Registration of the East Coast Fever Infection and Treatment Method vaccine (Muguga cocktail) in East Africa.

Registration of the East Coast Fever Infection and Treatment Method vaccine (Muguga cocktail) in East Africa.

A. R. Peters[1], P. Toye[2], P. Spooner[2], G. di Giulio[3] and the late G. (Lieve) Lynen[3]

[1]Supporting Evidence based Interventions-Livestock (SEBI), University of Edinburgh, UK (andy.peters@ed.ac.uk)
[2] International Livestock Research Institute, Nairobi, Kenya
[3] Merlino (Lynen) Veterinary Clinic, Arusha, Tanzania

Abstract

This paper describes the preparation and submission of the original registration dossier[1] for the East Coast fever vaccine ECF ITM 'Muguga cocktail' in Kenya, Tanzania, Uganda and Malawi between 2007and 2009. The process faced a series of challenges in that the 'vaccine' unconventionally comprises a formulation of three stocks of the live virulent *Theileria parva* parasite administered together with a long-acting formulation of oxytetracycline. Only two batches had been manufactured and the dossier was based on the first, FAO-1. Since there were no official guidelines to follow for the relevant countries the dossier was constructed using the official European Union format and guidelines, following the universal principles of quality, safety and efficacy. Specific protocols (SOPs) were prepared to describe the production process. There was a complete lack of specifically designed clinical studies so the published and grey literature were searched for evidence to support safety and efficacy and these were used for the relevant sections of the dossier. Registrations were granted in three of the four countries in 2008-2009.

Introduction

East Coast fever (ECF) caused by the apicomplexan parasite *Theileria parva* (Theiler, 1912) and transmitted by the brown ear tick *Rhipicephalus appendiculatus* is a fatal disease of cattle in eastern and southern Africa (see recent review by Nene *et al*., 2016). Along with other tick-borne diseases ECF poses a severe constraint to livestock production in sub-Saharan Africa. Control has relied mostly on regular acaricide treatment (Dolan, 1999) and anti–theilerial drugs (McHardy, 1989) but no effective conventional vaccine has yet been developed, largely due to the lack of a complete understanding of the necessary immune mechanisms and how to stimulate them (Morrison and McKeever, 2006; Nene *et al*., 2016). An unconventional vaccination procedure, known as the Infection and Treatment Method (ITM) was developed in

[1] This paper is a description of the original ECF ITM dossier submitted for first registrations in East Africa in 2008-2009 and how it was assembled. This together with the draft SPC in Appendix 1 have been superseded since that time.

the 1970's (see for example Radley *et al*. 1975a; b; c). The procedure relies on infection of animals with a potentially lethal dose of the live *T. parva* sporozoites with concurrent treatment with long-acting oxytetracycline to control clinical symptoms. Thus treated, animals apparently acquire life-long immunity to subsequent disease (see review by Morrison and McKeever, 2006).

It became evident during development of ITM that immunization with one isolate of the parasite did not necessarily confer immunity to challenge with other known isolates. However, an ITM formulation based on three different stocks of parasite conferred immunity to challenge with several heterologous stocks (Radley *et al*.,1975c). The three stocks (Muguga, Kiambu 5 and Serengeti transformed) are known collectively as the Muguga cocktail, which has been used to immunize cattle across broad areas of East Africa (di Giulio *et al.*, 2009). However other monovalent stabilates e.g. Chitongo, Katete and Marikebuni have been used in some regions with varying degrees of success (P. Spooner, personal communication).

Although ECF-ITM has been available for almost three decades, its implementation has been inconsistent due to various reasons. First, the complexity of manufacturing the vaccine stabilate raised doubts as to whether consistent, commercial-scale batches could be produced. Such batches have been produced in recent years (Patel *et al*., 2016). Secondly there were epidemiological concerns that the Muguga cocktail would not induce protection against field strains found in all geographical situations. Third, as immunization with live parasites can result in a persistently infected or carrier animals, the possibility exists for the vaccine strains to be introduced into areas previously free of them (de Castro,1997; Berkvens *et al*., 1998; de Castro *et al*., 1998; McKeever, 2008). Fourth, there are significant logistical challenges in distribution of a vaccine which requires storage in liquid nitrogen until administration into target animals. Fifth, the product has not been taken up by a private commercial organisation, which would be required to maintain sustainable distribution channels. Finally, there was the, as yet unfulfilled, promise of a more conventional, subunit vaccine which, in theory at least, is easier to manufacture and deliver (P. Spooner personal communication). The institutional history that has formed the backdrop to this fascinating story was recently documented by Perry (2016).

In 1996, the International Livestock Research Institute (ILRI) addressed the first issue above and produced two commercial scale batches of the Muguga cocktail, known as FAO-1 and FAO-2. In total, about 660,000 doses were manufactured and distributed on a fully commercial basis. The product was not formally registered but its use was allowed by special

sanction of the Directors of Veterinary Services in the respective countries. By 2006, almost all of the vaccine had been distributed, indicating, that despite the concerns listed above, and the relatively high retail price of US$ 8-12 per dose, there was a demand for the product. A meeting of stakeholders was organised in Nairobi in February 2007 by AU-IBAR where several key points were agreed at that meeting. Amongst these were that ILRI would produce a new batch of 1 million doses and that the newly formed GALVmed, a public private partnership and alliance, would fund production of a second batch of 500,000 doses subject to the condition that there was formal establishment of quality standards for the product in the form of a registration dossier and there should be formal product registration in four user countries - Kenya, Tanzania, Uganda and Malawi. Additionally, one or more commercial production sites should be established, for which the dossier could also act as a technology transfer and training manual. Subsequently this was selected as the Centre for Ticks and Tick Borne Disease (CTTBD) in Lilongwe, Malawi.

The development of the registration dossier presented several challenges, given the unique nature of the vaccine and the varying registration procedures in each of the user countries. There were very few formal documents on which to base the dossier particularly for the Quality (manufacturing) section. The manufacturing procedure developed by ILRI was largely based on research methodology with limited cognizance of regulatory processes and requirements. Similarly, there were no established, dedicated clinical study designs to assess either product safety or efficacy, resulting in significant deviation from normally accepted studies. Lastly there was generally a poor knowledge regarding the regulatory systems for veterinary vaccines in the target countries of Kenya, Tanzania, Malawi and Uganda.

This paper documents the approach used to develop the dossier, how each of the unique challenges was addressed and presents an outline of the final registration dossier itself.

Design of the dossier

International registration of medicines (including veterinary medicines) universally relies on the principles of product quality, safety and efficacy. For the ITM vaccine, it was decided to develop a registration dossier that could be used, either in full or its modular parts as necessary by any national regulatory authority to construct a document that would meet individual national requirements. The format for a European registration dossier was selected, (EudraLex - Volume 6 - Notice to applicants and regulatory guidelines for medicinal products for veterinary use https://ec.europa.eu/health/documents/eudralex/vol-6_en), as it was considered that this would most likely include all necessary requirements. However the

dossier was prepared in the knowledge that the ITM vaccine is an unusual if not unique product in veterinary medicine and was thus written with fitness for purpose always in mind.

Structure of the dossier

The dossier was structured approximately in line with the European Notice to Applicants with the parts shown in Table 1.

Table 1. Structure of the ECF ITM registration dossier

Section	Content
Part 1	Introduction and summary of product characteristics
Part 2	Quality: manufacturing and control Expert report (DACS) on quality section
Parts 3 and 4	Safety and efficacy Expert report (DACS) on safety and efficacy
Part 5	Appendices Standard operating procedures Documentation on constituents e.g. MSDS Batch production record

The preparation of the dossier is described below noting just the critical features.

Part 1. The summary of product characteristics (SPC; see Appendix 1)

The SPC (also known as the Data Sheet) was drafted initially in order to ensure clarity and alignment on the exact specifications (sometimes referred to as the product profile) of the product that was being produced and how it should be used. The SPC is based essentially on the data collected during development of the product. The main features of the SPC are the description of the composition, presentation, indications, contra-indications, storage, handling and administration and precautions.

Part 2. Quality (manufacture and quality control)

In outline, the Quality section includes the qualitative and quantitative composition of the product, a description of the manufacturing method, quality control of starting materials, control tests at both intermediate and final product stages, stability and further information on batch to batch consistency. These sections are shown in Table 2.

The manufacturing procedure including control tests was described specifically for batch FAO-1 and some details included for FAO-2 where these differed significantly. A description of the

manufacture of the next batch named ITM ECF MC ILRI 08 has recently been published by Patel *et al*. (2016). In essence the principles are the same as used for the earlier batches although certain modifications were made to improve the process and these are described in that paper. Thus the manufacturing process will not be described again here.

Table 2. Part 2 of the dossier. Chemical, pharmaceutical and biological / microbiological information		
A	Qualitative and quantitative particulars of the constituents	Active ingredient details, excipients, usual terminology (PhEur etc.), quantitative amounts of all actives and excipients
B	Description of the manufacturing method	Premises, methodology, validation
C	Control of starting materials	Provenance and QC tests on starting materials Packaging and closures TSE compliance
D	Control tests at intermediate stages	QC tests
E	Control tests on finished product	Specifications, compliance with specification. Safety tests e.g. contaminants endotoxins etc.
F	Stability	Shelf life of active, product both in storage and in-use
G	Further information	Batch to batch consistency

It should be noted that this section of a registration dossier is extremely detailed and can be somewhat repetitive. Thus in the interests of brevity what follows is just a summarised version of the salient features.

