THE IMPACT OF THE INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

EDITED BY JOHN McINTIRE AND DELIA GRACE
6 The Management and Economics of East Coast Fever

Philip Toye¹, Henry Kiara¹, Onesmo ole-MoiYoi², Dolapo Enahoro³ and Karl M. Rich⁴

¹International Livestock Research Institute, Nairobi, Kenya;
²Senior Visiting Scientist, International Centre of Insect Physiology and Ecology, Nairobi, Kenya; ³International Livestock Research Institute, Accra, Ghana; ⁴International Livestock Research Institute, Dakar, Senegal

Contents

Executive Summary 240
The problem 240
ILRI’s contribution in the global context 240
Impacts of ILRI research 241
Scientific impacts 241
Development impacts 241
Economic impacts 242
Policy impacts 242
Capacity building 242
Partnerships 242
Introduction 242
The Parasite 243
Control methods 244
Impacts of ECF 244
The Infection-and-Treatment Method (ITM) of Vaccination 245
Development of the ITM vaccine 246
ILRI’s contribution to the Muguga cocktail 246
Development and application of molecular tools 247
Monoclonal antibodies (mAbs) 248
DNA-based strain identification 249
Research to Develop a Subunit Vaccine 250
Protection of exposed animals 250
The anti-schizont vaccine 250
Lack of protection with serum 250
The central role of CTLs 250
Identification of CTL antigens 251
Antigenic diversity 251
Immunodominance 252
The anti-sporozoite vaccine 252
ITM: The Future 252
The buffalo problem 252
Executive Summary

The problem

East Coast fever (ECF) is a fatal bovine disease caused by the protozoan parasite *Theileria parva*. The disease occurs in 16 countries in eastern, central and southern Africa where the vector, the brown ear tick (*Rhipicephalus appendiculatus*), is found. ECF causes major economic losses by affecting both dairy cows and young Zebu cattle in pastoralist systems and ranches. It is among the most serious constraints to cattle productivity in the countries in which it is found.

The costs of ECF include both direct and indirect losses. Direct losses are due to cattle deaths, the stunting of calves, reduced milk production in survivors, and the costs of preventing and controlling the disease. Indirect losses include the lack of adoption of more productive breeds of cattle and the avoidance of areas of high infection risks. ECF affects households by reducing milk supplies, depleting assets and reducing incomes, all of which harm household food and nutritional security.

ECF has been controlled predominantly through acaricide application, but this treatment is expensive and not always successful. An alternative option is for farmers to keep local breeds of cattle, which tend to be more disease resistant but less productive than exotic breeds.

It is widely accepted that vaccination is the most attractive control option, and the development of a vaccine to protect cattle against ECF was one of the founding aims of the International Laboratory for Research on Animal Diseases (ILRAD).

At about the time of ILRAD's establishment in 1973, a vaccination procedure was being developed at the East African Veterinary Research Organization (EAVRO) at Muguga, Kenya. The infection-and-treatment method (ITM) is an immunization procedure against ECF. It involves inoculation of live sporozoites of *T. parva*, usually in the form of a semi-purified homogenate of *T. parva*-infected ticks, combined with simultaneous treatment with a dose of a long-acting formulation of the antibiotic oxytetracycline. Whilst safe and very effective when administered correctly, production and delivery of this live ECF vaccine is complicated, expensive and time consuming, and at the time of ILRAD's founding, there were doubts as to whether such a procedure was commercially viable.

ILRI's contribution in the global context

The International Livestock Research Institute (ILRI) has played a pivotal role in overseeing ECF management in the affected regions of Africa. From the beginning, ECF and trypanosomiasis
were central to the work of ILRAD. The institute has led activities in various vaccine development strategies, including commercial production of the ITM vaccine. ILRI has also overseen the development and application of molecular tools to characterize the vaccine and to address concerns by veterinary authorities about the risks of using ITM in the field. ILRI has also undertaken major research efforts to develop an alternative (subunit) vaccine and has furthered our understanding of the bovine immune response in support of these efforts.

**Impacts of ILRI research**

**Scientific impacts**

ILRI has generated important research findings in several aspects of ECF research. These are outlined as follows.

**ITM.** Scientific contributions to the development, production and use of ITM immunization have been significant; together, these contributions have enabled the immunization of hundreds of thousands of cattle in both the pastoralist and dairy sectors and have assisted the commercial production, distribution and use of the vaccine in eastern Africa.

**Immunology of T. parva infection.** In terms of scientific impacts, ILRI scientists provided convincing evidence that a response by cytotoxic T lymphocytes (CTLs) was the main effector mechanism deployed by immune cattle against T. parva infection; this work was important in the broader context of vaccine development as it was one of the first demonstrations that CD8+ CTLs could mediate protection against intracellular protozoan parasites. ILRI scientists made the first successful identification of T. parva proteins recognized by CTLs from immune cattle; the identification of these CTL antigens was a major achievement in vaccine research, and further examination of the antigens and the CTLs directed against them has yielded some very valuable insights into the immunobiology of the host–parasite relationship.

**Advances in bovine immunology.** As part of the work investigating the immunology of T. parva infection, significant advances were made in our understanding of the bovine immune system. These are discussed here and in Chapter 4 (this volume).

**Sequencing of the T. parva genome.** Sequencing of the T. parva genome was carried out by Gardner et al. (2005). This was the second apicomplexan to be sequenced and was essential in screening for CTL antigens. A related paper (Pain et al., 2005) compared the genome of Theileria annulata with that of T. parva.

**Strain characterization.** Scientists at ILRI in collaboration with many other researchers developed DNA-based methods to characterize Theileria spp. parasites, including restriction fragment length polymorphisms of repetitive regions of the T. parva genome, analysis of polymorphisms in ribosomal RNA genes, in telomere regions of the parasite chromosomes and in genes encoding T. parva antigens and the use of microsatellites and minisatellites.

**Sporozoite subunit work.** Scientists at ILRI demonstrated that immunity to T. parva could also be induced through vaccination with the p67 protein, which is present on the surface of the infective sporozoite stage of the parasite. The protection is believed to be mediated primarily by antibodies.

**Cellular proliferation.** A spillover impact of ILRI’s ECF research, which turned out to be important for the medical research community, was the discovery that upregulated casein kinase 2 is the cause of uncontrolled cell proliferation in cattle suffering from theileriosis. This discovery in this ‘bovine cancer’ research advanced our understanding of the role of this enzyme in the development of certain human cancers, and thus of potential targets for treatment regimes.

Epidemiological scientific impacts are described in Chapters 5 and 8 (this volume). A major ILRAD product was the book The Epidemiology of Theileriosis in Africa (Norval et al., 1992). This not only focused on epidemiology but also covered all aspects of the parasite and infection life cycles.

**Development impacts**

In 1996 at the request of the FAO, ILRI produced 600,000 doses of the ‘Muguga cocktail’, a version of the ITM vaccine, which was subsequently distributed commercially. In 2007, when stocks of this batch were depleted, ILRI was asked by
AU-IBAR to produce a second batch at its own cost. With no other institutions in the region with the facilities and expertise to oversee this task, ILRI again complied, producing 1.2 million doses of the ‘Muguga cocktail’ vaccine (‘ILRI08’ batch), almost all of which has been commercially distributed. In 2008, ILRI addressed concerns of smallholder dairy farmers regarding the large number of doses that were included in each vaccine straw. To make relatively few doses available to those keeping just a few cows, ILRI produced vaccine straws with just five to eight doses. The doses in these straws proved to be as safe and effective as those in the larger-dose straws. Use of the ECF ITM vaccine has had major development impacts. It has protected approximately 1.6 million cattle against the disease from 1997 to 2014, preventing the untimely deaths of some 400,000 animals over that period, assuming a typical annual calf mortality of 40% (Di Giulio et al., 2009). About 80% of the ECF ITM vaccine has been sold in the pastoral production systems of northern Tanzania, about 10% in the pastoral systems of Kenya and the remainder in the smallholder dairy systems of Kenya. ILRI also facilitated the transfer of production of the vaccine to the Centre for Ticks and Tick-Borne Diseases (CTTBD) in Malawi.

Economic impacts

The production and distribution of the ECF ITM vaccine has protected millions of cattle, preventing the deaths of thousands of valuable animals. Use of the vaccine also improved milk production and reduced stunting in calves and the costs of preventing and controlling the disease. Earlier studies estimated that adoption of multi-component ECF vaccines in affected countries would reduce the value of calf mortality annually (by US$10.1 million) and increase the value of milk production (by US$1.7 million), with estimated economic returns of US$9–17 for every dollar invested. Other economic modelling that considers vaccine adoption processes over time and a wide range of economic impacts is presented in this chapter. In Kenya, production of beef and dairy in 2030 is shown to increase by up to 40% and 56%, respectively, compared with baseline conditions of no new investments in ECF management. Changes such as these have potential implications for incomes obtained by farmers and prices paid by consumers but also on food imports, livestock feed demand, and land use.

Policy impacts

ILRI scientists helped obtain official registration of the ECF ITM Muguga cocktail vaccine in Kenya, Malawi and Tanzania and approval for use in Uganda pending registration. Until the vaccine was registered in each country, it had only been used with special permission given by the national veterinary authorities.

Capacity building

ILRI supported at least 34 graduate fellows in ECF studies, including 20 PhD degrees, 13 MSc degrees and one post-doctoral scientist. ILRI also contributed significantly to vaccine production capacity CTTBD, Malawi.

Partnerships

Development of the ECF ITM vaccine and overall management of ECF in the region has led to several ILRI partnerships with other institutions, including: the Africa Union–Interafrican Bureau for Animal Resources (AU-IBAR), Centre for Ticks and Tick-Borne Diseases (CTTBD, Malawi), Food and Agriculture Organization of the United Nations (FAO), GALVmed (UK), Kenya Agricultural and Livestock Research Organization (KALRO), United Nations Development Programme (UNDP), Vet Agro Limited (Tanzania), VETAID (Uganda) and Vétérinaire Sans Frontière-Germany (VSFG).

Introduction

ECF is a devastating tick-borne disease of cattle caused by a protozoan parasite, *Theileria parva* (Norval et al., 1992, pp. 64–97, on the classification of *Theileria* spp.; Coetzer et al., 1994). The majority of the discussion in this chapter and in Chapter 5 (this volume) is of *T. parva*. The form of theileriosis known as ECF is present in 12 countries in eastern, central and southern Africa where the vector, the brown ear tick (*Rhipicephalus appendiculatus*), is found (CABI, 2020). ECF causes major economic losses by affecting both dairy cows and young Zebu cattle in pastoralist systems and ranches. A clinically
similar disease, Corridor disease, is found in cattle infected with *T. parva* transmitted by ticks which have fed on buffalo. The chief distinguishing feature of Corridor disease is the low number of the piroplasm (blood) stage of the parasite. A milder form of ECF in Southern Africa with strong seasonal occurrence is referred to as January disease in Zimbabwe. It is among the most serious constraints to cattle productivity in the countries where it is found. Development of a vaccine to protect cattle against ECF was one of the founding aims of ILRAD (see Introduction chapter, this volume). *T. parva* also causes corridor disease if buffalo-adapted parasites are transmitted to cattle, and January disease in Zimbabwe. These have similar clinical signs to ECF but last for only a few days, and emaciation and diarrhoea are not seen. Turning sickness is an aberrant infection characterized by neurological signs caused by parasites in cerebral blood vessels.

*T. annulata* causes tropical theileriosis or Mediterranean Coast fever. Transmitted by hyalommid ticks, it occurs in North Africa, southern Europe, the Near and Middle East, India, China and Central Asia. It causes both mortality and reduced production, and has significant economic impacts as a result.

**The Parasite**

The *T. parva* parasite has a complex life cycle involving bovine hosts (African buffalo and domestic cattle) and the tick vector (Fig. 6.1).

The tick feeds on the host three times, as a larva, nymph and adult. *Theileria* sporozoites develop in the salivary glands of infected ticks and are passed to cattle along with tick saliva when the ticks feed. In cattle, these sporozoite forms of the parasite attach themselves to the animal’s white blood cells (lymphocytes): some sporozoites enter lymphocytes and develop into multinucleate parasite forms called macroschizonts. The infected bovine lymphocytes become enlarged.

![Fig. 6.1. Life cycle of *T. parva* (anon, ILRI).](image-url)
cells (lymphoblasts) that multiply in synchrony with the parasites, resulting in a rapidly expanding population of infected bovine cells. Some of the macroschizonts differentiate into microschizonts and then merozoites during infection. These merozoites are released into the bloodstream and invade red blood cells, where they further develop into forms called piroplasms. Ticks feeding on cattle become infected when they ingest red blood cells containing piroplasms. In the tick gut, the parasites differentiate into male and female gametes, which fuse to form zygotes. These develop further and eventually migrate to the tick’s salivary glands. Here, stimulated by tick feeding, 30,000–50,000 sporozoites develop in an average tick. These are introduced with tick saliva into a new bovine host, initiating a new cycle of parasite development.

The initial clinical signs of infection include pyrexia and enlargement of the superficial lymph nodes, most notably the parotid and precapular nodes. As the infection progresses, the animals become listless and anorexic and exhibit severe respiratory distress. There is often a progressive loss of white blood cells. On post-mortem examination, infected lymphocytes are found in several organs including the lymph nodes, lungs, liver, kidneys, gastrointestinal tract and sometimes the brain. A particularly prominent post-mortem finding is frothy exudate in the trachea and severe congestion of the lungs (Irvin et al., 1983). In contrast, T. parva-infected buffalo show few if any clinical signs of disease.

It is believed that T. parva co-evolved with the African Cape buffalo and has undergone a ‘host jump’ to cattle, where it causes the disease referred to as ECF (reviewed by Norval et al., 1992).

