The occurrence of *Ehrlichia ruminantium* and other haemoparasites in calves in western Kenya determined by reverse line blot hybridization assay, real-time PCR and nested PCR

by

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Declaration

I hereby declare that this dissertation reports original work done by me during the course of my MSc project. It has not been submitted before for any degree or examination in any other university.

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Abstract

Ehrlichia ruminantium is a tick-borne pathogen transmitted by ticks in the genus *Amblyomma*. This bacterial pathogen causes heartwater, a fatal disease affecting domestic and wild ruminants in sub-Saharan Africa. The prevalence of heartwater in western Kenya is not well documented. In this study, reverse line blot (RLB) hybridization and quantitative real-time PCR (qPCR) assays were used to detect *E. ruminantium* DNA in 545 blood samples collected from calves from twenty sublocations distributed across five agro-ecological zones of western Kenya. *Ehrlichia ruminantium* DNA was detected in 1.10% and 0.92% of the samples using RLB and qPCR, respectively. There were discrepancies in the detection of *E. ruminantium* by the RLB and the qPCR. Five samples were positive with the qPCR while six were positive with the RLB, but only three of the samples were positive by both tests. The occurrence of *E. ruminantium* in western Kenya appears to be low, but this might be attributed to the inability of the tests used to detect *E. ruminantium* carriers. The most prevalent haemoparasites detected by the RLB in the *Ehrlichia*/*Anaplasma* group were *Anaplasma* (formerly *Ehrlichia*) sp. Omatjenne and *Anaplasma bovis* at 37.98% each, while *Theileria mutans* (66.61%) was the most prevalent in the *Theileria*/*Babesia* group. In addition, a nested p104 PCR was used to detect *Theileria parva* in a subset of 86 of the samples; *T. parva* was detected in 32.56% (28/86) of these samples. The RLB detected *T. parva* in 27.91% (24/86) of the same sample subset, but only 17 were positive by both tests. The molecular tests used in this study suggest that, of the pathogenic haemoparasites known to cause disease in Kenya, *T. parva* occurs the most commonly in western Kenya, while *E. ruminantium*, *A. marginale*, *B. bigemina* and *B. bovis* are less frequently detected.

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Abbreviations

AEZ:	agro-ecological zone
B catch-all 1&2:	*Babesia* genus-specific probes 1 and 2
bp:	base pairs
E/A catch-all:	*Ehrlichia*/*Anaplasma* group-specific probe
ECL:	enhanced chemiluminescence
EDTA:	ethylene diamine tetra-acetic acid
EDAC:	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
ECF:	East Coast fever
ELISA:	enzyme linked immunosorbent assay
IDEAL:	Infectious Diseases of East African Livestock project
LM1:	Lower Midland 1
LM2m:	Lower Midland 2 middle
LM2s:	Lower Midland 2 south
LM3:	Lower Midland 3
MWM:	molecular weight marker
PCR:	polymerase chain reaction
qPCR:	quantitative real-time polymerase chain reaction
RLB:	reverse line blot
rRNA:	ribosomal ribonucleic acid
SDS:	sodium dodecyl sulphate
SSPE:	Sodium chloride-sodium phosphate EDTA

T/B catch-all:	*Theileria*/*Babesia* group-specific probe
T catch-all:	*Theileria* genus-specific probe
UDG:	uracil DNA glycosylase
UM3:	Upper Midland 3

1 Literature Review

Tick-borne infections in cattle in Africa are complex with many tick species interacting with different hosts and a wide range of infectious agents being transmitted. In endemically stable environments, cattle are continuously exposed to challenge by infected ticks (Medley et al., 1993), which hinders rearing of exotic breeds. A large number of cattle mortalities in Kenya are due to tick-borne diseases, the most important of which are theileriosis, anaplasmosis, babesiosis and heartwater (Wesonga et al., 2010). These livestock diseases therefore have a substantial negative impact on the economic well-being of farmers in Kenya.

East Coast fever (ECF) is an acute and often fatal disease of cattle in Eastern and Central Africa that causes substantial production losses (Mukhebi et al., 1992). It is caused by *Theileria parva* which is transmitted by the brown ear tick, *Rhipicephalus appendiculatus*. East Coast fever is prevalent in Kenya where it causes major economic losses through morbidity and mortality. Other tick-borne diseases that occur in cattle in Kenya include benign theileriosis caused by *Theileria mutans*, babesiosis caused by *Babesia bigemina*, anaplasmosis caused by *Anaplasma marginale* and cowdriosis, or heartwater, caused by *Ehrlichia ruminantium* (Wesonga et al., 2010). Heartwater is a major constraint to animal production in the sub-Saharan region and causes huge economic losses due to mortality and decreased production. It is an obstacle to upgrading of local cattle with more productive exotic breeds (Uilenberg et al., 1993). The parasite is transmitted transtadially by ticks of the genus *Amblyomma*. The prevalence of heartwater in western Kenya is not well known, but it is thought to be wide-spread.

In order to prioritize future research on the development of improved control measures against tick-borne diseases, it is essential to define the prevalence of tick-borne pathogens in target populations. The Infectious Diseases of East African Livestock (IDEAL) project was thus initiated in order to collect baseline epidemiological data on the diseases affecting cattle in East Africa (Dr. Mark Bronsvoort, personal communication). The project aimed at addressing the lack of baseline epidemiological data on the dynamics and impacts of infectious diseases of cattle in the tropics and also improving understanding of interactions between multiple infections and their sequelae. The project combined regular screening with monitoring episodes of clinical disease and their sequelae. A cohort of 552 calves was recruited into the project at birth and monitored for a period of one year.