A. Constituents

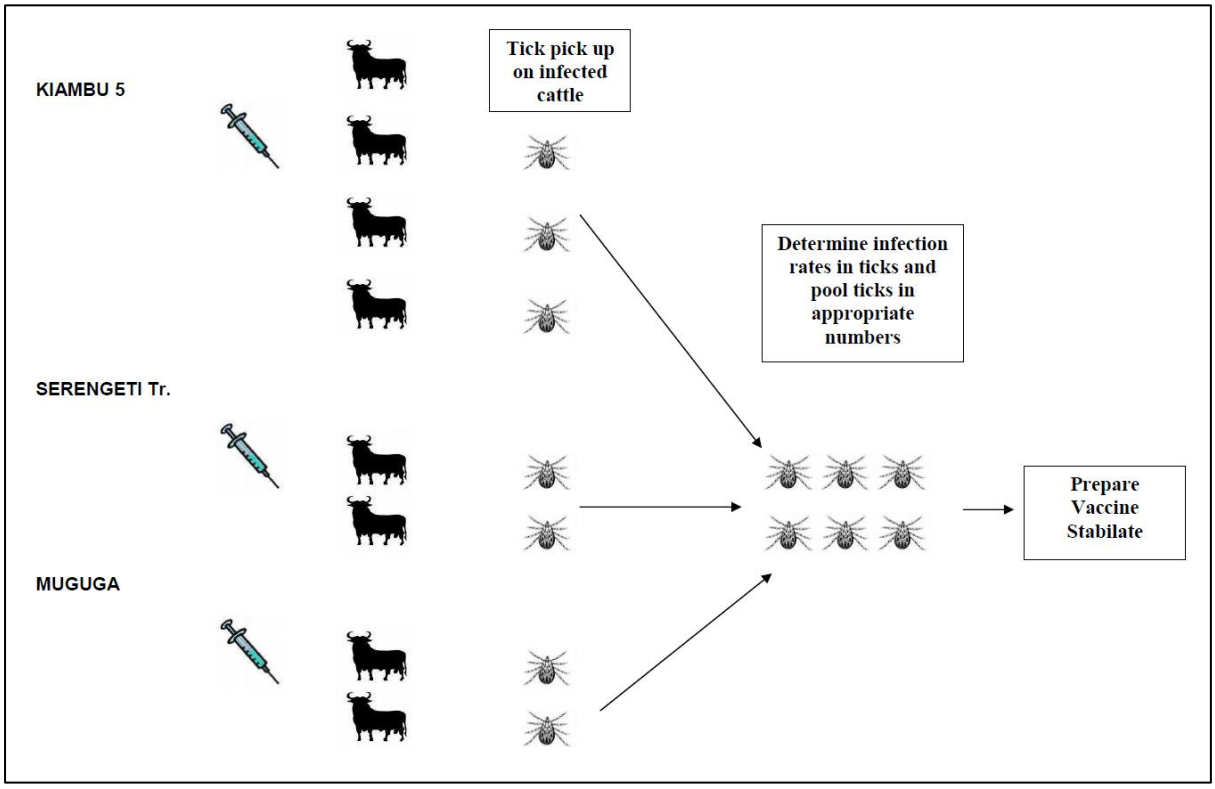
In addition to the qualitative challenges indicated above for Section 1, the quantitative aspects of the composition also required a unique approach. The sporozoite preparation is essentially a semi-purified homogenate of ticks harvested from cattle infected with one of the three component stabilates. One of the major aims of the manufacturing process is to ensure that the final vaccine stabilate comprises equal numbers of sporozoites from the three stabilates. As the level of parasitaemia in infected cattle can vary considerably (Patel et al., 2016), the most direct way of enumerating the numbers of sporozoites is to determine the mean number of infected acini (salivary gland cells) in the tick batches, and pool the batches accordingly. This does not account for differences in the number of sporozoites in each acinus, nor does it allow for loss or death of sporozoites during the remainder of the manufacturing process. To address this, the dossier provided the final concentration of infected acini in the undiluted

stabilate and acknowledged that, as losses could be expected during manufacture, the final recommended dose relied on subsequent *in vivo* testing of the stabilate in cattle.

B and C. Manufacturing method and starting materials

For the dossier, the manufacturing procedure including control tests was described specifically for batch FAO-1 and some details included for FAO-2 where these differed significantly. A detailed description of the manufacture of the subsequent batch named ITM ECF MC ILRI 08 has recently been published by Patel *et al*. (2015) and is shown in outline in Figure 1. In essence the principles are the same as used for the earlier batches although certain modifications were made to improve the process and these are described in that paper.

Figure 1. Summary of the ECF ITM Muguga cocktail production process



The procedure is briefly described here. Production cattle were inoculated with known doses of the three seed stabilates. After 12 days, uninfected nymphal *R. appendiculatus* ticks were placed onto the backs of the cattle (approximately 36,000 per animal). Engorged ticks dropping off the cattle were collected and subsequently fed on rabbits for 4 days to allow tick maturation. The salivary glands were removed from a sample of the ticks to estimate the number of infected acini per tick. Calculated aliquots of ticks were ground and homogenized and suspended in cryoprotectant medium. The supernatant containing the sporozoites

transferred into 0.5 ml artificial insemination straws which were then frozen by a stepwise process in liquid nitrogen. Each batch was then tested for infectivity followed by a safety and dose determination study before release for field evaluation and sale. A comprehensive record of all production processes is kept for each batch. The batch production record for FAO-1 was produced as part of the original dossier.

Two items which required specific attention were the use of live starting materials in the form of cattle, ticks and rabbits, and the need for specialized tick facilities and associated expertise. Of uppermost concern for the live starting materials was that they were free of extraneous pathogens to prevent the contamination of the final stabilate. Detailed protocols were provided to attain this. These included a description of the preferred source of the material (cattle – from areas unsuited to tick-borne infections; ticks and rabbits from closed, characterised laboratory colonies), and detailed procedures for screening for common pathogens. It was recognized that the screening could not cover every infectious agent potentially present on the cattle, and additional reliance was placed on the absence of untoward clinical signs in the animals used in the final testing of the stabilate, as described below.

The production and maintenance of the ticks used in the manufacture requires both expert knowledge in the biology of ticks, and equipment including dedicated incubators, dissecting apparatus and cattle holding facilities. ILRI had maintained a tick unit for several decades, which, although primarily established for research purposes, met all the requirements for the manufacture of the vaccine.

D. In-process Quality Control tests

Most of the tests described in this section comprised clinical and parasitological assessments of the production cattle following infection, to ensure adequate parasitaemias were attained, no extraneous agents were present and that the welfare of the cattle was monitored.

E. Quality Control tests on the finished product

The primary test to ascertain the fitness of the final product was the infection and challenge test described in the next section. Additional aspects which needed to be addressed and for which there was little information were the shelf life of the vaccine and its stability during use. The effective shelf life of the vaccine, essentially maintenance of the viability of the sporozoites stored in liquid nitrogen, had never been formally evaluated. Evidence for the longevity was therefore sought from field reports, in particular those concerning the FAO batches which had been used over a 10-year period at the time this dossier was prepared in 2008. The evidence

from the field was that they were still performing well, and three references were cited to support this.

1. Assessment of FAO-1 after storage for 2.5 years at the CTTBD, Lilongwe, Malawi, demonstrated that the infectivity of the ILRI VS had been maintained at a similar level as at initial storage (Anon, 1999a).
2. Results from immunisations in the Narok district of Kenya, with vaccine stabilate (VS) that had been stored for over 7 years showed it to be safe, with protection of cattle against ECF in pastoral systems (Turasha, 2005).
3. Immunisations with a live trivalent East Coast fever (ECF) vaccine in northern Tanzania, between 2000 and 2004, demonstrated a "robust and effective ECF control method". The vaccine stabilate used had been stored for 4-8 years (Lynen *et al.,* 2005).
4. In addition, ILRI undertook infectivity tests with the stabilates used in 1996 with similar results.

The Anon (1999a) reference above provides the best experimental evidence that the VS retains its potency for at least 2.5 years. The reports of Turasha (2005) and Lynen *et al.* (2005) are more anecdotal in nature but if there had been evidence of either safety or efficacy problems in the field, then these would no doubt have been reported in these publications. Based on experience of preservation of other biological materials in liquid nitrogen, it would be expected that VS stability would be maintained for many years.

For stability during use, one report indicated that stabilates may be diluted and stored on ice for up to 6 hours and used successfully to immunise cattle (Marcotty *et al.*, 2001). These immunisations were carried out under optimal conditions and it is considered that the sub-optimal field conditions may considerably shorten this effective period (Spooner, 2004). From this, it was clear that immunisations should be carried out as soon as possible after vaccine stabilate thawing and dilution, with the diluted stabilate stored on ice in a cooler box during immunisations. In practice there are likely to be delays in immunisations in the field and cattle should therefore be mustered in advance to reduce these. The latter applies particularly to small holder farms. The dossier included a recommendation that cattle are immunised within 2 hours of vaccine stabilate thawing and within a maximum period of 4 hours under optimal storage conditions following dilution of the stabilate.