Control methods

The dominant method for controlling ECF has been the application of acaricides to cattle to limit their tick infestations. The acaricides are applied as sprays, in dips and more recently as oil-based pour-ons. This control method has several disadvantages. After prolonged use, the ticks can develop resistance to the active ingredient in the acaricides (Abbas et al., 2014) and few if any novel acaricides are expected on the market in the immediate future. Ticks have developed resistance to all known classes of acaricides. The rate of development of resistance has ranged from 2 years (synthetic pyrethroids) to 40 years (arsenic) influenced by, among other factors, the class of acaricide and the frequency of application. Acaricides also expose users to potential health risks, which are exacerbated by lack of protective clothing and can also cause environmental contamination. Frequent use of acaricides is expensive – in high ECF-challenge areas, application might be needed every 5 days.

A second strategy to reduce the losses associated with ECF is to use less susceptible, but less productive, indigenous cattle rather than improved cattle breeds, which are more susceptible to the disease (Stobbs, 1966; Ndungu et al., 2005). As a third strategy, drugs are available for the treatment of clinical ECF, but to be effective, these must be used at an early stage of the disease and thus require constant monitoring of cattle, which presents difficulties, especially in pastoral systems. In addition, the cost of the drug treatment is high (US$40 per animal), which can be prohibitive, especially for less valuable Zebu animals. More recently, a vaccination protocol known as the infection-and-treatment method (ITM) has become available commercially and is increasingly being used, as discussed below.

Impacts of ECF

The impacts of the disease include direct losses due to cattle deaths, the stunting of calves, reduced milk production in survivors, and the cost of measures to prevent and control ECF. Indirect losses include the lack of adoption of more productive breeds of cattle and the avoidance of areas of high infection risk. ECF affects households by reducing milk supply, depleting assets and reducing incomes, all of which harm household food and nutritional security. Moreover, for smallholder dairy farmers with just one or two animals, the loss of a valuable cow can be a devastating blow. In one pastoralist area in northern Tanzania, overall calf mortality was shown to be 40–80%, with 75% of all deaths due to ECF (Di Giulio et al., 2009). Chapter 5 (this volume) reports more detailed epidemiological and economic studies of the impact of ECF.

It is estimated that 40 million of the 75 million cattle in the eastern, central and southern Africa region are at risk of contracting ECF.
The Management and Economics of East Coast Fever

(Morzaria and Williamson, 1999; McLeod and Randolph, 2000; FAO, 2014, 2017).

In 1992, an ILRI-led study estimated that ECF caused annual economic losses totalling US$170 million, including more than 1 million cattle deaths a year (Mukhebi et al., 1992). By 2005, cattle deaths alone, at 1.1 million head, accounted for more than US$300 million, based on the unit price of cattle. ECF-related cattle deaths in 2005 thus represented around 44% of the combined value of beef production in Burundi, Kenya and Rwanda in that year (FAO/AU-IBAR/ILRI, 2017).

Various studies have estimated the impact of the disease at national or local levels. In 1999, an ILRI-led study estimated that total losses due to ECF in Kenya were more than US$95 million a year, with mortality accounting for almost three-quarters of this amount; loss of milk production due to morbidity and the cost of acaricides made up most of the balance. For Tanzania, the estimated total annual loss was US$43.8 million, most of which was due to mortality. These estimates can be of significant economic importance. For example, the annual ECF-related losses in Kenya estimated in 1999 would have been equivalent to 11% of the total value of output from the livestock subsector, while the annual losses in Tanzania translated to up to 46% of the same.

The Infection-and-Treatment Method of Vaccination

ITM is an immunization procedure against ECF. The basis for the development of ITM lay in the observations, made shortly after the disease was characterized, that cattle that survived an episode of ECF were unlikely to experience a second clinical episode. Early experiments of Arnold Theiler showed that cattle infected ‘artificially’ with parasite preparations were subsequently resistant to field challenge. Proof of concept of the ITM approach as a practical means of vaccination was achieved during the 1970s by the group at EAVRO led by Matt Cunningham, as has recently been documented in detail by Perry (2016), and much of the credit for current field application of ITM against theileriosis belongs to these scientists, who long preceded ILRAD and ILRI.

ITM involves inoculation of live T. parva sporozoites, usually in the form of a semi-purified homogenate of T. parva-infected ticks, combined with simultaneous treatment with a dose of a long-acting formulation of the antibiotic oxytetracycline. Without the antibiotic treatment, the sporozoite inoculation would be lethal. The oxytetracycline suppresses the infection by inhibiting the development of sporozoites to schizonts in lymphocytes (Spooner, 1990). The outcome is an asymptomatic infection or a mild ECF episode followed by the development of a protective immune response. The immunity has been shown to last for at least 43 months in the absence of challenge (Burridge et al., 1972) and it is accepted that a single inoculation confers life-long immunity to the disease under field conditions. Cattle that have been immunized by ITM, or that have survived a natural challenge, may become ‘carriers’ of a persistent, potentially tick-transmissible, infection.

Adoption of the ITM vaccine has been reported to be associated with a range of benefits. Calf mortality rates have been dramatically reduced, such as in pastoralist areas of northern Tanzania where calf mortality rates dropped from 80% to as low as 2% in a study of 2178 crossbred calves (1434 immunized and 744 controls) in 167 smallholder households in two districts (Lynen et al., 2012). Pastoralists also report that cattle wearing the distinctive round ECF ear tag attract higher prices than non-vaccinated animals of equal size. In another study in Tanzania, it was found that households that had used the ITM vaccine sold twice as many animals as non-adopting households (Homewood et al., 2006).

Although acaricides may still be required for other tick-borne diseases, vaccination by ITM reduces the frequency of acaricide application. In both smallholder dairy and pastoralist areas of northern Tanzania, acaricide application is now often done once a month rather than once a week. Pastoralists also report that when their animals are vaccinated, they no longer have to avoid areas where they know cattle are at high risk of ECF. A study by Lynen et al. (2012) reported that 80% of the farmers reduced the frequency of acaricide treatment after immunization, while 38% reduced this treatment by more than 75%.

Other methods of controlling ECF are available. They include treating animals with anti-theilerial drugs when they fall sick. The therapeutic drugs are quite effective but require early diagnosis as the disease progresses very
quickly. The drugs also tend to be expensive, costing US$40–60, and are beyond the reach of many smallholder farmers. This drug treatment also has the disadvantage that once an animal suffers ECF, production losses, including stunting in calves, follow.

In smallholder dairy systems where cattle are kept confined rather than allowed to graze, exposure to ticks, and therefore diseases, is minimized. Where farmers can grow their own fodder, this is quite an effective disease control strategy. Quite often, however, farmers must get feed from outside the farms and this can introduce ticks, leading to disease outbreaks. As mentioned, another approach has been to maintain indigenous cattle breeds that are relatively tolerant to tick-borne diseases, including ECF. However, the local breeds are less productive than improved breeds, so this strategy is unsuitable for intensive smallholder dairy farming.

### Development of the ITM vaccine

Work to develop an ECF vaccine began in the 1960s at EAVRO, located in Muguga, Kenya, under the auspices of the East African Community. Following the collapse of the East African Community, it continued at the National Veterinary Research Centre, KARI (now the Veterinary Research Centre, KALRO). This culminated in the introduction of the ITM Muguga cocktail vaccine on a commercial basis in various eastern African countries for various classes of cattle between 1998 (Tanzania pastoral sector) and 2012 (Kenya dairy sector).

For close to 50 years, many partners have been involved in the testing, refinement, re-testing, production, registration and commercialization of the vaccine. Key among this team has been (in alphabetical order): the Africa Union–Inter-African Bureau for Animal Resources (AU-IBAR), CTTBD (Malawi), Food and Agriculture Organization of the United Nations (FAO), Global Alliance for Livestock and Veterinary Medicines (GALVmed, UK), KARI, UNDP, Vet Agro Limited (Tanzania), VETAID (Uganda) and VSFG. Financial support for more than four decades has been provided by many donors and investors, including the Bill & Melinda Gates Foundation (BMGF), Biotechnology and Biological Sciences Research Council (BBSRC), CGIAR Funders through ILRI, Danish International Development Agency (DANIDA), FAO, International Fund for Agricultural Development (IFAD), Netherlands government, Overseas Development Administration/Department for International Development (ODA/DFID) and Wellcome Trust, among others.

The ITM method was built on three key practical developments:

- The demonstration that animals could be reproducibly infected with a ground-up suspension of whole infected ticks.
- The observation that such tick preparations could be stored for extended periods in liquid nitrogen and were infectious when thawed.
- The use of antibiotics, particularly long-acting tetracycline, as a reliable means to prevent the clinical effects of a dose of the tick suspension without impairing the development of immunity.

One challenge that remained was that of parasite strain specificity, or the frequent inability of a single parasite strain when used in ITM to protect against all other strains of the parasite. Researchers at EAVRO assessed the protective capacity of several parasite isolates and showed that a combination of parasites from three isolates – Muguga, Serengeti-transformed and Kiambu 5 – offered the most complete protection to heterologous challenge (Radley et al., 1975). The combination was named the ‘Muguga cocktail’. Other forms of ITM have been produced and tested over the years using different T. parva isolates. The veterinary authorities in Kenya, Zambia and Zimbabwe favoured the locally derived, single isolates Marikebuni, Boleni and Katete/Chitongo, respectively, while the rest of the eastern African region chose the Muguga cocktail. Kenya later also adopted the Muguga cocktail.

### ILRI’s contribution to the Muguga cocktail

Production of the ITM vaccine is a long and complicated process, involving artificial infection of ‘production’ cattle, application of several hundred thousand ticks to the cattle and homogenization of the subsequently infected ticks (Patel et al., 2016). The clean ticks, which come from a selected population of highly susceptible ticks maintained by ILRI’s tick unit, are fed on production cattle infected with one of the three component stabilates. Infected ticks are analysed
for infection rates to ensure that equal numbers of sporozoites from each isolate are included when the ticks are combined before homogenization. The Muguga cocktail stabilate is evaluated in extensive in vivo trials to ensure that the vaccine is both safe and effective and to determine the appropriate dilution with respect to a fixed dose of oxytetracycline.

Given the complicated production process, one of the perceived obstacles to the widespread use of the ITM Muguga cocktail was the prospect of producing standardized, large-scale batches of several hundred thousand doses of the vaccine. In 1996, ILRI was asked by FAO to undertake this challenge, which resulted in the production of over 600,000 doses of a safe and effective vaccine. At the time, there was no other institution in the region with the facilities and expertise to undertake this task. The vaccine was provided to CTTBD, in Malawi, for subsequent sales and distribution. All the available vaccine was sold unsubsidized on a commercial basis, which provided strong evidence that a commercially viable demand for the vaccine existed.

In 2007, when the stocks of the FAO-requested ILRI batch were about to be depleted, ILRI was asked to produce a second commercial-scale batch. At its own expense, the institute produced a batch of about 1.2 million doses (named ‘ILRI08’). These were sold to distributors authorized by the respective national veterinary authorities. As in 1998, no other institution in the region could mass produce a quality-assured ITM Muguga cocktail vaccine, so ILRI’s production again ensured continued vaccine supply at a time when demand for the vaccine was gradually increasing across the region, especially in Tanzania. In association with GALVmed, ILRI also compiled the documentation required to support the registration of the ITM vaccine produced at ILRI. ILRI scientists continue to provide technical advice to Malawi.

Further production of the vaccine will be undertaken by CTTBD, in Malawi. In conjunction with GALVmed, ILRI facilitated the establishment of the process in CTTBD and supplied the vaccine seed stabilates, which were made at the same time as the ‘ILRI08’ vaccine batch, and well-characterized pathogen-free *Rhipicephalus appendiculatus* ticks. ILRI scientists continue to provide technical advice to Malawi.

One of the commonly voiced issues surrounding the ITM vaccine, especially in the smallholder dairy systems, is the number of doses in the vaccine straws (ILRI/GALVmed, 2015). The standard presentation of the ITM vaccine produced at ILRI is plastic straws of 32–40 doses, cryopreserved in liquid nitrogen. Once thawed and prepared for vaccination with diluent, the vaccine has a working life of only a few hours (Mbao et al., 2006); any vaccine unused by that time must be discarded. Whereas this is not a major problem when vaccinating large herds in pastoralist settings, use of the vaccine in smallholder settings, where each smallholder may have only two or three cattle, can lead to substantial wastage of the vaccine. To overcome this, ILRI demonstrated that the vaccine straws can be thawed, diluted, refrozen and repackaged with minimal loss of vaccine viability. An initial experimental production resulted in straws containing five to eight doses (Patel et al., 2019). In collaboration with Vet Agro Limited, in Tanzania, ILRI showed that the vaccine was safe and effective under both experimental and field conditions. Importantly, cattle vaccinated with the diluted vaccine are protected against challenge with the parent stabilate, suggesting that any important antigenic types are not lost during the process.

**Development and application of molecular tools**

Over the past 35 years or so, ILRI has developed and utilized a series of increasingly sophisticated molecular tools to investigate various aspects of *T. parva* infection. These have taken advantage of, and kept pace with, advances in the fields of immunology, molecular biology, genomics and biotechnology. Some of these tools, which were developed primarily to study the biology and epidemiology of the parasite or to facilitate the pursuit of a subunit vaccine against *T. parva*, have
found subsequent use in the analysis of the ITM vaccine. These tools have improved the quality control of the immunizing stabilate and have provided greater insights into the true genetic complexity of the Muguga cocktail and the efficacy and biological impact of ITM in the field. They have also helped to allay concerns of the veterinary authorities about the risks of using ITM in the field.

Monoclonal antibodies (mAbs)

mAbs were raised against the schizont stage of the parasite specifically to try to define the immunological heterogeneity that had been observed during the early stages of the development of the ITM vaccine. The first set of seven mAbs did indeed show differential reactivity in immunofluorescent assays with a set of schizont-infected cells, especially those derived from buffalo (Pinder and Hewitt, 1980). These observations were confirmed with a further nine mAbs tested on an extended array of parasite isolates (Minami et al., 1983). An additional observation from this work was that immunized cattle were susceptible to heterologous challenge from a parasite of different mAbs reactivity profiles, and that the ‘breakthrough’ parasites had the same profile as those comprising the heterologous challenge. In a companion publication, it was reported that cattle were protected from heterologous challenge from parasites having a similar mAb profile but not those with a different profile (Irvin et al., 1983).

