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Titrating *Theileria parva*: single stocks against combination of stocks.

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Abstract

Theileria parva is the causative agent of East Coast fever (ECF), an important cattle disease in East and Central Africa. One of the methods for control of ECF is 'infection and treatment', a procedure in which an animal is infected with the live parasite and at the same time treated with a long-acting oxytetracycline formulation, restraining the infection and allowing a protective cellular immune response to develop. Optimal immunizing doses were estimated using models of trichotomous response: dysimmunization (death or severe reaction during immunization), immunization failure (death or severe reaction during lethal challenge) and successful immunization (neither dysimmunization or immunization failure).

In this paper we present methods of interpreting immunization trials and apply these methods to previously unpublished data from two such trials: one with a mixture of three *T. parva* stocks and one with a single *T. parva* stock.

We explain why titration trials conducted with cocktail of antigens could predict a suboptimal immunization dose. Indeed it is possible for a combination of three individually efficient stocks to result in a mixture with which optimal immunization response might be difficult to achieve, because of averaging effects.

The corresponding interpretation provides insights into why standard immunization trials for *T. parva* have not yielded the results that might be expected of them. The results of this work may also have implications for the use of antigen cocktails in cancer, HIV and malaria vaccine trials.

Key words: *Theileria parva*; immunization, Statistical modelling.

Introduction

Theileria parva is the causative agent of East Coast fever (ECF), a cattle disease in East and Central Africa, which costs US $ 186 million annually (Mukhebi et al., 1992). Animals that recover from ECF show a long-lasting immunity against a homologous challenge (Burridge et al., 1972) but may become infected and even die, if challenged with a heterologous parasite stock (Cunningham et al., 1974). One of the methods of ECF control is immunization by 'infection and treatment' (Radley et al., 1975; Radley, 1981) in which an animal is infected with the live parasite and at the same time treated with a long-acting oxytetracycline formulation. In this way a protective cellular immunity is stimulated. The benefit of immunization lies in the long-lasting immunity that is engendered provided cattle are not subsequently exposed to heterologous challenge (Goddeeris et al., 1994). In Zambia, the most cost-effective method of controlling *T. parva* was reported to be by immunization (Minjauw et al., 1998).

Cross-protection between different stocks (e.g. from different parts in Africa) may be observed, but the patterns of protection are variable. Indeed, immunity appears to be strain-specific on heterologous cattle-derived parasite challenges., although some cross-immunity between stocks is likely (Goddeeris et al., 1994), and it can therefore be assumed that the sporozoites from one stock will result in different degrees of protection against those from another stock (Geysen, 2000). As yet, no master immunizing stock has been identified. (Radley et al., 1975) recommended the use of a mixture, or so-called "cocktail", of stocks in one immunizing dose to provide broad protection against ECF. Such an approach gave effective protection to cattle in the field when exposed to infections from many different areas (Uilenberg et al., 1977). Although no truly commercial vaccine for ECF exists, the "infection and treatment" method, using either a single stock or a cocktail, has been used extensively in the field (Lynen et al., 1992), usually in programs sponsored by donors or supported by governments. In "in vivo" titration trials, used to define the optimal immunizing dose, cattle are inoculated with 1 ml of

stabilate, diluted serially on a logarithmic scale. Later, the animals are challenged with a lethal dose to appraise their immunity.

Statistical methodology to analyze titration data from this sort of trial has never been formally presented. The use of multiple PD_{50}'s (PD = Protective dose) has been proposed (Musisi et al., 1992) to define an appropriate dose. The responses in such trials were originally considered dichotomous, and analysis was carried out by a logistic regression on the probability that an immunized animal develops serious clinical disease or dies following a lethal homologous ECF challenge, i.e. that the animal experiences "immunization failure ". However, the outcome following infection with *T. parva* is dose-dependent (Dolan et al., 1984): a dose needs to be increased sufficiently to acquire infection (Mutugi et al., 1988), but increases the risk that an animal shows a severe reaction following immunization requiring additional treatment (and which may result in death), i.e. that the animal experiences "dysimmunization" (Marcotty et al., 2001). Dysimmunization was initially neglected because the objective was to obtain immunity. Similar to immunization failure , dysimmunization can also be analyzed using logistic regression. There are thus three possible outcomes of a titration trial: dysimmunization, immunization failure and successful immunization. The purpose of titration trials in cattle thus should be to derive a dose of the stabilate(s) that is efficient, i.e., that is both safe (i.e. low dysimmunization) and efficacious (i.e. low immunization failure) and maximizes the chances of successful immunization.

In this paper we propose models which can assist in interpreting commonly practiced immunization trials. The three likely outcomes of a titration trial are best analyzed by means of a multi-category extension of logistic regression. We compare three of these models, for their ability to describe sample data sets obtained from immunizations of cattle and apply these methods to previously unpublished data from two titration trials: one with a single stock of infectious *Theileria* sporozoites (*T. parva* Katete, used in eastern Zambia) and one with a mixture of three different stocks of infectious *Theileria* sporozoites (i.e. the Muguga cocktail used in, for

example, parts of Tanzania). We indicate how results from titration trials using a mixture of three different stocks (i.e. a cocktail) can be hard to interpret and that the combination of individually efficient stocks can lead to an inefficient mixture.

Materials and Methods

An ECF control method is immunization by 'infection and treatment' (Radley et al., 1975; Radley, 1981) in which an animal is infected with live parasite and at the same time treated with a long-acting oxytetracycline.

The responses of cattle to immunization can be grouped into three categories: dysimmunization, successful immunization or immunization failure (immunized but not protected). Dysimmunization means that the animal shows a severe reaction to the immunizing procedure and requires an additional treatment; the animal may even die (Marcotty et al., 2001). Criteria for defining severe reactions are found in (Morzaria et al., 1987) and (Anonymous, 1989). Immunization failure is recorded when an immunized animal develops severe clinical disease or dies following a lethal ECF challenge. Finally, an efficient or successful immunization is one that results in neither dysimmunization nor immunization failure.