Parts 3 and 4. Safety and Efficacy

In a conventional European registration dossier, which had been selected as the template for present purposes, it is usual to present safety and efficacy as separate sections. However, for the ITM vaccine, the clinical evidence available to be used as the data package had evolved as a series of individual scientific studies without any specific regulatory pathway in mind and many of the studies were carried out simultaneously to address both safety and efficacy. Therefore for the purposes of the dossier it was decided that the two issues would be presented together in a combined section (i.e. Parts 3 & 4; see EudraLex - Volume 6 - Notice to applicants and regulatory guidelines for medicinal products for veterinary use.
https://ec.europa.eu/health/documents/eudralex/vol-6_en

In regulatory terms, Safety can be defined as a lack of local or systemic reactions and in statutory tests usually involves daily observations for a 14-day period post-vaccination. Vaccine Efficacy can be defined as a significant improvement in clinical signs, infection or transmission compared to unvaccinated controls in a series of different tests. The major sub-headings for Safety and Efficacy in a European dossier are shown in Table 3.

Table 3. The major safety and efficacy parameters to be addressed in vaccine registration

Safety / efficacy parameter	**Specific considerations**
Safety	Single dose, Repeated dose, Overdose Live vaccines – reversion, shed and spread, dissemination Field safety Pregnancy and lactation Environmental safety User safety
Efficacy	Experimental challenge Immunogenicity (usually based on seroconversion) Dose determination Onset of immunity Duration of immunity Field efficacy
Other	Safety and efficacy of oxytetracycline LA Economic benefit / acceptance

The available evidence for Safety and Efficacy was reviewed under these headings as considered relevant and comprised previously published reports and data sheets from the

testing of previous batches. Unless otherwise stated the studies on finished product were conducted with batch FAO-1. These were included in the dossier and are listed in Table 4. In addition, the relevance of each report to Safety or Efficacy or both is shown in Table 5. A benefit risk assessment was also included at the end of the dossier.

Table 4. [2]List of reports used in the Safety and Efficacy section
(the individual reports / publications were allocated a number SE for easy reference)

Report number	Report title	Reference
SE1	Determining a safe and protective immunizing dose for the FAO-1 *Theileria parva* composite stabilate for use in field vaccinations against East Coast fever.	Mutugi *et al*. (1997)
SE2	FAO-1 composite stabilate. Evaluation of efficacy at 1:80 and 1:100 dilutions and treatment with 30% oxytetracycline.	Anon (1998)
SE3	FAO-1-2 composite stabilate. Evaluation of efficacy at 1:80 direct dilution and treatment with 30% oxytetracycline.	Anon (1999b)
SE4	Titration of the FAO-1 stabilate	ILRI (1996)
SE5	The persistence of component *Theileria parva* stocks in cattle immunized with the 'Muguga cocktail' live vaccine against east Coast fever in Uganda.	Oura *at al*. (2004)
SE6	*Theileria parva* live vaccination: parasite transmission and heterologous challenge in the field.	Oura *et al*. (2007).
SE7	An outbreak of East Coast fever on the Comoros: A consequence of the import of immunised cattle from Tanzania?	De Deken *et al.* (2007).
SE8	Infectivity / viability test of FAO-1 stabilate after 2½ years storage at CTTBD, Lilongwe, Malawi.	Anon (1999a)
SE9	Efficacy of East Coast fever (ECF) vaccine on improved and indigenous cattle in Tanzania.	Magwisha *et al*.
SE10	Applying ITM immunisation in Tanzania using the FAO-1 vaccine batch (1998-2007).	Anon (2007).

[2] Some of these reports are not available in the scientific press. Copies can be obtained from the first author A. R. Peters (andy.peters@ed.ac.uk)

SE11	Deployment of a live ECF vaccine in pastoral areas: lessons learned from Tanzania.	Lynen *et al.* (2005).
SE12	The use of a 30% formulation of oxytetracycline long-acting in East Coast Fever immunisation in Tanzania.	Lynen *et al*. (unpublished data).
SE13	Use of two different dose rates of oxytetracycline in East Coast fever immunisation in Tanzania	Di Giulio *et al*.
SE14	Technical meeting on the Infection and Treatment Method of East Coast Fever immunization and the way forward in Kenya.	Turasha (2005)
SE15	Molecular and immunological characterization of *Theileria parva* stocks which are components of the 'Muguga cocktail' used for vaccination against East Coast fever in cattle.	Bishop *et al.* (2001)
SE16	The biological and practical significance of antigenic variability in protective T cell responses against *Theileria parva*.	Morrison (2007).
SE17	Current status of vaccine development against Theileria parasites.	Morrison and McKeever (2006).
SE18	East Coast fever: 1. Chemoprophylactic immunization of cattle against *Theileria parva* (Muguga) and five Theilerial strains. 2. Cross-immunity trials with a Kenya strain of *Theileria lawrencei*. 3. Chemoprophylactic immunization of cattle using oxytetracycline and a combination of theilerial strains.	Radley *et al.* (1975a; b; c).
SE19	Pharmacokinetics of two long-acting oxytetracycline products administered subcutaneously and intramuscularly	Clarke *et al.* (1999).
SE20	Perception of cattle farmers of the efficacy of East Coast fever immunization in Southern Zambia.	(Fandamu *et al*. 2006).
SE21	Financial analysis of East Coast fever control strategies in traditionally managed Sanga cattle in central province of Zambia.	Minjauw *et al*. (1999).
SE22	Epidemiological aspects and economic impact of bovine theileriosis (East Coast fever) and its control: A preliminary assessment with special reference to Kibaha district, Tanzania.	Kivaria *et al.* (2007).

Table 5. Relationship of individual reports to specific issues of safety and efficacy	
Safety / efficacy parameter	**Report no.**
Safety (lack of significant adverse local or systemic reactions)	
Single dose	SE1, SE2, SE3
Repeated dose	No data, see text
Overdose	SE1, SE4
Live vaccines – reversion, shed and spread, dissemination	SE5, SE6, SE7, SE15
Field safety	SE9, SE10, SE11, SE12, SE14
Pregnancy and lactation	No data, see text
Efficacy (Evidence of protection compared to controls)	
Experimental challenge	SE1, SE4, SE12
Seroconversion (immunogenicity)	SE2, SE3, SE8, SE12
Dose determination	SE1, SE4
Onset of immunity	SE2, SE3, SE5, SE8
Duration of immunity	SE5, SE17
Field efficacy	SE9, SE10, SE11, SE14
General safety and efficacy summary	SE16, SE17
Safety and efficacy of oxytetracycline LA	SE1, SE2, SE3, SE4, SE8, SE10, SE12, SE13, SE18, SE19
Economic benefit / acceptance	SE20, SE21, SE22

Safety

Safety of a single dose. Study **SE1** (Mutugi *et al*., 1997) describes the three-stage process which was used to determine the safe and protective dose of batch FAO-1. The investigation was done in three titration stages, with each stage consisting of immunization with the vaccine stabilate with oxytetracycline, followed after five weeks with a challenge with live sporozoites. The experimental design allowed the results from the first titration to form the basis for planning the second titration stage, which in turn led to planning and execution of the third and final titration step with the derivation of the recommended immunizing dose for the vaccine. A total of 72 cattle received varying dilutions of batch FAO-1 from 'concentrated' to 1:1024, along with oxytetracycline LA at 20 mg/kg. A dose of 1:80 was found to be safe in terms of survival and relatively few reactions to vaccination. This report provides evidence of safety of the selected dose 1:80 of the FAO-1 stabilate. Indeed the methodology described in the report was adopted as the method by which the safe and effective dose (dilution) was experimentally selected during manufacture of all subsequent batches.

In study **SE2** (Anon 1998), cattle were immunised with either a 1:80 or 1:100 dilution of FAO-1 along with oxytetracycline LA at 30 mg/kg. Animals were monitored for clinical reactions, rectal temperature and lymph node swelling. Serum samples were taken on days 0 and 30 for serology. The results are summarised in Table 6.

Table 6. Effect of 1:80 and 1:100 dilutions of batch FAO-1 ECF ITM MC (Anon, 1998)

Group	**A**	**B**
Number of animals	63	62
Vaccine dilution	1:80	1:100
Percent seroconverted day 0	13.6	18.3
Percent seroconverted day 30	93.9	84.0
Number with transient elevated rectal temperatures	4	3
Number with lymph node swellings	10	31

Elevations in rectal temperature were only slight and transient. Lymph node swellings were only slight. This study showed that the 1:80 and 1:100 dilutions of FAO-1 were safe and produced a high proportion of seroconversions in cattle after 30 days.

In study **SE3** (Anon, 1999b) two groups of *T. parva* – seronegative cattle aged 4 to 10 months were immunised with a 1:80 dilution of either FAO-1 (GR5; n=31) or FAO-2 (GR7; n=31). A high rate of seroconversion occurred by day 45 in both groups (see Table 7). There were negligible clinical reactions to the vaccine in either group.

Table 7. Effect of 1:80 dilutions of either batch FAO-1 or FAO-2 (Anon, 1999)

Group	Number of cattle	Vaccine	Percent seroconverted	Number of reactions
GR7	31	FAO-2	95	0
GR5	31	FAO-1	100	1

This study demonstrated the safety of both FAO-1 and FAO-2 at a dilution of 1:80 in conjunction with oxytetracycline LA at 30mg/kg. The above three studies (**SE1, 2 and 3**) were taken together to confirm that a dilution of 1:80 is safe to the target animal.