In this project, we investigated the occurrence of *E. ruminantium* in the IDEAL calves using a quantitative real-time polymerase chain reaction (qPCR) test as well as a reverse line blot (RLB) hybridization assay. A nested p104 PCR assay was compared to the RLB for specific detection of *T. parva* in these calves.

1.1 Heartwater

A disease believed to be heartwater was first reported in South Africa in 1838 by Louis Trichardt while trekking through the Limpopo province of South Africa. He observed that many of his sheep succumbed to a disease known locally as ‘nintas’ three weeks after they had suffered massive tick infestation (Provost and Bezuidenhout, 1987). The organism responsible for the disease, *E. ruminantium*, was discovered in the 1920s by Cowdry. He observed an intracellular rickettsial bacterium

in tissues from infected animals and ticks (Cowdry, 1925a, b, 1926). Cowdry named the organism *Rickettsia ruminantium*, which was later changed to *Cowdria ruminantium* (Moshkovski, 1947) and in 2001 the organism was reclassified as *Ehrlichia ruminantium* (Dumler et al., 2001). Since the early discovery of heartwater in South Africa, the disease has been described from almost all the African countries south of the Sahara as well as from Madagascar, Sao Tome, Reunion, Mauritius and a number of islands in the Caribbean (Provost and Bezuidenhout, 1987).

Heartwater occurs in many forms; peracute, acute, subacute and a clinically inapparent form. Acute heartwater is the most common form of the disease and mainly affects cattle between the ages of 3 and 18 months. It is characterised by fever of 40°C or higher which usually persists for 3 to 6 days, showing only small fluctuations before the body temperature falls to subnormal shortly before death. A mild mucoid diarrhoea is also thought to occur (Uilenberg, 1981). During the later stages of acute heartwater, nervous signs occur which range from mild inco-ordination to pronounced convulsions. The animals are hypersensitive when handled or exposed to bright light. Slight tapping with a finger on the forehead of the animal often evokes an exaggerated blinking reflex. They frequently show a peculiar high stepping gait that is usually more pronounced in the front limbs. Peracutely affected animals experience fever and convulsions and die within a few hours after the initial development of fever. The subacute form of heartwater is characterised by a fever which may remain high for 10 days or longer. Animals with the sub-acute form of heartwater exhibit clinical signs similar to the acute form of heartwater but less pronounced. Animals with the clinically inapparent form usually recover quickly.

Clinical signs associated with this form are a brief fever, apathy and slight tachypnea (Allsopp et al., 2004).

In most parts of Africa *Amblyomma variegatum* is the principal vector of heartwater. *Amblyomma hebraeum* is the only vector of the disease in the Republic of South Africa while *Amblyomma gemma* and *Amblyomma lepidum* have been reported to be involved in the transmission of heartwater in East Africa (Wesonga et al., 1993). In Kenya, *E. ruminantium* was identified in *A. gemma* ticks collected from various wild animals in a game ranch in a heartwater endemic area (Wesonga et al., 1993). After dissection, all the ticks were tested for the presence of *E. ruminantium* using the pCS20 DNA probe assay (Waghela et al., 1991). *Ehrlichia ruminantium* was also identified in ticks collected from eland and giraffe (Wesonga et al., 1993). An attempt was made to isolate *E. ruminantium* from three *Amblyomma* species (*A. variegatum*, *A. gemma* and *A. lepidum*) in eight districts of Kenya (Ngumi et al., 1997). In that study, *E. ruminantium* was isolated from *A. gemma* ticks and its transtadial transmission from nymphal to adult stage demonstrated.

Amblyomma ticks are large three host parasites, more or less brightly ornamented. They are confined to the tropics and subtropics. Larvae and nymphs become infected when they feed on domestic and wild ruminants and possibly also on certain game birds and reptiles when *E. ruminantium* is circulating in the blood of these hosts. The developmental cycle of *E. ruminantium* in the tick and the infectivity of successive stages are not well known. It is thought that after an infected blood meal, the organism's initial replication takes place in the epithelium of the intestine of the tick and that the salivary glands eventually become parasitized. The demonstration of *E.*

ruminantium in salivary glands of ticks suggests that transmission to vertebrate hosts takes place through the saliva of attached ticks and by their regurgitated gut content (Bezuidenhout, 1987). In the vertebrate host, initial replication of the organisms is thought to take place in reticulo-endothelial cells and macrophages in the regional lymph nodes. They are then disseminated through the blood stream and invade endothelial cells of blood vessels in various organs and tissues where further multiplication occurs. In domestic ruminants, *E. ruminantium* most readily infects endothelial cells of the brain (Du Plessis, 1970).

Control of heartwater is mainly through controlling the tick vector by use of acaricides, immunisation by infection and treatment, or by regular administration of prophylactic antibiotics (Allsopp, 2009). While prophylaxis is effective, it is very expensive especially where large herds of cattle are involved. Use of acaricides and immunization are also subject to failure because of seasonal changes influencing abundance, activity and intensity of tick control and change in tick infection rates.

1.2 East Coast fever

Theileria parva is an apicomplexan parasite which causes East Coast fever (ECF), the most important tick-borne disease of cattle in Eastern, Central and parts of southern Africa (Norval et al., 1992). The infection occurs in cattle and buffalo and is transmitted by the ixodid tick *Rhipicephalus appendiculatus* but *R. zambeziensis* and *R. duttoni* are also capable of transmitting the disease (Lawrence et al., 1994). Before 1900, ECF was enzootic to the East African coast where it had been well known for hundreds of years. In the early 1900s, the movement of cattle from the coastal regions

and the importation of exotic breeds through the coastal regions spread the disease to the inland areas of Kenya, Tanzania and Uganda. East Coast fever was introduced into South Africa in the early 1900s and was eventually eradicated in the 1950s. A different form of theileriosis, Corridor disease, persists in South Africa and the reservoir host is the African buffalo (*Syncerus caffer*) (Uilenberg, 1999).