One of the practical aims of developing the mAbs was to use them to characterize antigenically distinct strains for inclusion in an ITM stabilate for use in field immunization. This has not been possible, primarily because most of the anti-schizont mAbs recognize the same antigen and it is now known that several other antigens are the targets of the protective immune response induced by ITM (see below). A panel of nine of the mAbs was used as part of a study involving several approaches to characterize the component stocks of the Muguga cocktail (Bishop et al., 2001). The results showed that two of the stocks (Muguga- and Serengeti-transformed) displayed identical patterns of reactivity, whereas the Kiambu 5 stock did not react with three of the mAbs. The original 16 mAbs described above, plus an additional four generated against buffalo-derived parasites, were used to characterize infected lymphocytes from buffalo (Conrad et al., 1987b; Baldwin et al., 1988). Among other things, the results showed that parasites with different mAb reactivity patterns were present in a single isolate and that different parasite types were isolated from the same animal when sampled at different times but that cloned parasite lines did not alter their mAb reactivity profile. Although the possibility of antigenic variation (the ability of a single organism to express different antigens or forms of the same antigen at different times) in T. parva had been proposed earlier (Young et al., 1988), the last observation suggested that this was not the case. Thus, it became clear that the same animal can be infected with several parasite types concurrently, and that while an initial infection can result in protection against subsequent disease, it may not necessarily protect against subsequent infections. This observation has important implications in the ability of vaccinated animals to transmit parasites to other, non-vaccinated, animals.

The specificity of several of the mAbs has been determined and all were shown to react with a single antigen known as the polymorphic immunodominant molecule, or PIM (Shapiro et al., 1987; Toye et al., 1991). The differential reactivity with strains of T. parva is believed to be due to sequence variation of the epitopes located within the PIM antigen (Toye et al., 1995a). The size variation of the antigen among parasites from different isolates allowed an additional layer of discrimination. It was noted, for example, that analysis of cloned cell lines, all derived from the Marikebuni isolate, revealed four different parasite types (Goddeeris et al., 1990; Toye et al., 1991).

PIM was also shown to be the major antigen recognized by sera from infected cattle, which has led to its use in an enzyme-linked immunosorbent assay (ELISA) developed by ILRI scientists to detect antibodies to T. parva (Katende et al., 1998). This ELISA is used extensively during the production of the ITM vaccine to screen cattle to be used for production of the sporozoites for the stabilate and for assessing the safety and efficacy of the final product (Patel et al., 2016). In the field, the assay is relied upon to...
assess seroconversion levels following vaccination, and it has been used widely in epidemiology studies (Gitau et al., 1997; Okuthe and Buyu, 2006; Swai et al., 2009; Kivaria et al., 2012; Malak et al., 2012; Toye et al., 2013; Kiara et al., 2014).

Similar ELISAs were developed at ILRI for other tick-borne diseases caused by *Theileria* mutants (Katende et al., 1990), *Anaplasma marginale* (Morzaria et al., 1999) and *Babesia bigemina* (Tebele et al., 2000) and were transferred for commercial distribution to Svanova Biotech AB, now part of Boehringer-Ingelheim Animal Health. The *Theileria* assays were subsequently withdrawn from sale due to insufficient global demand. *T. parva* is limited to parts of eastern, central and southern Africa, and *T. mutans* is generally considered to be non-pathogenic. Nevertheless, the assays are still offered as kits and as a diagnostic service by ILRI.

**DNA-based strain identification**

Scientists at ILRI in collaboration with other researchers have developed a series of DNA-based methods to characterize *Theileria* parasites, including restriction fragment length polymorphisms of repetitive regions of the *T. parva* genome (Conrad et al., 1987a; Allsopp et al., 1989), analysis of polymorphisms in ribosomal RNA genes (Bishop et al., 1992), telomere regions (Morzaria et al., 1990) and genes encoding *T. parva* antigens (Geysen et al., 1999), and the use of microsatellites and minisatellites (Oura et al., 2003). An early application of these techniques to the ITM vaccine revealed that two of the component stabilates (Muguga and Serengeti-transformed) were remarkably similar and quite distinct from the Kiambu stabilate (Bishop et al., 2001). In a collaborative study with scientists from the University of Glasgow Veterinary School and the UK’s Institute of Animal Health at Pirbright, these markers were applied to analyse samples from cattle vaccinated in the field with the Muguga cocktail (Oura et al., 2004, 2007).

With the caution that these markers represent a very small but none the less highly polymorphic segment of the parasite genome, the work showed that the vaccine strains can persist in vaccinated animals for several years. Local parasite strains were also detected in animals after vaccination, indicating that the vaccinated animals can become re-infected but not show clinical disease. The studies also showed that non-vaccinated animals co-grazing with vaccinated animals could be infected with the vaccine parasites. This phenomenon has been put forward as a case against the use of live parasite vaccines, particularly those comprising parasites originating from areas outside those in which cattle are being vaccinated (McKeever, 2007). These concerns are allayed by observations that no deleterious effects from the use of ITM have yet to be reported, that such mixing of parasite strains has been occurring for millennia, particularly through the presumably unrestricted movement of infected buffalo, and that the parasite strains used in the vaccines originate from natural infections and are not ‘artificial’ or the result of genetic manipulation in the laboratory.

ILRI scientists were also part of a large international team that, in 2005, reported the sequencing of the *T. parva* genome. In the conclusion to the paper reporting this in *Science* (Gardner et al., 2005), the team described the genome data as ‘a critical knowledge base for a pathogen of significance to agriculture in Africa’. One immediate use of the genome sequence was the development of a panel of genome-wide mini- and microsatellite markers, which greatly enhanced the power and utility of molecular analyses, as described above. The application of the mini- and microsatellite markers coupled with high-throughput electrophoresis showed that the Muguga cocktail is more complex than previously thought, containing at least 14 different genotypes of *T. parva*, although they express a limited number of alleles (Patel et al., 2011). The study also showed, by comparing the genotypic composition between different batches of the vaccine, that the batches were very similar. High-throughput electrophoresis with the satellite markers has been shown to be a useful and reproducible approach that can be used to monitor the genetic composition of future ITM vaccine batches. Such a tool is essential to facilitate standardized, consistent, quality-assured vaccine production.

More recently, high-throughput sequencing has been used by ILRI scientists and collaborators to characterize the parasite more deeply. Norling et al. (2015) analysed the whole-genome
sequences derived from the three component stocks of the Muguga cocktail and showed that two of the stocks (Muguga- and Serengeti-transformed) were remarkably similar. Somewhat surprisingly, the total diversity residing in the three component stocks together represents only a small fraction of the *T. parva* genetic diversity observed in field isolates. This result was supported by satellite genotyping and high-throughput multilocus genotyping of genes encoding antigens recognized by cytotoxic T lymphocytes (CTLs) and believed to be important in the protective immunity seen in cattle (Hemminck et al., 2016). There was very limited diversity in many of the antigen gene or satellite loci and certainly far less than is seen in field populations of *T. parva*. The results raise the intriguing question of how the Muguga cocktail can protect cattle exposed to field challenge from *T. parva*. This is one of many questions yet to be addressed concerning this method of vaccination, as discussed below.

### Research to Develop a Subunit Vaccine

A subunit vaccine is one that avoids the use of a whole organism and instead relies on inoculation of those components that can stimulate, and are the target of, a protective immune response. The advantages of subunit vaccines are that they avoid the risk of infection with virulent organisms and, if they are manufactured by ‘synthetic’ means such as recombinant DNA technologies, are easier and cheaper to produce.

### Protection of exposed animals

A fundamental observation that serves as a basis for vaccine development is that animals that survive an infection are immune to the clinical effects of a subsequent exposure. For *T. parva*, this observation was made shortly after ECF was recognized (reviewed by Lawrence, 1992). The key knowledge gained from these and many subsequent experiments is that cattle have the capacity to prevent the clinical effects of *T. parva* infection, thus instilling confidence that an effective vaccine could be developed. What was required for the rational design of an ECF vaccine was an understanding of the mediators of this immunity and the corresponding parasite components that induced these mediators.

### The anti-schizont vaccine

#### Lack of protection with serum

The initial focus on the mediators of the protection seen in immune animals was on the role of serum. This is not surprising given the state of knowledge of, and tools available to study, mammalian immune responses at the time. The expectation was that infection with the parasite induced the production of protective serum antibodies. However, it was shown early on that cattle with high levels of anti-*T. parva* antibodies were nevertheless fully susceptible to infection (Wagner et al., 1974).

#### The central role of CTLs

Attention at ILRAD then turned to investigating a direct role for white blood cells as the primary mediators of immunity. The first evidence was obtained by Terry Pearson and co-workers, who showed that infected lymphocytes are specifically recognized and killed by effector cells from immune animals (Pearson et al., 1979). Shortly thereafter, Eugui and Emery (1981) showed that the cytotoxic (killing) activity was genetically restricted. In other words, the killing was only observed when the infected cells and the cytotoxic effector cells came from the same animal or closely related animals. This would be expected with killing mediated by classical CD8+ CTLs, where recognition of the infected cell is dependent on presentation of the parasite components by molecules of the class I major histocompatibility complex (MHC), which varies among animals. This was subsequently confirmed by Goddeeris et al. (1986a,b) who showed that the killing was mediated by cells of the CD8+ lineage and, by using a panel of mismatched and semimatched target cells, that it was restricted by a class I MHC molecule (KN104). Importantly, it was demonstrated that the killing was specific for strains of the parasite – the immune cells were derived from the Muguga isolate of *T. parva* and did not recognize cells infected with the
Marikebuni isolate. As this reflected earlier in vivo challenge experiments, such as the work undertaken in the development of the Muguga cocktail (discussed earlier), it provided convincing evidence that the CTL response was the main effector mechanism deployed by immune cattle. Further confirmation of this was provided by cell transfer experiments where a purified population of CD8+ lymphocytes from immune animals was shown to provide protection when transferred into a naïve twin recipient (McKeever et al., 1994).

This work was important in the broader context of vaccine development as it was one of the first demonstrations that CD8+ CTLs could mediate protection against intracellular protozoan parasites. The challenge was then to replicate the induction of the protective CTL response by using isolated parasite components (antigens) rather than the whole organism – in other words, a subunit vaccine.

**Identification of CTL antigens**

By the early 1990s, the techniques to identify, characterize, isolate and administer parasite components that are the targets of antibody responses were well advanced and commonplace. The same situation did not apply to those recognized by CTLs. In general, CTLs are induced by and recognize antigens when they are presented on the surface of infected cells in the context of the class I MHC molecule. The most efficient way to evaluate individual components from infectious organisms for CTL recognition is to introduce the gene that encodes the antigen into a cell expressing the appropriate MHC class I molecule, and one of the technologies that was emerging to do this was transfection technology.

Among the first uses of transfection technology at ILRI was the production of cells suitable for expressing candidate antigen genes. Thus, a widely used mouse cell line was transfected with two bovine class I MHC genes, which, at the same time, provided the first conclusive evidence that a second class I MHC locus existed in cattle (Toye et al., 1990; Bensaid et al., 1991). Transfection technology also proved useful in isolating the gene encoding an early candidate CTL antigen (PIM), which had proved recalcitrant to isolation by the traditional bacterial expression systems (Toye et al., 1995b), and in establishing the specificity of mAbs used to characterize lymphocyte subsets (Naessens et al., 1992; MacHugh et al., 1993).

The feasibility of using transient transfection technology to screen libraries of genes for candidate CTL antigens was also demonstrated (Toye et al., 1995c). Eventually the use of a random immunoscreen coupled with targeted gene analysis yielded the first successful identification of T. parva proteins recognized by CTLs from immune cattle (Graham et al., 2006). In further work, the short (9–11 amino acids) peptide regions precisely recognized by the CTLs were identified, together with the class I MHC molecule that presented the peptides on the cell surface (Graham et al., 2008). Unfortunately, the goal of inducing protective immune responses by using these antigens as vaccines was only partially achieved (Graham et al., 2006), and no method for consistently inducing CTLs in large mammals with isolated proteins is currently available. Nevertheless, the identification of these CTL antigens was a major achievement in vaccine research, and further examination of the antigens and the CTLs directed against them has yielded some very valuable insights into the immunobiology of the host–parasite relationship.

**Antigenic diversity**

Antigenic diversity is the phenomenon through which parasites and other infectious organisms evade a protective immune response by changing the sequence of the epitope recognized by an existing immune response such that the mutated organism can infect an already exposed host. Evidence for antigenic diversity in T. parva was first shown for two of the CTL antigens, Tp1 and Tp2 (MacHugh et al., 2009; Pellé et al., 2011). The diversity was particularly striking in the Tp2 gene, with single nucleotide polymorphisms identified at 61% of positions in the gene. Equally striking was the finding that there was much greater diversity in the T. parva parasites originating from buffalo than those from cattle, suggesting that the T. parva population transmitted among cattle may represent only a subset of the entire T. parva population, presumably that which is most fully adapted for transmission among cattle. The studies did not detect evidence among the currently identified epitopes that the mutations were driven by positive immune selection (Pellé et al., 2011), although there was a
lack of recognition by naturally derived CTLs of mutated epitope sequences in both Tp1 and Tp2 (MacHugh et al., 2009; Connelley et al., 2011).

**Immunodominance**

CD8+ CTLs recognize antigens presented by class I MHC molecules. The genes encoding the MHC molecules are carried on two genetic regions, or haplotypes, with each parent contributing one of the haplotypes. For CTLs specific for *T. parva*, it was noted very early on that the CTLs were restricted predominantly or even completely by only one of the MHC haplotypes (Morrison et al., 1987). More recent work led by Ivan Morrison at the University of Edinburgh, UK, and in collaboration with ILRI scientists has shown that the great majority of the CTLs in any given responding animal recognize the same peptide presented by the same MHC molecule (MacHugh et al., 2009). The precise peptide that is recognized is governed by the MHC type of the animal. This is a quite remarkable phenomenon, given that *T. parva* is predicted to encode over 4035 proteins (Gardner et al., 2005), each of which could, theoretically at least, contain hundreds of peptide epitopes. The focus of the CTL response on one or two epitopes, termed immunodominance, had been described previously in viral infections, but this was the first description of the phenomenon in a complex organism such as *T. parva*.

