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Optimisation and Rationalisation of Cattle Immunisation against *Theileria parva* in Eastern Zambia

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The most serious constraint of cattle rearing in Eastern Zambia has been East Coast Fever (ECF), and acute and often lethal tick-borne disease caused by the protozoan *Theileria parva*. A method of immunisation known as "infection and treatment" was developed in the seventies and has, so far, been the only efficient and available immuno- prophylactic control method. It is done by inoculating cattle with a stabilate of live *T. parva* sporozoites isolated from infected ticks, simultaneously to an injection of long-acting tetracycline. The method is based on the observation that animals recovering from ECF develop a strong immunity against subsequent homologous challenges. Unlike tick control, it needs to be applied only once in an animal's life span and, unlike chemotherapy, it is administered without emergency, in an organised manner.

At the beginning of the study, immunisation against ECF in Eastern Zambia was not sustainable. It was free of charge for the farmers. It was carried out by expatriate experts using four-wheel-drive vehicles to transport the vaccine stored in liquid nitrogen. Additionally, all vaccine titrations were done *in vivo*. The objective of this thesis was to confirm the suitability of immunisation to the epidemiological situation, to assess its efficiency and side-effects, to simplify its storage and distribution and to reduce its cost without diminishing its efficacy.

Two longitudinal studies allowed the characterization of the endemic and epidemic epidemiological states in the field. The effect of the climate on the tick phenology and on their vectorial capacity was demonstrated. The existence of a second tick generation p.a. seems to be important for the epidemiology of ECF since it allows the infecting stages of the vector and those prone to infection to feed simultaneously on the same host. Additionally, it shortens the interval between the infection of nymphs and the transfer of the infection by the adults. The deleterious effect of this interval on the infectivity of *T. parva* was confirmed.

Whilst endemicity seems unavoidable, it is imperative to protect the young stock against ECF given the severity of clinical reactions observed in the field. Immunisation by the infection and treatment technique seems to be the most appropriate method. The use of a univalent stock of *T. parva* seems preferable than the trivalent «Muguga cocktail». It appeared that mixing stocks that are appropriate for immunisation on their own might lead to an unsuitable cocktail. When trying to optimise one of the components of a cocktail, another one might become inefficient simply because the efficiency curves of the components are not in phase while the doses are linked. Furthermore, from a theoretical point of view, the use of foreign *T. parva* strains in the vaccine represents an additional risk as it might complicate the prevailing epidemiological situation.

This study also demonstrated that immunisation against ECF by the infection and treatment method using the Katete strain of *T. parva* induces a carrier state in cattle. This characteristic allows the infection to be picked up by both, the larvae and the nymphs of the vector tick. Such ticks were able to induce severe and even lethal clinical reactions in susceptible cattle. Larvae fed on carriers were however less infected than nymphs. The existence of a carrier state and the lethality of the reactions induced after transfer to susceptible cattle are important from the epidemiological point of view. They imply that immunisation by the infection and treatment method is not recommended in ECF free areas.

Maternal immunity does not seem to interfere with immunisation, but some data suggest that maternal antibodies reduce the seroconversion in calves.

The use of ice bath for field delivery of stabilates would make the cold chain less stringent as compared to liquid nitrogen containers. In experimental conditions, stabilates retained 95% of their initial efficiency after six hours of

storage at 2°C. A field trial indicated that stabilates kept for four to six hours on ice was even more efficient than stabilate thawed on the spot. This was probably due to the fact that deferred immunisation allows the use of more homogenous material. The transport of sporozoite stabilates on ice should allow a cheaper delivery, whereby motorbikes or bicycles are used instead of vehicles. Additionally, the use of a cheaper long-acting tetracycline was proposed since the efficacy of an acid formulation as chemotherapeutic agent for ECF immunisation was proven.

Three *T. parva* sporozoite stabilates were successfully lyophilised. The observation of schizonts and piroplasms in inoculated animals unequivocally proved that part of the sporozoites survived the process of lyophilisation, even if a great number of them were probably destroyed. The lyophilisation of stabilates seemed to allow a less stringent middle-term storage. The inoculation of lyophilised stabilate kept for two weeks in a fridge or for 12 weeks at -20°C induced clinical and parasitological reactions in susceptible animals.

Finally, a convenient method of *in vitro* titration of immunising stabilates was proposed. It is based on the *in vitro* infection and transformation of lymphocytes by *T. parva*. The fungal contaminations and the fragility of the cultures used to be the main constraints of the method. An experiment showed that fungi could be eliminated from stabilates by centrifuging the latter at 400 g for 10 minutes. Nystatin and flucytosine did not seem to interfere with the *in vitro* development of *T. parva* but their effect on fungal growth was limited. Titration was done in 96 well culture plates. Cultures were kept for 10 days at 37 °C in an atmosphere containing 5% CO₂ in air. The presence or absence of parasitic development was analysed in function of stabilate dilutions. *In vitro* titration turned out to be more sensitive than *in vivo* testing.

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List of articles published from thesis results:

1. Billiow M., Mataa L., Marcotty T., Chaka G., Brandt J. & Berkvens D., 1999, The current epidemiological status of bovine theileriosis in eastern Zambia. *Trop. Med. Int. Health*, 4: pp. A28-A33.
2. Marcotty T., Billiow M., Chaka G., Berkvens D., Losson B. & Brandt J., 2001, Immunisation against east coast fever by the infection and treatment method: evaluation of the use of ice baths for field delivery and appraisal of an acid formulation of long-acting tetracycline. *Vet. Parasitol.*, 99: pp. 175-187.
3. Marcotty T., Brandt J., Billiow M., Chaka G., Losson B. & Berkvens D., 2002, Immunisation against *Theileria parva* in eastern Zambia: influence of maternal antibodies and demonstration of the carrier status. *Vet. Parasitol.*, 110: pp. 45-56.
4. Marcotty T., Berkvens D., Besa R.K., Losson B., Dolan T.T., Madder M., Chaka G., Van den Bossche P. & Brandt J., 2003, Lyophilisation and resuscitation of sporozoites of *Theileria parva*: preliminary experiments. *Vaccine*, 22: pp. 213-216.
5. Marcotty T., Speybroeck N., Berkvens D., Chaka G., Besa R.K., Madder M., Dolan T.T. & Losson B., 2004, *In vitro* titration of *Theileria parva* tick derived stabilates. *Parasitology*, 128: pp. 131-137.

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