The character of an 'ideal' stabilate with nearly 100% successful immunization is shown in Figure 1 for the corresponding multicategory logistic model. Possible variability among and within production runs of the same vaccine stock(s) necessitates a broad successful immunization plateau. Both virulent and mild stocks exist. For example, a very virulent stock requires a high dilution—e.g. 1/1000—to avoid dysimmunization.

The natural logarithm of dose is used to investigate the relationship between dilution and the reaction of an animal. Galton (Galton, 1879) pointed out that variation between individuals in their susceptibility to biological material exhibits a geometrical (logarithmic) rather than an arithmetical (linear) distribution, a finding confirmed in the majority of pharmacological and

toxicological studies (Govindarajulu, 2001). Thus, logarithm of dose can be viewed as an index of the inherent susceptibility of the individual to the infective material.

The titration data

The first trial was conducted between March and September 1997 at the Vaccine Production Centre in Lilongwe, Malawi. The data were provided by the African Unity/Interafrican Bureau for Animal Resources (AU/IBAR) and the Centre in Malawi. A total of 62 Friesian steers aged between 6 and 9 months were used. They were purchased in an ECF-free area, with no obvious infectious disease and negative for *T. parva* antibodies using an Immuno Fluorescent Antibody Test (IFAT) and an Enzyme Linked ImmunoSorbent Assay (ELISA) (Katende et al., 1998). The stabilate tested was the trivalent FAO 1 (FAO = Food and Agriculture Organization) composite stabilate, produced at the International Livestock Research Institute in Nairobi, Kenya and stored in liquid nitrogen (Morzaria et al., 1999). This stabilate consists of a mixture of *T. parva* Muguga, *T. parva* Kiambu 5 and *T. parva* Serengeti. The composite stabilate was prepared ensuring an equal number of infected tick salivary gland acini (Buscher and Otim, 1986) from each stock so that the resultant mixture of stabilate contained an approximately equal number of *T. parva* sporozoites from each stock. Immunization was by the infection and treatment method (Radley et al., 1975). The treatment was by an injection with a long acting oxytetracycline hydrochloride formulation (Duocycline, Univet) at 20 mg/kg bodyweight deep in the gluteal muscles.

Each of the experimental cattle was inoculated subcutaneously in the mid-neck with 1 ml of the appropriate dilution of stabilate. A three-step titration process was used, narrowing in each step the range of dilutions used. We analyzed the merged data because the different steps were conducted one after the other under the same conditions. The following dilutions were used: 1/1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/60, 1/64, 1/80, 1/100, 1/120, 1/128, 1/180, 1/240, 1/256, 1/512 and 1/1024. Five weeks after the inoculation, the cattle received 1 ml of the neat stabilate (the same

as the one used during the immunization) containing all three stocks to provide a known lethal challenge.

Titration data for single stocks are scarce. Therefore the data on the Katete stabilate (the second data set) have been compiled from three trials conducted on three occasions between 1986 and 1991 at Chipata, Zambia. The Katete "trial" consists of a combination of the titration results of stocks called V2 and V5 (tested together), V8, and V10, conducted on three occasions between 1986 and 1991. We included only data that were generated using standard stabilate production and titration protocols (e.g. one particular cohort (V6) was excluded as some animals had been administered levamisole during the pick-up). The conditions under which these experiments were carried out were kept as constant as possible using different batches of the same single strain. It is thus assumed valid to group the results together. In total, 66 Friesian steers aged between 6 and 9 months were used. They were healthy and had no obvious infectious disease and were negative for *T. parva* antibodies using IFAT and blood smears. Immunization was by the infection and treatment method (Radley et al., 1975) and the long acting oxytetracycline used was Terramycin (Pfizer Inc.) at 20 mg/kg bodyweight. The animals were inoculated subcutaneously with the stabilates below the ear. The following doses were used: 1/300, 1/100, 1/50, 1/20 and 1/10. Five weeks after the inoculation, the cattle received 1 ml of the neat stabilate to provide a known lethal challenge (the same as the one used during the immunization).

In all trials, animals undergoing severe reactions, as defined in (Morzaria et al., 1987) and (Anonymous, 1989), were euthanized. It is acknowledged that the two trials were done separately. However, in both situations cattle were exotic (i.e. Friesian), managed under good husbandry and feeding conditions and the trials were conducted under similar climatic conditions, the two study areas being physically quite close.

Multi-category logit models

The outcome considered in the titration trials was immunization response, according to the following categories: *dysimmunization*, *successful immunization* and *immunization failure.* Three models were used and compared for this categorical response: a baseline category logit model, a continuation ratio model, and a proportional odds model (Agresti, 1996). These models are explained in more details in an Appendix. Two of the models assume an ordered response.

Although at first, there might not seem to be any natural ordering for these categories, they can be thought of as decreasing response intensity: 3 = “too severe reaction” (*dysimmunization*), 2 = ”appropriate reaction” (*successful immunization*) and finally 1 = ”weak reaction” (*immunization failure*). Consequently, the continuation ratio model can be specified by postulating two models for

log [P(successful immunization or immunization failure) / P(dysimmunization)]

and

log [P(successful immunization) / P(immunization failure)].

The first logit leads to a model for the immunization survival probability (defined as successful immunization or immunization failure), while the second one describes the conditional probability of successful immunization given the survival of the immunization process. On the other hand, the proportional odds model leads to simultaneous modeling of

log [P(successful immunization or immunization failure) / P(dysimmunization)]

and

log [P(immunization failure) / P(dysimmunization or successful immunization)].

The first logit corresponds to its continuation ratio analogue. The second leads to a model for the probability of immunization failure.

The goodness-of-fit of the models was assessed using the deviance (the difference between the log-likelihood of a specific model with the saturated model) (McCullagh and Nelder, 1989). All three models were fitted using the statistical software package SAS.