Safety of a repeated dose. There did not appear to be any published reports of administration of repeated doses of MC to cattle. This is primarily because a single dose of the vaccine is believed to provide life-long immunity, so there is no practical reason to repeat the immunisation. However, it was argued in the dossier that there was no reason to assume that a repeated dose would be harmful. Nevertheless, the importance of identifying vaccinated cattle by the recommended ear tagging procedure, as described in the SPC to avoid the possibility of repeat vaccination, was emphasised.

Safety of an overdose (SE1 and SE4). In study SE1 (Mutugi *et al*., 1997), the three stage titration study cattle received doses between 'concentrated' and 1:1024. Although there were severe reactions and deaths at higher concentrations there were no such reactions or deaths at dilutions greater than 1:32. In study SE4 (ILRI, 1996)**,** four groups each of two cattle received dilutions of batch FAO-1 at 1:10, 1:20, 1:40 and 1:80 respectively, together with oxytetracycline LA at 20 mg/kg. The immunised cattle showed some elevation in rectal temperatures and evidence of parasitosis but none were severe and all recovered and seroconverted. Although this study only included two animals per group, doses of FAO-1 as high as 1:10 were found to be safe in all animals. Assuming a standard dose of FAO-1 is a 1:80 dilution, then it was concluded that the above two studies (SE1 and SE4) show that the vaccine is safe at least at double that concentration and probably higher.

Live vaccines – reversion, shed and spread (SE 5, 6 and 7). Since the ITM vaccine is a formulation of virulent parasites, reversion to virulence is not appropriate for this product as it is known to be already highly virulent in susceptible cattle.

With regard to shed and spread, three studies were cited which examined the persistence of the vaccine strains in vaccinated cattle.

In study **SE5** (Oura *et al*., 2004), Kiambu 5 (K5) stock was found to behave quite differently from Muguga (M) or Serengeti transformed (ST) stocks. K5 persisted in vaccinated cattle for up to two years while M and ST had been largely eliminated by 3 months. There was little evidence of transmission to in-contact unvaccinated animals over a 1-year period. Similarly, study **SE6** (Oura *et al*., 2007) showed that K5 persisted in vaccinated cattle for up to 4 years. Bishop *et al.* (2001) **SE15** had undertaken molecular characterisation studies confirming that K5 is quite distinct from the other two stocks which appear to be closely related. These conclusions have more recently been confirmed by more detailed genomic studies (Norling *et al*., 2015; Hemmink *et al*., 2016), which suggest that the ST stock may have become contaminated with the Muguga one.

Study **SE7** (DeDeken *et al*., 2007) describes the investigation of a new clinical syndrome on Grand Comore. The disease was identified as ECF, which had not previously occurred there with such severity. Molecular characterisation revealed profiles identical to Muguga and Kiambu stocks of the Muguga cocktail. *R. appendiculatus* was also found, which had hitherto not been present on Grand Comore. The outbreak had occurred shortly after illegal importation of cattle from Tanzania. The cattle were not tagged as is required after ECF-ITM administration. It is apparent that this severe outbreak of ECF was due at least in part, to shedding of the Muguga (± ST) and K5 parasites from ITM vaccinated cattle. Therefore it is also apparent that vaccinated animals can shed both of these stocks. This is somewhat in contrast to the findings of Oura *et al*. (2004, 2007; see above). It is likely that the cattle on Grand Comore were more susceptible to these stocks than the Ugandan cattle investigated in Oura's work. In addition to suggesting that vaccinated cattle can transmit the parasite to uninfected cattle in non-endemic areas, this study (De Deken *et al*., 2007) underlines the importance of correct tagging of ITM-vaccinated animals.

Thus the evidence concerning transmission from vaccinates to non-vaccinates was found to be equivocal and it was argued in the dossier that this may depend on innate immunity of the in-contact animals. Therefore it was recommended in the SPC that extreme care should be exercised when co-mingling vaccinates and non-vaccinated animals, particularly those which may be naive / very susceptible to the MC stocks.

Field safety (SE9, 10, 11, 12, 14). In study **SE9** (Magwisha *et al*.**,** 2006) a total of 1,216 cattle from 4 regions in Tanzania, immunised with MC about one year previously, were

sampled for serum *T. parva* antibody levels. During this process a sample of farmers were questioned about their opinion of safety and efficacy of the vaccine. A very high proportion indicated that there had been few clinical problems after use of the vaccine in their cattle. This is taken as anecdotal evidence of safety of the vaccine in the field.

Study **SE10** (Anon, 2007) is an update of field use of ECF-ITM between 1998 and 2007 in Tanzania and reported that 278,677 cattle had been immunised over that period. It was concluded that the product was well accepted there as safe and efficacious.

Study **SE11** (Lynen *et al*., 2005) reported two trials carried out in pastoral areas of Tanzania. In the first study 110 calves aged between 2 and 6 months were either vaccinated (n=50) or not (n=50). All animals were monitored for a period of 16 months. In the second study a total of 1038 animals were included in the trial but the relative numbers of vaccinated and non-vaccinated are not specified. The results are shown in Table 8. In summary there are highly significant reductions in mortality in vaccinated animals in both studies.

Table 8. The effect of ECF ITM MC in field studies in Tanzania (Lynen *et al*., 2005)

Location	**Treatment**	**Number of animals**	**Mortality %**
Endulen	Vaccinated	50	4
	Controls	50	50
Engare Naibor	Vaccinated	1038 total	2
	Controls		46

It was reported that 80% of all mortalities in control cattle were due to ECF. It was concluded that ECF-ITM (MC) is both safe and effective under field conditions in Tanzania.

In study **SE12** (Lynen *et al*., unpublished data), two experimental trials were carried out comparing the use of 30% oxytetracycline LA with 20% oxytetracycline LA. Also the results of 1500 field immunisations are reported. In the first experiment, 28 seronegative cattle were immunised with MC and 14 were given oxytetracycline LA 30% (Alamycin) at 30mg/kg and 14 given a 20% oxytetracycline LA formulation (Coopertet) at 20mg/kg. The animals were challenged with a live virulent preparation of homologous *T. parva* 45 days after immunisation. Five unvaccinated control animals were also challenged. Two of the five controls died and

the other 3 developed chronic ECF. There were no severe reactions in the 28 vaccinated animals but there was a significant difference in the percentage of mild reactors as shown in Table 9.

In the second experiment, 104 seronegative dairy animals on farm were immunised with MC and half received each of the two oxytetracycline LA formulations. There was a highly significant difference between the percentages of severe reactors in the two dose groups also shown in Table 9.

Table 9. The effect of oxytetracycline formulation and dose on safety of the Muguga cocktail

Expt.	Treatment	Number	Oxytetracyc. LA formulation	Oxytetracyc. LA dose	Percent mild reactors	Number died
1.	Vaccinated	14	30%	30 mg/kg	7.1	0
	Vaccinated	14	20%	20 mg/kg	21.4	0
	Controls	5	-	-	0	2
2.					Percent severe reactors	
	Vaccinated	52	30%	30mg/kg	7.4	
	Vaccinated	52	20%	20mg/kg	44.0	

All vaccinated animals in the two experiments seroconverted following vaccination.

Thirdly the experience of 1500 immunisations using oxytetracycline (Alamycin) 30% is reported. It is stated that the number of immunisation reactors decreased from around 15% to less than 1% after the introduction of the 30% formulation. Although some clinical reactions occurred which were described as severe, there were no mortalities and the proportion of severe reactions was clearly reduced by the use of oxytetracycline LA at 30mg/kg.

In study **SE14** (Turasha, 2005), 4000 cattle were immunised in Kenya, using oxytetracycline LA at 30 mg/kg. Total reactions were 87 (2.2%) with a mortality of 46 (1.1%). These figures are total mortality and not just that due to ECF and compare to historical mortality rates of 20-40%. This study demonstrates the safety and efficacy of MC under Kenyan field conditions.

Safety in pregnancy and lactation. There were no available data on the use of ECF ITM MC in pregnant and lactating animals but in view of the many thousands of animals vaccinated

and the practice of whole herd vaccination, it was concluded that many pregnant and lactating animals must have been vaccinated in the field without reports of specific safety issues.

Efficacy

Experimental challenge (SE1, 4, 12). In **SE1** (Mutugi *et al*., 1997) a three-stage titration study of the FAO-1 vaccine was carried out at the Vaccine Production Centre, Malawi between March and October 1997. A total of 72 Friesian steers, aged between 6-9 months sourced from areas certified free of infectious diseases were used. Specifically, cattle used in the titration were negative for *T. parva* antibodies using both the IFAT and ELISA tests. Each animal was inoculated subcutaneously with the appropriate dilution of the FAO-1 stabilate and treated simultaneously with oxytetracycline LA at 20 mg/kg bodyweight. Immunized cattle were challenged using 1 ml of undiluted vaccine stabilate (a potentially lethal dose) 35 days after immunisation. Susceptible cattle from the same batch were used as experimental controls.

In the first titration pairs of cattle were inoculated with varying dilutions of the FAO-1 stabilate ranging from (1:2 to 1:1024 dilution). Results of the titration identified stabilate dilutions between 1:64 and 1:256 as the range within which broad protection was provided to cattle by the vaccine. The second stage focused on a narrower range of dilution for the optimal immunizing dose of between 1:60 and 1:100 of FAO-1 stabilate dilutions. The third titration focused the investigation around the predicted optimal range of stabilate concentrations between 1:60 and 1:100 identified in stage two. The focus was three stabilate dilutions; (i) the predicted optimal immunizing dose (1:80) (ii) a dose more concentrated than the predicted optimal dose (1:100) in order to determine the safety margin of the vaccine, and (iii) a dilution that was less concentrated than the predicted optimal dose (1:60) to determine the vaccine efficacy. The optimal dose for the FAO-1 stabilate was found to be 1: 80 dilution. This report provides direct evidence of efficacy of the selected dose 1:80 of the FAO-1 stabilate against experimental challenge.

