East Coast Fever

Disease Monograph Series – 13

Parasite | Protozoa | *Theleria parva* | Cattle | Water Buffalo

IDRC | Bartay

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Acronyms

AU	African Union
AU-IBAR	African Union Inter-African Bureau for Animal Resources
BBRSC	Biotechnology and Biological Sciences Research Council
BMGF	Bill and Melinda Gates Foundation
CTTBD	Center for Tick and Tick Borne Diseases
CVO	Chief Veterinary Officer
DALY	Disability-adjusted life year
DIVA	Differentiate infected from vaccinated animals
DVS	Director Veterinary Services
ECF	East Coast Fever
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
IAEA	International Atomic Energy Agency of the United Nations
ILRI	International Livestock Research Institute
IM	Intramuscular
ITM	Infection and Treatment method
NGO	Non-governmental organization
OIE	World Animal Health Organization

PCR	Polymerase chain reaction
PIM	Polymorphic Immunodominant Molecule
SHF	Small holder farmer
TPP	Target Product Profile
WHO	World Health Organization of the United Nations

Executive Summary

Etiology and relevance

Theileriosis results from infection with protozoa in the genus Theileria of the suborder Piroplasmorina. Theileria spp. are obligate intracellular parasites. The two most important species in cattle and water buffalo are T. parva, which causes East Coast fever (ECF), and T. annulata, which causes tropical theileriosis. The present monograph focuses on T. parva and ECF. ECF is considered to be the most economically devastating tick-borne disease of cattle in Africa.

Epidemiology and transmission

While T. parva occurs in 13 Eastern and Southern African countries, only 11 of them have ECF, South Africa and Swaziland having exclusively Corridor disease.

T. parva is transmitted transstadially by the three-host ticks, Rhipicephalus appendiculatus, Rhipicephalus zambeziensis and Rhipicephalus duttoni. R. appendiculatus is the most important vector. The African buffalo (Syncerus caffer) is the natural reservoir host of T. parva and infections in buffalo are usually asymptomatic, but are acute and usually fatal in cattle. The African buffalo is an indigenous bovine of sub-Saharan Africa and has lived in harmony with T. parva and its vector R. appendiculatus long before cattle were introduced into the region. Other reservoir hosts of T. parva are water buffalo (Bubalus bubalis) and waterbuck (Kobus defassa).

Clinical signs

The clinical signs of ECF include generalized lymphadenopathy, fever, anorexia and loss of condition with decreased milk yield. Petechia and ecchymosis may be found on the conjunctiva and oral mucous membranes. Terminally ill animals often develop pulmonary oedema, severe dyspnoea and a frothy nasal discharge. Some cattle have a fatal condition called “turning sickness.” In this form of the disease, infected cells block capillaries in the central nervous system and cause neurological signs. Animals that recover from ECF often become asymptomatic carriers, but some animals have poor productivity and their growth is stunted.

Diagnosis

Diagnosis of ECF is principally based on clinical signs, knowledge of disease and vector distribution, and identification of parasites in Giemsa-stained blood and lymph node smears.

Clinically ECF should be suspected in tick–infested animals with a fever and enlarged lymph nodes. Terminal pulmonary edema and a high mortality rate in introduced breeds are also suggestive. Indigenous animals with tropical theileriosis may be in poor condition, with wasting and signs of anemia.

For laboratory diagnosis in live animals, ECF can be diagnosed by finding schizonts in Giemsa-stained thin smears from blood or lymph node biopsies. At necropsy, schizonts may be found in impression smears from many internal organs. Piroplasms occasionally occur in the blood of carrier animals, but in many cases, they cannot be detected by direct examination. The diagnosis must be confirmed by detecting schizonts.

The most widely used diagnostic test for T. parva has been the indirect fluorescent antibody (IFA) test. However, because of the problems of cross-reactivity among some Theileria species, the test has limitations for large-scale surveys in areas where species distribution overlaps. The IFA test for T. parva does not distinguish among the different immunogenic stocks. ELISA tests based on both crude and specific antigens have been developed and can detect antibodies to Theileria antigens approximately 20 days after infection. Molecular tests, especially PCR assays and sequencing also have been developed and are often used for characterising species and parasite polymorphisms, defining population genetics and generating epidemiological data.

ECF is the most economically important tick-borne disease in Africa due to the mortality, morbidity and production losses it causes. Indirectly it also impacts on other economic aspects, affecting agriculture, mining and commerce, all of which still used ox-drawn transport.

With more than 38% of the African total bovine population affected and an estimated mortality of 1.1 million cattle per year, ECF remains probably the most important cattle disease in terms of economic losses and restriction of livestock development in affected countries of eastern, central and parts of southern Africa.

Control

In endemic regions, the main methods in the control of ECF include tick control, host immunization, chemotherapy and integrated control that combines any of the methods. Chemical control of ticks with acaricides is still the most practical and widely used method for the control of ECF. However, tick control practices are not always fully effective for a number of reasons, including development of acaricide resistance, the high cost of acaricides, poor management of tick control, and illegal cattle movement in many countries.

Several drugs, legal and substandard, are widely used in the treatment of clinical disease, sometimes to good effect, but they have not proved to be completely reliable therapeutic agents.

The only immunisation method that has been possible to use to date is the infection and treatment Method (ITM) in which cattle are given a subcutaneous dose of tick-derived sporozoites and a simultaneous treatment with a long-acting tetracycline formulation. This treatment results in a mild or inapparent ECF reaction followed

by recovery. Recovered animals demonstrate a robust immunity to homologous challenge, which usually lasts for the lifetime of an animal. The most widely used and studied ITM is the Muguga cocktail, developed in Kenya and comprising 3 stocks (T. parva Muguga and T. parva Kiambu 5 and buffalo-derived T. parva Serengeti transformed) in order to provide broader immunity to different isolates.

Through extensive efforts of several organisations and program few batches of the ECF ITM Muguga cocktail were produced at ILRI and made available to livestock keepers in East African countries the most affected by the disease. Although produced at an approximate cost of $USD2, the cost to the farmer has been around $USD10 – $USD15 per animal. The high cost is associated with storage requirements of stabilate for transport (liquid nitrogen), oxytetracycline for the treatment (from $USD2-8) and veterinary professional care required for the ITM process. Despite the cost, the farmers recognize the benefit of protecting their herds and pay for treatment by selling a few of their animals. The major disincentive for use of ITM by the farmers has been the unreliable and inconsistent supply. Through a GALVmed funded program, the CTTBD in Malawi was supported to produce more batches of the vaccine, and is still being supported for the process and product improvement.

Although the ITM vaccine is credited with saving 620,000 cows since its use in the most affected countries, the need for more immunisation is still not being met, and the vaccine has several shortcomings. One of the major problem associated with the ITM is the inoculation of live parasites, which makes it difficult to provide a product that is safe and which can be easily delivered to farmers. In addition, cross-immunity studies and field challenge of animals immunised by this method have shown that different immunological types of the parasite exist, and that the immunity is strain- or stock-specific. Furthermore, the ITM Muguga cocktail has been shown not to be protective on buffalo-derived T. parva.

The presence of sporozoite neutralizing antibodies in cattle repeatedly exposed to field infection, or to sporozoites by stabilate inoculation, provided the rationale for the development of a potential vaccine based on a recombinant sporozoite surface antigen of T. parva, known as recombinant p67. The cloned gene encoding p67, and expressed bacterially or insect cells induced protection in cattle against experimental needle challenge with a lethal dose of sporozoites. However, the results showed consistently approximately 70% protection against lethal needle challenge with sporozoite stabilates in susceptible taurine cattle in the laboratory. In subsequent field trials conducted in Kenya, with different p67 vaccines in order to determine protective efficacy against tick challenge, within different production systems and epidemiological situations, could demonstrate reduction in severe disease of only 47–50% relative to un-immunized cattle. More peptides had been identified and characterized which are believed to enhance the protection level afforded by p67, when administered as a cocktail.

Based on these initial results, a new initiative has been funded by the BMGF for the establishment of an ILRI-led ECF consortium made of all global institutions involved in a way or another in ECF vaccine research. This consortium, funded for 4 years to the tune of USD11million, will look into improvement of the ITM Muguga cocktail and also new generation vaccines, building on work conducted to date.

To date there are two programs looking into ECF vaccine: the work through the ILRI-led ECF consortium and the ITM production process improvement work at CTTBD.

The future of ECF vaccines and vaccination

The funding of ECF vaccine research through the ECF consortium gives hope for a possible better vaccine against this devastating disease. There is a need meanwhile to improve availability and access in the field of the current ECF ITM to as many affected livestock keepers as possible. It would also be important to intensify the vaccine matching effort of different isolates from different part of affected regions.

Clinical disease overview

There are a number of species of *Theileria spp.* that infect cattle; the two most pathogenic and economically important are *T. parva and T. annulata. Theileria parva* occurs in 13 countries in sub-Saharan Africa causing East Coast fever (ECF), whilst *T. annulata* (Tropical/Mediterranean theileriosis) occurs in southern Europe as well as North Africa and Asia [21]. The present monograph will focus on *T. parva* and East Coast Fever (ECF).

ECF, also referred to as Theileriosis, in its classical form is a usually fatal disease of cattle caused by *Theileria parva*. It is transmitted principally by the brown ear tick *Rhipicephalus appendiculatus* and is characterized by the proliferation of lymphoblasts infected with theilerial schizonts throughout the body, particularly in the lymph nodes, lymphoid aggregates, spleen, kidneys, liver and lungs. The disease occurs widely through the range of its main vector in eastern, central, and southern Africa [21][9].

Etiology

Theileria is a genus comprising tick-transmitted parasitic protozoa in the family Theileriidae, order Piroplasmida, subclass Piroplasmia, phylum Apicomplexa. Theileriae are obligate intracellular protozoan parasites that infect both wild and domestic Bovidae throughout much of the world (some species also infect small ruminants). They are transmitted by ixodid ticks, and have complex life cycles in both vertebrate and invertebrate hosts.

Besides the already mentioned *T. parva* and *T. annulata*, there are some mildly pathogenic and benign species of *Theileria* that infect cattle and buffalo in Africa such as *T. mutans*, a species which is usually non-pathogenic, although it may cause severe or fatal disease in East Africa, *T. buffeli*, which was originally identified in Asiatic buffalo and also is found in African buffaloes [9], *T. velifera, T. bujJeli, Theileria taurotragi* and *Theileria* sp. (buffalo) [1][4]. See Table 1 for the parasites, vectors and diseases.

Originally described as a single species, it was for a period divided into three subspecies: *T. parva parva* (the cause of classical East Coast fever); *T. parva lawrencei* (the cause of Corridor disease); and *T. parva bovis* (the cause of Zimbabwe theileriosis). This subdivision has now been abandoned [18][21].