Results

The results of both trials are presented in Table 1.

Trial 1: Mixture of three strains.

The deviance of the baseline category logit model (a), the continuation ratio model (b), and the proportional odds model (c) were 29.28 (p-value based on the chi-square distribution with 30 deegress of freedom (df), p=0.503), 29.6689 (df=30, p=0.483), 29.2567 (df=31, p=0.556). The statistics show that all models provided an adequate fit to the data (a small p value is indicative of a significant deviation of the model from the observations).

The observed and fitted relationship between the proportion of dysimmunization, immunization failure and successful immunizations versus the dose is illustrated for the baseline category in Figure 2 (numbers shown in Table 1). The dose generating the most successful immunizations corresponds to the maximum of the middle (i.e. heavy solid) curve, a dose which can be obtained mathematically from the equations of the different models. For example, for the baseline category model this corresponds to a $dose_{max}$ of 1/100.

Trial 2: Katete Strain.

The deviances for the general cumulative model (d), the proportional odds model (c), the continuation ratio model (b), and the baseline category logit model (a) were equal to respectively 3.8700 (df=6, p=0.694), 3.9330 (df=6, p=0.686) and 4.0297 (df=7, p=0.776). Here too, the models provide a good fit to the data (a small p value is indicative of a significant deviation of the model from the observations). The observed and fitted relationship between the proportion of dysimmunization, immunization failure and successful immunizations versus the dose is illustrated for the baseline category in Figure 3 (numbers shown in Table 1). In this case, the optimal immunizing dose for the Katete stabilate is the lowest dose tested in this titration trial (1/300). The optimal dose for the Katete stabilate is 1/300 which is different from the one used for field immunization 1/100 (Lynen et al., 1992), supporting that a wide range of dilutions result in an appropriate successful immunization

Discussion

The predictions of the three different multinomial models considered in this paper are very similar. The deviance statistics also suggest that all models provide a good fit to the data. This might be seen as an argument in favour of the simplest model, i.e. the proportional odds model with three parameters. The two other models, the baseline category model and the continuation ratio model have four parameters. The proportional odds model assumes an ordering of the response categories. Although there might not seem to be any ordering in the outcomes of the titration trial, they can be thought of in terms of decreasing response intensity: 3 = "too severe reaction" (*dysimmunization*), 2 = "appropriate reaction" (*successful immunization*) and finally 1 = "weak reaction" (*immunization failure*). One could argue that, for instance, to observe a *immunization failure* or a *successful immunization* the animal has to survive, thus it cannot be *dysimmunized.*

The intention of this paper is not to offer a defense of the advantages of using single strains over mixtures for vaccination. Instead, we focus on the titration trial results indicating that determining an optimal vaccinating dose based on a titration with multiple strains of *T. parva* can be difficult. The proportion of animals with a successful immunization using the Katete stabilate (about 90%) stands in contrast with the relatively low proportion found with the cocktail (about 60%). This is not necessarily due to a low efficacy of the individual stabilates but rather can be viewed of as a consequence of mixing. As illustrated in Figure 4, dysimmunization (immunization-related mortality) for a virulent stock in the cocktail increases rapidly when increasing the dose, while a mild stock allows a much higher dose before dysimmunization becomes critical. Due to the presence of the virulent stock, the mild stock cannot be sufficiently increased to obtain an optimal immunization failure. Indeed, if the dose of the cocktail is increased so as to decrease immunization failure of the weakest components, the most virulent components will necessarily induce more dysimmunization. This situation has been reported (Di Guilio et al., 2000), where a 50% higher tetracycline dose than the common

one had to be used to control the dysimmunisation. In further support of the titration trial results, Nambota (1989) reported that in Southern Province (SP) of Zambia from 834 animals immunised with Muguga cocktail, 45 animals died (of which 15 confirmed theileriosis). From 116 animals immunised with T. parva (Mandali-stock), 1 died (confirmed theileriosis). The high mortality with the Muguga cocktail was explained by under dosing with oxytetracyclines or overdosing with Theileria sporozoites. Both trials precisely underline difficulties related to dysimmunization. It would of course be useful to further confront the results reported in this paper with additional results based on field data and especially new titration trials results. Other unpublished reports indeed reveal that once the immunization is induced and, if required, the side effects (including mortality during immunization) controlled, high levels of protection can be achieved using both single and multiple strains. We highlight again that in the past the term immunization "efficiency" was used in different ways. The objective used to be the induction of immunity only. Today, it is (or should be) the induction of immunity without side effects. We therefore indicate that it is necessary to determine whether a balanced cocktail can indeed reach a good efficiency.

Mixing three stocks each characterized by a high immunization success but at different doses can therefore result in a cocktail in which similarly successful immunization rates can never be achieved, merely because of averaging effects across the distinct response curves (i.e. the need to find a balance between the different stocks). Indeed, it is possible for a combination of three individually efficient stocks to result in a mixture with which an optimal immunization response can never be achieved because of averaging effects. It might therefore be foreseen that titration trials conducted with cocktails of antigens of differing virulence will in general predict a sub-optimal immunizing dose.

The titration results of the Muguga cocktail defy conclusions that could be drawn based on the individual stock results. There are in fact a large number of possible combinations of the three stocks which could result in the observed data. If each of the probabilities can be characterized

as a function of dose, the probability of a successful immunization for stock 1 is: $\Pr(Successful_{stock1}) = f_1(dose)$. However, only one general relation $\Pr(Successful_{cocktail}) = f(dose)$ can be estimated in practice. This means that the parameters for two of the stocks can be chosen freely (i.e. are not determined, not identified). Once the stabilates are mixed, the stock causing dysimmunization or immunization failure can no longer be identified unless molecular characterization is used. If cocktails must be used, to ensure efficient mixtures, individual components should be titrated separately before combining, and a dosage for each single isolate and the optimum ratio of each stock should be tested. This calls for an analysis of each stock before constructing a cocktail. That this has not been done in the case of *Theileria* vaccine is partly due to constraints of time and money, but also to other complicating factors, such as interaction between stocks and cross-immunity: since different stocks are not 100% immunologically independent, this would complicate any careful study.