In study **SE4** (ILRI, 1996) four groups each of two cattle received dilutions of FAO-1 at 1:10, 1:20, 1:40 and 1:80 respectively together with oxytetracycline (Terramycin) LA 20% at 20 mg/kg. All animals plus two untreated controls received a homologous challenge a few weeks later. The immunised cattle showed no clinical responses to challenge, with no pyrexia or schizonts detected. The untreated controls reacted severely and were euthanized on days 13 and 14 after challenge. Although this study only included two animals per group, it demonstrated that dilutions of FAO-1 from 1:10 to 1:80 resulted in sero-conversion and protection against homologous challenge of all animals. However only the 1:80 dilution result

was immediately relevant here as the other dilutions all represent higher doses than are used in the field.

In study **SE12** (Lynen *et al*., unpublished data), 28 seronegative cattle were immunised with MC and 14 were given oxytetracycline 30% (Alamycin) at 30 mg/kg and 14 given Coopertet a 20% oxytetracycline LA formulation at 20 mg/kg. The animals were challenged with a live virulent preparation of homologous *T. parva* 45 days after immunisation. Five unvaccinated control animals were also challenged. Two of the five controls died and the other 3 developed chronic ECF. There were no severe reactions in the 28 vaccinated animals but there was a significant difference in the percentage of mild reactors as shown in Table 7. This study provides further evidence of protection of vaccinated animals against experimental challenge with *T. parva.*

Efficacy based on seroconversion (Immunogenicity; SE 2, 3, 8). Although it is believed that immunity to *T. parva* is due to cell mediated immune mechanisms (see Morrison, 2007 **SE16**), seroconversion is widely accepted as an indirect indication of a protective response. This is based on the premise that animals which recover from *T. parva* infection are immune to subsequent disease and that seroconversion indicates prior infection.

Study **SE2** (Anon, 1998) was carried out at Mwanza, Tanzania starting in June 1998. Cattle were immunised with either a 1:80 or 1:100 dilution of batch FAO-1. Animals were monitored for clinical reactions, rectal temperature and lymph node swelling. Serum samples were taken on days 0 and 30 for serology. The results are summarised in Table 6. This study shows that both the 1:80 and 1:100 dilutions of FAO-1 produce a high proportion of seroconversions in cattle after 30 days.

In study **SE3** (Anon, 1999) two groups of *T. parva* – seronegative Friesian x Ayrshire cattle aged 4 to 10 months were immunised with a 1:80 dilution of either FAO-1 (GR5; n=31) or FAO-2 (GR7; n=31), subcutaneously near to the parotid gland. A high rate of seroconversion occurred by day 45 in both groups (see Table 7).

Study **SE8** (Anon, 1999a) examined the infectivity of FAO-1 stabilate after 2½ years storage at -20^{0}C at CTTBD, Lilongwe. Three groups of 5 cattle were used. They received either 1ml concentrated FAO-1 stabilate, 1ml of 1:80 dilution or 1ml 1:80 dilution plus oxytetracycline LA at 20 mg/kg bodyweight. The results are shown in Table 10 below. Serum from surviving cattle on days 28 and 35 showed high antibody titres to *T. parva.*

Table 10. Response of cattle to different concentrations of Muguga cocktail with or without oxytetracycline			
Number of cattle	Treatment	Mortality	Time of death
5	Concentrated stabilate	5/5	Before day 21
5	1:80 dilution	4/5	Days 21-28
5	1:80 dilution plus OTC	0/5	-

Dose determination (SE1, 4). The two studies cited (Mutugi *et al*., 1997; ILRI, 1996) where dose determination was carried out have been reviewed above. A dilution of 1:80 was found to be an effective dose.

Onset of immunity (SE2, 3, 5, 8). Sero-conversion in cattle immunised with MC had occurred by 30 days (SE2, Anon, 1998), 45 days (SE3, Anon, 1999) 48 days (SE5, Oura *et al*., 2004)) and 28-35 days (SE8, Anon, 1999). Thus it was concluded that onset of immunity occurs within 28-30 days after immunisation. There are earlier studies which support this conclusion which are indicated in the Discussion.

Duration of immunity (SE5, SE17). We could find no data on duration of immunity beyond the presence of specific antibodies at day 48 (SE5, Oura *et al.*, 2004). However it is generally accepted that ECF-ITM produces lifelong immunity in vaccinated cattle or at least for several years (see **SE17;** Morrison and McKeever, 2006*).* Also there would be expected to be a 'trickle challenge' by ticks in the field, particularly if the frequency of acaricide treatment is reduced after vaccination. This would probably have the effect of continually boosting immunity to *T. parva*. As discussed above, the vaccine stabilate persists in animals for some time after vaccination. The lack of disease in these animals suggests the presence of a protective immune response. For these reasons, it was argued in the dossier that immunity would last for several years and in the field it is generally held that immunity is effectively lifelong.

Field efficacy (SE9, 10, 11, 14). In study **SE9**, (Magwisha *et al*., 2006) a total of 1216 cattle from 4 regions in Tanzania, immunised with MC about one year previously were sampled for serum *T. parva* antibody levels. A value of 20 was taken as the threshold for sero-conversion. Overall 70.1% of the vaccinates and 46% of non-vaccinates had antibody levels above 20, suggesting protection against disease (see Table 11).

Table 11. Serum antibody responses of cattle approximately 1 year after vaccination with Muguga cocktail (Magwisha *et al*., 2006).					
Region	**Total number of animals**	**Antibody titre <20**		**Antibody titre >20**	
		Number	**Percent**	**Number**	**Percent**
Arusha	225	153	68	72	32
Coast	208	57	27	151	73
Mara	311	56	18	255	82
Morogoro	472	97	21	375	79
Total / mean	1216	363	29.9	853	**70.1**
Controls	67	36	54	31	**46**

In report **SE10** (Anon, 2007), a total of 278,677 cattle had been immunised with ECF-ITM MC between 1998 and 2007. It was concluded that there was a high degree of confidence in the procedure and that ECF-ITM is well accepted as a safe and efficacious product in Tanzania.

Study **SE11** (Lynen *et al*., 2005) comprised two field studies were carried out in pastoral areas of Tanzania, one in Endulen and one in Engare Naibor. In the first study 110 calves aged between 2 and 6 months were either vaccinated (n=50) or not (n=50). All animals were monitored for a period of 16 months. In the second study a total of 1038 animals were included in the trial but the relative numbers of vaccinated and non-vaccinated are not specified. The results are shown in the Table 8. In summary there are highly significant reductions in mortality in vaccinated animals in both studies. 80% of all mortalities in control cattle were due to ECF. It is concluded that ECF-ITM with Muguga cocktail is both safe and effective under field conditions in Tanzania.

In study **SE14** (Turasha, 2005), 4000 cattle were immunised and 30% oxytetracycline LA was used. Total reactions were 87 (2.2%) with a mortality of 46 (1.1%). These figures are total mortality and not just that due to ECF and compare to historical mortality rates of 20-40%. This report demonstrates the safety and efficacy of Muguga cocktail under Kenyan field conditions.

It is concluded that the above four reports provide good evidence of the efficacy of ECF-ITM MC under field conditions.

Further support for the general safety and efficacy of the product are provided in **SE16 and 17** (Morrison 2007; Morrison and McKeever, 2006).

Safety and efficacy of oxytetracycline

Since ITM is a live, virulent vaccine, concurrent treatment with oxytetracycline long acting (LA) is necessary to reduce severe clinical signs and even death after vaccination. However the concentration and dose of oxytetracycline has been the subject of some debate. The publications of Radley *et al*. (1975a, b, c; **SE18**) were amongst the first to describe the use of oxytetracycline, but preceded the availability of an LA formulation and therefore used a series of daily injections. When the first LA formulation became available, it was used at a dose rate of 20 mg/kg. Indeed it is apparent that it was used at this dose in a series of studies including the titration of the FAO-1 batch to determine the optimal therapeutic dose (**SE1,** Mutugi *et al*., 1997; **SE4,** ILRI, 1996**; SE3,** Anon 1999). However it became apparent from field reports that on occasion, the frequency and severity of adverse clinical reactions to immunisation were unacceptably high. Higher dose rates were used e.g. 30 mg/kg but with the 20% formulations available, the injection volume was often very large in heavier cattle. The availability of a 30% formulation was welcomed and was shown to be preferable in terms of reducing the frequency and severity of clinical reactions when used at 30 mg/kg as opposed to 20% at 20 mg/kg (**SE10,** Anon, 2007**; SE12** Lynen *et al*., unpublished data). Unfortunately these studies were somewhat confounded by using a different dose per unit-bodyweight as well as a different formulation. Nevertheless other reports have concluded that the use of 30 mg/kg is preferable (**SE2,** Anon 1998**; SE3,** Anon 1999**; SE14** Turasha 2005).