Another important point worth noting is the fact that isolates and vaccine strains are generally named or referred to based on the geographic area where they were isolated. It has been suggested by several experts that a genotypic-based nomenclature would be preferable, as it will also dissipate some of the current conflicts and perceived fears of introducing foreign isolates in some regions believed to have different isolates (personal communication with T. Musoke, F. Musisi and S. Morzaria).

To date the most studied isolates are those included in the Muguga cocktail, which includes the cattle-derived *T. parva* Muguga and *T. parva* Kiambu 5 and buffalo-derived *T. parva* Serengeti transformed.

More recent sequencing work by Norling et al [15] has demonstrated points often noted that, the Serengetitransformed stock is very similar genetically to the Muguga stock.

From studies of the Muguga isolate, immunodominant antigens of the parasite have been identified as falling into the two categories of potential candidate vaccine antigens: p67 and PIM as sporozoite antigens [14] and Tp1, Tp2, Tp4, Tp5, Tp8, Tp9, Tp10 and Tp12 as schizont antigens.

Epidemiology

Susceptible animal species

T. parva can infect cattle, African buffalo (*Syncerus caffer*), water buffalo (*Bubalus bubalis*), and waterbucks (*Kobus spp*.). Symptomatic infections are common only in cattle and water buffalo.

T. parva is probably originally a parasite of African buffalo which has become adapted to cattle. It has been found to infect waterbuck (*Kobus defassa*) under natural conditions, and the Asiatic buffalo (*Bubalus bubalis*) under experimental conditions [9]. It is not infective to other ungulates, nor to any species of laboratory animal. There is no evidence that wildlife plays any role in the epidemiology of the disease.

There is a marked variation in the susceptibility of cattle to infection. Taurine breeds are generally more susceptible than Zebu and Sanga breeds. In addition, there are variations within breeds depending on the epidemiological state of East Coast fever.

Vector of ECF

The occurrence of ECF closely follows the geographic distribution of the main vector *Rhipicephalus appendiculatus*, a three host ixodid tick (Figures 1 & 2). *R. appendiculatus* is widely distributed throughout the warmer, more humid areas of eastern, central and southern Africa. Within its range its abundance varies considerably, being governed by soil moisture and temperature, presence of suitable habitat and availability of hosts. The distribution of climatic suitability has been plotted on the basis of ecoclimatic indices.[9] Interestingly, areas of ecoclimatic suitability exist where the presence of the tick has yet to be recorded [16] illustrating that other factors also play a role in determining distribution. Among these is vegetation cover, which influences the

microclimate of the habitat of the tick; overgrazing has a marked adverse effect on the tick population [12][9]. Also important is the livestock production system employed, particularly with respect to how management affects exposure to ticks. Thus in Kenya there is a considerable difference in tick infestation levels and corresponding infection prevalence between open grazing and stall-feeding (zero grazing) management systems [5].

Table 1: The theilerioses of livestock in eastern, central and southern Africa [9]

PARASITE	VECTORS	DISEASE	SPECIES AFFECTED	PATHOGENICITY	DISTRIBUTION
Theileria parva	*Rhipicephalus appendiculatus***	East Coast fever	Ox, Asiatic domestic buffalo (experimental)	Usually fatal (benign in resistant Zebu calves)	Endemic throughout most of the range of *R. appendiculatus* north of Zambezi River
	*R. duttoni**				
	*R. zambeziensis**		African buffalo	Benign	
	R. capensis		Waterbuck	Benign	
	R. carnivoralis				
	R. compositus				
	R. evertsi evertsi				
	R. kochi	Corridor disease	Ox	Usually fatal	Localized, in association with African buffalo
	R. pravus		African buffalo	Benign	
	R. pulchellus				
	R. simus				
	Hyalomma anatolicum	Zimbabwe theileriosis	Ox	Variable – often fatal	Zimbabwe highveld
	H. dromedarii				
	H. impressum				
Theileria taurotragi	*R. appendiculatus***		Ox	Usually benign – rarely fatal	Widespread (endemic)
	R. evertsi evertsi				
	R. pulchellus		Eland	Sometimes fatal	
	R. zambeziensis				
Theileria mutans	*Amblyomma hebraeum***		Ox	Usually benign – some strains pathogenic	Widespread (endemic)
	*A. variegatum***				
	A. astrion		African buffalo	Benign	
	A. cohaerens				
	A. gemma				
Theileria velifera	*A. hebraeum***		Ox	Benign	Widespread (endemic)
	*A. variegatum***		African buffalo	Benign	
	A. astrion				
	A. lepidum				
Theileria sp. near *T. buffeli*	*Haemaphysalis* spp.		Ox	Benign	Widespread
Theileria separata	*R. evertsi evertsi*		Sheep	Benign	Widespread (endemic)

Figure 1: *Rhipicephalus appendiculatus* - Brown ear tick

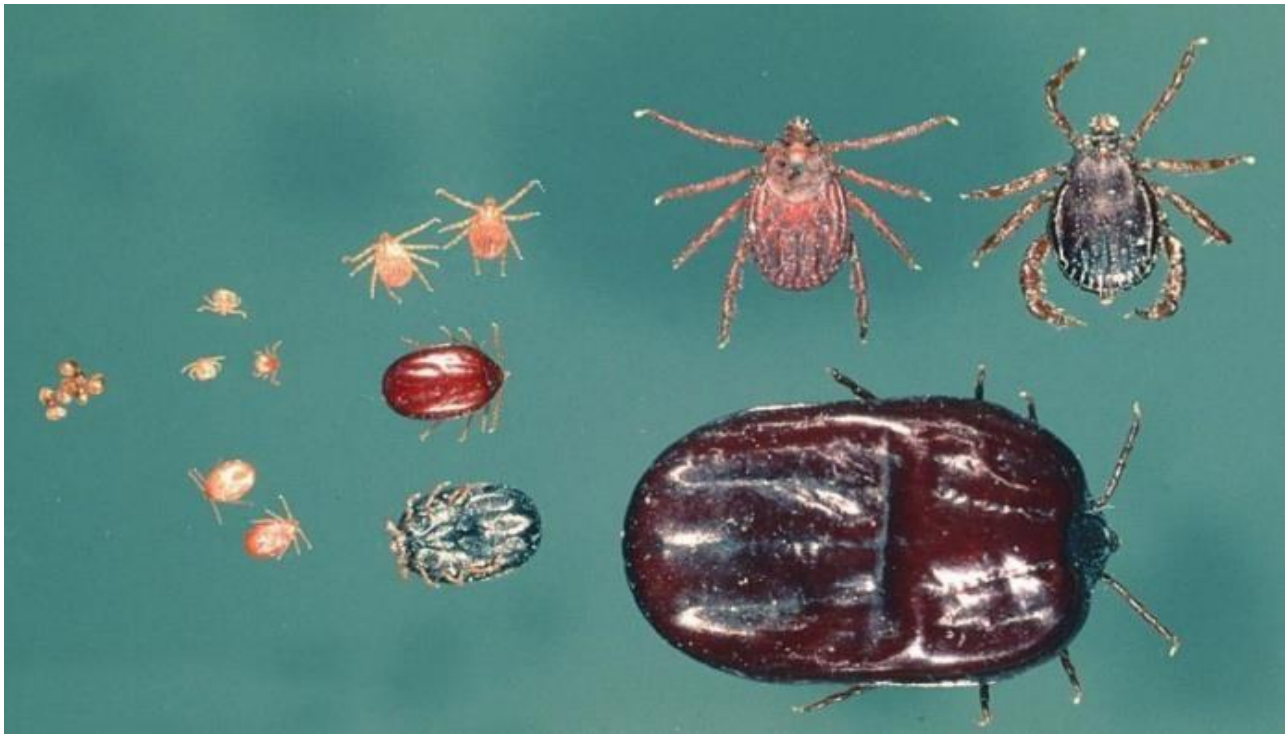

Figure 2: ***Life cycle of Rhipicephalus appendiculatus hard tick. Eggs at far left, then unfed and engorged larvae, unfed and engorged nymphs, unfed female and male top right, fully engorged female bottom right;*** *https://commons.wikimedia.org/wiki/File:Rhipicephalus-appendiculatus-life-cycle.jpg*

Life cycle of Theileiria parva

T. parva has a typical apicomplexan life cycle (Figure 3) with an alternation of sexual and asexual stages that are found in the mammalian and tick host. *T. parva* alternates between cattle and ticks and its life cycle involves the sequential invasion of two different cell types in the cattle host, and a sexual cycle in the vector. Ticks become infected with *T. parva* when they feed on cattle or buffalo carrying in their erythrocytes the piroplasm stage of the parasite. Piroplasme are released within the tick gut and differentiate into male and females gametes. Pairs of gametes fuse to produce diploid zygotes. These will invade cells in the gut wall and undergo a two-step meiotic division, resulting in large number of developmental stages which develop into motile kinetes and migrate to the salivary glands through the heamolymph. The kinete is now called a sporont. During tick feeding, sporoblasts are produced in a sporogony process of nuclear mitotic division. Maturation is then stimulated and haploid sporozoites are produced after three to four days feeding. These infective sporozoites are subsequently released into the saliva and injected into the mammalian host when ticks are taking their blood meal. Inoculated sporozoites invade bovine T and B lymphocytes by a complex receptor-mediated process and begin to develop.

The parasite completes the first stage of its lifecycle in lymphocytes which consists in a logarithmic multiplication phase (schizogony) and the formation of the invasive forms (merogony) for the next stage. [1]

Resulting merozoites are liberated from the lymphocytes and invade the erythrocytes in which they are referred to as piroplasms. The erythrocytic stages are infective to ticks and are characterised by limited proliferation. This is in marked contrast to most of the other members of the *Piroplasmia* in which the main multiplication phase occurs in the erythrocytic phase. [1][9]

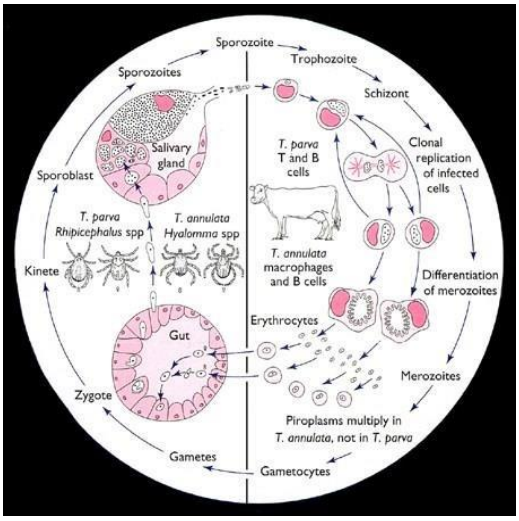

Figure 3: Lifecycle of *Theileria parva*

Distribution

T. *parva* are found only in Africa, in 13 Eastern, Central and Southern African countries: Burundi, Democratic Republic of Congo, Kenya, Malawi, Mozambique, Rwanda, South Sudan, South Africa, Swaziland, Tanzania, Uganda, Zambia and Zimbabwe [24], as shown in Figure 4 below. ECF on the other hand is considered to occur in 11 countries, since South Africa and Swaziland have rather Corridor disease.