East Coast fever is a big constraint in the keeping of improved breeds of cattle in the areas where it is endemic (Gitau et al., 2001). It has been shown amongst other calf illnesses to exert a temporal effect on calf growth at the height of illness and immediately after. In other studies conducted in central Kenya, ECF was found to be the major cause of both calf morbidity and mortality relative to other causes and especially in open grazing (Gitau et al., 1999; 2001).

Theileria parva sporozoites are produced in the salivary glands of nymph or adult ticks and subsequently inoculated into a susceptible animal during feeding. Once in the host, the parasites attach to and enter the lymphocytes where sporozoites develop into schizonts. The infected lymphocytes grow bigger and begin to divide resulting in a large number of infected lymphocytes. The infection spreads throughout the lymphatic system leading to the widespread destruction of the lymphatic cells. As the infection progresses, some of the schizonts differentiate into merozoites that are released into the blood stream where they invade red blood cells. Here they change to forms called piroplasms which are infective to ticks (Mehlhorn and Schein, 1984). When a tick feeds on an infected animal, it ingests red blood cells that are infected with piroplasms. These are released into the gut lumen and give rise to macro and micro gametes which undergo syngamy to form diploid zygotes. After invading gut

epithelial cells, zygotes undergo reduction division to yield kinete forms which access the hemocoel and migrate to the salivary gland where they invade cells of type III acini. The parasite then undergoes a process of sporogony to produce cattle-infective sporozoites and this completes the life cycle (Neitz, 1957; Katzer et al., 2006).

East Coast fever has an incubation period of seven to twenty-five days. Initial signs include anorexia, pyrexia and enlargement of the draining lymph nodes. Other symptoms include lacrimation, nasal discharge, corneal opacity, increased respiratory rate and diarrhoea. There is a rapid deterioration of body condition, prior to death the animal is recumbent with a drop in body temperature. At post-mortem, animals will have severe pulmonary oedema with froth in the trachea and nares. Lymph nodes are enlarged and may be hyperaemic, hemorrhagic and edematous (Jura and Losos, 1980).

In areas where ECF is endemic, control is based on dipping or spraying cattle with an acaricide to kill the vector ticks. Chemotherapeutic drugs are also used but they are expensive and their successful application requires diagnosis of the disease at its early stage of development which is beyond the capacity of many smallholder farmers. Currently, the only practical method of immunization is by the infection and treatment method. Animals are inoculated with a dose of sporozoites harvested from ticks and then treated with a chemotherapeutic drug (Radley, 1981).

1.3 Other tick-borne haemoparasites

Three other species of *Theileria* are recognized in cattle in Kenya. *Theileria mutans*, *Theileria velifera* and *Theileria taurotragi* infections usually cause at most a mild transient fever and anaemia, and are not reported as theileriosis in the field, however, they may confuse diagnosis of *T. parva* infections. *Theileria mutans* and *T. velifera* are transmitted by *Amblyomma* species while *T. taurotragi* is transmitted by *R. appendiculatus* and *Rhipicephalus pulchellus*.

Several other *Theileria* species have been identified in cattle in other African countries. *Theileria* sp. (sable) has been isolated from healthy animals such as the African short-horn zebu and the African buffalo (Nijhof et al., 2005). It causes fatal clinical disease in roan and sable antelopes in South Africa. Its main vectors are likely *Rhipicephalus evertsi evertsi* and *R. appendiculatus*. *Theileria bicornis* was originally described from healthy black rhinoceroses in South Africa (Nijhof et al., 2003); it has been described in 3.2% of cattle in western Uganda (Muhanguzi et al., 2010b) and is not known to be pathogenic. *Theileria buffeli* is a benign parasite common in cattle. It has a wide range of tick vectors and a worldwide occurrence. *Haemophysalis*, *Amblyomma* and *Dermacentor* species have been implicated in the transmission of this parasite (Stewart et al., 1992; Georges et al., 2001; Cossio-Bayugar et al., 2002; Garcia-Sanmartin et al., 2006; Salih et al., 2007; Altay et al., 2008).

Babesia bigemina and *B. bovis* are intra-erythrocytic protozoan parasites that cause babesiosis. *Babesia bigemina* has been isolated from cattle in Kenya and has been used in the production of tick derived stabilates (Morzaria et al., 1977). The major vectors for *B. bigemina* and *B. bovis* are *Rhipicephalus (Boophilus) microplus* and *R.*

(B.) annulatus. Bovine babesiosis can be found wherever the tick vectors exist with *B. bigemina* being more widely distributed than *B. bovis.*

Bovine anaplasmosis is a tick-borne disease of cattle caused by the intra-erythrocytic rickettsia, *Anaplasma marginale* (Potgieter and Stoltsz, 2004). The disease is characterized by fever, progressive anaemia and icterus, and has a case fatality rate of 36% (Losos, 1986). Anaplasmosis is widely distributed around the world. In Kenya it is transmitted by *Rhipicephalus (Boophilus) decoloratus* (Maloo, 1993).

Anaplasma (formerly *Ehrlichia*) sp. Omatjenne was initially isolated from *Hyalomma truncatum* ticks and is generally thought to be apathogenic (Du Plessis., 1990). *Anaplasma bovis* is transmitted by *R. appendiculatus* in southern Africa but *Amblyomma*, *Hyalomma* and other *Rhipicephalus* species are also thought to transmit it. It is the cause of benign bovine rickettsiosis and has been reported from South America and several parts of Africa (Sumption and Scott, 2004). *Anaplasma phagocytophilum* is an emerging human pathogen of public health importance that is transmitted to humans by tick bites (Dumler et al., 2001). It has been detected in various species of *Ixodes* ticks around the world (Woldehiwet, 2010) and has also been isolated from wild and domestic animals. In cattle, the disease is known as pasture fever in many parts of Europe and occurs as an annual minor epidemic (Woldehiwet, 2006).