Unfortunately, the problem might not be solved solely by titrating the individual stocks separately and then finding an appropriate mixing, since mixing three immunoprotective stocks can increase the total number of sporozoites inoculated, and thereby increase the risk of dysimmunisation.

To see this, assume (without loss of generality) that x1, x2 and x3 are the optimal doses of stocks 1, 2 and 3 respectively, and that the stocks are equally virulent. To avoid dysimmunisation, it follows that a maximum of x1/3+x2/3+x3/3 sporozoites can be injected.

The cocktail therefore contains a suboptimal dose of stock 1 (x1/3 instead of he required x1). Fortunately, however, some cross-immunity between stocks is likely (Goddeeris et al., 1994), and it can therefore be assumed that the sporozoites from one stock will result in some protection against those from another stock. Nevertheless, only if stocks show a high level of cross-immunity will the problem of suboptimal dosing per stock be minimal.

The lack of perfect cross-immunity, therefore, will inevitably lead to a suboptimal number of sporozoites, of each of the separate stocks, in the cocktail (offering protection against a lethal challenge of the same stock). This is evidently in conflict with the initial objective of mixing antigenetically different stocks to produce a universal cocktail.

It can be noted that it is not certain that all three stocks in the Muguga cocktail are different, and it has been suggested that the so-called *T. parva lawrencei* (Serengeti transformed) is in fact identical to the Muguga stock (Bishop et al., 2001; Oura et al., 2004). However, this possibility does not undermine the essential point analysed here, as the presence of two strains instead of three would not eliminate the identification problem inherent in trials with cocktails.

In conclusion, titration and determination of the immunizing dose of a cocktail of *T. parva* stocks for vaccination is more difficult than has been appreciated until now. A separate titration should be carried out on the different components of a cocktail and not on the cocktail itself as has been suggested (Uilenberg, 1999).

Additional controlled or planned studies would be useful in attempting to understand the mechanisms behind the conclusions, but are beyond the scope of our paper. However, we conclude that the current practice cannot identify an optimal immunizing dose for a combination of stocks since the results of trials using cocktails cannot distinguish between the effects of the individual stocks. These findings raise questions that have not been identified previously and which are important in veterinary vaccination research.

Although in both situations cattle were exotic (i.e. Friesian), managed under good husbandry and feeding conditions and the trials were conducted under similar climatic conditions, the two study areas being physically quite close, it is acknowledged that the two trials were done separately. For example in the Katete trial only an IFAT and blood smear were used to assess a negative status of the animals. In both trials PCR might have been more appropriate. We therefore note that the comparison of the results of the two trials in this study must be taken with some caution because different protocols, and oxytetracyclines were used. However, there are no

clear indications that the differences can explain the differences in protection between the Katete stock and the FAO1 stocks. The methodology presented here for multi-component vaccination-dose estimation could be adapted for use in other multi-therapy situations in which there are narrow therapeutic indices, such as for cancer and AIDS/HIV. The combination of individual doses resulting in an optimal pharmacological effect with minimal side effects can then be determined. The findings in this paper also provide a possible warning regarding the dangers of combining stocks when trying to find a malaria vaccine (Clyde et al., 1973; Rieckmann et al., 1979; Pombo et al., 2002) and suggest that a better strategy might be to focus on protection based on a vaccine for a specific area, region or epidemiological situation. *Theileria parva*, an apicomplexan like the parasites that cause malaria, with a similar complex lifestyle. Furthermore, just as the long-term immune response of humans to malaria infection is highly tuned to the invading strain and not to other strains, so is the immune response of the cattle to *Theileria* infection.

Although a relationship exists between *Theileria* and malaria vaccines, it can be noted that in malaria vaccine development efforts, radiation attenuated sporozoites have been tried in animals and humans, in which sporozoites are weakened even before injecting into humans or animals. Also the dose of radiation which renders the malaria sporozoites attenuated enough to invade the hepatocytes, express livers stage specific protein(s) and stops further development, has been repeatedly shown to protect humans without any breakthrough infection. At least in one clinical trial, immunization with the irradiated *Plasmodium falciparum* sporozoite of one strain has been shown to provide sterile protection in human volunteers challenged with another stain originating from another geographical area (Hoffman et al., 2002).

It would of course be useful to further confront the results reported in this paper with additional results based on field data and especially new titration trials results. Other unpublished reports indeed reveal that once the immunization is induced and the side effects (including mortality

during immunization) controlled, high levels of protection can indeed be achieved using both single and multiple strains.

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Appendix: Statistical models for analyzing *Theileria parva* titration trials

The outcome considered in the titration trials was immunization response, according to the following categories: *dysimmunization*, *successful immunization* and *immunization failure*.

In the baseline-category model, every outcome category is considered as an independent (nominal) group and compared separately to the reference category. Precisely, for each response *r* different from the reference response the model posits that:

$\log [P(\text{response } r) / P(\text{reference response})] = \alpha_r + \beta_r x,$

where x is the vector of covariates and α_r and βr are the unknown coefficients to be estimated.

The reference response in the baseline-category model is the *dysimmunization* category. Consequently, this model leads to a system of simultaneous logistic regression models.

Continuation ratio and proportional odds models are appropriate for ordered multi-category responses. Continuation ratio models are of use when responses are such that category *r* can be observed only after progressing through all previous *r-1* categories. Thus, for each response *r* the model specifies that

$\log [P(\text{response} \leq r\text{-}1) / P(\text{response } r)] = \alpha_r + \beta_r x.$

The above equation can be interpreted as a model for the conditional probability of observing responses *1* to *r-1*, given that the response is to be found among category *1* to *r*. The continuation ratio model also leads to a system of simultaneous logistic regression models.