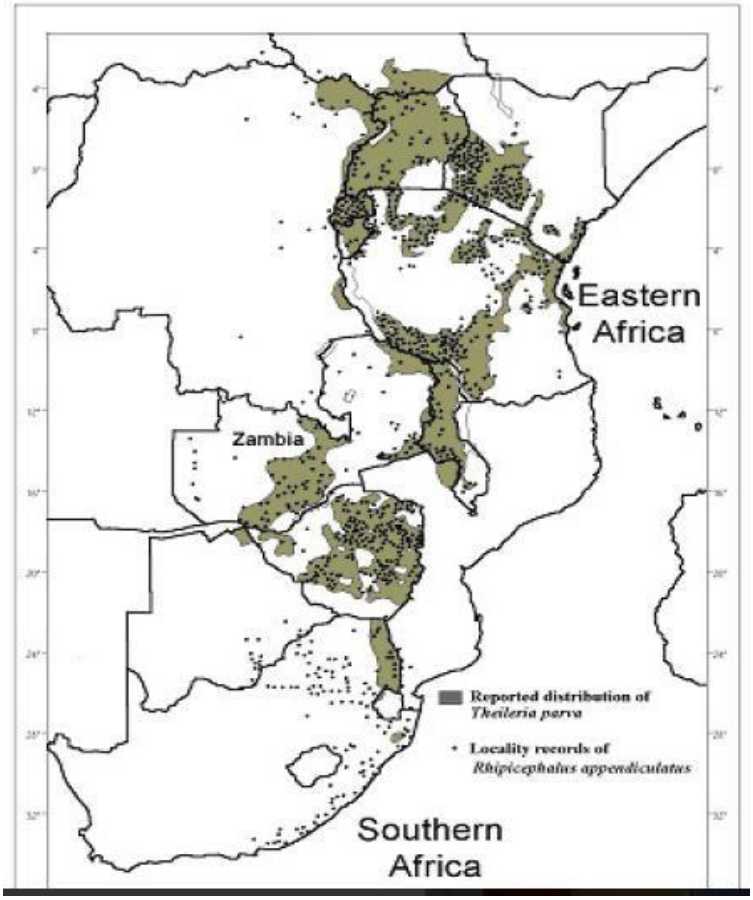

Figure 4: Distribution range of *T. parva* and distribution of *R. appendiculatus* [11]

Endemic stability and instability

Three major epidemiological forms or states of East Coast fever are recognized: endemic stability, endemic instability and epidemic. Under certain specific circumstances, ECF can exist in an endemic form in which it has little or no influence on morbidity and mortality in the herd. According to Lawrence et al. [9] this situation, which was first recognized in Uganda, is now commonly referred to as endemic stability. However, endemic stability to *T. parva* infection appears to be relatively limited in its distribution and may not be achieved easily. The more common situation seen in the region is that of endemic instability, in which varying degrees of clinical disease are experienced.

Endemic stability: implies an ecological balance between cattle, tick and parasite in which regular transmission of *T. parva* occurs in all age groups of the cattle population and high levels of population immunity are achieved, with minimal effects in terms of morbidity and mortality. This is most commonly achieved in areas where indigenous Zebu cattle are kept on pasture in areas highly suitable for the vector, and where major seasonal

fluctuations in vector abundance do not occur. Infection prevalence in ticks may be in the region of 1 or 2 per cent, infection intensity in infected ticks is low, infection of calves takes place early in life, and reinfection throughout life is common.

Endemic stability is characterised by a high (70%) seroprevalence but low incidence of theileriosis, and low theileriosis fatality rates.

It is most common in the smallholder systems prevalent in the humid areas surrounding the Lake Victoria basin, where the presence of endemic *T. parva* infection in cattle prohibits or severely limits the keeping of more productive taurine breeds of cattle.

Endemic instability: Endemic instability is found in two forms, low incidence and high incidence. Low incidence instability is found in areas of very low infection challenge, either in areas marginally suitable for the vector or on properties where acaricides are applied intensively. High incidence instability is characterized by an infection challenge that is insufficient to induce population immunity. This can be as a result of ineffective tick control, or intermediate levels of infection challenge.

Low incidence endemic instability is found on the large-scale commercial beef ranches of the entire region, and in the small-scale dairy systems of Kenya [5].

High incidence instability is characteristic of newer commercial ventures, in which an upgrading of indigenous cattle is undertaken through crossbreeding, and tick control is ineffective. Under such circumstances, and in epidemic ECF, the case fatality may be extremely high [5].

Epidemic ECF occurs when the disease is introduced to areas previously free of the disease, and often occurs on a seasonal or secular basis at the margins of *R. appendiculatus* distribution.

Clinical Signs

East Coast fever in the classical form is characterized by pyrexia, enlargement of the superficial lymph nodes, severe pulmonary oedema and wasting. It usually terminates in death. The period and the course of the disease become shorter as the challenge is increased. The first clinical signs are fever and increases in pulse and respiration rates. There may be a sharp decline in milk production.

The parotid lymph nodes, which drain the ear to which the infected tick has attached, are enlarged. After a few days the animal becomes depressed and lethargic. The temperature continues to rise, often to 41 or 42 °C. Anorexia may develop but is not inevitable. Lachrymation commonly occurs together with oedema of the eyelids, and may be accompanied by photophobia. The animal is often constipated. There is a generalized enlargement of the superficial lymph nodes; the prescapular and precrural nodes become very prominent [6].

The disease usually progresses over a period of about 15 days, but may terminate after five days or be prolonged to 25 days. Pregnant cows may abort. [6][9].

In the terminal stages of the disease dyspnoea develops, with an increased respiratory rate, a watery cough and the discharge of frothy fluid from the nostrils. The discharge is copious both when the animal is recumbent and immediately before death. Sternal and submandibular oedema may be present. The enlarged superficial lymph nodes begin to regress, the rectal temperature falls to subnormal levels, and the animal becomes recumbent and dies in a coma.

A small number of animals, usually about 5 per cent, may recover, but convalescence is prolonged and the animals may remain emaciated and unproductive for monthsThe disease may assume a less severe form in animals with partial immunity or inherent resistance, but pyrexia and enlargement of superficial lymph nodes remain constant features. Mild disease has also been reported occasionally following infection of fully susceptible animals with strains of *T. parva* of reduced virulence.

In the Asiatic buffalo the disease resembles that in the ox, but in the African buffalo infection is invariably subclinical or mild.

Immunity to T. parva

The immune response to theileriae parasites is complicated. Cell-mediated immunity is thought to be the most important protective response in *T. parva* and *T. annulata*. In *T. parva,* the principal protective responses are mediated through the bovine major histocompatibility complex (MHC) class I-restricted cytotoxic T lymphocytes [12][22]

Infection with the parasite results in an acute lymphoproliferative disorder with high mortality, but animals which survive infection are solidly immune to homologous challenge. Such immunity can be reproduced by infecting cattle with the parasite and treating them with tetracyclines or theilericidal drug: this characteristic is the basis of the infection and treatment immunisation method used for ECF.

These have provided evidence that immunity to the parasite can operate at two levels: blocking of the infection by antibody specific for sporozoite surface antigen and killing of parasitized leucocytes by cytotoxic T cells.

Humoral immunity

- Animals in endemic areas respond to *T. parva* infection by mounting humoral responses that decline over months in the absence of challenge. The serum from immune cattle contains antibodies against all stages of the *T. parva* parasite [12].

- The most relevant antibody responses are those directed against sporozoite surface antigens [14]. Antibodies against sporozoites appear to recognise a wide range of *T. parva* isolates and are correlated with some protection [1].
- *In vitro* studies have showed that a highly conserved 67-KDa protein (p67) and to a lesser extent the polymorphic immunodominant molecule (PIM) were the major neutralising antigens [16]. Immunisation of cattle with *Escherichia-coli* derived recombinant p67 gave approximately 70% protection of cattle against severe disease after laboratory challenge [14]. However, only a small proportion of immunised cattle were able to show complete neutralization of infection while the rest showed schizont parasitosis of varying severity.

Cell-mediated immunity

- There is strong evidence that the long lasting protective immune mechanisms in *T. parva* are cellmediated and targeted against the schizont-infected lymphocytes [12]. Animals subjected to a single immunisation by infection and treatment produce little or no sporozoite- neutralizing antibodies [14].
- The principal effectors of the protective cellular immunity are CTLs directed against the schizontinfected lymphocyts [12][13].
- The immune cells responsible for protection through lysis of infected cells belong to CD8+ T-cell subpopulation and are MHC class-I (MHC-I) restricted and parasite specific. The strain specificity of CTL response induced is consistent with the existence of immunological heterogeneity in populations of *T. parva* [13], which is maintained through sexual recombination in the vector [1].
- Other cellular mechanisms may also contribute to protection of immune cattle against challenge. Baldwin et al. [13] isolated parasite-specific CD4+ T cell clone with cytotoxic activity but their role in the immune response had not been determined.

There seem to be evidence of resistance in some breed, such as the Ankole. This was demonstrated in a study published in 1991, where following challenges of Ankole cattle from Rwanda and cross-bred animals, it was concluded that the partial Theileria tolerance of the Ankole is, to a great extent, genetic. The basis of this partial tolerance seems to be their ability to limit the explosive multiplication of macroschizonts during the acute phase of the disease [20]

Diagnosis

The diagnosis of classical ECF is based on the characteristic clinical signs and lesions, and may be confirmed by demonstration of schizonts and, in the later stages of the disease, piroplasms.

Clinical Diagnosis

East Coast fever should be suspected in tick–infested animals with a fever and enlarged lymph nodes. Terminal pulmonary edema and a high mortality rate in introduced breeds are also suggestive. Indigenous animals with tropical theileriosis may be in poor condition, with wasting and signs of anemia.

Pathology and post-mortem diagnosis

In the dead animal, smears prepared from the cut surface of the spleen and any enlarged lymph nodes should be examined. Smears must be of good quality and should be stained with Giemsa or any other Romanowsky stain. The presence of large hyperplastic lymphoblasts should be an incentive to continue the search for schizonts if they are scanty [9].

The most prominent feature seen in a carcass, is often the severe accumulation of fluid in the lungs, frequently also accompanied by large amounts of froth in the trachea and bronchi.

Laboratory diagnosis

In live animals, theileriosis can be diagnosed by finding schizonts in Giemsa-stained thin smears from peripheral blood or fine-needle aspirates of superficial lymph nodes, or lymph node biopsies. The piroplasmic stage follows the schizont stage and, it is usually less pathogenic and is thus often found in recovering or less acute cases [24].