Immunodominance has significant implications on the performance of a potential subunit vaccine. The focus of the response on one or two epitopes leaves the animal vulnerable to infection with a second parasite that carries mutations in those epitopes. Given the large number of MHC haplotypes that are likely to be present in outbred populations of cattle, there may be a similarly large number of epitopes presented by those MHC molecules. For there to be a subunit vaccine of practical use, it may rely on the existence of a few antigens of limited diversity that can induce CTLs specific for epitopes present on those antigens when given in isolation and that can still recognize and kill infected cells.

**The anti-sporeozoite vaccine**

While the CTL response was being examined, another approach, led by Tony Musoke, was being taken within ILRI, which was aimed at developing a vaccine to prevent the entry of sporozoites into infected cells. This was based on evidence that sera of animals from endemic areas or animals that had been hyperimmunized against *T. parva* were able to neutralize the entry of sporozoites into lymphocytes *in vitro* (Musoke et al., 1982). The availability of neutralizing mAbs led to the identification of the p67 surface molecule as the target of the protective antibodies (Dobbelaere et al., 1985). These observations culminated in the remarkable demonstration that cattle vaccinated with a recombinant version of the p67 antigen were immune to subsequent challenge with *T. parva*, which raised the prospects of a commercial vaccine against ECF (Musoke et al., 1992). However, subsequent field trials have returned mixed results in terms of the protective ability of the vaccine (reviewed by Nene et al., 2016) and the goal of a vaccine based on the p67 antigen is yet to be realized.

**ITM: The Future**

ILRI’s scientific contributions to the development, production and use of the ITM vaccine have been significant. Together, these contributions have enabled the ITM Muguga cocktail to be used to immunize hundreds of thousands of cattle in both extensive (pastoralist and ranch) and dairy sectors. They have also helped pave the way for commercial production, distribution and use of the vaccine in much of eastern Africa.

New research and development problems must be addressed, however, as discussed below. Many of these challenges and opportunities are also described by Perry (2016), based on a review commissioned by CGIAR.

**The buffalo problem**

Although the Muguga cocktail appears to provide good protection against cattle-derived parasites in both laboratory and field conditions, the group at EAVRO observed that this protection did not extend to cattle exposed to buffalo-derived parasites (Radley et al., 1979). Breakthrough infections were recently observed in
Kenya, where immunized cattle graze with or near buffalo (Sitt et al., 2015), although the vaccine appeared to perform well in northern Tanzania, where buffalo and cattle graze together (Homewood et al., 2006). To combat this, an obvious solution is to produce a vaccine stabilate containing buffalo-derived parasites, although this presents two additional challenges. First, given the extensive heterogeneity in the parasite population found in buffalo, it may be difficult if not impossible to select a ‘buffalo’ parasite stabilate that will protect against all buffalo challenges. Second, the very low parasitaemia found in cattle infected with buffalo-derived parasites will increase considerably the number of cattle required in the production process, which may render this approach economically unsustainable. The current alternative is to provide clear guidelines as to where the ITM Muguga cocktail should and should not be used.

Molecular tools

The powerful molecular tools developed by ILRI and other scientists can now be used to track quantitative variation in the parasite composition of stabilates. The availability of these tools means that formal procedures must be developed and documented as standards to evaluate production batches of stabilate to ensure the production of a consistent, standardized, safe and effective product. More sophisticated and powerful molecular tools are likely to be needed in the future, for example to detect parasite components that might be important for immunity or transmission but that are present at low, currently undetectable, levels. Also, more studies are needed to identify which antigens are responsible for the protection conferred by the Muguga cocktail vaccine.

Cold chain

The need for a liquid nitrogen cold chain is inconvenient andlogically challenging. Attempts to date to lyophilize (or freeze-dry) T. parva sporozoites to make them stable at or near room temperature have resulted in low and variable rates of recovery of viable parasites. Lyophilization of organisms as large and complex as sporozoites is likely to present a significant challenge and may not be possible with the technology available today.

Vaccine production

The production and testing of batches of stabilate is expensive, demanding and time consuming, and raises animal welfare issues: production of 1 million doses requires 18 months, 130 cattle, 500 rabbits and at least 600,000 nymphal ticks. A further problem in the production of the ITM vaccine is that the process includes the sexual stage of the T. parva life cycle. Because of this, recombination can occur, causing stabilates to vary both qualitatively and quantitatively, complicating efficacy and safety testing. Studies have begun at ILRI to develop an in vitro correlate of potency, which may reduce or eliminate the need for the extensive in vivo testing that is now required. In addition, in vitro production (growing of parasites in vessels under laboratory conditions) could minimize the need for cattle, rabbits and ticks, thereby simplifying production and reducing the opportunity for interbatch variation to occur.

Performance monitoring

As use of the ITM vaccine increases, it will be important to continue to monitor the dynamics of local T. parva populations and to investigate apparent vaccine failures and breakthroughs. Standard protocols should be developed to guide such studies and the molecular and other tools as well as capacity building and technical support to national laboratories charged with this responsibility.

Irradiation of sporozoites: potential lessons from candidate malaria vaccine

Encouraging results have recently been announced for a malaria vaccine based on malaria sporozoites attenuated by exposure to radiation. The resulting experimental vaccine is similar to the ITM vaccine, although it does not require simultaneous treatment with drugs.

In August 2013, an article in Science found that six adult volunteers who received the highest dose of an experimental malaria vaccine, PISPZ, were protected from subsequent challenge with malaria parasite-infected mosquitoes.
This is the first time that 100% protection has ever been achieved for a malaria vaccine, albeit in a very small-scale phase I safety trial. This outperforms the results reported in 2012 for the other leading malaria vaccine candidate, RTS.S/AS01, a subunit vaccine, which protected just 31% of young infants and 56% of older babies and toddlers.

The promising result for PfSPZ was achieved using an approach that is similar to ITM: Plasmodium falciparum sporozoites are attenuated by radiation and the weakened but live parasites are injected intravenously. Antibodies to other stages in the parasite life cycle were undetectable, indicating that the irradiated sporozoites were effectively attenuated and did not develop beyond the early liver stage. The malaria team believes that the result has established proof of concept for the vaccine and has demonstrated that PfSPZ is safe and meets regulatory standards.

There would be significant advantages in terms of cost and safety if an ECF vaccine comprising irradiated parasites were to be developed. The possibility of using irradiated sporozoites to immunize cattle was explored by Cunningham’s group at EAVRO (Cunningham et al., 1973). The experiments were unsuccessful and the EAVRO scientists concluded that vaccination against ECF was unlikely to be achieved using irradiated parasites. Nevertheless, researchers at ILRAD pursued the use of irradiated T. parva sporozoites in the late 1980s (ILRAD, 1989). Cattle inoculated with irradiated sporozoites showed weak antibody and cytotoxic responses and were not protected on challenge. Further advances in this area will require methods to quantitate sporozoites with much greater precision and to expose them more uniformly to the irradiation dose.

The issues discussed above raise new questions for vaccination against ECF:

- Is a subunit vaccine attainable or could a better use of available resources be achieved if these were redirected to making the ITM vaccine easier and cheaper to produce and deliver?
- Why are reactors and vaccine failures seen in Kenya in areas where buffalo-derived parasites are present but not in northern Tanzania?
- How does the Muguga cocktail provide such broad protection in the field?

### The Proliferative Response in T. parva-infected Lymphocytes

#### Casein kinase 2

ILRI’s molecular biology laboratory sought to determine the underlying molecular mechanisms that drove bovine lymphocytes to divide uncontrollably in theileriosis. The goal of understanding the mechanisms was to enhance the ability to create a vaccine against the disease. The results of this investigation, however, had a much broader significance, which became evident only several years later (ole-MoiYoi, 1989, 1995; ole-MoiYoi et al., 1988, 1989, 1992, 1993).

### Background

The generation time of T. parva-infected lymphocytes in vitro varies from 16 to 27 h. The intracellular form of the parasite, the macroschizont, is considered cancerous because it induces uncontrolled growth and clonal expansion of the infected lymphocytes, which are pleomorphic and show alterations in surface phenotype. The mode of death of the infected animals is very much like that of acute leukaemia in people, i.e. massive tissue and organ infiltration in the lung, kidneys and other organs. T. parva, together with its Mediterranean counterpart T. annulata, is unique among intracellular protozoan parasites in causing this bovine leukaemia. The disease is unusual because if infected cattle are treated in a timely manner with anti-theilerial drugs, the uncontrolled cell division stops with the death of the parasite. This is the reason why this disease is often described as reversible lymphocyte transformation (leukaemia).

### Experimental approach

Initial experiments to determine the driving force for lymphocyte division in theileriosis focused on small segments of DNA known as tumour viruses or oncogenes. Many of these were originally identified in diseases caused by viruses, such as src of Epstein–Barr virus associated with Burkett’s lymphoma or v-myc of bird myelocytomatosis. None of the oncogenes that were available at the time showed any significant hybridization signals with materials...
from *T. parva*-infected lymphocytes separated according to their size in gels.

The next set of experiments employed conventional gel electrophoresis, which separates proteins according to their size. Using various inhibitors and activators of enzymes called protein kinases, which attach phosphate groups to certain amino acids in proteins, the researchers could determine the class of protein kinase that was predominant in the *T. parva*-infected cells. There are two classes of protein kinase called tyrosine kinase oncogenes and serine/threonine oncogenes. There was little or no information about serine/threonine oncogenes in the late 1980s when these experiments were carried out. It was therefore surprising to find casein kinase 2 (CK2), which preferentially phosphorylated only serine and threonine amino acid residues in the *T. parva*-infected cells. There were no detectable tyrosine oncogene signals in the *T. parva*-infected materials. CK2 was not known to function as an oncogene at that time. That it was discovered to be the cause of uncontrolled cell proliferation in theileriosis prompted medical studies that confirmed it was also involved in some human cancers, which advanced understanding of their potential treatment. Follow-up experiments could have included the generation of lysates from *T. parva*-infected lymphocytes or such lysates from purified macroschizonts to determine what the parasite may secrete into the cytoplasm of the infected lymphocytes that activates CK2. It would have been interesting to have treated infected animals with a CK2 inhibitor, such as trace doses of heparin, to test whether such charged molecules could enter the cells. However, at this time, ILRAD merged with the International Livestock Centre for Africa (ILCA) to become ILRI and there was a change in the institute’s mandate.

**Current implications of CK2 overproduction in human medicine**

Although the implications of CK2 as an oncogene were not known, and this work did not lead to a vaccine against *T. parva*, CK2 has recently become a major target for the treatment of acute, chronic leukaemia in humans using competitive inhibitors of the adenosine triphosphate (ATP) molecule. These studies are in phase II trials and show promise. In addition, CK2 is also being tested for the treatment of other white blood cell cancers as well as cancers of solid tissues such as those of the breast, lung, kidney and prostate, and metastatic tumours. CK2 has been shown to be a ‘pro-life’ enzyme. It protects cells from natural death (apoptosis). As such, it minimizes the effectiveness of chemotherapy in many human cancers. Surprisingly, CK2 inhibition also shows great promise in slowing down degenerative neurological diseases such as Alzheimer’s disease and Parkinson’s disease. Scientists at ILRAD/ILRI were vaguely aware of this potential while doing their experiments because CK2 is the kinase that phosphorylates proteins in the brain that somehow become denatured and precipitate out as fibrillary tangles, compromising brain function.

**The Economic Impacts of ECF Research**

Earlier studies have estimated various aspects of the economic benefits of ECF disease control. According to McLeod and Randolph (2000), adoption of a multi-component ECF vaccine in small dairy and large commercial livestock systems across endemic countries in southern, central and eastern Africa would reduce the value of cattle mortality by US$10.1 million, while increasing value of milk production by US$1.7 million annually. Mukhebi et al. (1992) looked at the economics of immunization using ITM in ECF-affected countries in Africa. This analysis showed high potential economic returns, with a benefit:cost ratio in the range of 9–17 under various assumptions. Minjauw (1999) demonstrated the cost-effectiveness of ITM as an ECF control strategy in traditionally managed crossbred cattle (*Bos indicus × B. taurus*) in Zambia. Other work has focused on the economic burden of ECF in the livestock sector and general economy. For example, ECF-related spending in Kenya was estimated at US$10 million in 1987 (Young et al., 1978), while Zimbabwe expended an estimated US$9 million on ECF-related bills in the 1988/89 fiscal year (Perry et al., 1990). Although these costs included control of other tick-borne diseases, ECF is arguably the major disease requiring acaricide application in the region (Cunningham, 1977).
Modelling the economic impacts of ECF

ECF causes a range of economic impacts associated with the morbidity and mortality of animals. Following the classifications of disease impact developed by Rich et al. (2014) and expanded by Rich and Niemi (2017), we can categorize the distinct levels of impacts – and associated modelling needs – of ECF to inform our modelling approach. Rich et al. (2014) identified impacts taking place from the micro-level (i.e. household or farm level) to different types of meso-level impacts (species, sector and value chain) and to aggregate macro-impacts on the local, regional or global economy. Their framework also considered externalities that could come from control strategies themselves (e.g. the effects of acaricides on the environment) as well as spillovers from regional and international trade.

The impacts of ECF are primarily through high mortality affecting the assets and incomes of farmers. In smallholder settings that are less commercially oriented, this can severely restrict the ability of farmers to cope with market shocks or to meet irregular livelihood needs such as school fees and family emergencies. As with other diseases of livestock, the risk of disease itself can lead to stocking patterns that are suboptimal from the standpoint of market productivity, such as maintaining older, less productive (from a commercial standpoint) herds that are less disease prone. For more commercially oriented farmers, ECF reduces sales of meat and milk and thwarts investment into scale economies, such as fencing for beef and buildings, processing for dairy or expanding herd sizes to meet growing demand. Substitution effects with other sources of protein could also arise, causing changes in supply and demand in other livestock markets.