Another confounding factor was that different studies used different brands of oxytetracycline LA. Study **SE19** (Clarke *et al*., 1999) compared the pharmacokinetics of two long-acting oxtetracycline preparations, manufactured by Merial and Boehringer Ingelheim respectively given at 20mg/kg by the subcutaneous or intramuscular route in Hereford steers. Although there were marginal differences, the pharmacokinetics in all four situations were very similar and it was concluded that the two products were bio-equivalent. Although not directly relevant to the use of oxytetracycline in East Africa this study demonstrated that two generic oxytetracycline preparations of equivalent concentrations (20%) exhibit similar pharmacokinetics and it is inferred that most available generic oxytetracycline LA products are formulated to exhibit similar pharmacokinetic profiles.

Study **SE13** (di Giulio *et al.* unpublished data) compared the same formulation (30%) oxytetracycline LA at 30 vs. 40 mg/kg and concluded that while reducing the number of reactions, the higher dose also resulted in fewer seroconversions to the vaccine. Therefore it

was concluded that 30 mg/kg was the optimal dose for use with ECF-ITM and it is apparent that this is widely accepted.

The dossier proposed that while very few brands of oxytetracycline LA had been directly evaluated for this indication experimentally, it should be administered at 30mg/kg in the vaccination programme. If a lower dose of 20mg/kg is chosen then the vaccinated cattle should be more closely monitored for several days for signs of adverse reaction so that they can be further treated if necessary.

Economic benefit

Three studies were reviewed concerning the economic impact of ECF-ITM. Study **SE20** (Fandamu *et al*., 2006) used a structured questionnaire to assess the perception of 179 cattle farmers of the efficacy of ECF-ITM vaccination in southern Zambia. The majority (85%) regarded immunization as very effective and about half (51.4%) preferred immunization to other ECF control strategies. The study showed that the number of calves immunized was strongly associated with the farmer's perception of the benefits of immunization. It is concluded that ITM is regarded as effective by the majority of farmers in the study.

In study **SE21** (Minjauw *et al*., 1999) the financial consequences of five different ECF control strategies were analysed as follows:

- ECF immunisation and no tick control (INT)
- ECF immunisation and seasonal treatment for ticks (IS)
- ECF immunisation and weekly treatment for ticks (IW)
- No ECF immunisation and weekly treatment for ticks (NIW)
- No ECF immunization and no tick control (NINT)

Input and output data were calculated to construct discounted cashflows for each group. It was concluded that seasonal spraying with acaricide plus immunization (IS) gave the highest net present value and that no control (NINT) gave the lowest. It was shown that ITM costs could rise to US$ 25.90 per head before profitability was affected. Therefore ITM combined with seasonal tick control was the most cost effective measure to control ECF.

Study **SE22** (Kivaria *et al*., 2007) comprised a cross sectional study based on clinical examination, inspection of herd health records and a questionnaire designed to determine the epidemiology, economics and potential impact of immunization against theileriosis in Tanzania. The results showed annual theileriosis costs to be US$ 205.40 per head, whereas

the introduction of immunization reduced this by 40-68% depending on the post-immunisation dipping strategy. It was concluded that farmers who have immunized their cattle may cautiously reduce acaricide application by 50-75% depending on the level of tick challenge at the herd level. It was concluded that ECF-ITM immunization can effectively reduce costs associated with theileriosis but should be combined with strategic acaricide treatment post-immunisation.

Taken together these studies it was concluded that there is economic benefit real and perceived in the field application of ECF-ITM.

Benefit – risk analysis

It was argued in conclusion that the following aspects had been demonstrated in the reports presented in this dossier:

- The ECF-ITM MC vaccine as exemplified by batches FAO-1 and 2 is safe and effective at a final dilution of 1:80, when used according to instructions in the Summary of Product Characteristics (Product Data Sheet; see Appendix 1).
- Whilst systemic clinical reactions can occur following vaccination, these can be minimised by the concurrent use of oxytetracycline LA at a dose of 30 mg/kg.
- If oxytetracycline is given at 20 mg/kg then vaccinated animals should be monitored intensively for several days in case further anti-theilerial treatment is necessary.
- Muguga cocktail should only be used in areas where epidemiological data indicate that Muguga, Serengeti transformed and Kiambu 5 stocks are appropriate for that region.
- There is some evidence of differential shedding and transmission by the 3 stocks in MC. Although evidence of clinical disease in in-contact non-vaccinates is equivocal every effort should be made to protect susceptible animals from close contact with newly vaccinated cattle.

Dossier submission to national authorities

Due to its large file size the dossier was prepared as a CD-Rom and multiple copies prepared for distribution to the agencies and individuals closely involved in the registration process. In the case of Kenya, this was submitted by an academic consultant on behalf of GALVmed. For Tanzania a local technical representative (Drs Lynen and Di Giulio) was used in-country and for Malawi the dossier was submitted by the management of the CTTBD Lilongwe. For Uganda an in-country technical representative was appointed to submit and negotiate approval of the dossier. The registrations were processed by the respective competent authorities in the respective countries as shown in Table 12.

Table 12. Details of ECF ITM MC dossier submission and review by national regulatory authorities			
	Dossier submitted by	National regulatory 'competent authority'	Registration granted
Kenya	Academic consultant	Pharmacy and Poisons Board	2009
Tanzania	In-country technical representative	Tanzania Food and Drugs Agency	2009
Malawi	CTTBD management	National Drug Authority	2008
Uganda	In-country technical representative	Ministry of Health	Not registered

The registration process in Uganda was originally refused due to failure of GMP inspection by the Ugandan authorities, although this had been previously approved by PANVAC. It became clear that national standards could vary substantially between countries and the need for closer regional harmonisation on regulatory issues was identified.

Discussion

This paper describes the process of assembly of the original dossier and the challenges which were faced during the registration of the ITM live vaccine. The product is an unusual vaccine whose target users are predominantly poor livestock owners in eastern, central and southern Africa. The vaccine was manufactured by ILRI, an institute whose primary focus is research and not the production of commercial vaccines. These factors were taken into account when the registration was prepared, with an emphasis on 'fitness for purpose'. Subsequent sales of the vaccine established that, despite the limited geographical location of the disease, there is a high demand for the vaccine. Several issues emerged during the registration process, and these are discussed below.

Definition of the composition of the vaccine was not straightforward, as the components were prepared from ill-defined field isolates prepared several years earlier. Subsequent to the submission of the dossier, it was reported that that the component stabilates are composed of at least 14 different parasite types, based on genotypes established with satellite markers (Patel *et al*., 2011), although there is limited diversity in the satellite loci among the genotypes (Hemmink, 2016). It has also been shown that the composition of the FAO batch from 1996 and the ILRI-08 batch made in 2007 were very similar, suggesting that the manufacturing procedure provides consistency in the vaccine stabilate (Patel *et al*., 2011). An additional

study showed that there is a remarkable degree of similarity in the genomic composition of two of the stabilates (Muguga and Serengeti-transformed) to the extent that it is now suspected that some contamination occurred during the several tick-cattle passages that preceded establishment of the current stabilates.

A distinctive feature of the production process is the use of live animals, with the associated risks of inconsistent reactions and the introduction of extraneous pathogens into final vaccine stabilate. To minimize these risks, the dossier included specifications of where the type of animals to be used and where they should be, and a series of tests to detect such pathogens. In addition, the dossier describes the extensive clinical and parasitological examinations which were undertaken to ensure the ticks are applied at the right time and that the level of infection of the ticks is correctly estimated.

A key feature of the successful production of the ITM vaccine is the availability of suitable tick facilities and expertise in the biology of ticks. Such facilities are not common and it is a major reason why ILRI was initially asked to produce the commercial-scale batches of the vaccine. It is clear that the primary purpose of tick facilities is to make possible research on ticks and tick-borne pathogens, and the dossier had to make allowance for this. It is also a major challenge to the full privatization of the production and distribution of the ITM vaccine and other vaccines requiring a tick phase in the production process.

Because of the otherwise lethal nature of the vaccine stabilate and the potential for variation in its quantitative and qualitative aspects, the paramount quality test on the finished product is the series infection and challenge experiments. The dossier contained the results of several such tests. It is recognised that these are expensive and time-consuming, and are a consequence of the lack of a method for assessing the potency of the vaccine *in vitro*. The most direct method would involve the counting of viable sporozoites. Such a method does not currently exist, due in part to the tendency of sporozoites to form aggregates. DNA-based methods do not distinguish between live and dead parasites. A simple estimation of the number of sporozoites would also fail to allow for variations in the virulence and antigenic specificity of the parasites. Until such methodology is developed, registration of each batch of the vaccine will rely of the data from such trials, although it is hoped the accumulating experience with production will reduce the number of iterations of the experiment.

It is questionable how accurately the immunization and challenge experiments can discern a difference in clinical reactions or protection between dilutions of, say, 1:60 and 1:80. The

variability in response of individual animals and the need to limit the number of animals in any experiment, suggest that there is likely to be as much variation within a dosage group as there is between groups. The dosage which was finally recommended is a balance between one which is clearly safe and clearly effective.

The European dossier format used as a template for the current submission requires several other post production tests, such as stability, for which no tests were available. In lieu of these, the dossier presented evidence from published articles and field reports to articulate support the various claims. These are supported by additional publications which were not included in the dossier. For example, Morzaria *et al*. (1997) showed that animals could survive an otherwise lethal challenge as soon as five days after immunization. With regard to the duration of immunity, Burridge *et al*. (1972) reported that animals that had recovered from infection with the *T. parva* (Muguga) stabilate and kept under ECF-free conditions were protected against lethal homologous challenge up to 43 months following the initial infection.