Microscopic examination and PCR constitute tests aimed at the identification of the agent. o Serology is used in cross-sectional surveys or longitudinal studies to detect the presence of *T. parva* infection and to determine the prevalence, incidence and seasonality of infection and disease. Circulating antibodies were first demonstrated in recovered animals by the complement fixation test and since then various techniques, for detection of the immune response, have been developed using schizonts or piroplasms as antigen. Serological tests used are either the ELISA or the IFAT Table 2 below summarizes the different tests and circumstances of their use [24]. Important to note that the ELISA is currently used as an indication of immune response post vaccination both in the field and vaccine QC despite the fact that it is not ideal.

Microscopy

Multinucleate intralymphocytic and extracellular schizonts can be found in Giemsa-stained biopsy smears of lymph nodes, and are a characteristic diagnostic feature of acute infections with *T. parva*. Both intracellular and free-lying schizonts may be detected, the latter having been released from parasitised cells during preparation

of the smears. It is important to note that schizonts, which are the pathogenic stage of T. parva, and piroplasms of different theilerias are very difficult to discriminate in Giemsa-stained smears.

Piroplasms of most species of Theileria may persist for months or years in recovered animals, and may be detected intermittently in subsequent examinations. However, negative results of microscopic examination of blood films do not exclude latent infection. Relapse parasitaemia can be induced with some Theileria species by splenectomy. Piroplasms are also seen in prepared smears at post-mortem, but the parasites appear shrunken and their cytoplasm is barely visible.

Table 2: Diagnostic of ECF

Method	Population freedom from infection (nonvaccinated animals)	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases [2]	Prevalence of infectionsurveillance	Immune status in individual animals or populations postvaccination
	Agent identifictaion [3]					
Microscopy examination	-	+++	-	+++	-	-
PCR	+	++	++	+++	+	-
	Detection of Immune response [4]					
IFAT	+	+++	++	-	+++	-
ELISA	+	+	-	-	+	-

Key: +++ = recommended method; ++ = suitable method; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose. Although not all of the tests listed as category +++ or ++ have undergone formal validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable. PCR = polymerase chain reaction; IFAT = indirect fluorescent antibody test; ELISA = enzyme-linked immunosorbent assay

Molecular diagnostics

The need for an improved method of detection of *T. parva* carrier animals has led to the development of a number of PCR techniques using *T. parva*-specific primers based on single copy genes (pl04 and PIM antigen genes) or a repetitive gene sequence (TpR).

The tests developed to date include: o Conventional PCR followed by agarose gel electrophoretic analysis [10] o Nested-PCR,

PCR followed by dot blotting, capillary blotting or slot-blotting and hybridisation using radioisotope labelled probes [10]

Detection by molecular methods allow for direct confirmation of the presence of parasite genomic material, with the inference that live parasites are present in the animal at the moment of sampling. Developments from conventional to nested to real-time PCR has allowed improvement in sensitivity, quantification and speed of detection, while methods such as reverse line blot, bead arrays, pan-FRET assays and highresolution melt analysis hold the promise of detection of multiple species or genotypes at the same time [10].

Real-time melting profile based assays also hold the advantage that variation in probe regions may be detected by differences in melting profiles that may be related to genotypic or species differences.

Whereas many of these assays require specialized equipment, LAMP assays hold the advantage of functioning at isothermal conditions, with possible application under field conditions.

Indirect Fluorescent Antibody Test

The IFA test is robust, easy to perform and provides adequate sensitivity and specificity for use in the field for detection of prior infection with *T. parva* and *T. annulata* infections under experimental situations and in a defined epidemiological environment where only one theilerial species is present. [21].

The IFAT remains the gold standard assay recommended by the OIE for international trade. o The IFAT can use either schizont or piroplasm antigen, but that using schizont antigen is the most widely used serological test for both species. It has been used extensively in ECF endemic areas, both to improve understanding of the epidemiology of theileriosis and to assess seroconversion in immunisation trials (Minjauw et al.; 2003). The schizont IFAT for *T*. *parva* cross-reacts with *T*. *annulata* and *T*. *taurotragi*, but not with *T*. *mutans*. [24]. *ELISA*

The IFAT utilises whole-body antigen, but the realisation that a select number of antigens are responsible for the dominant immune response against most Theileria parasites has stimulated interest in ELISA.

Tests used are indirect ELISAs based on parasite-specific PIM antigen, found on the surface of sporozoites and schizonts. These ELISAs provide higher (over 95%) sensitivity than the IFA tests [24][10].

The ELISA detects antibody of IgG1 isotype and antibody levels remain positive after a single infection with *T. parva* for longer than with the IFAT.

Differential Diagnosis

In endemic areas ECF must be differentiated from other febrile conditions which usually terminate fatally after a course of one to two weeks and which feature progressive emaciation, respiratory distress, diarrhoea, corneal opacity and lymphoid hyperplasia [9].

Bovine virus diarrhoea/mucosal disease and bovine malignant catarrhal fever may all be distinguished by the presence of ulcerating or necrotizing inflammatory reactions in buccal and nasal mucosae, and conjunctivitis.

CBPP: difference based on characteristic coughing and evidence of pain on respiration.

Acute trypanosomosis does not exhibit diarrhoea or severe respiratory distress.

Babesiosis and anaplasmosis: anaemia and icterus are the major presenting signs.

Incidence and Prevalence in Selected Countries

Global

In the 11 countries where ECF is endemic, outbreaks and fatalities due to the disease occur yearly, and are not always reported or recorded. Below are data from official reporting at the OIE and the AU-IBAR, as well as prevalence data from publications.

Prevalence is usually low (63%) in the endemic instability state (Deem et al 1993; Medley et al., 1993 and Perry and Young, 1995). Endemic stability is likely to exist where the prevalence of serum antibodies to infection is equal or greater than 70% (Lynen et al., 1999). Animals in endemic areas respond to *Theileria parva* infection by mounting humoral responses that decline over months in the absence of challenge.

Incidence data by country

Table 3: Number of Theileriosis outbreaks reported to the OIE between 2005-2015 (Numbers given only for the target countries). Source: OIE.
http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail

Country	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
East Africa											
Ethiopia	0	0	0	0	0	0	0	0	0	0	-
Kenya	+	>28	39	+	>10	10	+	>5	4	27	21
Rwanda	-	+	+	+	+	+	+	+	+	-	-
Tanzania	+	93	437	110	70	80	54	29	31	50	>19

Uganda	+	2	+	+	+	+	+	+	+	+	-
Southern Africa											
Malawi	>5	+	>3	-	>1	2	0	0	0	-	-
Mozambique	5	+	16	>4	+?	0	0	0	-	-	-
Zambia	-	63	65	143	206	180	+	82	151	275	-

- No information, + Present but quantitative data not known, ? Disease suspected

AU-IBAR: The number of outbreaks reported to AU-IBAR is included in the Pan African Animal Resources Year Book. (**http://www.au-ibar.org/pan-african-animal-resources-yearbook?showall=&limitstart=**) and can be seen for the countries of interest in Table 4 below.

Table 4: Number of Theileriosis outbreaks reported to the AU-IBAR from 2005 to 2015 (numbers given only for the target countries). Source: AU-IBAR Year Books.

Country	2005*	2006*	2007	2008	2009	2010	2011	2012	2013	2014	2015
East Africa											
Ethiopia	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Kenya			263	81	134 cases	3540 cases	1356	791	260	34	
Rwanda											
Tanzania			531	2246 cases	1065 cases	1068 cases	14,700 cases	28	28	44	
Uganda			4	10	12	45	45		11	6	
Southern Africa											

Country	2005*	2006*	2007	2008	2009	2010	2011	2012	2013	2014	2015
Madagascar											
Malawi					1			37			
Mozambique			2	17	4	10	10		8	6	
South Africa**			4	10	2			11		11	
Zambia			65	2	2585 cases	9	9	43	141	121	

*AU-IBAR didn't start yet producing data for ECF
**Report on South Africa refer to Corridor disease

Regional

No data or not recent prevalence data was found for Ethiopia, Malawi and Mozambique.

Kenya

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2014	Western Kenya	Indigenous cattle	453	12.9% (Reverse line blot hybridization)	Njiri et al, 2015
2014	Western Kenya	Indigenous cattle	548	77% (serology)	Kiara et al, 2014
2011	Ngong and Machakos	Cattle	Ngong: 154 Marchakos: 38	Ngong: 33.8% Theileria spp Machakos: 39.5% Theileria spp	Adjou Moumouni et al, 2015

2003	Mbeere District	Cattle	440 from 80 farms	19.3%	Skilton et al, 2003

Region/Province	District	Prevalence (cattle ages sampled)	Annual incidence rate	Case-fatality rates	Epidemiological factors
Lake Victoria basin/ Nyanza region	Rusinga island	>70%	NA	NA	Region very suitable for the tick vector
	Rusinga island	NA	22%	21%	
	Kisumu, Siaya and Bondo	60% (4–18 months)	NA	NA	
Coastal lowlands/ Coast	Kaloleni/Kilifi	22% - 85% (4–18 months)	NA	NA	Region very suitable for the tick vector
Western Kenya highlands	Uasin Gishu	60%[a], 73%[b]	32%[a], 39%[b]	NA	Farm management practices influenced epidemiology
Central highlands (Central Kenya)	Murang'a (AEZ: UM4*)	72% (6–18 months)	90%	16%	AEZ suitability for tick vector differs, age, breed, grazing system
Western province	Busia district	7% - 8%[c]	NA	NA	-
Southern Rift Valley (Maasailand)	Trans Mara	~ 100% <6 months	NA	3%	Age
Eastern Province (Arid-semi arid region)	Mbeere District	All age categories 4% – 48%	NA	NA	AEZ suitability for tick vector differs, presence of vector tick on the farm, calf tick control frequency, herd size
	Machakos District	All age categories 60%	NA	NA	-
Southern Rift Valley (Maasailand)	Kenya-Tanzania border	NA	NA	30% to 60%	Precipitation levels

a: Rural area; b: Peri-urban; * Upper midlands 4; c: parasitological data.