ECF can induce important spillover effects outside the immediate livestock sector. For instance, where animals are used as draught labour, ECF can have strong, negative impacts on the productivity of staple crops such as maize. Mukhebi et al. (1992) estimated that the negative impacts of ECF on animal traction comprised 13% (US$21 million) of the total annual loss associated with ECF in 11 African countries in 1989. This in turn has indirect effects on prices of other food crops. Land and labour markets can also be affected by ECF, the former through the changing use of pasture land and/or reduced land for crops based on the unavailability of draught labour, and the latter from reduced throughput in local abattoirs and milk processors that diminishes the demand for labour (see Rich and Wanyoike, 2010, for a similar discussion of Rift Valley fever in Kenya). While macroeconomic impacts outside the agricultural and livestock sectors associated with ECF outbreaks are likely to be small, important environmental spillovers such as water and land contamination exist with the use of acaricides for dipping (Mukhebi and Perry, 1992).

Most economic analyses of ECF are derived from farm budget data. Various authors estimated the regional impacts of ECF on meat and milk based on a combination of available secondary data on livestock production and primary survey data (Mukhebi et al., 1992, 1995; Martins et al., 2010; Kivaria et al., 2012). An exception to these partial budget approaches is that of Nyangito et al. (1996), who developed a whole-farm simulation model with linkages outside the livestock sector, including crops and products derived and used by livestock (e.g. feed, draught power, manure). Financial and economic impacts derived from these simulations were computed over a 10-year horizon. The model allowed the simulation of alternative investments and technologies and was used to develop ECF control scenarios based on combinations of ITM and acaricides. Based on smallholder farm data from Kilifi in Kenya, they found that a control strategy that used ITM alongside a 75% reduction in acaricide use generated an internal rate of return of over 34% and a benefit-cost ratio of 5.18.

A drawback of this approach (and of the partial budget models cited above) is that prices and other market parameters are exogenous to the system and thus fail to capture market dynamics related to disease control. Moreover, most of the available economic analyses do not directly consider the adoption process over the time involved in the implementation of ECF control strategies. Partial or slow uptake of ITM could reduce its impact both on the disease and on markets over time. This suggests the need to use more robust modelling frameworks that capture a wider range of economic phenomena over longer periods.
Methods

To assess the economic impacts of ECF, Rich and Perry (2011) investigated the impacts of ECF on production, prices, trade and livelihoods. First, they used the DynMod model (http://livtools.cirad.fr/dynmod; accessed 13 February 2020) developed by Lesnoff (2007) to assess the impact of ECF on herd demography. DynMod traces livestock herd growth, based on exogenously defined births, fecundity and mortality; exogenously defined purchases and offtake rates; and the initial structure of herd populations by age and gender. These parameters calibrate the evolution of herd growth based on a state-transition (age-cohort) model. Animals move between age cohorts based on pre-defined parameters associated with their time (in months) spent in a particular age class (juveniles, subadults or adults).

Rich et al. (2014) used DynMod to develop scenarios of alternative rinderpest control regimes in which varying levels of rinderpest-associated mortality were generated to define herd populations under different control regimes. Rich et al. (2014) adopted a similar approach for computing the ex ante benefits of ECF control. Based on a review of the literature (Gachohi et al., 2012) and expert consultations, the authors first derived an average of annual incidence and fatality associated with ECF in four different production systems (intensive dairy (ID), open-grazing dairy (OD), agropastoral (AP) and pastoral (P)), as shown in Table 6.1. The product of incidence (I, with units of new annual cases of ECF/total population) and case fatality (CF, with units of annual deaths from ECF/new annual cases of ECF) rates gives the percentage of the population dying annually from ECF. This product was weighted by the population share (w) in each system to give a number for the national ECF mortality (MECF; Equation 6.1). A triangular distribution comprising a minimum, most likely and maximum level of ECF mortality was generated for sensitivity analysis.

\[ M_{ECF} = \sum_{i=ID,OD,AP,P} w_i I_i CF_i \]  

(6.1)

Next, we developed alternative scenarios of adoption patterns of ECF control through ITM. It is instructive to first review some of the issues governing adoption of ITM to inform our choice of parameters.

The Muguga cocktail ITM vaccine has been commercially available for the past 15 years. Over that period, close to 1.8 million doses of the vaccine have been distributed. About 80% of these have been sold in the pastoral production systems of northern Tanzania and about 10% in the pastoral systems in Kenya. The rest have been sold in smallholder dairy systems across Kenya. Although considerable effort has been put into the intensive dairy systems, adoption of ITM in dairying has been low.

Many reasons have been advanced for this apparent contradiction, because when the vaccine was first developed the main target was smallholder dairy farmers who, it was then believed, had the incentive to protect their high-value dairy animals and were more commercially oriented than other livestock keepers (Perry, 2016).

Some of the reasons suggested include alternative disease control methods in dairy systems. Acaricides are very effective in controlling ECF when correctly used. They are also relatively economical because one can buy enough for just one spray per week, and they also protect against other tick-borne diseases. Curative drugs are also available because most private animal health service providers are in the high agricultural potential areas where most smallholder dairying is located.

The reluctance of the smallholder dairy farmers to experiment with new untested methods on their valuable animals has also played a role. As much as acaricides have their shortcomings, most farmers have used them and know they work. A novel approach would have to prove itself before most farmers want to risk using it.

Strong marketing of acaricides and anti-theilerial drugs by pharmaceutical companies in the dairy areas has also contributed. Unfortunately, they may sometimes give incomplete information, and the current distributors of the vaccine are small local companies whose staff may lack capacity to present scientifically correct information.

The packaging of the last several batches of the vaccine in 30–40-dose packages is also a disincentive to smallholder dairy farmers. A single straw of the current batch of the vaccine covers 40 calves, and to collect this number from smallholder farms that on average keep one or two animals is problematic. Had small-dose packages...
Table 6.1. Parameters used for deriving population trajectories in DynMod. (Calculations from DynMod.)

| System                | Cattle population share (%) | ECF incidence (%) | Case-fatality rate (%) | Adoption rate (%) | Predicted adoption by 2026 (%) |
|-----------------------|----------------------------|-------------------|------------------------|-------------------|-------------------------------|
|                       |                        | Base | Low | High | Base | Low | High | 2007 | 2016 | Base | Low | High | 2007 | 2016 | Base | Low | High | 2007 | 2016 | Base | Low | High | 2007 | 2016 |
| Intensive dairy       | 8.7                      | 20   | 10  | 30   | 10   | 8   | 15   | 0    | 3    | 20   | 15  | 25   |       |      |      |      |
| Open-grazing dairy    | 10.5                     | 20   | 10  | 30   | 15   | 10  | 20   | 0    | 5    | 40   | 30  | 50   |       |      |      |      |
| Agropastoral          | 27.3                     | 20   | 10  | 30   | 4    | 3   | 5    | 0    | 0    | 20   | 15  | 25   |       |      |      |      |
| Pastoral              | 22.5                     | 20   | 10  | 30   | 30   | 20  | 40   | 1    | 15   | 33   | 20  | 40   |       |      |      |      |
| Not affected by ECF   | 31.5                     | 0    | 0   | 0    | 0    | 0   | 0    | 0    | 0    | 0    | 0   | 0    |       |      |      |      |
| Population-weighted incidence |                | 13.7 | 6.9 | 20.6 |       |      |      |      |      |      |      |      | 10.1 | 7.0 | 13.6 |      |
| Population-weighted case fatality |                |       |      |      |       |      |      |      |      |      |      |      |      |      |      |      |
| Population (number of cattle in millions) |                |       |      |      |       |      |      |      |      |      |      |      | 17.5 |      |      |      |


been available for smallholder dairy systems, adoption of the vaccine might have been greater.

The reason for the dose packaging is related to vaccine production. Because of the need to maximize on the number of sporozoites and reduce the amount of tick material in the stablate, ticks with high infection rates are normally selected. The estimation of the sporozoites is also a crude estimate based on the estimated number of infected ticks in a batch and the number of infected acini in an infected tick based on a random sample of ticks assessed. The final dose was therefore only determined at the end of the production run and there was no easy way to dilute the stablate once it was produced. Recently, Patel et al. (2019) demonstrated that, once made, the stablate can be thawed, diluted and repackaged without loss of efficacy. Another attempt to reduce the number of doses in a straw has been to predilute the stablate based on the expected concentration (based on the number of sporozoites/ml) and then determine the final dose afterwards. New techniques to more accurately count the number of sporozoites in the stablate are being developed. If these studies are successful, it may be possible in the future to produce vaccines of a desired number of doses.

**ITM price**

The price of ITM relative to other veterinary vaccines is quite high. Although smallholder dairy farmers make money from the sale of milk, raising at once US$8–12 for each animal to be immunized is not easy. Unlike pastoralists who can sell one animal to raise enough money to vaccinate all their calves, the smallholder dairy farmer does not have a surplus animal to sell. This can work only in areas where there are strong cooperatives that can advance farmers money for vaccination.

**Grazing type**

Zero grazing dominates the intensive dairy systems. This involves keeping cattle in stalls and bringing fodder to them. This method reduces the risk of tick-borne diseases, including ECF, because animals move less in tick-infested pastures. In some highland areas where animals are constantly kept in stalls, many farmers can go for up to 3 months without using acaricides. Only when farmers get external hay do they experience increased tick-borne disease risk.

Many factors that block tick-borne disease in dairy systems are the opposite in pastoral systems. First, pastoralists do not have alternative disease control options. Second, they keep large herds, and any one herd can easily include the 40 calves required for immunization with one straw of the vaccine. Third, whereas the vaccine is relatively expensive, it is easier for pastoralists to sell one animal to raise enough money to vaccinate their calves. Fourth, in many pastoral areas, the risk of ECF is increasing as a result of upgrading indigenous stock with breeds that are more productive but also more susceptible to ECF.

Few studies have investigated adoption trends for ITM, partly because the vaccine has not been used extensively other than in the pastoral systems. Attempts at predicting future adoption trends are therefore speculative, based on projected developments in the three production systems.

Intensification of smallholder dairy farming is likely to increase, driven by population increases and land pressure. As pointed out, the risk of disease declines with intensification. Provision of animal health services in these systems is also likely to improve. Although more acaricide resistance is projected to develop generally, because acaricides are not intensively applied in intensive smallholder dairy systems, acaricide resistance in these dairy systems is unlikely to be a problem in the immediate future. Demand for the vaccine in the intensive systems will arguably remain low in the near future.

In the more open-grazing dairy systems, demand for ITM is likely to rise due to increasing acaricide resistance. However, this will be contrasted by the trend towards intensification and zero grazing in some of the currently medium-sized farms. Overall, there is likely to be a moderate increase in demand for the ITM vaccine in open-grazing dairy systems.

The greatest potential of ITM is expected from agropastoral systems, which have the most cattle and which are spread across areas most suitable for ticks. Currently, the disease risk is low due to an endemically stable situation. The indigenous breeds kept and a scarcity of ticks
due to overgrazing leads to low incidence and rare fatalities. However, many farmers are upgrading their cattle by crossing their local animals with exotic breeds to produce greater amounts of milk that they can sell for high prices. This trend is likely to continue, and as more and more farmers begin keeping ECF-susceptible breeds, the disease risk might rise, and with it, demand for the ITM vaccine.

Demand for the vaccine in pastoral systems is unlikely to change significantly. As more farmers become aware of the vaccine, more are likely to adopt its use until a peak is reached. After that, only newly born calves that have not been vaccinated in the past and very few unvaccinated adults will be vaccinated. The trend towards upgrading animals is also likely to continue, which will lead to more disease-susceptible animals and a greater demand for the vaccine. It is unlikely that animal health services in the pastoral systems will improve significantly in the near future and that there may not be alternative methods for controlling ECF other than vaccination.

Given these dynamics in the different production systems, the authors developed a variety of different paths of adoption that are summarized in Table 6.1 and in Fig. 6.2 in scenarios of low, most likely (shown as ‘base’) and high levels of adoption. For dairy systems during 2007–2016, it was assumed that adoption took place in the last 3 years only (starting from 1% in 2014), while for pastoral systems, a gradual linear increase in the adoption range was assumed. For 2017–2026, roughly linear rates of adoption were assumed, albeit with slightly slower rates of increase in the first part of the period, rising steadily in the latter part and levelling off by 2026. Each of these adoption levels was weighted in its respective system to obtain a national weighted average.

The estimated impact on ECF mortality of adoption of ITM was then computed. Adoption of ITM in each period (t) was assumed to result in perfect control of ECF, consequently reducing population-weighted ECF mortality by the rate of incremental adoption relative to the first period (2007) of the simulation. This percentage is defined as avoided mortality (AM: Equation 6.2) from ECF for each period:

$$AM_i^{ECF} = \sum_{i=0}^{t} w_i AR_i I_i^{CF}$$  \hspace{1cm} (6.2)

The new population mortality rate per age class and time step ($M_{t+1}^C$; Equation 6.3) is thus the difference between the original age-cohort mortality rate ($M_0^C$) and the avoided mortality (AM) achieved from the adoption of ITM:

$$M_{t+1}^C = M_0^C - AM_i^{ECF}$$  \hspace{1cm} (6.3)

This new path of mortality rates was subsequently put into DynMod for simulation and compared against a counterfactual (baseline) scenario of control measures.

The International Model for Policy Analysis of Agricultural Commodities and Trade (IMPACT) was then employed to compute impacts on the agricultural economy and livelihoods (the model is documented in Robinson et al., 2015). IMPACT is a system of simulation models incorporating economic, crop, livestock, hydrology and climate change components. IMPACT uses a partial equilibrium economic model that represents global supply, demand and trade of agricultural commodities among open economies. The demand for agricultural commodities is simulated for 159 countries. Agricultural production is modelled at a subnational level known as food production units (FPUs). There are 320 FPUs in IMPACT that represent intersections among 159 nations and 154 water basins. Solving the system of country demand and FPU supply equations in IMPACT produces measures of each country’s crop and livestock production and land use, as well as measures of prices and trade of agricultural commodities consistent with trade balances in global agricultural markets. Model results are then translated into indicators of agricultural incomes, food security, nutrition and environmental health, among others.