In recent years regulatory systems for veterinary medicines in East African countries have developed considerably including a mutual recognition system for vaccines. This has happened at least in part due to the investment by the Bill and Melinda Gates Foundation through funding of agencies such as the Global Alliance for Livestock Veterinary Medicines (GALVmed). This has considerably improved the process of veterinary medicine registration in the region.

Acknowledgements

This program was carried out by AP, then consultant Chief Scientific Adviser for GALVmed, Drs P. Toye and P. Spooner of ILRI and with the valued clinical contributions of Drs Lieve Lynen and B di Giulio. GALVmed was funded by the Bill and Melinda Gates Foundation and the Department for International Development UK. This paper is dedicated to Dr Lieve Lynen, a much-respected pioneer and advocate of ECF ITM. The photographs below show her preparing doses of Muguga cocktail in the field and vaccinating a calf.

References

Anon (1998). FAO-1 composite stabilate (comprising the Muguga cocktail stocks). Evaluation of efficacy at 1:80 and 1:100 dilutions and treatment with 30% oxytetracycline.
Report prepared for OAU/IBAR by ITTBDCP, Arusha and ILRI, Nairobi.

Anon (1999a). FAO 1-2 composite stabilate (comprising the Muguga cocktail stocks). Evaluation of efficacy at 1:80 direct dilution and treatment with 30% oxytetracycline.
Report prepared for OAU/IBAR by ITTBDCP, Arusha and ILRI, Nairobi.

Anon (1999b). Infectivity/Viability test of FAO-1 stabilate after 2½ years storage at CTTBD, Lilongwe. Report by Organisation of African Unity/Inter-African Bureau for Animal Resources - Centre for Ticks and Tick-Borne Diseases.

Anon (2007). Applying ITM immunisation in Tanzania using the FAO-1 vaccine batch (1998-2007). Report Central veterinary laboratory, Dar es Salaam, Tanzania.

Berkvens, D. L., Geysen, D. M. and J. R. A. Brandt (1998). Comments on the paper ‘‘Sustainable tick and tickborne disease control in livestock improvement in developing countries’’. Veterinary parasitology 77, 211.

Bishop, R., Geysen, D., Spooner, P., Skilton, R. Nene, V. and Dolan, T. and S. Morzaria (2001). Molecular and immunological characterization of *Theileria parva* stocks which are components of the ‘Muguga cocktail’ used for vaccination against East Coast fever in cattle. Veterinary Parasitology 94 227.

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

Clarke, C. R., Wang, Z., Cudd, L., Burrows, G. E., Kirkpatrick, J. G. and M. D. Brown (1999). Pharmacokinetics of two long-acting oxytetracycline products administered subcutaneously and intramuscularly. *Journal of Veterinary Pharmacology and Therapeutics* 22, 65.

De Castro, J. J. (1997). Sustainable tick and tickborne disease control in livestock improvement in developing countries. *Veterinary Parasitology* 71, 77.

De Castro J. J. (1998). Reply to comments on the paper ‘‘Sustainable tick and tickborne disease control in livestock improvement in developing countries’’. *Veterinary Parasitology* 77, 213.

De Deken, R., Martin, V., Saido, A., Madder, M., Brandt, J. and D. Geysen (2007). An outbreak of East Coast fever on the Comoros: A consequence of the import of cattle from Tanzania? *Veterinary Parasitology* 143, 245-253.

Di Giulio, G. Lynen, G., Morzaria, S., Oura, C. and R. Bishop (2009). Live immunization against East Coast fever – current status. Trends in parasitology 25, 85.

Di Giulio, G. Lynen, G., Ulicky, E., Katende, J. and S. Morzaria, Use of two different dose rates of oxytetracycline in East Coast fever immunisation in Tanzania (unpublished data).

Dolan T. (1999). Dogmas and misunderstandings in East Coast fever. *Tropical Medicine and International Health* 4, A3-A11.

Fandamu, P., Thys, E., Duchateau, L. and D. Berkvens (2006). Perception of cattle farmers of the efficacy of East Coast fever immunization in Southern Zambia. *Tropical Animal Health and Production* 38, 9–16.

Hemminck, J. D., Weir, W., MacHugh, N. D., Graham, S. P., Patel, E., Paxton, E, Shiels, B., Toye, P. G., Morrison, W. I. and R. Pelle (2016). Limited genetic and antigenic diversity within parasite isolates used in a live vaccine against *Theileria parva. International Journal for Parasitology* 46, 495.

ILRI (1996). Titration of the FAO-1 composite stabilate. Experiment K83K (4/2).

Kivaria F. M., Ruheta, M. R., Mykonyi P. A. and P.C. Malamsha (2007). Epidemiological aspects and economic impact of bovine theileriosis (East Coast fever) and its control: A preliminary assessment with special reference to Kibaha district, Tanzania. *The Veterinary Journal* 173, 384.

Lynen, G., Di Giulio, G. Homewood, K., Reid, R. and A. Mwilawa. (2005). Deployment of a live ECF vaccine in pastoral areas: Lessons learned from Tanzania. In Proceedings of the 4th All Africa Conference on Animal Agriculture and the 31st Annual Meeting of the Tanzania Society for Animal Production (TSAP).The role of biotechnology in animal agriculture to address poverty in Africa: Opportunities and challenges 20–24 September 2005 Arusha, Tanzania. Edited by J.E.O. Rege, A.M. Nyamu and D. Sendalo.

Lynen, G. The use of a 30% formulation of oxytetracycline long-acting in East Coast Fever immunisation in Tanzania (unpublished data).

Magwisha, H. B., Ruheta, M. R., Mbwambo, H. A., Ranfa, E.E. and L. S. Makungu (2006). Efficacy of East Coast fever (ECF) vaccine on improved and indigenous cattle in Tanzania. Report Central veterinary laboratory, Dar es Salaam, Tanzania.

Marcotty, T., Billiouw, M., Chaka, G., Berkvens, D., Losson, B. and 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, 175-187.

McKeever, D. J. (2008). Live immunisation against *Theileria parva:* Containing or spreading the disease? *Trends in Parasitology* 23, 565.

McHardy, N. (1989). Multinational research on the use of buparvaquone (Butalex) for the control of theileriosis. International Symposium on Mycoplasmosis and Theileriosis, 11-13 October, 1989, Pendik, Turkey, 114-126.

Minjauw, B., Rushton J., James A. D. and M. Uptond (1999). Financial analysis of East Coast Fever control strategies in traditionally managed Sanga cattle in Central Province of Zambia. *Preventive Veterinary Medicine* 38, 35.

Morrison, W. I. (2007). The biological and practical significance of antigenic variability in protective T cell responses against *Theileria parva*. *Veterinary Parasitology* 148, 21.

Morrison, W. I. and D. J. McKeever (2006). Current status of vaccine development against *Theileria* parasites. *Parasitology* 133, S169–S187.

Morzaria, S., Spooner, P., Bishop, R and S. Mwaura (1997). The preparation of a composite stabilate for the immunization against East Coast fever. In: Proceedings of a joint OAU, FAO and ILRI workshop held at ILRI, Nairobi, Kenya 10-12 March, pp58-61.

Mutugi, J. J., Musisi, F. L., Njuguna, L. M., Morzaria, S. P., Spooner, P. R. and L Duchateau (1997). Determining a safe and protective immunizing dose for the FAO-1 *Theileria parva* composite stabilate for use in field vaccinations against East Coast fever. FAO Technical Report AG:GCP/RAF/291/DEN & GCP/RAF/293/BEL.

Nene V, Kiara, H., Lacasta, N., Pelle, R., Svitek, N. and L. Steinaa (2016). The biology of Theileria parva and control of East Coast fever - Current status and future trends. Ticks and tick borne Disease 7, 549-564.

Norling, M., Bishop, R. P. *et al.* (2015). The genomes of three stocks comprising the most widely utilized live sporozoite *Theileria parva* vaccine exhibit very different degrees and patterns of sequence divergence. *BMC Genomics* 16,729.

Oura, C. A. L., Bishop, R., Wampande, E. M., Lubega, G. W. and A. Tait (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, 27.

Oura, C. A. L., Bishop, R., Asiimwe, B. B., Spooner, P., Lubega, G. W. and A. Tait (2007). *Theileria parva* live vaccination: parasite transmission, persistence and heterologous challenge in the field. *Parasitology* 134, 1205.

Patel, E. H., Lubembe, D. M., Gachanja, J., Mwaura, S., Spooner, P. and P. Toye (2011) Molecular characterization of live *Theileria parva* sporozoite vaccine stabilates reveals extensive genotypic diversity. *Veterinary Parasitology* 179, 62.

Patel, E. Mwaura, S., Kiara, H., Morzaria, S., Peters, A. and P. Toye (2015). Production and dose determination of the Infection and Treatment Method (ITM) Muguga cocktail vaccine used to control East Coast fever in cattle. *Ticks and Tick borne disease* 7, 306.

Perry, B. D. (2016). The control of East Coast fever by live parasite vaccination: a science-to-impact narrative. *One Health* 2, 103.

Radley, D. E., Brown, C. G. D., Burridge, M. J., Cunningham, M. P., Kirimi, I. M., Purnell R. E. and A. S. Young (1975a). East Coast fever: 1. Chemoprophylactic immunization of cattle against Theileria parva (Muguga) and five theilerial strains. *Veterinary Parasitology*, 1, 35.