The proportional odds model investigates effects of covariates on the odds of observing a higher category of response *versus* a lower one. Specifically, for each response *r,* the model specifies that log [P(response $\leq$ *r-1*) / P(response $\geq$ *r*)] = $\alpha_r + \beta$ x.

Note that here a common covariates effect β is assumed for all responses. Thus, unlike the baseline category logit and continuation ratio models, the proportional odds model does not lead to a set of simultaneous sub-models for each response category. An attractive property of the model is the invariance of the parameter when response categories are collapsed, a feature not incorporated within the continuation ratio model.

TABLES

Table 1: The observed and predicted proportions for dysimmunization, succesfull immunization and breakdown in the Cocktail titration trial.

	Observed			Predicted			
ln(dilution)	Dysimmunisation	Succesful Immunisation	Breakdown	Dysimmunisation	Succesful Immunisation	Breakdown	Total number of animals
-6.932721	0.00	0.50	0.50	0.00	0.04	0.95	2
-6.239449	0.00	0.00	1.00	0.01	0.15	0.84	2
-5.546177	0.50	0.00	0.50	0.04	0.39	0.56	2
-5.481626	0.00	0.20	0.80	0.05	0.42	0.53	5
-5.193891	0.20	0.20	0.60	0.09	0.53	0.38	5
-4.852905	0.00	1.00	0.00	0.16	0.62	0.23	2
-4.788354	0.40	0.40	0.20	0.17	0.62	0.20	5
-4.606	0.20	0.80	0.00	0.22	0.63	0.14	5
-4.382816	0.20	0.80	0.00	0.29	0.62	0.09	10
-4.159633	0.50	0.50	0.00	0.36	0.58	0.06	2
-4.095082	0.10	0.80	0.10	0.39	0.57	0.05	10
-3.46636	1.00	0.00	0.00	0.60	0.39	0.01	2
-2.773088	1.00	0.00	0.00	0.79	0.21	0.00	2
-2.079816	1.00	0.00	0.00	0.90	0.10	0.00	2
-1.386544	1.00	0.00	0.00	0.96	0.04	0.00	2
-0.693272	1.00	0.00	0.00	0.98	0.02	0.00	2
0	1.00	0.00	0.00	0.99	0.01	0.00	2

Table 2: The observed and predicted proportions for dysimmunization, succesfull immunization and breakdown in the Katete titration trial.

	Observed			Predicted			
ln(dilution)	Dysimmunisation	Succesful Immunisation	Breakdown	Dysimmunisation	Succesful Immunisation	Breakdown	Total number of animals
-5.7	0.00	1.00	0.00	0.05	0.92	0.03	10
-4.61	0.14	0.82	0.04	0.12	0.86	0.02	28

-3.91	0.25	0.75	0.00	0.19	0.79	0.01	12
-3	0.17	0.83	0.00	0.34	0.65	0.01	6
-2.3	0.50	0.50	0.00	0.47	0.52	0.00	10

FIGURES

Figure 1: Proportion of dysimmunized animals (dysimmunization, long dashed line), proportion of animals succumbing to a field challenge (or an artificial lethal challenge) (immunization failure, dotted line) and proportion of animals successfully immunized, as a function of the dose (full line).

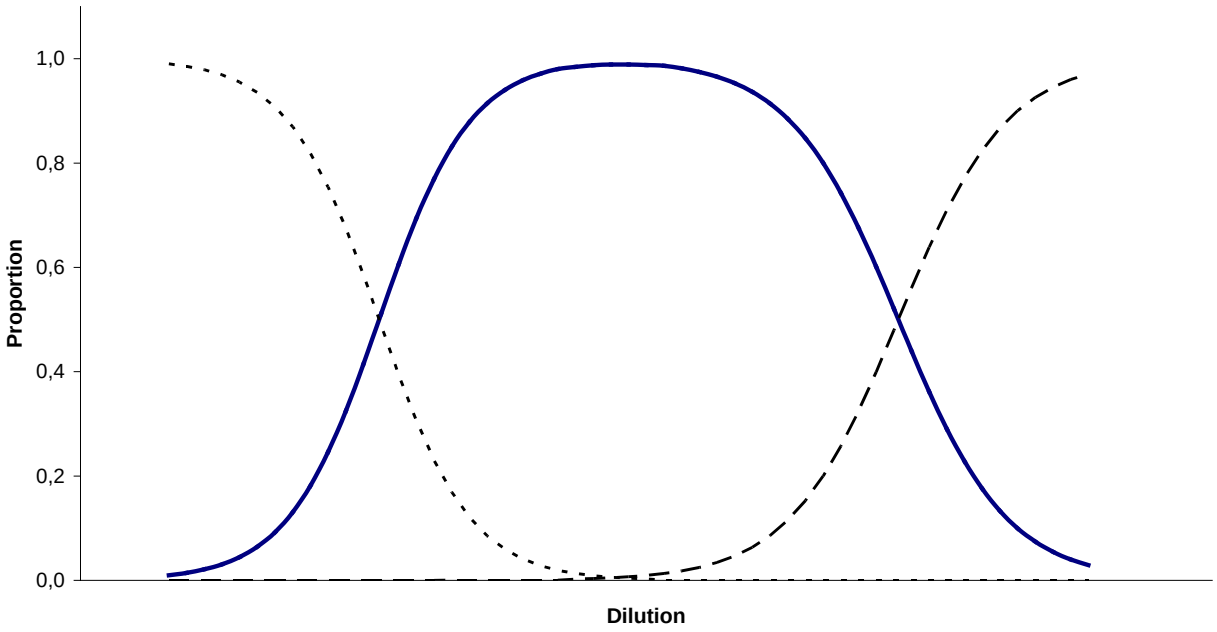


Figure 2: The observed and predicted proportions for dysimmunization, successful immunization and immunization failure in the Cocktail titration trial.