The IMPACT model has been used to analyse global food security, rural development and natural resource management to 2050, including different scenarios of policy, technological, economic and climatic change. Some of the model’s applications are relevant to assessment of transformations in global livestock. These applications highlight the drastically changing roles of developing countries in the demand and production of livestock products globally (Delgado et al., 1999); a more recent study assessing the potential impacts on food prices and the management of natural resources of changing demand and supply of meat and milk in fast-growing regions.
(Rosegrant et al., 2013); and an assessment of trajectories of livestock demand and supply in Africa and South Asia that explores the potential impacts of improving livestock productivity in key livestock-producing countries (McDermott et al., 2013).

The IMPACT model allows for international trade, providing a framework to assess the sub-national factors of ECF and its management that are driving dynamics of the demand and production of livestock products and the global context of food systems. As such, the model is useful in assessing the effects of ECF control on livestock production and food security, and on the potential (including through higher imports) for offsetting shortfalls in the supply of animal-sourced foods. Furthermore, the impact effects are captured over the long run, providing a tool useful for more long-term planning. IMPACT may thus offer a much more comprehensive approach to measuring impacts of ECF control in the affected regions than has been offered by previous approaches (e.g. Mukhebi et al., 1992; Minjauw, 1999). In addition, by introducing reliably informed estimates of the parameters depicting incidence and control of a specific disease, this work builds directly on the earlier analysis of livestock futures by McDermott et al. (2013).

The current study applies the IMPACT model to medium- to long-run impacts of the

![Projected ECF vaccine adoption rates by adoption scenario and production system, 2007–2026. Constructed from DynMod model for ECF in Kenya. Intensive dairy cattle were about 8.7% of the cattle population in 2007, open dairy were 10.5%, agropastoral were 27.3%, pastoral were 22% and cattle unaffected by ECF approximately 31.5% of the national cattle herd of 17.5 million head.](image-url)
ITM vaccine for ECF control. Only the main aspects of the model structure that are most relevant to the current context are presented below.

As mentioned, FPU is the unit of analysis in IMPACT, with FPU production of livestock calculated as the product of an assumed ‘average’ yield (AY) per head and the number of animals in the FPU. Animals are distinguished by species and include beef cattle, dairy cattle, sheep and goats, poultry and pigs, while livestock product types (j) accounted for in the model are beef, milk, lamb, poultry meat, eggs and pork. Livestock yield or production per animal (AY; Equation 6.4) is driven by factors of improved animal and animal management practices that are exogenous (Int) to the system of demand and supply equations in IMPACT:

\[ AY_{j,pu} = AY_{Int_{j,pu}} \times AY_{Int_{2,j,pu}} \]  

(6.4)

Animal numbers (AN; Equation 6.5) are functions of endogenous and exogenous factors. These are species and system specific and are a function of endogenously determined input (c) and output (j) price indices (PNET) and feed costs (PC), country-specific (cty) parameters of feed efficiency (Feeds). The exogenous component of the equation adjusts year-to-year changes in herd populations through a herd expansion rate (ANInt2) that denotes system-level differences.

\[ AN_{j,pu} = AN_{Int_{j,pu}} \times AN_{Int_{2,j,pu}} \times \left( \frac{PNET_{c,cty}}{PNET_{0,j,cty}} \right)^{ANC_{c,cty}} \times \prod_{feeds} \left( \frac{PC_{c,cty}}{PC_{0,c,cty}} \right) \]  

(6.5)

Equation 6.5 provides a convenient entry point for modelling production system growth in the context of ECF, as cattle mortality, a major disease effect, can be factored into the animal population growth rates. The effects of ITM vaccine use were thus simulated in IMPACT using adjustments to the historical and projected growth rates (ANInt2) of beef and dairy cattle herds. These adjustments are based on the mortality and population growth rates generated in DynMod for the different scenarios of ECF control, as described above. Animal mortality rates in turn reflect incidence, fatality and adoption rates related to ECF disease and vaccine use, as presented in Table 6.1.

**Returns to ECF Investment**

Data from DynMod and IMPACT were used to derive the benefits associated with ECF control under different scenarios. To measure the returns to investment for the ITM vaccine, we compared these benefits with the costs of achieving them to derive benefit:cost ratios associated with investments in ECF control.

Two sets of costs were generated based on two different sources. First, data on expenditures on research by ILRAD/ILRI from 1975 to 2015 were obtained to quantify the sunk costs associated with research expenditures on vaccine development, etc. It is worth highlighting that these costs are global costs that are wholly attributed to the case study of Kenya. As such, they overestimate the costs incurred in Kenya. Second, for each scenario, costs were generated on vaccine delivery based on expert opinions. These costs included the number of doses deployed per scenario, distributor costs and training costs for vaccinators. As delivery costs differ by production system, a weighted-average vaccine cost was derived based on the share of animals in different systems. The weighted-average current cost of vaccine dose plus delivery was estimated at US$6.70. It was assumed that the costs of a new distributor of vaccines was US$100,000 per 250,000 doses administered in the first 5 years of the scenario, and US$100,000 per 500,000 doses from year 6. We assumed these costs were recurrent costs based on the number of vaccines delivered. Finally, we assumed that for every 10,000 doses of vaccine delivered, a training cost of US$300 was incurred. This was assumed as a one-time cost in the year when this occurred. All scenario costs were assumed to increase by a 5% inflation rate annually.

ITM was assumed to confer life-long immunity to ECF. Therefore, the number of doses administered had to account for the natural survival and presence of animals that had been vaccinated in previous years. A simple Markov chain was constructed over the 20-year scenario period to adjust the number of administered vaccinations for animals that had already been vaccinated and either survived or had not exited the system through offtakes. The Markov chain was age-cohort weighted to account for different mortality and sales transitions by age and sex of animal.
Sunk research costs and scenario costs were added to generate a stream of current costs from 1975 to 2026. These were discounted at a 5% discount rate to generate net present values for each scenario. Benefit:cost ratios compare additional agricultural production value derived from IMPACT/DynMod with the added costs from the different scenarios, with the counterfactual assuming no expenditure on ECF at all.

**Model results**

First, results are presented of the projected paths of cattle production from DynMod under nine scenarios of ECF control. These are contrasted with a baseline scenario that assumes no ITM adoption from 2007, thus providing us with an *ex post* impact of adoption of ITM to date (2007–2016) and *ex ante* projections against such a baseline from 2017 to 2026. As summarized in Fig. 6.3, there are higher impacts on herd growth from ITM adoption than in our most likely and low mortality scenarios. In these scenarios, we see an increase in cattle stocks of 60% over 2007–2026 versus a no-control baseline growth of just over 20% over the same period. Taking our most likely scenarios into account, cumulative herd numbers remain about 10% higher relative to the baseline.

Different rates of animal mortality simulated using DynMod were translated into cattle populations in Kenya for scenarios of ECF control using IMPACT. These scenarios include a

![Projected cattle herds by vaccine adoption and mortality avoided, 2007–2026. (Constructed from DynMod model for ECF in Kenya.)](image-url)
baseline that assumes that current levels of ECF control are maintained over the simulation period (2005–2026) and nine plausible situations of ECF management. The baseline condition accounts for the current management of ECF in Kenya, including acaricides and low rates of adoption of the ITM vaccine. Because future adoption is uncertain, the ECF control scenarios necessarily cover a range of adoption rates. Because there is insufficient information on current rates of cattle mortality due to ECF (particularly as distinct from other disease-related animal deaths), the scenarios also use a range of ECF mortality rates. In applying the data on animal growth rates as input into the IMPACT model, impacts were simulated of interactions among ECF, herd dynamics, markets and socio-economic variables.

Table 6.2 presents IMPACT projections of the supply of animal-source foods associated with the baseline and ECF scenarios in Kenya. Compared with the status quo in which beef production increases from 392,000 t in 2005 to 706,000 t in 2026, beef output in 2026 is projected to increase to between 726,000 and 990,000 t under various assumptions of ECF mortalities. These scenarios correspond to low to high levels of vaccine adoption countrywide. The estimates of beef production under the ECF control scenario represent increases of 3–40% over year 2026 production under baseline conditions. Dairy production similarly increases 6% to 56% in 2026 under alternative ECF management.

Looking at the baseline trend for lamb, eggs, poultry meat and pork, it is observed that national production increases by 80%, 19%, 113% and 44%, respectively, for these product types, from 2005 to 2026. Furthermore, relative to the baseline in 2026, production may decline very slightly under the ECF control scenarios for lamb, for poultry and for eggs, with no change observed in pork production to 2026. The decline in production of lamb, eggs and poultry meat is probably explained by market substitution effects. As cattle meat and dairy supplies increase under ECF management, the prices of these products decline, causing a weakening in the demand (and subsequent production) of comparable products. The market shifts are, however, small. Related to the expansion in cattle production, the aggregate supply of meat, milk and eggs increases by 48% from 2005 to 2026. Compared with the baseline in 2026, supply increases under the ECF control scenarios in a range of 0.5–5%.

Figure 6.4 presents an index of the value of national agricultural production associated

Table 6.2. Production and net imports of selected commodities in Kenya, 2005–2026. (Calculations from DynMod and from IFPRI IMPACT; Robinson et al., 2015).

|          | 2005 | 2026: status quo | 2026: low mortality, medium adoption | 2026: medium mortality, medium adoption | 2026: high mortality, medium adoption |
|----------|------|------------------|------------------------------------|----------------------------------------|-------------------------------------|
| Production in 1000 t |
| Beef     | 392  | 706              | 732                                | 768                                    | 942                                 |
| Dairy    | 2178 | 5018             | 5200                               | 5254                                   | 6694                                |
| Lamb     | 77   | 140              | 140                                | 140                                    | 140                                 |
| Eggs     | 61   | 73               | 73                                 | 73                                     | 73                                  |
| Poultry meat | 19 | 43               | 43                                 | 43                                     | 42                                  |
| Pork     | 14   | 21               | 21                                 | 21                                     | 21                                  |
| Feedsa   | 286  | 461              | 475                                | 494                                    | 590                                 |
| Net imports in 1000 t |
| Beef     | 0.83 | 36.68            | 9.26                               | 0.00                                   | 0.00                                |
| Dairy    | 3.30 | 0.00             | 0.00                               | 0.00                                   | 0.00                                |
| Lamb     | 0.10 | 0.00             | 0.00                               | 0.00                                   | 0.00                                |
| Eggs     | 0.11 | 52.33            | 52.54                              | 52.55                                  | 52.56                               |
| Poultry meat | 0.00 | 10.76           | 10.77                              | 10.77                                  | 10.79                               |
| Pork     | 0.00 | 0.00             | 0.00                               | 0.00                                   | 0.00                                |

*aFeed grain for livestock production, not quantity of feeds produced.*
with the vaccination scenarios. Relative to the reference case, agricultural incomes improve by between 0.8% and 12% in 2027. On a commodity-by-commodity basis, revenue from beef and dairy production increases by 3% to 40% over the baseline. Benefits also accrue to the consumers, but these are more modest. Aggregate household expenditures on food are lowered by 0.01% to 0.07% in 2027 under the ECF scenarios compared with the reference case. Food expenditure as a share of national income remains the same or declines (by 0.01% at most) relative to the base case in 2027.

The vaccination scenarios have faint effects on international trade in livestock commodities. The scenarios lead to reduced imports in both volume and value. Under assumptions of low animal mortality, the net import of beef is between 5000 and 15,000 t in 2026, or roughly 1–2% of the demand in that year. Net dairy imports at 3300 t, are only 0.1% of national demand in 2005. ECF control in cattle does influence the demand and supply of other livestock commodities, probably through competition for feeds, but these effects are also small.

The model projections on crop use for feed are substantial. Baseline feed demand increases 61% from 2005 (285,000 t) to 2026 (461,000 t). Demand for cereals is 29% of this demand in 2005 and 35% of the same in 2016. Under the ECF control scenarios, demand for livestock feeds in 2026 is 2.3–34% higher than the baseline volume. However, there is little or no increase in cultivated area. This suggests that

---

**Fig. 6.4.** Index of agricultural production in Kenya by vaccine adoption and mortality avoided, 2026–2027. (Constructed from DynMod model for ECF in Kenya.)
cropland is being reallocated (e.g., from use as food) to the production of livestock feeds. As cattle production expands and meat and milk prices fall, households are probably able to replace staples in their diets with the higher nutrient animal-source foods, so that no additional land is needed to support the growth in feed demand.

Figure 6.5 illustrates benefit:cost ratios associated with the use of the ITM vaccine in Kenya. The ITM vaccine, under a wide range of hypotheses about vaccine adoption rates across livestock systems and about avoided mortality, would produce a high return to ILRI’s historical investment in all types of ECF research. This example of a proven technology against ECF is unlike the projections of Kristjanson et al. (1999) of the returns to a trypanosomiasis vaccine, which does not exist.

Model results indicate modest impacts of ECF control through ITM. There was a steady increase in domestic supply of cattle, meat and milk and an associated reduction in net imports. The value of production in the agricultural sector rises by about 12% relative to the baseline by 2026. Agricultural revenue is increased, mostly through expanded livestock production, while food expenditures are lower in 2026 under the ECF scenarios than in the baseline case. The implication is that producer welfare is improved in the near to medium term but not at the expense
of consumer welfare. Impacts on nutrition and food security are relatively small, suggesting that increased production probably reduces reliance on food imports and improves the supply of key macro- and micronutrients in human diets but does not create new food consumption.

**Model gaps**

An important research gap is ECF’s impact on morbidity and productivity. Our analysis focuses on mortality avoided by ECF vaccination and does not consider disease effects on meat and milk productivity or possible rising costs of treatment. We may have therefore understated the potential benefits from ITM in Kenya.

Another model gap is that IMPACT does not consider the power output of cattle. We expect that improved control of ECF would have a positive impact on the use of cattle as an input to crop production, which would raise producer incomes. However, as Kenyan agriculture mechanizes, draught power will become less important and potential benefits from increasing draught power will become smaller, so it is not possible to project a net effect of omitting power benefits from the present model.