Radley, D. E., Young, A. S., Brown, C. G. D., Burridge, M. J., Cunningham, F. L., Musisi, F. L. and R. E. Purnell (1975b). East Coast fever: 2. Cross-immunity trials with a Kenya strain of *Theileria lawrencei. Veterinary Parasitology*, 1, 43.

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. and A. S. Young (1975c). East Coast fever: 3. Chemoprophylactic immunization of cattle using oxytetracycline and a combination of theilerial strains. *Veterinary Parasitology*, 1, 51.

Spooner P. (2004). Controversial issues. ICTTD Newsletter on Ticks and Tick-borne diseases of Livestock in the Tropics 26, 33.

Theiler, A. (1912). The Immunisation of Cattle against East Coast Fever. *Ondersterpoort Journal of Veterinary Research*. Second report of the Director of Veterinary Research pp 266-314.

Turasha, G. (2005). Technical meeting on the Infection and treatment method of East Coast fever immunization and the way forward in Kenya. Report to meeting at Naivasha country club March 7th 2005.

Appendix 1

Summary of product characteristics (Product Data Sheet)

East Coast Fever – Infection & Treatment Method (Muguga cocktail)

Composition

This is a live East Coast Fever (ECF) vaccine produced from 3 *Theileria parva* stocks: *T. parva* Muguga, Kiambu 5 and Serengeti-tranformed. The parasites are suspended in Eagle's Minimum Essential Medium, which contains 3.5% bovine serum albumin (Fraction V), 7.5% glycerol to protect the parasites during freezing and thawing, penicillin (150 units/ml) and streptomycin (150 µg/ml).

Presentation

The vaccine is presented as:

- Concentrated Vaccine Stabilate in coloured 0.5ml straws sealed at one end.
- Vaccine Stabilate Diluent in glass serum bottles containing the required volume for dilution of VS (following the procedure described below). The bottles are closed with grey butyl rubber stoppers and sealed with aluminium tear-off seals.

On thawing the concentrated VS is an opaque dark brown finely particulate suspension. As a consequence upon thawing the VS should be carefully mixed, but without shaking, before addition to the VS diluent. Dilution of concentrated VS to provide the immunisation dose provides a light orange-brown solution, finely particulate.

Pharmacological Action

Immunisation with the vaccine allows the controlled establishment of the 3 component parasite stocks in cattle. Overt disease is prevented by simultaneous treatment with oxytetracycline. Immunised cattle develop full immunity within 4 weeks to the component- and immunologically- related stocks of *T. parva.*

Indications

The ECF vaccine is indicated in the following situations:

- For *Bos indicus* calves in ECF endemic areas. It can be used safely in calves over 1 month of age.
- For pure and cross-bred cattle susceptible to ECF in endemic areas, or for cattle to be moved to endemic areas.
- Other cattle not immune to ECF.

The vaccine will not protect against theileriosis caused by *Theileria annulata* or *Theileria mutans*.

Contra-indications

Cattle should not be immunised with this vaccine in the following circumstances:

- Cattle incubating or showing symptoms of ECF. These cattle should be treated with anti-theilerial drugs e.g. Butalex.
- Calves less than 1 month of age.

- Cattle in poor condition and or suffering from other disease, especially those undergoing infection with Foot and Mouth Disease or Lumpy Skin Disease. There is a risk of adverse ECF immunisation reactions in these cattle.
- Cattle in the last 3 months of pregnancy.
- Cattle treated with levamisole within one month of planned immunisation. There is a risk of ECF immunisation reactions in these cattle.
- Cattle in draught work.

Storage and handling of the vaccine

- Vaccine Stabilate concentrate must be kept frozen in liquid nitrogen until immediately before use.
- Vaccine Stabilate diluent must be kept frozen below -20C until required for dilution of Vaccine Stabilate concentrate. Refer to details below.
- In the field, the Vaccine Stabilate diluent must be kept in a coolbox on ice, but not submerged in ice/water.

Directions for use – Vaccine Stabilate dilution and immunisation procedure

Thawing, dilution and handling of Vaccine Stabilate and diluent

(a) Vaccine Diluent

Thaw the diluent using warm water, as necessary, but below 40^{o}C, taking care to avoid immersing the bottle cap, since water may enter the bottle. Carefully mix the diluent bottle during thawing, but do not shake to avoid frothing. Check to ensure the diluent is completely thawed, dry the bottle and place immediately on ice. The temperature of the diluent during this process should not rise above about 10^{o}C.

Do not use thawed diluent if colour is deep red or bright yellow.

Once thawed, unused diluent should not be frozen and re-used.

(b) Vaccine Stabilate concentrate

1. Take out the number of straws required (bearing in mind time available to immunise with the prepared vaccine is 2 hours - 4 hours) and the number of diluent bottles to be used (1 straw = 32 doses of current Vaccine Stabilate – minus handling losses). Thaw the straws by rolling them in between palms for 1-2 minutes. Normal safety precautions should be observed in handling liquid nitrogen.

2. Remove the contents of the straw into a pre-cooled sterile serum tube e.g. Nunc 2 ml tube. If 2 or more straws are to be used it is advisable to pool the straw contents.

3. Draw 0.4 ml of Vaccine Stabilate concentrate into a 1 ml syringe, and inject into one diluent bottle, but firstly drawing some diluent into this syringe, allowing for mixing of diluent and stabilate and injecting the mixture into the diluent bottle. This process should be repeated to ensure that the full volume of Vaccine Stabilate has been transferred. Keep diluent bottle inverted during this mixing to avoid air bubbles and frothing of the diluent bottle contents.

4. Put prepared vaccine on ice in a suitable cool box, it can now last for 2 hours, with 4 hours maximum under optimal conditions. Do not allow cap of bottle to come into contact with or be immersed in ice-water.

5. Do not use prepared vaccine if colour is deep red or bright yellow. Normal colour should be in the range orange-red to orange-yellow.

6. Mix the prepared vaccine gently and avoid shaking the vaccine bottle as this will cause foam and loss of usable vaccine volume. Draw 1 ml of prepared vaccine in a suitable 1ml syringe from the diluent bottle and inoculate immediately as above. Return the vaccine bottle to storage on ice as quickly as possible. Do not fill the syringe unless the animal can be inoculated immediately, as keeping the filled syringe in the environment can result in deterioration of the vaccine.

Immunisation procedure

1. Tag the animal and record the number.

2. Weigh the animal and record the weight.

3. Injection of long acting oxtetracycline (OTC) 30% (1 ml/ 10 Kg), volumes of drug exceeding 15 ml should be injected in different sites, calves below 50 kg receive a standard 5 ml dose.

4. Inject 1 ml of prepared Vaccine Stabilate, diluted as below, subcutaneously close to the parotid gland (i.e. slightly below and in front of the base of the ear). Use this opportunity to check for possible gland swelling due to ECF and if detected do not immunise, but treat with anti-theilerial drugs e.g. Butalex.

5. Monitor the animals for 10-20 minutes after immunisation in order to observe possible allergic reaction (skin rash, lacrimation, salivation, swollen eyelids, rapid breathing). If severe allergic reactors are observed treat the animal with 2-4 ml of adrenaline (1:1000 dilution).

6. Monitor animals after immunisation in close collaboration with the livestock owner. Animals showing severe signs of ECF 2-3 weeks after immunisation must be treated with anti-theilerial drugs.

Note: Any stabilate left after the last animal has been immunised must be discarded.

Safety (adverse reactions in cattle to immunisation)
The VS has been thoroughly tested in large numbers of cattle before release to provide a safe immunising dose. The use of 30mg/Kg OTC has been shown to eliminate or minimise untoward reactions in cattle.

The vaccine supplier/immunising team will instruct livestock owners about monitoring of immunised cattle and on the action to take in the rare case of an adverse reaction to immunisation, as follows:

- Cattle should be monitored for 10-20 minutes after immunisation in order to observe possible allergic reactions
    - These could include: skin rash, lacrimation, salivation, swollen eyelids.
    - If severe allergic reactors are observed cattle should be treated with 2-4 ml of adrenaline (1:1000 dilution).

- Cattle should also be monitored for 2-3 weeks following immunisation in close collaboration with the livestock owner. Animals showing severe signs of ECF must be treated with anti-theilerial drugs e.g. Butalex ®

Operator safety

Humans are not at risk of infection with ECF.

Efficacy

The trivalent live ECF vaccine will protect cattle against field challenge from a wide range of *Theileria parva* stocks, but the possibility of unknown and different immunogenic stocks breaking through this immunity cannot be excluded.

It has also been demonstrated to protect cattle in areas with presence of African buffalo, such as in Northern Tanzania and in the Loita District of Kenya. However, the vaccine may not protect against all *Theileria parva* stocks derived from buffalo.

Additional precautions

Oxytetracycline (OTC) brands for immunisation

The brand and concentration of OTC administered as treatment during immunisation can influence the response of cattle to immunisation and therefore the quality of the immune response. Only approved brands and drug concentrations should be used during immunisation.

At present only one brand and dose of OTC has been evaluated for use with this vaccine. Certain other OTC brands and doses have been used with different vaccines, but the determination of the Vaccine Stabilate dose to provide safe and protective immunisation has only been carried out with one brand of OTC.

Warnings

- Standard safety precautions must be followed for the handling of liquid nitrogen.
- This vaccine may lose efficacy if the conditions for handling as described above are not followed precisely.

Manufacturer and distributor details

TBD