Table: ECF prevalence, incidence and case-fatality rates from studies conducted in traditional crop-livestock and livestock-dependent systems in Kenya [5]

Region	District /area	Prevalence (cattle ages sampled)	Annual incidence rates	Case-fatality rates	Epidemiological factors
Central highlands	Kiambu	41%-55%			Age
	Murang'a	18%[a], 72%[b] (6–18 months)	54%[c] 74%[d] 86%[e], 110%[f]	6%[c], 5%[d] 9%[e], 16%[f]	AEZ suitability for tick vector, age, breed, grazing system
Coastal lowlands	Kaloleni/ Kilifi	57%[g], 79%[h] (adult)			Age, AEZ, grazing system
	Kaloleni/ Kilifi	18%[g] 48%[h] (<18 months)	6.0%[g] - 50.4%[g], 10.8%[h] - 87.6%[h]	13%[g], 31%[h]	Age, AEZ, grazing system
	Kwale		23%*	11%*	Age, grazing system
Central Rift Valley	Nakuru		22%[j], 33%[k]		Grazing system

Malawi

The table below includes unpublished data from the CTTBD for *T. parva* (TP) and *T. mutans* (TM)

AREA /SITE SAMPLED	NO. OF SAMPLES	Positive cases per parasite		AREA /SITE SAMPLED	NO. OF SAMPLES	Positive cases per parasite	
2011		**TP**	**TM**	**2013**		**TP**	**TM**
Natural Resources College	23	0	1	Mpalo MBG [Ntchisi]	9	3	1
Central African Cattle	39	1	5	Mwera Mkaka MBG [Ntchisi]	21	11	7
Katete Dairy Farm	65	0	4	Mchinji	28	2	2
Likasi Livestock Centre	30	3	2	Chitedze Research Station	17	2	2
Mapanga Dairy Farm [Blantyre]	27	0	0	Natural Resources College	33	2	3
Mpemba [Blantyre]	28	0	0	Mzuzu ADD	17	0	0
Shire Highlands RDP [Blantyre]	58	2	3	Blantyre	238	5	31
Blantyre	61	0	5	Kalata Farm [Lilongwe]	6	0	4
Rujeri [Mulanje]	6	0	0	Ntchewu	83	0	0
Chimbiya [Dedza]	12	1	0	**2014**		**TP**	**TM**
Ntchewu	2	0	0	Katete Dairy Farm	82	7	18
2012		**TP**	**TM**	Mpalo MBG [Ntchisi]	49	1	7
Natural Resources College	20	0	3	Blantyre	145	0	20
Katete Dairy Farm	40	0	0	Magomero MBG [Lilongwe]	40	0	6

Lik asi Livestock Centre	10	2	0	Chitedze Research Station	8	2	3
Mapanga Dairy Farm [Blantyre]	26	0	1	Golden Peacock [Salima]	12	0	1
Mikolongwe area - [Chiradzulu]	14	0	2	Dzalanyama Ranch	10	0	0
Blantyre	35	1	2	Chitipi	22	4	0
Mbulumbuzi - [Chiradzulu]	30	1	8	Mchinji	56	11	13
Chileka and Lunzu [Blantyre]	37	0	1	Kasungu	4	0	0
Chigumula and Soche [Blantyre]	53	0	3	**2015**		**TP**	**TM**
Mpemba [Blantyre]	76	1	8	Dzalanyama Ranch	6	0	4
State House [Lilongwe]	32	0	0	Katete Dairy Farm	27	3	4
Bvumbwe	18	1	1	Bunda College [Lilongwe]	10	9	0
2013		**TP**	**TM**	Magomero MBG [Lilongwe]	24	7	13
Katete Dairy Farm	96	12	24	Natural Resources College	14	5	0
Magomero MBG [Lilongwe]	65	8	4	Likasi Livestock Centre	7	6	0
Namwiri MBG [Dowa]	17	0	4	Chilikhanda Farm	10	0	4

Rwanda

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2007?	Throughout the country	Cattle		83-85%*	Bazarusanga, 2008

***** This was calculated using a Bayesian model which integrates PCR and serological methods. However, agreement between the sensitivity of PCR and serological methods was only achieved in the agro-ecological

zone of the mountainous region in the northern highland at 1,900 m altitude above sea level, where the lowest tick challenge was found (< 20 ticks per animals).

Tanzania

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2013	Kilosa district (Morogoro)	Indigenous cattle	382	8.1	Tarimo, 2013

Uganda

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2015	Whole country	Cattle	564	Disease predominates in Southwestern and Northeastern Uganda	Colli et al, 2015
2013-2014	15 Districts (Central and Western region)	Cattle	295	47.4%	Kasozi et al, 2014
2011-2012	10 agroecological zones	Indigenous cattle	925	30% For more details, see Table and Figure below	Kabi et al, 2014
2011	Tororo District	Cattle	2,658	Individual: 5.3 Herd: 0 - 21	Muhanguzi et al, 2014

Prevalence of T. parva per district. Source: **Kabi et al, 2014**

Districts sampled	n = 925	Non-clinical *T. parva* occurrence (95% CI)
Pader, Kitgum, Katakwi, Abim	127	17 (0.1 - 0.23)
Northeastern Kotido, eastern Kitgum, northern Nakapiripiriti	61	18 (0.08 - 0.27)
Iganga, northern Bugiri, Tororo, Kaberamaido	134	22 (0.14 - 0.28)
Adjumani, western Nebbi, Arua, Yumbe, northern Gulu, northern Apac	155	25 (0.17 - 0.31)
Eastern Nebbi, southwestern Gulu, western Masindi	16	25 (0.03 - 0.46)
Hoima, Kibaale, Kyenjonjo,	113	26 (0.17 - 0.33)
Southern Masaka, Bukomansimbi, Buikwe, Mpigi, Jinja, Mayuge	56	27 (0.15 - 0.38)
Masindi, Nakasongola, southern Mubende, eastern Mbarara, southern Ntungamo	166	36 (0.28 - 0.43)
western Mbarara, northern Ntungamo, Rukungiri	36	39 (0.22 - 0.62)
Kabale, Kasese, western Kyenjonjo	61	43 (0.3 - 0.73)

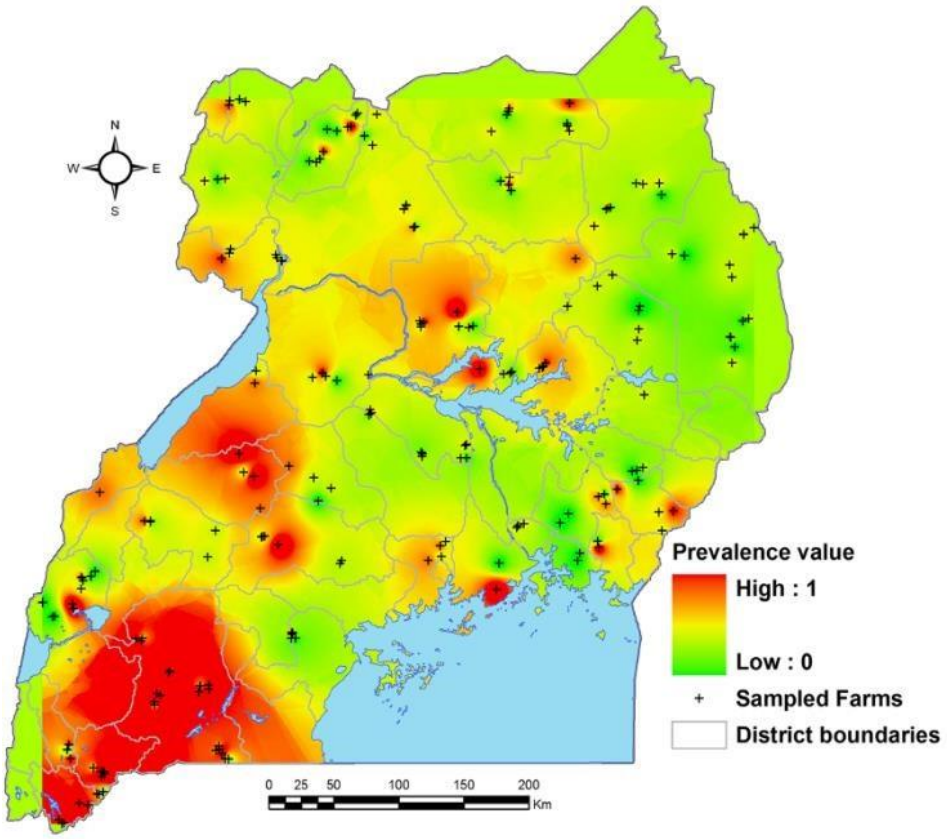

Figure 5: The spatial distribution of non-clinical *T. parvo* infection among indigenous cattle populations in Uganda: January 2011-April 2012. Source: Kabi et al, 2014.

Zambia

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2008	Kanyelele, Kalembe (Isoka district – Northern province) and Saukani (Petauke district)	Indigenous and mixed breeds of cattle	142 Kanyelele: 62 Kalembe: 34 Saukani: 46	54.9% Isoka: 44.8 Petauke: 76.1 (PCR)	Muleya et al, 2012

Economic and Social Impacts at Global and Regional Levels, and in Selected Countries

ECF has a devastating impact on pastoralists and smallholder farmers. The economic impact of the disease includes cattle morbidity and mortality, and production losses in all production systems, as well as the costs of the measures taken to control ticks and the disease.

The costs of acaricide applications, which is the primary means of tick control, is estimated to range between USD 6 -36 per adult animal in Kenya, Tanzania and Uganda. The disease further prevents the introduction of the ECF susceptible but more productive exotic breeds of cattle, hampering development in the livestock sector **(Demessie and Derso, 2015)**. The financial losses incurred, directly and indirectly by ECF are extremely high.

Figures widely used in expressing the impact of ECF is that in the 11 affected countries, 28 million cattle are at risk, with the disease killing annually over one million cattle resulting in annual losses exceeding widely $300 million [5][14]. According to the BMGF, the overall annual economic losses due to ECF are estimated to be USD 382.64 million, with losses to small holder farmers estimated to be representing USD 286.98 million.

In their study of the economic impact of Tick borne diseases on the livelihood of poor livestock keepers, Minjauw et al [14] generated data shown in figure 6 and 7 below for *T. parva* in Africa and *T. annulata* in India