1.4 Diagnosis and detection of *E. ruminantium* and other tick-borne pathogens

Successful control of tick-borne pathogens depends on early and accurate diagnosis. Diagnostic methods include microscopy, serological tests such as enzyme linked immunosorbent assay (ELISA), and DNA-based molecular detection methods such as polymerase chain reaction (PCR) and reverse line blot (RLB) hybridization assay.

Microscopy is commonly used for diagnosis of *E. ruminantium* because it is both easy to carry out and cheap. Brain squash smears prepared in such a way that segments of capillaries remain more or less intact are stained in Giemsa and observed under a light microscope. The heartwater organisms stain purplish blue (Allsopp et al., 2004). However, this method is relatively insensitive; the organisms are difficult to find in the stained smears and may be missed in some cases. Although microscopic examination of Giemsa-stained brain smears is still employed in heartwater diagnosis, newer and more sensitive techniques have been developed. Currently, several PCR and ELISA based diagnostic techniques are employed in the detection of *E. ruminantium* in infected ticks and ruminants in addition to microscopy. As opposed to serological methods like the MAP1-B ELISA (Van Vliet et al., 1995), molecular methods such as PCR could potentially help in the detection of carrier animals without detectable antibodies especially if used in combination with clinical signs observed (Faburay et al., 2007).

1.4.1 PCR assays for the detection of *E. ruminantium*

DNA probes that have been developed to detect and characterize PCR products amplified from *E. ruminantium* isolates include small subunit rRNA (16S) probes (Allsopp et al., 1997), the pCS20 probe (Waghela et al., 1991; Van Heerden et al., 2004), and *map*1 probes (Allsopp et al., 1998; 1999). The pCS20 probe is specific for *E. ruminantium* and is the most sensitive of the probes for *E. ruminantium* detection (Collins et al., 2002). The pCS20 PCR/probe assay has been used to detect exposure to *E. ruminantium* infection in heartwater endemic areas (Mahan et al., 1992; 2004; Van Heerden et al., 2004). A comparison of the pCS20 PCR/probe assay and the MAP1-B ELISA indicated that the pCS20 PCR was more reliable because it detected more infections and would be the method of choice for the detection of *E. ruminantium* infection (Simbi et al., 2003).

A quantitative real-time PCR (qPCR) assay based on the pCS20 region of *E. ruminantium* has recently been developed (Steyn et al., 2008). Real-time PCR is advantageous over other PCR methods because it allows a visual indication of target amplification throughout the PCR. Consequently, it has higher sensitivity and detection rates than other PCR methods. The pCS20 qPCR assay was found to detect as few as seven copies of pCS20 per microlitre of DNA extracted from cell culture. When compared to the pCS20 PCR/probe test, it detected significantly more positive field samples (Steyn et al., 2008).

1.4.2 Reverse line blot hybridization assay

A reverse line blot (RLB) hybridization assay for simultaneous detection and differentiation of all the *Theileria* and *Babesia* species that infect cattle was described by Gubbels et al. (1999) and has been widely used in the detection of tick-borne haemoparasites. It is based on sequence differences in the haemoparasites' small subunit ribosomal RNA (rRNA) gene. Besides being a versatile technique for the simultaneous detection of all known bovine tick-borne protozoan parasites, the RLB hybridization assay has the advantage of detecting carrier states of most parasites. This *Theileria*/*Babesia* RLB hybridization assay was successfully applied in the identification of haemoprotozoa in Minorcan cattle (Almeria et al., 2002). It allowed the simultaneous detection and identification of the *Theileria* and *Babesia* species in carrier cattle. Bekker et al. (2002) described a further development of this assay that enabled simultaneous detection of all the *Anaplasma* and *Ehrlichia* species that infect ruminants. In East Africa, a combination of these techniques has been applied in a field situation for the identification of tick-borne haemoparasites including *Theileria*, *Anaplasma*, *Babesia* and *Ehrlichia* species, in an endemic region in Uganda (Oura et al., 2004). In their study, the RLB assay was assessed for the ability to detect the principal tick-transmitted protozoan and rickettsial cattle pathogens in indigenous and crossbred cattle and more importantly to identify carrier animals. This assay was able to identify *T. parva* at a level comparable with previously developed PCR methods and well below conventional microscopic detection.

1.4.3 p104 nested PCR for detection of *T. parva*

A p104 nested PCR assay has been developed for the sensitive detection of *T. parva* (Skilton et al., 2002). It has been found to provide enhanced sensitivity for detection of *T. parva* infections in bovine blood samples in carrier animals. When previously compared to two alternative detection systems, p104 based PCR and RLB hybridization, it was found to present a highly sensitive tool for the detection and monitoring of asymptomatic carrier state infections of *T. parva* in the blood of cattle (Odongo et al., 2009).

1.5 Objectives of this study

1.5.1 General Objective

To determine the occurrence of *E. ruminantium* and other tick-borne haemoprotozoans in five agro-ecological zones of western Kenya using the pCS20-specific quantitative real-time PCR (qPCR) test and the reverse line blot (RLB) hybridization assay as diagnostic methods.

1.5.2 Specific Objectives

1. To compare the pCS20 qPCR test and the RLB hybridization assays for specific detection of *E. ruminantium*.
2. To determine whether co-infections of tick-borne pathogens occur in the IDEAL calves.
3. To compare the RLB hybridization assay and the nested p104 PCR test for specific detection of *T. parva*.

