feasibility of vaccination had to be considered carefully. In the first instance it was necessary to establish a small colony for the production of *C. bezziana*, both as a source of antigenic material in the development of prototype vaccines and also as a source of challenge material for vaccination trials, both *in vivo* and *in vitro*. Then, it was necessary to develop assay systems for vaccine effects. Both *in vitro* and *in vivo* assays have been developed (PARTUOTOMO *et al.*, 1998; SUKARSIH *et al.*, 2000) largely by adapting assay techniques developed for the sheep blowfly *Lucilia cuprina* (EISEMANN *et al.*, 1989).

There is a great deficiency of scientific information relevant to the development of such a vaccine. The only close parallel was *L. cuprina*, another myiasis fly and a major problem for the Australian sheep industry. Some decades of research, largely in Australia, had examined immunological responses to natural blowfly infestation and the possibility of vaccination. Briefly, the conclusions from this research were as follows. Firstly, there was little evidence for naturally acquired immunity, even to repeated infestations with fly larvae (EISEMANN *et al.*, 1990). Immune responses were induced and they had some, usually marginal effects on the growth of larvae, but the responses were frequently transient and not sufficiently protective to be an effective control measure. A number of experimenters had looked at vaccination with excretory/secretory material from *L. cuprina* and also at the proteases contained in excretory/secretory material (TELLAM and BOWLES, 1997). Here results were more encouraging, but highly variable. Finally, a large project which began by the pragmatic fractionation of larval material had led to the identification of a series of novel antigens and a novel immune mechanism. The gut of *L. cuprina*, as in most insects, is lined by a peritrophic membrane, a semi-permeable membrane which separates the ingested meal from the gut epithelium of the feeding larvae. Vaccination of sheep either with this membrane or with some of the proteins embedded in the membrane induces an antibody response in the sheep. Subsequent ingestion of the antibody by feeding larvae led to blockage of the membrane and dramatically decreased larval size (WILLADSEN *et al.*, 1993). If the inhibition of growth was sufficiently strong, larval mortality occurred.

The project recognised from the beginning the danger of extrapolating results from one species, *L. cuprina*, to another, *C. bezziana*. It was also recognised from the beginning that a comparison of vaccination results with these two species, which superficially share similar biology, could give a great deal of information about the generic nature of vaccine results obtained to date.

Recognising the limitations and also the opportunities offered by the prior work on *L. cuprina*, three strategies were adopted in parallel. Firstly, the project undertook a *de novo* search for antigens from the accessible crude materials or dissectable tissues. These were the first and third instar larvae and the cardia. Secondly, in view of the encouraging results with *L. cuprina* and some other parasites, it was decided to investigate the vaccine efficacy of the major class of proteases contained in larval secretory/excretory material. Thirdly, in view of the results with *L. cuprina*, it was decided to investigate in more detail the efficacy of the peritrophic membrane as a vaccine antigen and subsequently of proteins obtained from the peritrophic membrane. This third strategy itself was divided into two components. Firstly the peritrophic membrane was fractionated and native proteins from that membrane were tested as vaccine antigens. Secondly, since complete gene sequences were available for a number of peritrophic membrane antigens from *L. cuprina*, it was decided to look for homologous gene products in *C. bezziana* and to assess these as potential antigens, thus circumventing the need for the difficult process of protein purification, characterisation and gene isolation.

The following collection of papers reports the outcomes of this strategy.

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