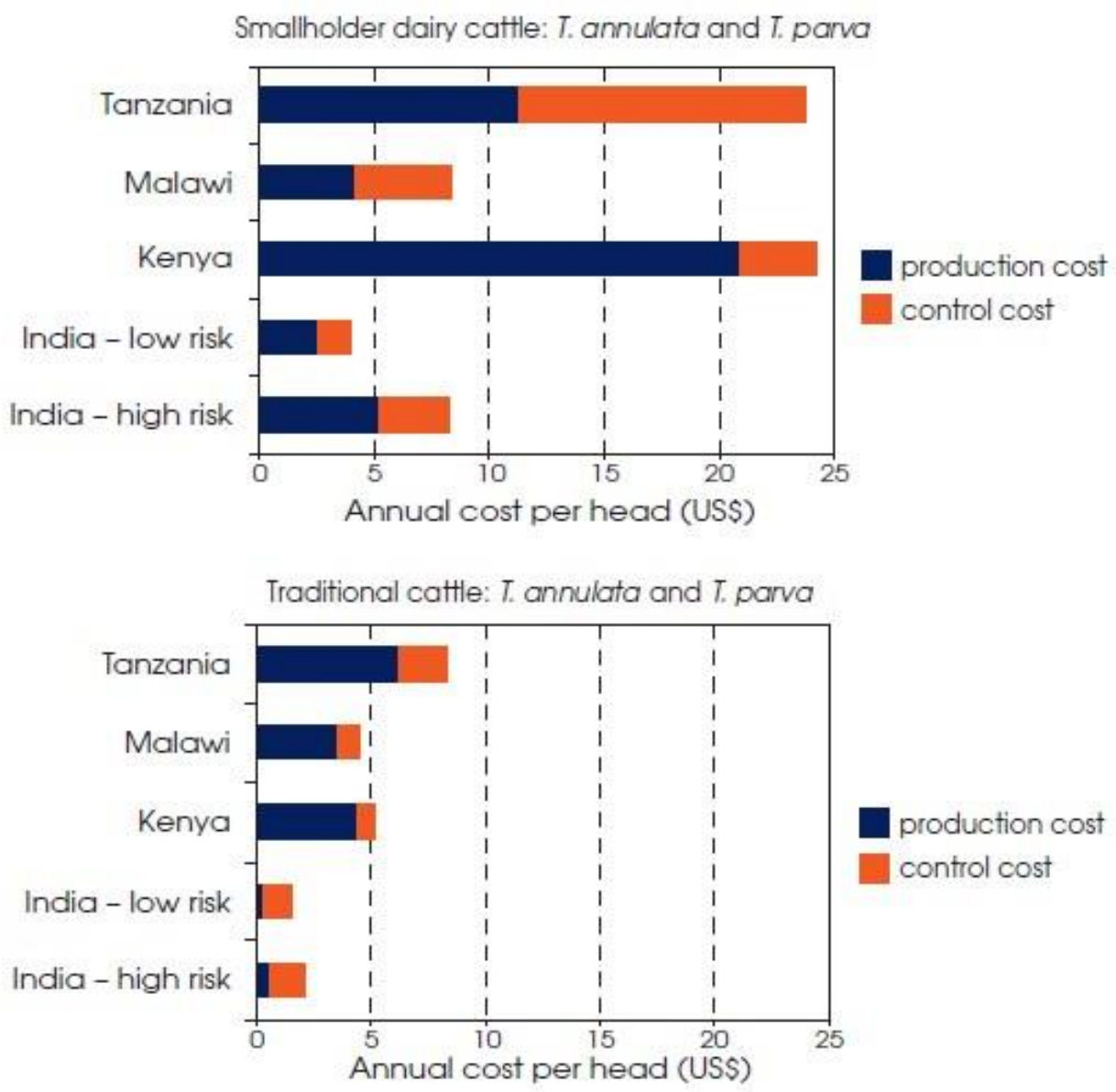

Figure 6: Annual costs per head of *T. parva* and *T. annulata* in cattle systems [14]

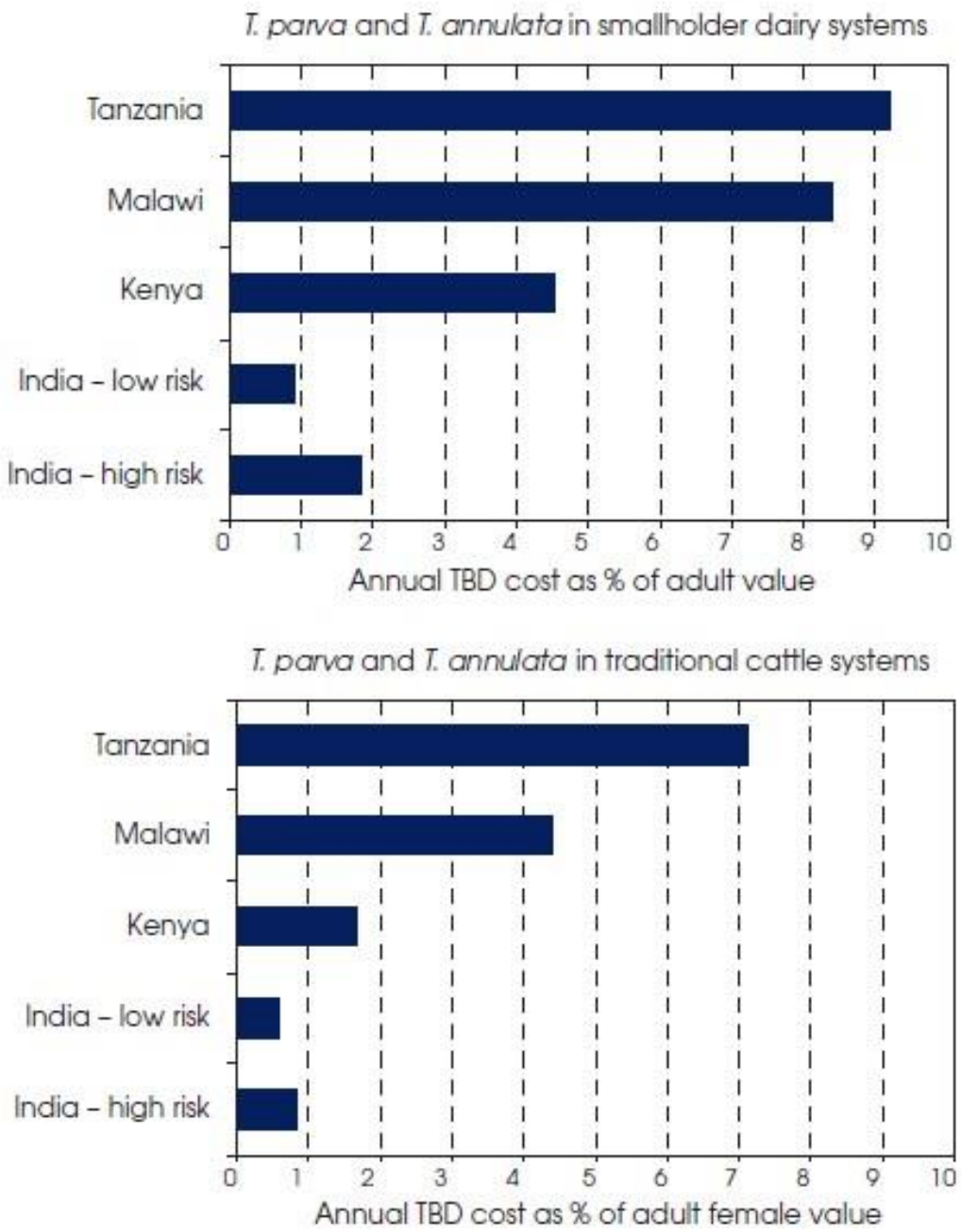

Figure 7: Annual costs per head of theileriosis compared to adult female value

In Tanzania, ECF accounts for more than 70% of all cattle death annually [8], resulting in total costs assessed to be more than USD247 million annually (Table 5).

Table 5: Combined estimated annual direct costs associated with tick-borne diseases in Tanzania (million USD). Source: 8

	Disease			
Costs	Anaplasmosis	Babesiosis	Cowdriosis	Theileriosis
Control				
Acaricides	7.56	5.04	2.52	35.30
ITM	NA	NA	NA	2.21
Chemotherapy	8.58	8.60	4.76	55.41
Production losses				
Mortality	21.48	21.54	8.84	128.44
Milk loss	1.52	1.58	0.80	16.28
Live weight	8.99	9.06	5.51	10.06
Total	48.13	45.82	22.43	247.70
Grand total		364.08		

Disease Prevention and Control Methods

Treatment (Control)

The main methods in the control of ECF include tick control, host immunization and chemotherapy and integrated control that combines any of the methods. The most practical and widely used method for the control of theileriosis is the chemical control of ticks with acaricides. However, tick control practices are not always fully effective for a number of reasons, including development of acaricide resistance, the high cost of acaricides, poor management of tick control, and illegal cattle movement in many countries.

- Chemotherapy is widely used in the treatment of clinical disease, sometimes to good effect, but they have not proved to be completely reliable therapeutic agents.
- The only immunisation method that has been possible to use to date is the infection and treatment Method (ITM) described later in Section 6.
- Other methods that have been tried in specific cases include Isolation of susceptible cattle maintained in a closed herd on properly fenced pastures; and Destocking infected pastures, by keeping them free of cattle for 15 to 18 months, leading to a disappearance of the infection (these methods are impractical in endemic areas).

Vector control

In endemic areas, the tick burden can be decreased with acaricides and other methods of tick control such as rotational grazing.

Tick control methods include direct application of acaricides to cattle through dipping, spray races, hand spray, pour-ons, and hand dressing.

Although acaricides constitute the most widely used method for the effective control of ticks in most part of affected regions, the use has not been always efficient and is more and more resulting on increasing cases of tick resistance (ref.). In addition, acaricides are expensive and can be detrimental to the environment: their use should be minimised and integrated with alternative approaches.

In order to optimise the use of acaricides, farmers need to understand the biology and ecology of the tick species on their land, since an effective control strategy should use the available compounds at the most appropriate application frequency [14].

Depending on the abundance and importance of the various tick species, strategies such as seasonal treatments at the peak of tick activity, or intensive dipping at the beginning of the tick season, may be sufficient to avoid economic losses due to ticks and TBDs.

The use of immunisation and of tick-resistant breeds of cattle can also considerably reduce dependence on acaricides. For example, it is widely recognised that, compared to breeds of *Bos taurus, Bos indicus* has a much greater natural ability to acquire protective immunity against ticks [14].

A brief description of the main methods of applying acaricides to cattle and of the types of acaricides available is given below (adapted from FAO, 1984 **ftp://ftp.fao.org/docrep/fao/010/ag014e/ag014e05.pdf**)

Dipping tanks

The dip tank is an efficient, practical and convenient means of applying acaricide to a herd of livestock. The characteristics of each dip tank will depend on the type of animals to be treated, but several important features should be included

The advantages of a dipping tank are [2] :

- The wetting of the animals is good.
- The stripping rate is lower than in the spray race: the dip concentration is more stable during dipping. The disadvantages are:
- An adequate source of clean water must be readily available to fill the dip initially and to ensure that top-ups can be made when required. This is often not possible in arid and semi-arid regions.
- It requires a large initial capital outlay to fill the tank with acaricide.
- Acaricides cannot be changed regularly.
- The dipping fluid is often dirty and old and the strength is not known.
- The concentration in the dip tank may change as a result of flooding or evaporation.
- The suspension settles in the interval between dippings. Therefore 20 animals must be sent through to mix the fluid and these animals must be re-dipped. Alternatively, relatively large holes can be drilled in a circular plowshare, the centre of which is then welded to a metal pipe. The plowshare is then dunked to the bottom of the dip and pulled up again and the procedure repeated until the dip is well mixed.
- The animals may be injured.

Spray race

A spray race is a corridor or series of crushes permanently fixed in an infrastructure similar to a dip tank, in which animals passing through are sprayed with acaricides pumped at low pressure (but high volume) through nozzles placed along the race. Drained acaricide solution is filtered before being returned to a reservoir for re-use. Spray races are very economical in their use of acaricide (2–3 l per adult bovine) and are also quick to operate (600 head per hr). However, because they incorporate various mechanical parts (engine, pumps, nozzles, etc.), they are more expensive and more difficult to maintain than dip tanks, a fact that has prevented them from being adopted by small-scale farmers in most developing countries The advantages of a spray race are:

- The dip wash is always at the correct strength and fresh and evenly suspended in the fluid because it is made up at each dipping.
- The cost of the acaricide is spread evenly throughout the year or season.
- Acaricides may readily be alternated.
- Injuries to the animals are unlikely to occur. The disadvantages of a spray race are:
- Mechanical failure of the pump.
- Wetting is not always complete, particularly of the ears and under the tail.
- The stripping rate is high, resulting in a lower concentration of acaricide by the time the last animals are treated. Stripping implies that a higher concentration of acaricide clings to the animals and that the concentration of acaricide in the fluid that drips off the animals after they have been sprayed and that flows back into the sump is lower than that in the original spraying suspension.