A second indirect effect of ITM adoption that we did not capture is the potential benefit of controlling high-mortality diseases such as rinderpest and ECF. This would, in theory, allow farmers to stock herds in a more efficient manner and one less dictated by risk preferences (e.g. stocking older animals instead of more productive younger ones, or keeping less productive but disease-resistant breeds; Rich et al., 2014). These shifts could have significant effects by providing farmers with higher prices for the sales of younger, more productive animals, and might allow farmers greater access to commercial markets that demand younger stock.

**Conclusions and the Future**

ILRI and its historical partners have had significant scientific and development impact on our understanding of theileriosis and on management of ECF. The demonstration that commercial-scale batches of the ITM vaccine could be produced was a major achievement and underpinned the sale of the vaccine in Tanzania. In a more direct sense, it also resulted in the immunization of more than 1.5 million cattle. The many years of basic research in the search for a subunit vaccine have not only resulted in a greater understanding of the biology and immunology of *T. parva* infection but have also had a much broader scientific impact on our general knowledge of protozoan–host interactions and of the bovine immune system, especially the role and function of the MHC and CTLs. The scientific outcomes unexpectedly found application in human tumour biology. The numerous serological and nucleic acid-based tools that were generated during this time later proved immensely valuable in the characterization and quality control of the ITM vaccine, and in dissecting the functioning of the bovine immune system.

The future for ILRI in the pathology and immunoparasitology of theileriosis will be guided by the degree of success in the uptake of the ITM vaccine, balanced against the evolving prospects for a subunit vaccine. The future in the epidemiology and economics of ECF management will be developing and evaluating current or novel control methods.

**References**

Abbas, R.Z., Zaman, M.A., Colwell, D.D., Gillear, J. and Iqbal, Z. (2014) Acaricide resistance in cattle ticks and approaches to its management: the state of play. *Veterinary Parasitology* 203, 6–20.

Allsopp, B.A., Carrington, M., Baylis, H., Sohal, S., Dolan, T. and Iams, K. (1989) Improved characterization of *Theileria parva* isolates using the polymerase chain reaction and oligonucleotide probes. *Molecular and Biochemical Parasitology* 35, 137–147.

Baldwin, C.L., Malu, M.N. and Grootenhuis, J.G. (1988) Evaluation of cytotoxic lymphocytes and their parasite strain specificity from African buffalo infected with *Theileria parva*. *Parasite Immunology* 10, 393–403.
Bensaid, A., Kaushal, A., Baldwin, C.L., Clevers, H., Young, J.R., et al. (1991) Identification of expressed bovine class I MHC genes at two loci and demonstration of physical linkage. *Immunogenetics* 33, 247–254.

Bishop, R., Baylis, H., Allsopp, B., Toye, P., Nene, V., et al. (1992) Genomic polymorphisms in *Theileria parva*. In: Morzaria, S. (ed.) *Genome Analysis of Protozoan Parasites*. Proceedings of a Workshop held at ILRAD Nairobi, Kenya, 11–13 November 1992. ILRAD, Nairobi, pp. 70–76.

Bishop, R., Geysen, D., Spooner, P., Skilton, R., Nene, V., et al. (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, 227–237.

Burridge, M.J., Morzaria, S.P., Cunningham, M.P. and Brown, C.G.D. (1972) Duration of immunity to East Coast fever (*Theileria parva* infection of cattle). *Parasitology* 64, 511–515.

CABI (2020). *Invasive Species Compendium*: distribution table. www.cabi.org/isc/datasheet/62109#todistributionTable (accessed 30 April 2020).

Coetzer, J.A.W., Thomson, G.R. and Tustin, R.C. (eds) (1994) *Infectious Diseases of Livestock with Special Reference to Southern Africa*, Vols I–III. Oxford University Press, Cape Town.

Connelley, T.K., MacHugh, N.D., Pellé, R.P., Weir, W. and Morrison, W.I. (2011) Escape from CD8+ T cell response by natural variants of an immunodominant epitope from *Theileria parva* is predominantly due to loss of TCR recognition. *Journal of Immunology* 187, 5910–5920.

Conrad, P.A., Iams, K., Brown, W.C. and Sohanpal, B. (1987a) DNA probes detect genomic diversity in *Theileria parva* stocks. *Molecular and Biochemical Parasitology* 25, 213–226.

Conrad, P.A., Stagg, D.A., Grootenhuis, J.G., Irvin, A.D., Newson, J., et al. (1987b) Isolation of *Theileria* parasites from African buffalo (*Syncerus caffer*) and characterization with anti-schizont monoclonal antibodies. *Parasitology* 94, 413–423.

Cunningham, M.P. (1977) Immunization of cattle against *Theileria parva*. In: Henson, H.B., and Campbell, M (eds) *Theileriosis*. Report of a Workshop, 7–9 September, Nairobi, Kenya. IDRC, Ottawa, pp. 66–75.

Cunningham, M.P., Brown, C.G.D., Burridge, M.J., Musoke, A.J., Purnell, R.E. and Dargie, J.D. (1973) East Coast fever of cattle: 60Coirradiation of infective particles of *Theileria parva*. *Journal of Protozoology* 20, 298–300.

Delgado, C.L., Rosegrant, M.W., Steinfeld, H., Ehui, S. and Courbois, C. (1999) *Livestock to 2020*: the next food revolution. Food, Agriculture, and Environment Discussion Paper 28. IFPRI, Washington, DC.

Di Giulio, G., Lynen, G., Morzaria, S., Oura, C. and Bishop R. (2009) Live immunization against East Coast fever – current status. *Trends in Parasitology* 25, 85–92.

Dobbelare, D.A., Shapiro, S.Z. and Webster, P. (1985) Identification of a surface antigen on *Theileria parva* sporozoites by monoclonal antibody. *Proceedings of the National Academy of Sciences USA* 82, 1771–1775.

Eugui, E.M. and Emery, D.L. (1981) Genetically restricted cell-mediated cytotoxicity in cattle immune to *Theileria parva*. *Nature* 290, 251–254.

FAO (2014) *Global Livestock Production and Health Atlas* (GLiPHA). FAO, Rome.

FAO (2017) FAOSTAT Statistics Database of the Food and Agricultural Organization of the United Nations. FAO, Rome.

FAO/AU-IBAR/ILRI (2017) Regional strategy for the control of African swine fever in Africa. FAO, Rome.

Gachohi, J., Skilton, R., Hansen, F., Ngumi, P. and Kitala, P. (2012) Epidemiology of East Coast fever (*Theileria parva* infection) in Kenya: past, present and the future. *Parasites & Vectors* 5, 194.

Gardner, M.J., Bishop, R., Shah, T., de Villiers, E.P., Carlton, J.M., et al. (2005) Genome sequence of *Theileria parva*, a bovine pathogen that transforms lymphocytes. *Science* 309, 134–137.

Geysen, D., Bishop, R., Skilton, R., Dolan, T.T. and Morzaria, S. (1999) Molecular epidemiology of *Theileria parva* in the field. *Tropical Medicine & International Health* 4, A21–A27.

Gitau, G.K., Perry, B.D., Katende, J.M., McDermott, J.J., Morzaria, S.P. and Young, A.S. (1997) The prevalence of tick-borne infections in smallholder dairy farms in Murang’a district; a cross-sectional study. *Preventive Veterinary Medicine* 30, 95–107.

Goddeeris, B.M., Morrison, W.I., Teale, A.J., Bensaid A. and Baldwin, C.L. (1986a) Bovine cytotoxic T-cell clones specific for cells infected with the protozoan parasite *Theileria parva*: parasite strain specificity and class I major histocompatibility complex restriction. *Proceedings of the National Academy of Sciences USA* 83, 5238–5242.

Goddeeris, B.M., Morrison, W.I. and Teale, A.J. (1986b) Generation of bovine cytotoxic cell lines, specific for cells infected with the protozoan parasite *Theileria parva* and restricted by products of the major histocompatibility complex. *European Journal of Immunology* 16, 1243–1249.
Goddeeris, B.M., Morrison, W.I., Toye, P.G. and Bishop, R. (1990) Strain specificity of bovine *Theileria parva*-specific cytotoxic T cells is determined by the phenotype of the restricting class I MHC. *Immunology* 69, 38–44.

Graham, S.P. Pellé, R., Yamage, M., Mwangi, D.M., Honda, Y., *et al.* (2006) *Theileria parva* candidate vaccine antigens recognized by immune bovine cytotoxic T lymphocytes. *Proceedings of the National Academy of Sciences USA* 103, 3286–3291.

Graham, S.P., Pellé, R., Yamage, M., Mwangi, D.M., Honda, Y., *et al.* (2008) Characterization of the fine specificity of bovine CD8 T cell responses to defined antigens from the protozoan parasite *Theileria parva*. *Infection and Immunity* 76, 685–694.

Hemmink, J.D., Weir, W., MacHugh, N.D., Graham, S.P., Patel, E., *et al.* (2016) Limited genetic and antigenic diversity within parasite isolates used in a live vaccine against *Theileria parva*. *International Journal for Parasitology* 46, 495–506.

Homewood, K., Trench, P., Randall, S., Lynen, G. and Bishop, B. (2006) Livestock health and socioeconomic impacts of veterinary intervention in Masailand: infection-and-treatment vaccine against East Coast fever. *Agricultural Systems* 89, 248–271.

ILRAD (1989) Bovine immune responses to irradiated *Theileria parva* sporozoites. In: *ILRAD Annual Scientific Report*. ILRAD, Nairobi, pp. 11–13.

ILRI/GALVmed (2015) Distribution, delivery and improvement of the infection and treatment method vaccine for East Coast fever. Report of a Workshop, Nairobi, Kenya, 19–20 August 2014. ILRI, Nairobi.

Irvin, A.D., Dobcelaere, D.A.E., Mwamachi, D.M., Minami, T., Spooner, P.R. and Ocama, J.G.R. (1983) Immunisation against East Coast fever. Correlation between monoclonal antibody profiles of *Theileria parva* stocks and cross immunity *in vivo*. *Research in Veterinary Science* 35, 341–346.

Katende, J., Morzaria, S., Toye, P., Skilton, R., Nene, V., *et al.* (1998) An enzyme-linked immunosorbent assay for the detection of *Theileria parva* antibodies in cattle using a recombinant polymorphic immuno-dominant molecule. *Parasitology Research* 84, 408–416.

Katende, J.M., Goddeeris, B.M., Morzaria, S.P., Nkonge, C.G. and MusoKE, A.J. (1990) Identification of a *Theileria mutans*-specific antigen for use in an antibody and antigen detection ELISA. *Parasite Immunology* 12, 419–433.

KiarA, H., Jennings, A., Bronsvoort, B.M. de C., Handel, I.G., Mwangi, S.T., *et al.* (2014) A longitudinal assessment of the serological response to *Theileria parva* and other tick-borne parasites from birth to one year in a cohort of indigenous calves in western Kenya. *Parasitology* 141, 1289–1298.

Kivaria, F.M., Kapaga, A.M., Mbassa, G.K., Muli, P.F and Wani, R.J. (2012) Epidemiological perspectives of ticks and tick-borne diseases in South Sudan: cross-sectional survey results. *Onderstepoort Journal of Veterinary Research* 79, E1–E10.

Kristjanson, P.M., Swallow, B.M., Rowlands, G.J., Kruska, R.L. and de Leeuw, P. (1999) Measuring the costs of African animal trypanosomosis, the potential benefits of control and returns to research. *Agricultural Systems* 59, 79–98.

Lawrence, J.A. (1992) History of bovine theileriosis in southern Africa. In: Norval, R.A.I., Perry, B.D. and Young, A.S. (eds) *The Epidemiology of Theileriosis in Africa*. Academic Press, London, pp. 27–29.

Lesnoff, M. (2007) *DynMod – A Tool for Demographic Projections of Ruminants Under Tropical Conditions*. User’s Manual. ILRI, Nairobi.

Lynen, G., Yrjo-Koskenen, S.E., Bakunane, C., Di Giulio, G., Mlinga, N., *et al.* (2012) East Coast fever immunisation field trial in crossbred dairy cattle in Hanang and Handeni districts in Northern Tanzania. *Tropical Animal Health and Production* 44, 567–572.

MacHugh, N.D., Connelly, T., Graham, S.P., Pellé, R., Formisano, P., *et al.* (2009) CD8+ T cell responses to *Theileria parva* are preferentially directed to a single dominant antigen: implications for parasite strain-specific immunity. *European Journal of Immunology* 39, 2459–2469.

MacHugh, N.D., Taracha, E.L. and Toye, P.G. (1993) Reactivity of workshop antibodies on L cell and COS cell transfectants expressing bovine CD antigens. *Veterinary Immunology and Immunopathology* 39, 61–67.

Malak, A.K., Mpoke, L., Banak, J., Muriuki, S., Skilton, R.A., *et al.* (2012) Prevalence of livestock diseases and their impact on livelihoods in Central Equatoria State, southern Sudan. *Preventive Veterinary Medicine* 104, 216–223.

Martins, S.B., Di Giulio, G., Lynen, G., Peters, A. and Rushton, J. (2010) Assessing the impact of East Coast Fever immunisation by the infection and treatment method in Tanzanian pastoralist systems. *Preventive Veterinary Medicine* 97, 175–182.
Mbao, V., Berkvens, D., Dolan, T., Speybroeck, N., Brandt, J., et al. (2006) Infectivity of Theileria parva sporozoites following cryopreservation in four suspension media and multiple refreezing: evaluation by in vitro titration. Onderstepoort Journal of Veterinary Research 73, 207–213.

McDermott, J., Enahoro, D. and Herrero, M. (2013) Livestock futures to 2020. In: Barrett, C.B. (ed.) How Will They Shape Food, Environmental, Health, and Global Security? Food Security and Sociopolitical Stability. Oxford University Press, Oxford.

McKeever, D.J. (2007) Live immunisation against Theileria parva: containing or spreading the disease? Trends in Parasitology 12, 565–568.