2 Materials and methods

2.1 Experimental procedures

A cohort of 552 calves was recruited into the IDEAL project at birth and monitored for a period of one year. They were chosen from twenty randomly selected sub locations in western Kenya, which were in turn distributed across five agro-ecological zones (AEZs). The AEZs were defined according to climate, altitude and agricultural activities (Figure 1).

The AEZs were defined as Lower Midland 1 (LM1), Lower Midland 2 middle (LM2m), Lower Midland 2 south (LM2s), Lower Midland 3 (LM3) and Upper Midland 3 (UM3). LM1 is a sugar cane zone with an altitude ranging from 1,300 to 1,500 m above sea level with an annual mean temperature from 20.8 to 22.0°C and annual rainfall from 1,800 to 2,000 mm. LM2 differs from LM1 only in rainfall which ranges from 1,550 to 1,950mm. LM3 is a low midland cotton zone with annual mean temperatures of 21 to 22°C and an annual average rainfall of 900 to 1,100 mm. UM3 is a marginal coffee zone with an annual average rainfall of 1,000 to 1,250 mm and annual mean temperatures of between 18 to 21°C.

For the purposes of the current study, 545 of the 552 IDEAL calves were sampled. LM1 had 217 calves, LM2m and LM2s had 82 calves each, while there were 80 calves from LM3 and 84 from UM3. The RLB assay was used to detect the presence of tick-borne haemoparasites in these samples, and the pCS20 qPCR assay and the

p104 nested PCR assay were used for the specific detection of *E. ruminantium* and *T. parva*, respectively.

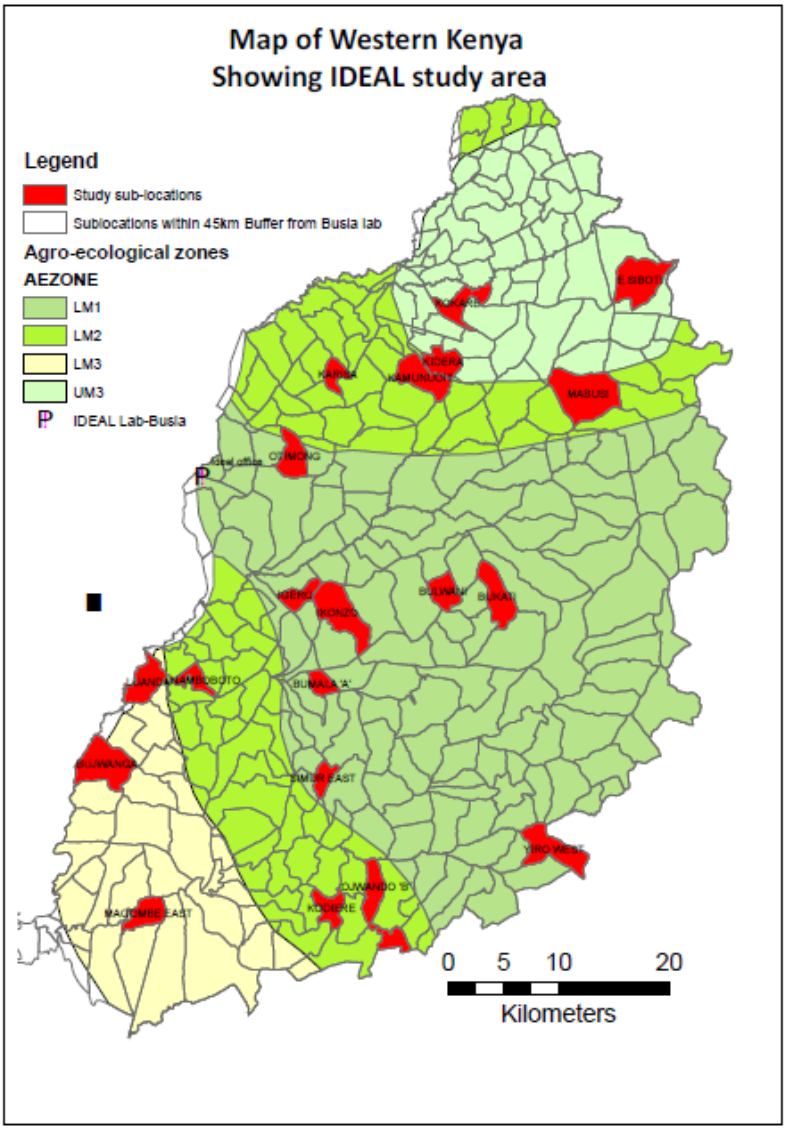


Figure 1: Map of western Kenya showing the study sites and agro-ecological zones.

2.2 Blood samples

Five hundred and forty-five calves were sampled, 459 of which were one year old and 86 of which died before they attained one year. Five millilitres of blood was collected at one year of age for the calves that survived, and at the last visit for the calves that died before attaining one year. The blood was collected into sterile vacutainer tubes containing EDTA as anticoagulant and stored at -80°C. DNA was extracted from 250 µl of each blood sample at the International Livestock Research Institute (ILRI) using a Blood DNA extraction kit (Invitrogen, Germany) according to the manufacturer's instructions, and eluted in 100 µl of elution buffer. The concentration of DNA in each sample was determined by spectrophotometric analysis using a Nanodrop 1000 spectrophotometer (Thermo scientific). The DNA was shipped to South Africa for analysis by qPCR and RLB.

2.3 Reverse line blot (RLB) hybridization

The RLB hybridization assay was performed as previously described (Gubbels et al., 1999; Bekker et al., 2002; Nijhof et al., 2003; 2005). The RLB test involved a number of steps commencing with preparation of the membrane followed by a PCR. Genus- and species-specific probes were covalently linked to a Biodyne^{R} nylon transfer membrane (Pall Corporation, Port Washington, NY, USA), and PCR products were hybridized to the probes with the help of a miniblotter apparatus. The procedure is described below.