Hand spray

Most small-scale farmers who own only a small number of animals cannot afford a dip tank or spray race, and many communal dips have fallen into disrepair as operational funding for veterinary services has declined. Handspraying is therefore often the method of choice.

However, although this is the simplest method of treating livestock with acaricides, it is also potentially the least effective. The animals have to be tethered to posts or held in a crush so that the spray nozzle can be held sufficiently close to allow all parts of the body to be treated. Each animal should be sprayed with 10 litres of solution to ensure full and effective coverage. The procedure is time-consuming and its efficacy is highly dependent on the degree of care taken to ensure that all parts of the animal are treated. It is therefore only suitable for very small numbers of animals.

Pour-on

The acaricide, in a formulation containing effective spreaders, is administered along the top-line from the poll to the tail base. Pour-ons can be used when there are few or many cattle to be treated and require only a crush pen for handling the animals. Some of the endectocides administered as pourons are absorbed through the skin and reach their targets in this way.

Pour-on formulations are expensive, but have the advantage of not requiring water or costly equipment for their

application. They also have a persistent effect and protect animals against both ticks and biting flies Available and recommended acaricides include:

- **Organophosphates and carbamates:** the most common being chlorphenvinphos, coumaphos, diazinon, dioxathion and carbaryl (carbamate). Important to note that several ticks, and particularly *B. microplus,* have developed resistance to organophosphates in many parts of the world
- **Pyrethroids :** The synthetic pyrethroids are a highly effective group of acaricides that includes such compounds as permethrin, decamethrin, deltamethrin, cyhalothrin, cyfluthrin and flumethrin. Resistance to pyrethroids is widespread in 1-host ticks such as *B. microplus* and *B. decoloratus* [14]
- **Amidines:** These compounds also show prolonged residual activity (7–10 days) and no residues are found in meat or milk. The only amidine compound commercialised for tick control is Amitraz
- **Macrocyclic lactones:** Macrocyclic lactones (which include ivermectin, moxidectin, and doramectin) are not acaricides as such but are active against a variety of endo- and ectoparasites. A single treatment with one of the macrocyclic lactones will protect against ticks for up to 7–10 days. However, since ticks are only affected when they suck the blood of a treated animal, these compounds do not prevent tick damage or rapid inoculation with tick-borne pathogens. In addition, residues of these products can occur in the milk and meat of treated animals for several weeks after application.
- **Benzoylphenylureas:** Benzoylphenylureas are growth regulators: they do not kill the ticks but disrupt their development and stop the moulting process. The best-known product, Fluazuron®

(difluorobenzoyl urea) acts systematically and is applied to cattle as a pour-on, but has a long residual life in tissue and milk. These products are very effective against *B. microplus* and may be a solution where resistance to other acaricides is high (Ref).

Treatment

ECF proved refractory to treatment for many years until Neitz, in 1953, found that certain tetracyclines had a marked suppressive effect on the schizonts if administered during the incubation period. Tetracyclines have been widely used in the treatment of clinical disease, sometimes to good effect, but they have not proved to be completely reliable therapeutic agents. They have been used successfully, however, in immunization against East Coast fever by an infection-treatment technique [9]

An important breakthrough in the control of East Coast fever was achieved in the late 1970s by the development of two highly effective therapeutic agents. Parvaquone, a napthoquinone, known during its development trials as 993C, proved to be a valuable therapeutic agent when administered by intramuscular injection at a rate of 10 mg/kg, repeated after 48 hours. Recovery rates of 90 per cent or better were recorded in field trials. The drug is effective against both schizonts and piroplasms, but treatment does not achieve a parasitological cure, and recovered animals may remain intermittent carriers and some may take several months to return to normal productivity. An analogue of parvaquone (Clexon®), named buparvaquone, has been developed[91] and has replaced parvaquone on the commercial market [9]

The most largely commercially available drug in use against *Theileria* today are therefore halofuginone lactate (Terit®), Parvaquone (Clexon®), and subsequent more active analogue of Parvaquone, Buparvaquone (Butalex®). All three drugs are currently available but are extremely expensive, so that the cost of treating small indigenous cattle of low productivity may be equal to the value of the animals. Treatment in any case is only likely to be successful if carried out early enough to limit the development of the schizont stage of the parasite and subsequent damage to the immune system. Furthermore, none of these compounds will eliminate carrier infections induced by *Theileria spp*. [14]

Prophylaxis (Prevention)

Immunization

The only commercially available vaccine against *T. parva* is an infection-and-treatment method (ITM) which consists of a live sporozoite challenge together with simultaneous treatment of the resultant infection with oxytetracycline. This procedure induces a controlled disease reaction which results in a mild infection and a solid protective immunity to homologous parasite challenge. In the absence of further challenge, this immunity lasts for over 36 months, but with regular natural exposure to the parasite, it should persist for the entire life of the animal. The ITM is further discussed in section 6 below.

Disease situation and government policies by country

Tables 6 and 7 below have been completed with the information received so far from the questionnaires sent to the DG and DVS. This information will be updated and completed once the results from the different countries are received.

Table 6 covers the disease situation (if it is notifiable or not), the presence of official surveillance and/or control programs, and the treatment situation. Table 7 refers to vaccination.

The definitions that were given to the respondents are:

[1]Surveillance: is the systematic ongoing collection, collation and analysis of data and the timely dissemination of information to those who need to know so that action can be taken.

[2]Control: a program which is approved, and managed or supervised by the Veterinary Authority of a country for the purpose of controlling a vector, pathogen or disease by specific measures applied throughout that country, or within a zone or compartment of that country.

Table 6: Official status, official programs for ECF in the countries of interest

Country	Notifiable (yes/no)	Official surveillance[1] program (yes/no) If yes, active or passive	Official control[2] program (yes/no)	Treatment (Chemotherapy)	
				Treatment authorised (yes/no)	Frequently practiced (yes/no)
Kenya	Yes	Yes, passive	No	Yes	Yes
Madagascar					
Malawi					
Mali	Yes	Yes, passive	Yes	Yes	Yes
Mozambique					
Rwanda	-	-	-	-	-
South Africa					
Tanzania	No	Yes, passive	Yes	Yes	Yes
Uganda	No	No	No	Yes	Yes
Zambia	Yes	Yes, passive	Yes	Yes	Yes

Table 7: Vaccination for ECF in the countries of interest

Country	Vaccination			
	Compulsory vaccination (yes/no)	Who pays for the vaccine (Government, farmers, combination, others-specify)	Who delivers the vaccine (official, private vaccinators or both)	Species vaccinated (cattle, sheep, goats, pigs, poultry)
Kenya	No	Farmers	Both	Cattle
Madagascar				
Malawi	No	Combination	Both	Cattle
Mozambique				
Rwanda	-	-	-	-
South Africa				
Tanzania	No	Farmers	Private	Cattle
Uganda	No	Farmers	Private	Cattle
Zambia	No	Farmers	Official	Cattle

Vaccines Available

The only commercially available vaccine against *T. parva* is an infection-and-treatment method (ITM) which involves the inoculation of potentially lethal dose of sporozoites and treatment with long acting oxytetractine (OTC). This procedure induces a controlled disease reaction which results in a mild infection and a solid protective immunity to homologous parasite challenge. In the absence of further challenge, this immunity lasts for over 36 months, but with regular natural exposure to the parasite, it should persist for the entire life of the animal.

The method was developed by a team of scientists working in Kenya on an FAO project, inspired by the observation made by Neitz in 1953 that chlortetracycline (Aureomycin) during the ECF incubation period had a marked suppressive effect on the development of infection and permitted the establishment of a solid immunity [9].

As illustrated in Figure 8 below, a crude suspension of sporozoites is prepared from adult ticks which have fed as nymphs on infected cattle, have moulted, and have been pre-fed on rabbits for three to four days to allow the sporozoites to mature. The suspension is mixed with glycerol as a cryoprotectant and stored, deep-frozen at −70°C or in liquid nitrogen. Aliquots are titrated in susceptible cattle to determine an optimum dose for immunization.

While ITM provide good immunity against homologous immunizing strains, it is not consistently successful against unrelated strains. This situation led to efforts directed towards finding a broad spectrum stick or combination of stocks that could provide protection against a wide range of antigenically distinct *T. parva* parasites. On the basis of extensive cross-immunity trials, three isolates, identified as cattle-derived

T. parva Muguga and T. *parva* Kiambu 5 and buffalo-derived *T. parva* Serengeti transformed, have been combined in the so-called Muguga cocktail [9].

The Muguga cocktail provides protection against most isolates from outbreaks of East Coast fever in eastern Africa and is used in Tanzania, Kenya, Uganda and Malawi. It has also been found to be suitable and used for use in South Sudan, Eastern DR Congo and Rwanda.

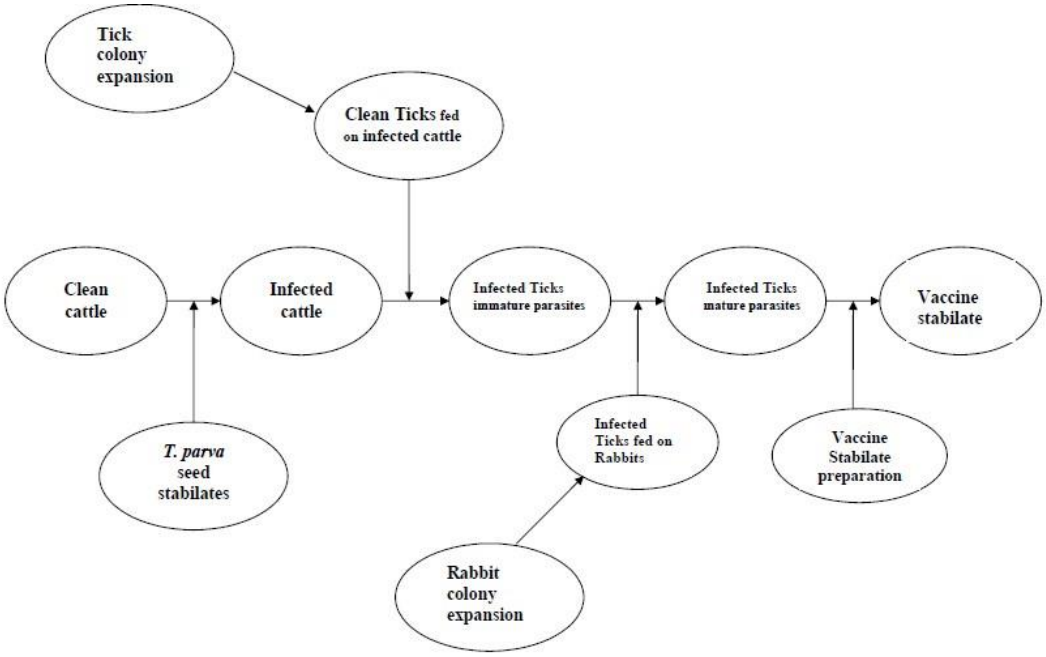

Figure 8: Flow diagram illustrating the steps in production of individual stabilates of the Muguga cocktail ECF ITM (source Di Giulio et al. 2009)