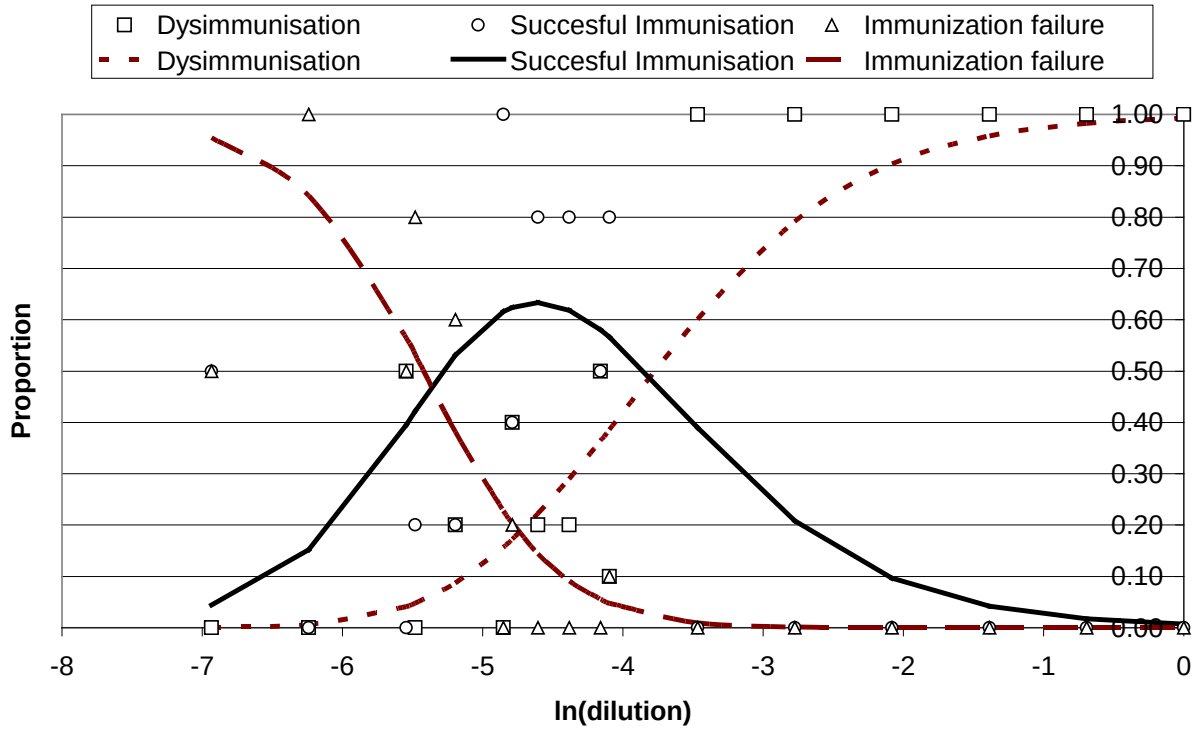


Figure 3: The observed and predicted proportions for dysimmunization, successful immunization and immunization failure in the Katete titration trial.