2.3.1 Membrane preparation

The Biodyne^{R} nylon transfer membrane was prepared by measuring a piece of membrane according to the size of the miniblotter apparatus support cushion. It was then activated by incubating in 10 ml freshly prepared 16% EDAC (1-ethyl-3-(-3-dimethylamininopropyl) carbodiimide) for 10 min. Each probe was diluted in 0.5 M $NaHCO_3$, pH 8.4 to a final concentration of 2 pmol/µl and 200 µl was loaded onto the membrane using the miniblotter. *Anaplasma, Ehrlichia, Theileria* and *Babesia* genus- and species-specific probes that were included on the membrane are shown in Table 1. The membrane was incubated for 2 min at room temperature and inactivated with 100 mM freshly made NaOH for 8 min at room temperature on a shaker. The membrane was then washed in 100 ml 2 X SSPE/0.1% SDS at 60°C for 5 min and it was ready for use.

Table 1: Genus- and species-specific RLB oligonucleotide probes that were used in this study. The degenerate position R denotes either A or G, W denotes either A or T and Y denotes either C or T.

Pathogen	**Sequence (5'--> 3')**
Ehrlichia/Anaplasma group-specific probe ("E/A catch-all")	GGG GGA AAG ATT TAT CGC TA
Anaplasma bovis	GTA GCT TGC TAT GRG AAC A
Anaplasma centrale	TCG AAC GGA CCA TAC GC
Anaplasma marginale	GAC CGT ATA CGC AGC TTG
Anaplasma phagocytophilum	TTG CTA TAA AGA ATA ATT AGT GG
Anaplasma (formerly *Ehrlichia*) sp. Omatjenne	CGG ATT TTT ATC ATA GCT TGC
Ehrlichia canis	TCT GGC TAT AGG AAA TTG TTA
Ehrlichia chaffeensis	ACC TTT TGG TTA TAA ATA ATT GTT
Ehrlichia ruminantium (Welgevonden)	AGT ATC TGT TAG TGG CAG
Theileria/Babesia group-specific probe ("T/B catch-all")	TAA TGG TTA ATA GGA RCR GTT G
Babesia genus-specific probe 1 ("B catch-all 1")	ATT AGA GTG TTT CAA GCA GAC
Babesia genus-specific probe 2 ("B catch-all 2")	ACT AGA GTG TTT CAA ACA GGC
Babesia bicornis	TTG GTA AAT CGC CTT GGT C
Babesia bigemina	CGT TTT TTC CCT TTT GTT GG
Babesia bovis	CAG GTT TCG CCT GTA TAA TTG AG
Babesia caballi	GTG TTT ATC GCA GAC TTT TGT
Babesia canis	TGC GTT GAC CGT TTG AC
Babesia divergens	ACT RAT GTC GAG ATT GCA C
Babesia felis	TTA TGC GTT TTC CGA CTG GC
Babesia gibsoni Japan	TAC TTG CCT TGT CTG GTT T
Babesia gibsoni USA	CAT CCC TCT GGT TAA TTT G
Babesia leo	ATC TTG TTG CTT GCA GCT T
Babesia major	TCC GAC TTT GGT TGG TGT
Babesia microti	GRC TTG GCA TCW TCT GGA
Babesia rossi	CGG TTT GTT GCC TTT GTG

Babesia vogeli	AGC GTG TTC GAG TTT GCC
Theileria genus-specific probe ("T catch-all")	ATT AGA GTG CTC AAA GCA GGC
Theileria annae	CCG AAC GTA ATT TTA TTG ATT TG
Theileria annulata	CCT CTG GGG TCT GTG CA
Theileria bicornis	GCG TTG TGG CTT TTT TCT G
Theileria buffeli	GGC TTA TTT CGG WTT GAT TTT
Theileria equi	TTC GTT GAC TGC GYT TGG
Theileria lestoquardi	CTT GTG TCC CTC CGG G
Theileria mutans	CTT GCG TCT CCG AAT GTT
Theileria ovis	TTT TGC TCC TTT ACG AGT CTT TGC
Theileria parva	GGA CGG AGT TCG CTT TG
Theileria sp. (buffalo)	CAG ACG GAG TTT ACT TTG T
Theileria sp. (kudu)	CTG CAT TGT TTC TTT CCT TTG
Theileria sp. (sable)	GCT GCA TTG CCT TTT CTC C
Theileria taurotragi	TCT TGG CAC GTG GCT TTT
Theileria velifera	CCT ATT CTC CTT TAC GAG T

2.3.2 Preparation of the PCR mastermix

Primers RLB-F2 (5'-GAC ACA GGG AGG TAG TGA CAA G-3') and RLB-R2 (5'-biotin-CTA AGA ATT TCA CCT CTG ACA GT-3') were used for the specific amplification of the V4 hypervariable region of the 18S rRNA gene of *Theileria* and *Babesia* species (Nijhof et al., 2003). Primers EHR-F (5'-GGA ATT CAG AGT TGG ATC MTG GYT CAG-3') and EHR-R (5'-biotin-CGG GAT CCC GAG TTT GCC GGG ACT TYT TCT-3') were used for the specific amplication of the V1 hypervariable region of the 16S rRNA gene of *Anaplasma* and *Ehrlichia* species (Bekker et al., 2002). In each case, the reverse primer was labeled with biotin to allow for detection of the PCR product during the hybridization process. The PCR reaction mixture was prepared using Platinum® Quantitative PCR SuperMix-UDG

(Invitrogen, Celtic Molecular Diagnostics, South Africa). Reactions were performed in a 25 µl volume with a final concentration of 3 mM $MgCl_2$, 20 pmol of each primer, 0.5 U UDG, 200 mM dNTPs, 0.75 U Platinum® Taq DNA polymerase and 5 µl of template DNA (containing between 50 and 100 ng DNA).