McKeever, D.J., Taracha, E.L.N., Innes, E.L., MacHugh, N.D., Awino, E., et al. (1994) Adoptive transfer of immunity to Theileria parva in the CD8+ fraction of responding efferent lymph. Proceedings of the National Academy of Sciences USA 91, 1959–1963.

McLeod, R. and Randolph, T. (2000) Product development plan: East Coast fever vaccine for Africa. ILRI, Nairobi (unpublished report).

Minami, T., Spooner, P.R., Irvin, A.D., Ocama, J.G.R., Dobbelaere, D.A.E. and Fujinaga, T. (1983) Characterisation of stocks of Theileria parva by monoclonal antibody profiles. Research in Veterinary Science 35, 334–340.

Minjauw, B. (1999) The economic impact of heartwater in Tanzania, Zambia, Mozambique, and of its control through the use of new inactivated vaccines. ILRI, Nairobi (unpublished report).

Morrison, W.I., Goddeeris, B.M., Teale, A.J., Groocock, C.M., Kemp, S.J. and Stagg, D.A. (1987) Cytotoxic T-cells elicited in cattle challenged with Theileria parva (Muguga): evidence for restriction by class I MHC determinants and parasite strain specificity. Parasite Immunology 9, 563–578.

Morzaria, S.P., Katende, J., Musoke, A., Nene, V., Skilton, R. and Bishop, R. (1999) Development of serodiagnostic and molecular tools for the control of important tick-borne pathogens of cattle in Africa. Parasitologia 41, 73–80.

Morzaria, S.P., Spooner, P.R., Bishop, R.P., Musoke, A.J. and Young, J.R. (1990) Stil and NotI polymorphisms in Theileria stocks detected by pulsed field gel electrophoresis. Molecular and Biochemical Parasitology 40, 203–211.

Morzaria, S. and Williamson, S. (1999) Live Vaccines for Theileria parva: Deployment in Eastern, Central and Southern Africa. Proceedings of an FAO/OAU-IBAR/ILRI workshop, 10–12 March 1997. Nairobi.

Mukhebi, A.W. and Perry, B.D. (1992) Economic implications of the control of East Coast fever in Eastern, Central, and Southern Africa. In: Kategile, J.A. and Mubi, S. (eds) Future of Livestock Industries in Eastern and Southern Africa: Proceedings of the workshop held at Kadoma Ranch Hotel, Zimbabwe, 20–23 July 1992. ILCA, Addis Ababa, pp. 107–112.

Mukhebi, A.W., Perry, B.D. and Krukska, R. (1992) Estimated economics of theileriosis control in Africa. Preventive Veterinary Medicine 12, 73–78.

Mukhebi, A.W., Kariuki, D.P., Mussukuya, E., Mullins, G., Ngumi, P.N., et al. (1995) Assessing the economic impact of immunisation against East Coast fever: a case study in coast province, Kenya. Veterinary Record 137, 17–22.

Musoke, A.J., Nantulya, V.M., Buscher, G., Masake, R.A. and Otim, B. (1982) Bovine immune response to Theileria parva: neutralizing antibodies to sporozoites. Immunology 45, 663–668.

Musoke, A., Morzaria, S., Nkonge, C., Jones, E. and Nene, V. (1992) A recombinant sporozoite surface antigen of Theileria parva induces protection in cattle. Proceedings of the National Academy of Sciences USA 89, 514–518.

Naessens, J., Sileghem, M., MacHugh, N., Park, Y.H., Davis, W.C. and Toye, P. (1992) Selection of BoCD25 monoclonal antibodies by screening mouse L cells transfected with the bovine p55-interleukin-2 (IL-2) receptor gene. Immunology 76, 305–309.

Ndungu, S.G., Brown, C.G.D. and Dolan, T.T. (2005) In vivo comparison of susceptibility between Bos indicus and Bos taurus cattle types to Theileria parva infection. Onderstepoort Journal of Veterinary Science 72, 13–22.

Nene, V., Kiara, H., Lacasta, A., Pellé, R., Svitik, N. and Steinaa, L. (2016) The biology of Theileria parva and control of East Coast fever – current status and future trends. Ticks and Tick-borne Diseases 7, 549–564.

Norling, M., Bishop, R.P., Pellé, R., Qi, W., Henson, S., 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.
Norval, R.A.I., Perry, B.D. and Young, A.S. (eds) (1992) *The Epidemiology of Theileriosis in Africa*. Academic Press, London.

Nyangito, H.O., Richardson, J.W., Mundy, D.S., Mukhebi, A.W., Zimmel, P. and Namken, J. (1996) Economic impacts of East Coast Fever immunization on smallholder farms, Kenya: a simulation analysis. *Agricultural Economics* 13, 163–177.

Okethe, O.S. and Buyu, G.E. (2006) Prevalence and incidence of tick-borne diseases in smallholder farming systems in western Kenya highlands. *Veterinary Parasitology* 141, 307–312.

ole-MoiYoi, O.K. (1989) *Theileria parva*: An intracellular protozoan parasite that induces reversible lymphocyte transformation. *Experimental Parasitology* 69, 204–210.

ole-MoiYoi, O.K. (1995) Casein kinase II in theileriosis. *Science* 267, 834–837.

ole-MoiYoi, O.K., Iams, K.P., Nayar, A., Brown, W.C., Sugimoto, C., et al. (1988) Protein kinase activation in *Theileria*-infected cells. In: Lonsdale-Eccles, J.D. and Lenahan, J.K. (eds) *Protein Traffic in Parasite and Mammalian Cells. Proceedings of an International Workshop*. ILRAD, Nairobi, pp. 127–129.

ole-MoiYoi, O.K., Nayar, A., Iams, K.P., Musoke, A.J. and Yilma, T. (1989) Molecular aspects of *Theileria parva* and approaches to vaccine development for animals. *Annals of the New York Academy of Sciences* 569, 174–182.

ole-MoiYoi, O.K., Brown, W.C., Iams, K.P., Nayar, A. and Macklin, M.D. (1993) Evidence for the induction of casein kinase II in bovine lymphocytes transformed by the intracellular protozoan parasite *Theileria parva*. *EMBO Journal* 12, 1621–1631.

ole-MoiYoi, O.K., Sugimoto, C., Conrad, P.A. and Macklin, M.D. (1992) Cloning and characterization of the casein kinase II alpha subunit gene from the lymphocyte-transforming intracellular protozoan parasite *Theileria parva*. *Biochemistry* 31, 6193–6202.

Oura, C.A.L., Bishop, R., Asiimwe, B.B., Spooner, P., Lubega, G.W. and Tait, A. (2007) *Theileria parva* live vaccination: parasite transmission, persistence and heterologous challenge in the field. *Parasitology* 134, 1205–1213.

Oura, C.A.L., Bishop, R., Wampande, E.M., Lubega, G.W. and 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, 27–42.

Oura, C.A.L., Odongo, D.O., Lubega, G.W., Spooner, P.R., Tait, A. and Bishop, R.P. (2003) A panel of microsatellite and minisatellite markers for the characterisation of field isolates of *Theileria parva*. *International Journal for Parasitology* 33, 1641–1653.

Pearson, T.W., Lundin, L.B., Dolan, T.T. and Stagg, D.A. (1979) Cell-mediated immunity to Theileria-transformed cell lines. *Nature* 281, 678–680.

Pellé, R., Graham, S.P., Njahira, M.N., Osaso, J., Saya, R.M., et al. (2011) Two *Theileria parva* CD8 T cell antigen genes are more variable in buffalo than cattle parasites, but differ in pattern of sequence diversity. *PLoS One* 6, e19015.

Perry, B.D. (2016) The control of East Coast fever of cattle by live parasite vaccination: a science-to-impact narrative. *One Health* 2, 103–114.

Perry, B.D., Mukhebi, A.W., Norval, R.A.I. and Barrett, J.C. (1990) A preliminary assessment of current and alternative tick and tick-borne disease control strategies in Zimbabwe. ILRAD, Nairobi.

Pinder, M. and Hewitt, R.S. (1980) Monoclonal antibodies detect antigenic diversity in *Theileria parva* parasites. *Journal of Immunology* 124, 1000–1001.

Radley, D.E., Brown, C.G.D., Cunningham, M.P., Kimber, C.D., Musisi, F.L., et al. (1975) East Coast fever: 3. Chemoprophylactic immunization of cattle using oxytetracycline and a combination of Theilerial strains. *Veterinary Parasitology* 1, 51–60.
Radley, D.E., Young, A.S., Grootenhuis, J.G., Cunningham, M.P., Dolan, T.T. and Morzaria, S.P. (1979) Further studies on the immunization of cattle against *Theileria lawrencei* by infection and chemoprophylaxis. *Veterinary Parasitology* 5, 117–128.

Rich, K.M. and Niemi, J. (2017) Economic impact of a new disease: same impact in developed and developing countries? *Revue Scientifique et Technique de l’Office International des Epizooties* 36, 115–124.

Rich, K.M. and Perry, B.D. (2011) The economic and poverty impacts of animal diseases in developing countries: new roles, new demands for economics and epidemiology. *Preventive Veterinary Medicine* 101, 133–147.

Rich, K.M. and Wanyoike, F. (2010) An assessment of the regional and national socio-economic impacts of the 2007 Rift Valley fever outbreak in Kenya. *American Journal of Tropical Medicine and Hygiene* 83, 52–57.

Rich, K.M., Roland-Holst, D. and Otte, J. (2014) An assessment of the ex-post socio-economic impacts of global rinderpest eradication: methodological issues and applications to rinderpest control programs in Chad and India. *Food Policy* 44, 248–261.

Robinson, S., Mason-d’Croz, D., Islam, S., Suler, T.B., Robertson, R., et al. (2015) The International Model for Policy Analysis of Agricultural Commodities and Trade (IMPACT): model description for version 3. IFPRI Discussion Paper 01483. IFPRI, Washington, DC.

Rosegrant, M.W., Tokgoz, S. and Bhandary, P. (2013) The new normal? A tighter global agricultural supply and demand relation and its implications for food security. *American Journal of Agricultural Economics* 95, 303–309.

Seder, R.A., Chang, L.J., Enama, M.E., Zephir, K.L., Sarwar, U.N., et al. (2013) Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. *Science* 341, 1359–1365.

Shapiro, S.Z., Fujisaki, K., Morzaria, S.P., Webster, P., Fujinaga, T., et al. (1987) A life-cycle stage-specific antigen of *Theileria parva* recognized by anti-macroschizont monoclonal antibodies. *Parasitology* 94, 29–37.

Sitt, T., Poole, E.J., Ndambuki, G., Mwaura, S., Njoroge, T., et al. (2015) Exposure of vaccinated and naive cattle to natural challenge from buffalo-derived *Theileria parva*. *International Journal for Parasitology: Parasites and Wildlife* 4, 244–251.

Spooner, P.R. (1990) The effects of oxytetracycline on *Theileria parva in vitro*. *Parasitology* 100, 11–17.

Stobbs, T.H. (1966) The introduction of Boran cattle into an E.C.F. endemic area. *East African Agricultural and Forestry Journal* 31, 298–304.

Swai, E.S., Karimuribo, E.D., Kambarage, D.M. and Moshy, W.E. (2009) A longitudinal study on morbidity and mortality in young stock smallholder dairy cattle with special reference to tick borne infections in Tanga region, Tanzania. *Veterinary Parasitology* 160, 34–42.

Tebele, N., Skilton, R.A., Katende, J., Wells, C.W., Nene, V., et al. (2000) Cloning, characterization, and expression of a 200-kilodalton diagnostic antigen of *Babesia bigemina*. *Journal of Clinical Microbiology* 38, 2240–2247.

Toye, P.G., MacHugh, N.D., Bensaid, A.M., Alberti, S., Teale, A.M. and Morrison, W.I. (1990) Transfection into mouse L cells of genes encoding two serologically and functionally distinct bovine class I MHC molecules from a MHC-homozygous animal: evidence for a second class I locus in cattle. *Immunology* 70, 20–26.

Toye, P.G., Goddeeris, B.M., Iams, K., Musoke, A.J. and Morrison, W.I. (1991) Characterization of a polymorphic immunodominant molecule in sporozoites and schizonts of *Theileria parva*. *Parasite Immunology* 13, 49–62.

Toye, P., Gobright, E., Nyanjui, J., Nene, V. and Bishop, R. (1995a) Structure and sequence variation of the genes encoding the polymorphic, immunodominant molecule (PIM), an antigen of *Theileria parva* recognized by inhibitory monoclonal antibodies. *Molecular and Biochemical Parasitology* 73, 165–177.

Toye, P.G., Metzelaar, M.J., Wijngaard, P.L.J., Nene, V., Iams, K.P., et al. (1995b) Characterization of the gene encoding the polymorphic immunodominant molecule (PIM), a neutralizing antigen of *Theileria parva*. *Journal of Immunology* 155, 1370–1381.

Toye, P., Wijngaard, P., MacHugh, N. and Clevers, H. (1995c) An assay for the identification of antigens recognized by cytotoxic T cells based on transient transfection of COS cells. *Journal of Immunological Methods* 187, 95–101.

Toye, P., Handel, I., Gray, J., Kiara, H., Thumbi, S., et al. (2013) Maternal antibody uptake, duration and influence on survival and growth rate in a cohort of indigenous calves in a smallholder farming system in western Kenya. *Veterinary Immunology and Immunopathology* 155, 129–134.
Wagner, G.G., Duffus, W.P.H. and Burridge, M.J. (1974) The specific immunoglobulin response in cattle immunized with isolated *Theileria parva* antigens. *Parasitology* 69, 43–53.

Young, A.S., Brown, C.G.D., Burridge, M.J., Grootenhuis, J.G., Kanhai, G.K., *et al.* (1978) The incidence of theilerial parasites in East African buffalo (*Syncerus caffer*). *Tropenmedizin und Parasitologie* 29, 281–289.

Young, A.S., Groocock, C.M. and Kariuki, D.P. (1988) Integrated control of ticks and tick-borne diseases of cattle in Africa, *Parasitology* 96, 403–432.