Given the fact that the ITM leads to a persistent infection with specific strains included in the vaccine, which may not be present in all areas, there is always a risk of introducing parasites of an antigenic type against which local cattle may have no immunity, and also complicating the situation of these areas. This situation had brought certain countries to use local stocks for immunization within affected regions:

- In Eastern Zambia a local isolate known as *T. parva* Katete is used; and the Chitongo strain is used in Southern Zambia
- In Kenya, an isolate from the Coastal region called Marikebuni has been used countrywide
- In Zimbabwe a cattle-derived *T. parva* called Boleni has been used. It is a mild strain that has decreased in virulence through passage. As a result it was used in immunization without treatment [14]. According to Lawrence et al., Theileria parva Boleni has also been shown to protect cattle against several cattlederived *T. parva* stocks from Kenya and two buffalo-derived strains. This raises, according to the authors, the possibility of using *T. parva* Boleni more widely as a vaccine against ECF, although the fact that it induces the carrier state in immunized animals may make some reluctant to use it.

Due to the very complex, time consuming, unpredictable and expensive production process, the ITM has not been available to cover most of the needs. As an illustration, production and laboratory testing of a batch of the trivalent Muguga Cocktail (MC) stabilate for immunization of a million cattle requires approximately 130 cattle

and 500 rabbits. In addition, application of at least 600,000 nymphal ticks to infected cattle is required to provide sufficient infected ticks for monitoring of infection levels and stabilate production [3]. The production of a batch takes around 18 months.

On the efficacy side, recent work by Sitt et al. has added to the widely observed fact that the Muguga cocktail doesn't protect against buffalo derived *T. parva* [22]

It is also important to note the genetic similarity found by Norling et al. between the Serengeti-transform and Muguga stocks of the Muguga cocktail 51[18]

On the vaccination side, the ECF ITM has a number of challenges that has made its use quite complex

- Firstly the requirement for transport in liquid nitrogen has always created serious logistical challenge for its wider distribution,
- The complexity of the infection and treatment process creates a requirement for skilled veterinarians for effective delivery.
- The price of the immunization and treatment operation is generally quite high, generally between USD 10 and 20 per animal (Uganda dissertation). Experience in affected countries however has shown that price is not generally an issue for livestock owners

Commercial vaccines manufactured in Africa and Asia

Table 8: Vaccination for ECF in the countries of interest

Manufacturer	Country	Name & Strain	Vaccine Type	Countries distribution
Centre for Ticks and Tick borne diseases	Malawi	Muguga cocktail	ITM	Malawi, Tanzania, Uganda, Kenya?
		Chitongo	ITM	Zambia
ILRI	Kenya	Marikebuni	ITM	Research purposes

Although KARI has been involved in the development and production of the Marikebuni stock of ECF, there is no evidence that production is still ongoing.

There is almost no information whether Zimbabwe still produces the Boleni vaccine.

Commercial vaccines imported into Africa and Asia

The information summarised in Table 9, is based on a questionnaire send to the Director of Veterinary Services office and regulators of the countries of interest. Note that some vaccines might have been imported under DVS dispensation, and they are not necessary licensed in the country.

Table 9: Vaccine imported into the different countries.

Country	Vaccine name	Strain or type	Country of origin	Doses imported 2015	Doses imported 2014	Doses imported 2013	Doses imported 2012
Kenya	-	-	-	-	-	-	-
Madagascar							
Malawi	-	-	-	-	-	-	-
Mozambique							
Rwanda	-	-	-	-	-	-	-
South Africa							
Tanzania*†				250	215	500	
Uganda*	Muguga Cocktail		Kenya	0	45,000		
Zambia	-	-	-	N/A	N/A	N/A	

* Source: Local regulatory agencies
† The figures provided by the regulatory authority seem underestimated

Characteristics of Ideal Vaccine Candidates for Smallholders

Table 10: Vaccine imported into the different countries.

	Attribute	Minimum (current available vaccine)	Ideal
1	Antigen	Immunogen with protective antigens of T. parva	Immunogen capable of providing full protection in cattle against T. parva infection
2	Indication for use	For active immunization of cattle	For active immunization of cattle
3	Recommended species	Cattle	All T. parva susceptible livestock
4	Recommended dose	1 ml	1 ml
5	Pharmaceutical form	Reconstituted injectable solution/suspension (freeze-dried vaccine) or ready to use solution (inactivated vaccine)	Ready to use solution/suspension
6	Route of administration	SC	SC, Intramuscular or pour on
7	Regimen - primary vaccination	Single dose	Single lifetime dose
8	Regimen - booster	Single annual booster	Lifelong immunity after primary vaccination

9	Epidemiological relevance	Protection against all geographically distinct strains of *T. parva*	Protection against T. parva and prevention of transmission
10	Recommended age at first vaccination	Animals over 3 months: one injection	From 1-2 months of age
11	Onset of immunity	2-3 weeks following primary vaccination	One week following primary vaccination
12	Duration of immunity	At least 1 year	Lifelong immunity
13	Expected efficacy	To prevent disease & prevent mortality.	To prevent infection and transmission. No disease & no mortality in vaccinated animals after virulent challenge.
14	Expected safety	In animals under 6 months of age, a transient pyrexia reaction can occur. A transient nodular reaction of varying importance, may appear at the injection site, it progressively disappears within 1 to 2 months. Only vaccinate pregnant animals on emergency.	No post-vaccinal reactions at any age. Safe for pregnant animals. No carrier form in vaccinated animals
15	Withdrawal period	Nil	Nil
16	Special requirements for animals	Do not vaccinate un-healthy animals	DIVA
17	Special requirements for persons	None	None
18	Package size	50 doses	Multiple pack size from 10 doses
19	Price to end user	Not more than $0.50/dose	$0.20/dose at end user

20	Storage condition and shelf-life as packaged for sale	12 months at 4-8° C	24 months 4-8° C and/or 48 hours at 30° C
21	In-use stability	1 hour	24 hours

Overall conclusion for improved ECF control through vaccination

The activities included in the ECF Consortium project covers all possible areas that could lead to a better vaccine or immunisation approach. All groups with some expertise on ECF are involved in the consortium.

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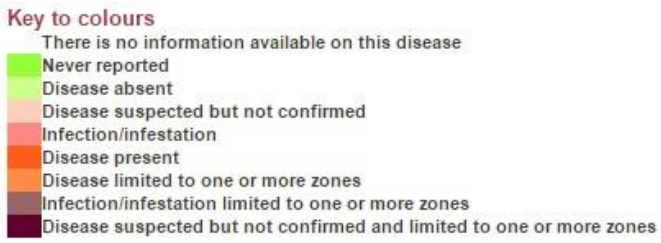
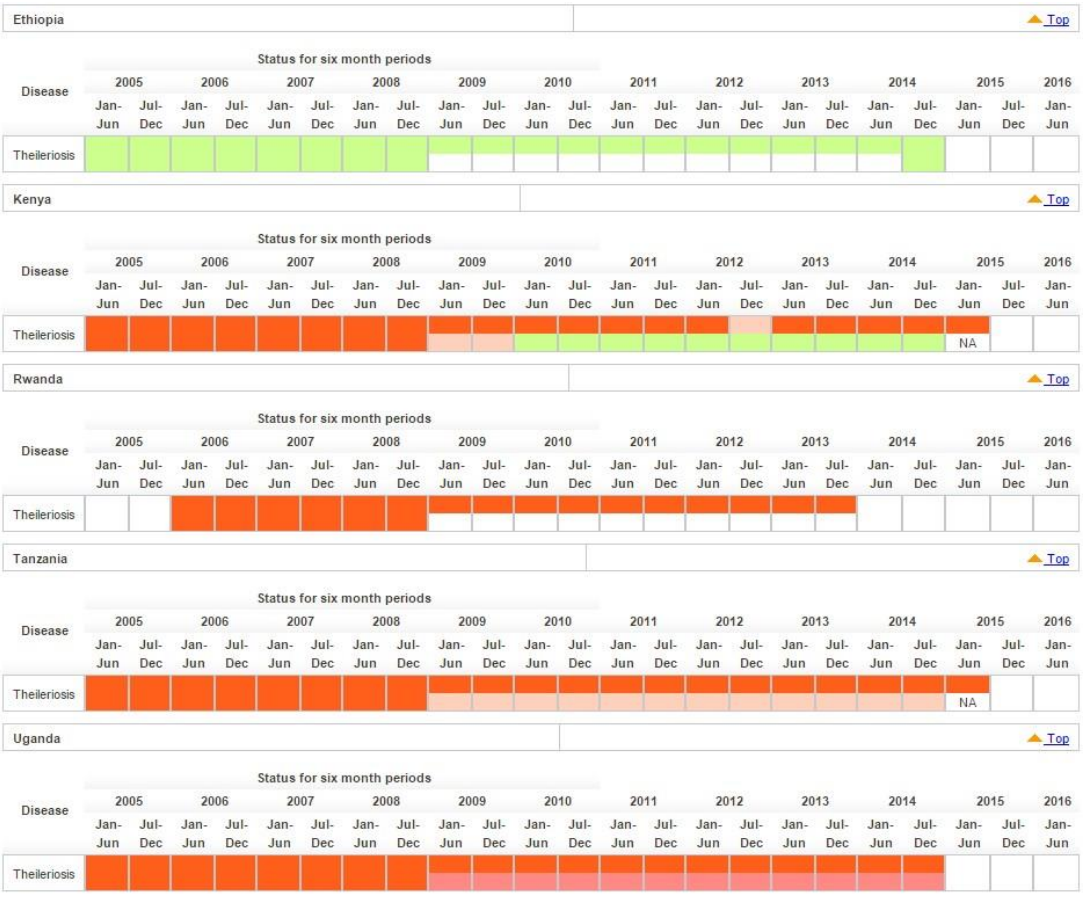
Annex 1: Additional data on disease presence and incidence

Reports to OIE on Theileriosis:



When different animal health statuses between domestic and wild animal population are provided, the box is split in two: the upper part for domestic animals, and the lower part for wild animals.

Theileriosis in Eastern Africa: Ethiopia, Kenya, Rwanda, Tanzania and Uganda



Theileriosis in Southern Africa: Malawi, Mozambique and Zambia