Separate PCR master mix reactions were prepared for amplification of *Theileria* and *Babesia* species, and for amplification of *Ehrlichia* and *Anaplasma* species. For the *Theileria*/*Babesia* PCR reaction mix (18S rRNA), RLB-F2 and RLB-R2 primers were used while for the *Ehrlichia*/*Anaplasma* PCR master mix (16S rRNA) EHR-F and EHR-R primers were used. A touchdown thermal cycling programme (Table 2) as described by Nijhof et al. (2005) was used.

Mastermix with no DNA template (negative control), and known *A. centrale* and *B. bigemina* DNA samples (positive controls) were included to monitor the occurrence of false positive or false negative results.

Table 2: Thermocycling program for *Theileria*/*Babesia* and *Ehrlichia*/*Anaplasma* touchdown PCR.

Cycle	Time	Temperature	Purpose
1 cycle	3 min	37°C	Activate UDG
1 cycle	10 min	94°C	Inactivate UDG & activate *Taq*
	20 sec	94°C	Denature double stranded DNA template
2 cycles	30 sec	67°C	Anneal primers
	30 sec	72°C	Extension of PCR products by *Taq* polymerase
	20 sec	94°C	
2 cycles	30 sec	65°C	
	30 sec	72°C	
	20 sec	94°C	
2 cycles	30 sec	63°C	
	30 sec	72°C	
	20 sec	94°C	
2 cycles	30 sec	61°C	
	30 sec	72°C	
	20 sec	94°C	
2 cycles	30 sec	59°C	
	30 sec	72°C	
	20 sec	94°C	
40 cycles	30 sec	57°C	
	30 sec	72°C	
1 cycle	7 min	72°C	Final extension

2.3.3 Hybridization

Hybridization was performed as previously described (Nijhof et al., 2005). The Biodyne^{R} nylon transfer membrane containing genus- and species-specific oligonucleotide probes was activated in approximately 50 ml 2 X SSPE/0.1% SDS in

a plastic container at room temperature for 5 min. The PCR products were prepared for hybridization by adding 130 µl of 2 X SSPE/0.1% SDS to a 25 µl aliquot of each PCR product, denaturing for 10 min at 99.9°C on a thermal cycler machine and cooling on ice immediately. Denatured PCR products were hybridized to genus- and species-specific oligonucleotide probes covalently linked to the activated membrane using a miniblotter apparatus. Hybridization was done at 42°C for 60 min, subsequently samples were removed by aspiration and the membrane removed from the blotter. The membrane was washed twice in preheated 2 X SSPE/0.5% SDS for 10 min at 50°C, then incubated with 10 ml 2 X SSPE/0.5% SDS and 12.5 µl streptavidin-POD (peroxidase labelled) conjugate (1.25 U) for 30 min at 42°C. The membrane was further washed twice in preheated 2 X SSPE/0.5% SDS for 10 min at 42°C and twice with 2 X SSPE for 5 min at room temperature. All incubations and washes were performed in the incubator with gentle shaking. For detection of hybridized PCR products by enhanced chemiluminiscence (ECL), 10 ml of ECL buffer (5 ml ECL1 + 5 ml ECL2) was added onto the membrane and mixed by shaking the container gently for 1 min at room temperature and keeping the membrane covered with ECL buffer. The membrane was exposed to an X-ray film (Separation Scientific, South Africa).

2.3.4 Stripping of the membrane

The membrane was stripped immediately after use, by washing twice with 1% SDS (preheated to 80ºC) for 30 minutes with gentle shaking, followed by one wash with 20 mM EDTA at room temperature for 15 minutes with gentle shaking. After stripping, the membrane was stored at 4ºC in 20 mM EDTA, pH 8 in a plastic container.

2.4 pCS20 quantitative real-time PCR (qPCR)

The pCS20 qPCR was performed as previously described (Steyn et al., 2008), using amplification primers CowF (5'-CAA AAC TAG TAG AAA TTG CAC A-3') and CowR (5'-TGC ATC TTG TGG TGG TAC-3'), and TaqMan probe Cow^{TM} (5'-6FAM-TCC TCC ATC AAG ATA TAT AGC ACC TAT TA XT-PH-3'). The LightCycler® FastStart DNA Master^{PLUS} HybProbe kit was used (Roche Diagnostics). Reactions were performed in a final volume of 20 µl with a final concentration of 1.2 mM $MgCl_2$, 0.2 µM of each primer, 0.2 µM probe, 0.1 U Uracil DNA N-Glycosylase (UDG), 4 U Taq polymerase (Roche) and 5 µl of template DNA (containing between 100 and 200 ng DNA). UDG activation was performed at 40°C for 10 min followed by activation of the FastStart DNA polymerase at 95°C for 10 min. This was followed by 38 cycles of denaturing at 95°C, 10 s with a 20°C/s slope, annealing at 48°C, 10 s with a 20°C/s slope and elongation at 58°C, 30 s with a 20°C/s slope, and a final cooling step to 40°C. Fluorescence data at 520 nm was acquired at the end of the extension step of each cycle. Five microliters of Mastermix pure grade water was used as a negative control and an *E. ruminantium* positive sample was included to serve as a positive control.