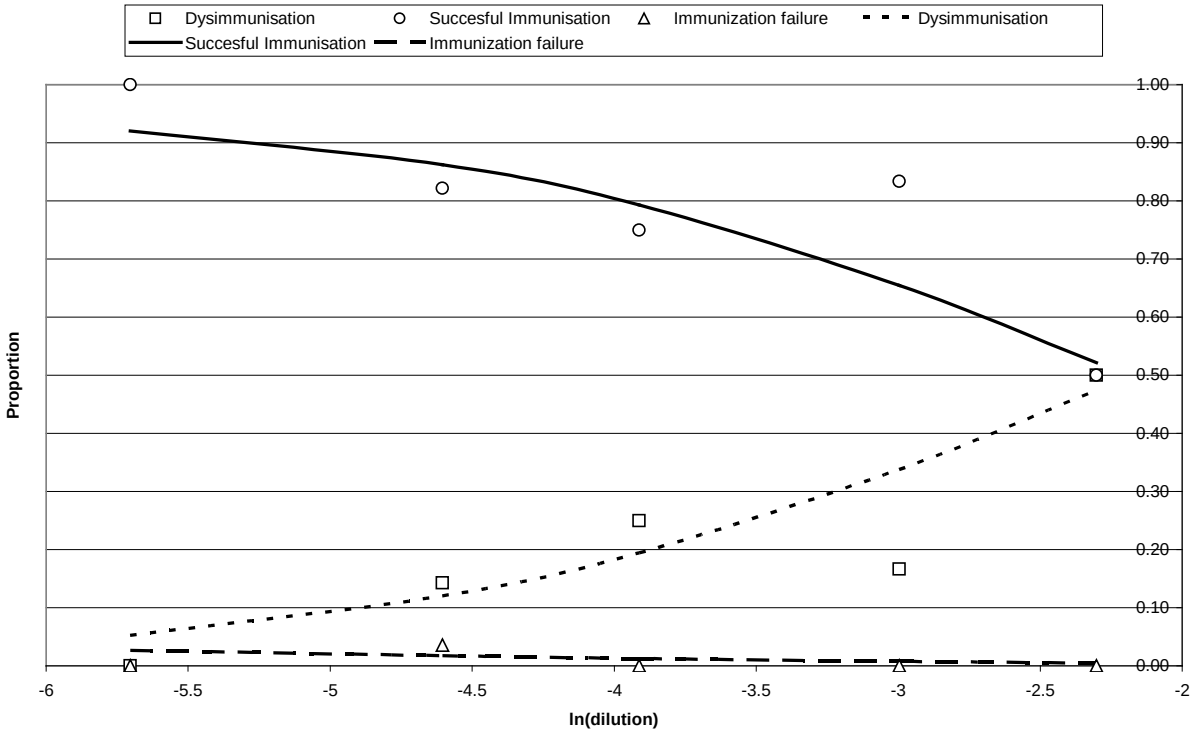
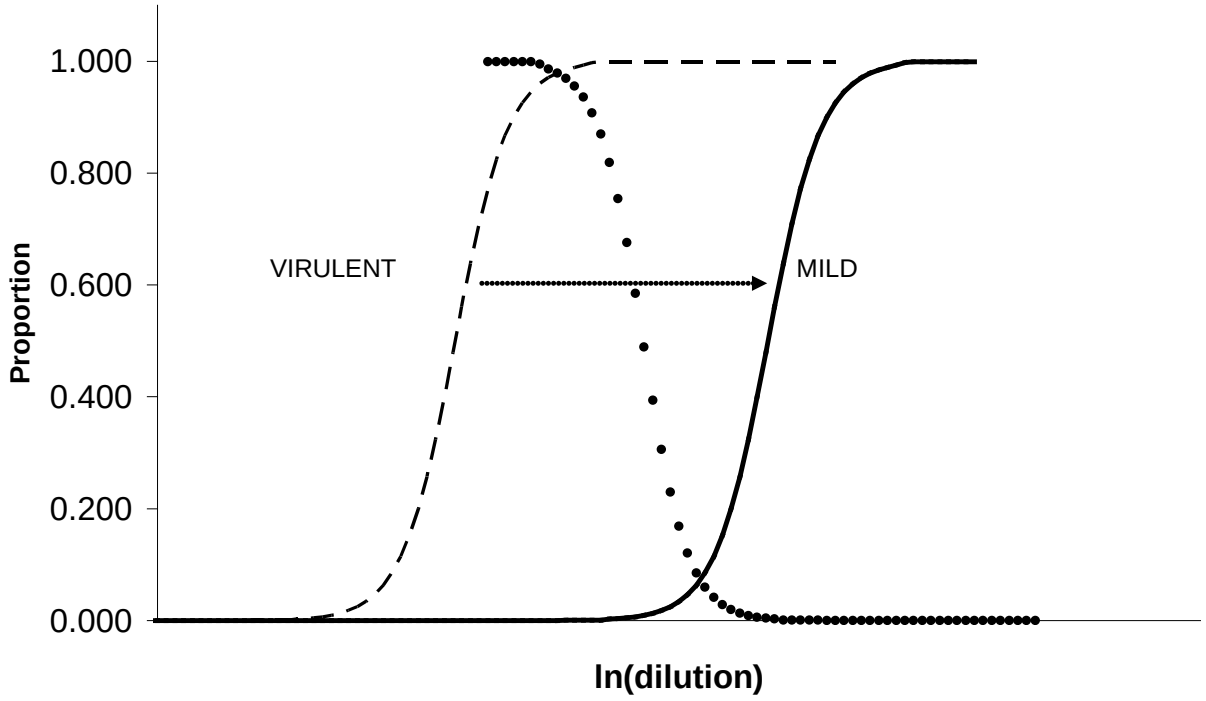


Figure 4: Dysimmunization for a virulent stock of T. parva (dashed line) and a mild stock (full line) and immunization failure in the mild stock (dotted line). Increasing the dose results in high dysimmunization due to the virulent stock, which does not allow a sufficiently low immunization failure of the mild stock.



Reference List

Agresti, A., 1996. An introduction to categorical data analysis. Wiley, New York.

Anonymous, 1989. Classification of *Theileria parva* reactions in cattle. In: Dolan, T.T. (Ed.), Theileriosis in eastern, central and southern Africa. English Press Ltd, Nairobi, pp. 187-188.

Bishop, R., Geysen, D., Spooner, P., Skilton, K., Nene, V., Dolan, T., Morzaria, S., 2001. Molecular and immunological characterisation of Theileria parva stocks which are components of the 'Muguga cocktail' used for vaccination against East Coast fever in cattle. Veterinary Parasitology 94, pp. 227-237.

Burridge, M.J., Brown, C.G.D., Cunningham, M.P., Morzaria, S.P., 1972. Duration of Immunity to East Coast Fever (Theileria-Parva Infection of Cattle). Parasitology 64, p. 511-&.

Buscher, G., Otim, B., 1986. Quantitative Studies on Theileria-Parva in the Salivary-Glands of Rhipicephalus-Appendiculatus Adults - Quantitation and Prediction of Infection. International Journal for Parasitology 16, pp. 93-100.

Clyde, D.F., Most, H., Mccarthy, V.C., Vanderberg, J.P., 1973. Immunization of Man Against Sporozite-Induced Falciparum Malaria. American Journal of the Medical Sciences 266, pp. 169-177.

Cunningham, M.P., Brown, C.G., Burridge, M.J., Irvin, A.D., Kirimi, I.M., Purnell, R.E., Radley, D.E., Wagner, G.G., 1974. Theileriosis: the exposure of immunized cattle in a *Theileria lawrencei* enzootic area. Tropical Animal Health and Production 6, pp. 39-43.

Di Guilio, G., Lynen, G., Ulicky, E., Katende, J., Morzaria, S., 2000. Use of two dose rates of oxytetracyclines in East Coast fever immunisation in Tanzania. In: Kazimirova, M., Labuda, M., Nutall, P.A. (Eds.), Proceedings of the 3rd International Conference "Ticks and Tick-borne Pathogens: Into the 21st Century". Slovak Academy of Sciences., Bratislava, pp. 121-123.

Dolan, T.T., Young, A.S., Losos, G.J., McMilian, I., Minder, C.E., Soulsby, K., 1984. Dose dependent responses of cattle to Theileria parva stabilate. Int.J.Parasitol. 14, pp. 89-95.

Geysen, D., 2000. The application of molecular biology techniques to analyse diversity in theileria parva populations in Zambia (PhD thesis). Department of Biology and Biochemestry, Brunel University,UK.

Goddeeris, B.M., Morrison, W.I., Taracha, E.L.N., McKeever, D.J., 1994. East Coast Fever (*Theileria-parva*) - cell-mediated immunity and protection. In: Goddeeris, B.M., Morrison, W.I. (Eds.), Cell-mediated immunity in ruminants. CRC Press, London, pp. 143-156.

Hoffman, S.L., Goh, L.M.L., Luke, T.C., Schneider, I., Le, T.P., Doolan, D.L., Sacci, J., de la Vega, P., Dowler, M., Paul, C., Gordon, D.M., Stoute, J.A., Church, L.W.P., Sedegah,

M., Heppner, D.G., Ballou, W.R., Richie, T.L., 2002. Protection of humans against malaria by immunization with radiation-attenuated Plasmodium falciparum sporozoites. Journal of Infectious Diseases 185, pp. 1155-1164.

Katende, J., Morzaria, S., Toye, P., Skilton, R., Nene, V., Nkonge, C., Musoke, A., 1998. An enzyme-linked immunosorbent assay for detection of *Theileria parva* antibodies in cattle using a recombinant polymorphic immunodominant molecule. Parasitology Research 84, pp. 408-416.

Lynen, G., Makala, L., Pas, W., 1992. Eats Coast fever Immunisation in Eastern Province, Zambia. In: Dolan, T.T. (Ed.), Ticks and Tick-borne Disease Control: Proceedings of a joint OAU, FAO and ILRAD Workshop Held in Kampala, Uganda, 12-14 September 1991. The International Laboratory for Research on Animal Diseases, Nairobi, pp. 16-21.

Marcotty, T., Billiouw, 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. Veterinary Parasitology 99, pp. 175-187.

McCullagh, P., Nelder, J.A., 1989. Generalized linear models. Chapman and Hall, London.

Minjauw, B., Otte, M.J., James, A.D., 1998. Epidemiology and control of East Coast Fever in Zambia - A field trial with traditionally managed sanga cattle. Tropical Veterinary Medicine 849, pp. 219-225.

Morzaria, S., Spooner, P., Bishop, R., Mwaura, S., 1999. The preparation of a composite stabilate for immunisation against East Coast fever. In: Morzaria, S., Williamson, S. (Eds.), Live Vaccines for *Theileria parva*: Deployment in Eastern, Central and Southern Africa. Proceedings of a Joint OAU, FAO and ILRI Workshop held at ILRI. The International Laboratory for Research on Animal Diseases, Nairobi, Kenya, pp. 56-61.

Morzaria, S.P., Irvin, A.D., Voigt, W.P., Taracha, E.L.N., 1987. Effect of timing and intensity of challenge following immunization against East Coast Fever. Veterinary Parasitology 26, pp. 29-41.

Mukhebi, A.W., Perry, B.D., Kruska, R.L., 1992. Estimated economics of theileriosis control in Africa. Preventive Veterinary Medicine 12, pp. 73-85.

Musisi, F.L., Quiroga, J.C., Mutugi, J., Jacobsen, J., de Castro, J., Di Guilio, G., 1992. Immunisation against East Coast Fever: Recent experiences with the trivalent vaccine in East and Central Africa. In: Bovine Theileriosis, 3rd symposium on Tropical and Animal Health and Production. Office for International Co-operation, Utrecht, pp. 26-32.

Mutugi, J.J., Young, A.S., Maritim, A.C., Ndungu, S.G., Stagg, D.A., Grootenhuis, J.G., Leitch, B.L., 1988. Immunization of cattle against theileriosis using varying doses of *Theileria parva lawrencei* and *T. parva parva* sporozoites and oxytetracycline treatments. International Journal for Parasitology 18, pp. 453-461.

Oura, C.A.L., Bishop, R., Wampande, E.M., Lubega, G.W., Tait, A., 2004. The persistence of component Theileria parva stocks in cattle immunized with the 'Muguga cocktail' live vaccine against East Coast fever in Uganda. Parasitology 129, pp. 27-42.

Pombo, D.J., Lawrence, G., Hirunpetcharat, C., Rzepczyk, C., Bryden, M., Cloonan, N., Anderson, K., Mahakunkijcharoen, Y., Martin, L.B., Wilson, D., Elliot, S., Eisen, D.P., Weinberg, J.B., Saul, A., Good, M.F., 2002. Immunity to malaria after administration of ultra-low doses of red cells infected with *Plasmodium falciparum*. The Lancet 360, pp. 610-617.

Radley, D.E., 1981. Infection and treatment method of immunisation against theileriosis. In: Irvin, A.D., Cunningham, M.P., Young, A.S. (Eds.), Advances in the control of theileriosis. Martinus Nijhoff Publishers, The Hague, pp. 227-237.

Radley, D.E., Brown, C.G.D., Cunningham, M.P., Kimber, C.D., Musisi, F.L., Payne, R.C., Purnell, R.E., Stagg, S.M., Young, A.S., 1975. East Coast Fever. 3. Chemoprophylactic immunisation of cattle using oxytetracycline and a combination of theilerial strains. Veterinary Parasitology 1, pp. 51-60.

Rieckmann, K.H., Beaudoin, R.L., Cassells, J.S., Sell, K.W., 1979. Use of attenuated sporozoites in the immunization of human volunteers against *Falciparum* malaria. Bulletin of the World Health Organization 57, pp. 261-265.

Uilenberg, G., 1999. Immunization against diseases caused by *Theileria parva*: a review. Tropical Medicine and International Health 4, p. A12-A20.

Uilenberg, G., Silayo, R.S., Mpangala, C., Tondeur, W., Tatchell, R.J., Sanga, H.J.N., 1977. Studies on Theileriidae (Sporozoa) in Tanzania. X. A large scale field trial on immunisation against cattle theileriosis. Tropenmedizin und Parasitologie 28, pp. 499-506.