2.5 Nested p104 PCR

A nested p104 PCR was used to detect *T. parva* in the 86 samples from animals which had died before they attained one year. The nested p104 PCR was done as previously described (Skilton et al., 2002). Briefly, oligonucleotide primers IL755 (5'-TAA GAT GCC GAC TAT TAA TGA CAC C-3' and IL759 (5'-CCG TTT GAT CCA TCA

TTC AAG G-3') were used to amplify bases 2396-3223 of the *T. parva* p104 gene from genomic DNA. For the secondary PCR, internal primers were used to amplify a 277 bp internal fragment located between bases 2784 and 3061 of the p104 gene. The sequences of the nested forward and reverse primers were 5'-GGC CAA GGT CTC CTT CAG ATT ACG-3' and 5'-TG GTG TGT TTC CTC GTC ATC TGC-3' respectively (Iams et al., 1990).

Reagents were thawed at room temperature but kept on ice after thawing; the UV lights were turned on in the PCR hoods prior to commencement of the PCR set-up to destroy contaminating DNA in the working area. The primary PCR amplifications were performed in a total volume of 25 µl containing 0.25 U of Taq DNA polymerase (Promega), 1X PCR buffer (500 mM KCl/100 mM Tris- HCl), 200 mM of each dNTP, 25 ng of primers, 1.5 mM of $MgCl_2$ and 2.5 µl of DNA (containing between 50 and 100 ng DNA). The reactions were performed in a hot lid MJ-PCR thermal cycler (Applied Biosystems) with an initial denaturation step of 95°C for 5 min followed by 40 cycles of 95°C for 1 min, 60°C for 1 min and 72°C for 1 min and a final extension of 9 min at 72°C followed by a 20°C incubation for 1 min with a holding stage at 4°C. Positive (*T. parva* DNA) and negative controls (Master mix without DNA template) were included to monitor false positive and false negative results.

Secondary PCR amplification reactions were set up as described above, except that 1 µl of primary PCR product was used as template. Cycling profile conditions were as described above except that the reaction was run for 30 cycles and the annealing temperature was 55°C for 1 min (Odongo et al., 2009). Strict precautions were taken to avoid PCR product contamination between samples.

The PCR products were visualised by UV trans-illumination in a 2% agarose gel following electrophoresis and staining with GelRed™ (Biotium, Hayward, CA, USA).

2.6 Statistical analysis

The Statistical Packages for Social Sciences (SPSS) program was employed in the calculation of the Pearson's chi-squared test and the Fisher's exact test values. The RLB hybridization assay was compared to the pCS20 qPCR in detection of *E. ruminantium*. Similarly, the RLB hybridization assay was compared to the nested p104 PCR for specific detection of *T. parva*. Cross tabulations were done and the Pearson's chi-squared test value and the Fisher's exact test values were determined at $P<0.05$. For any given set of data, the SPSS program computes values for both the Pearson's chi-squared and the Fisher's exact tests. When the expected count values are small, the Fisher's exact test value is more accurate than the Pearson's chi-squared test value. When the expected count values are large, the Pearson's chi-squared test value is more accurate.

3 Results

3.1 Reverse line blot hybridization assay

Four hundred and eighty-seven (487) of the samples tested (89.36%) gave a positive reaction for haemoparasites using RLB. More *Theileria* and *Babesia* (90.76%) species were identified than *Ehrlichia* and *Anaplasma* species (69.61%). Mixed infections were found in many samples (94.05%). An example of an RLB result is shown in Figure 2.

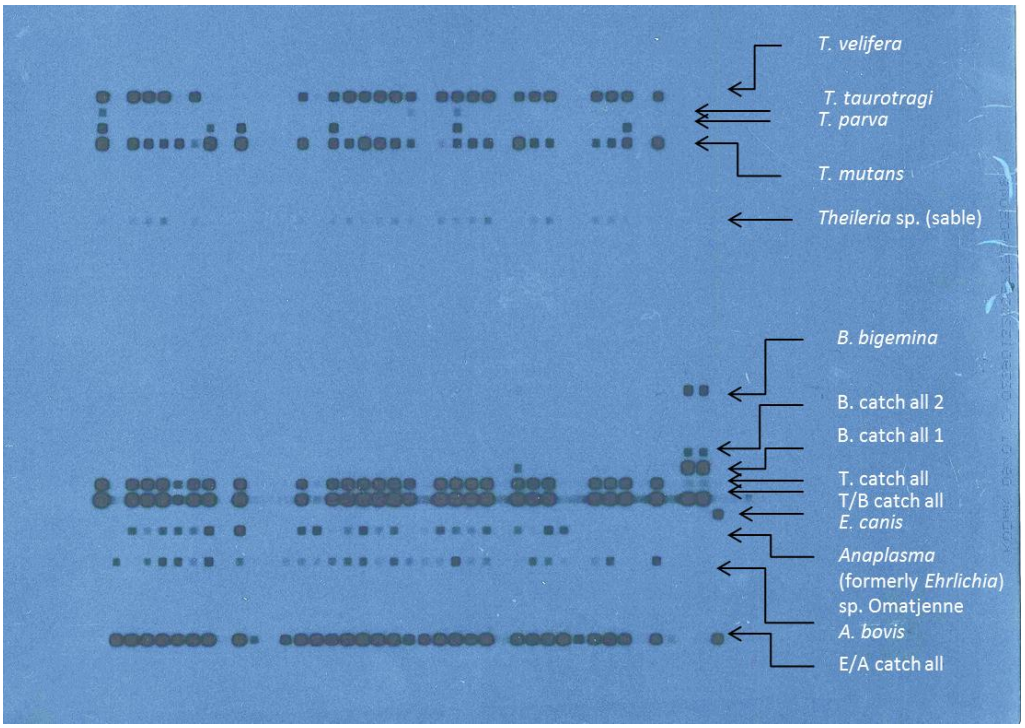
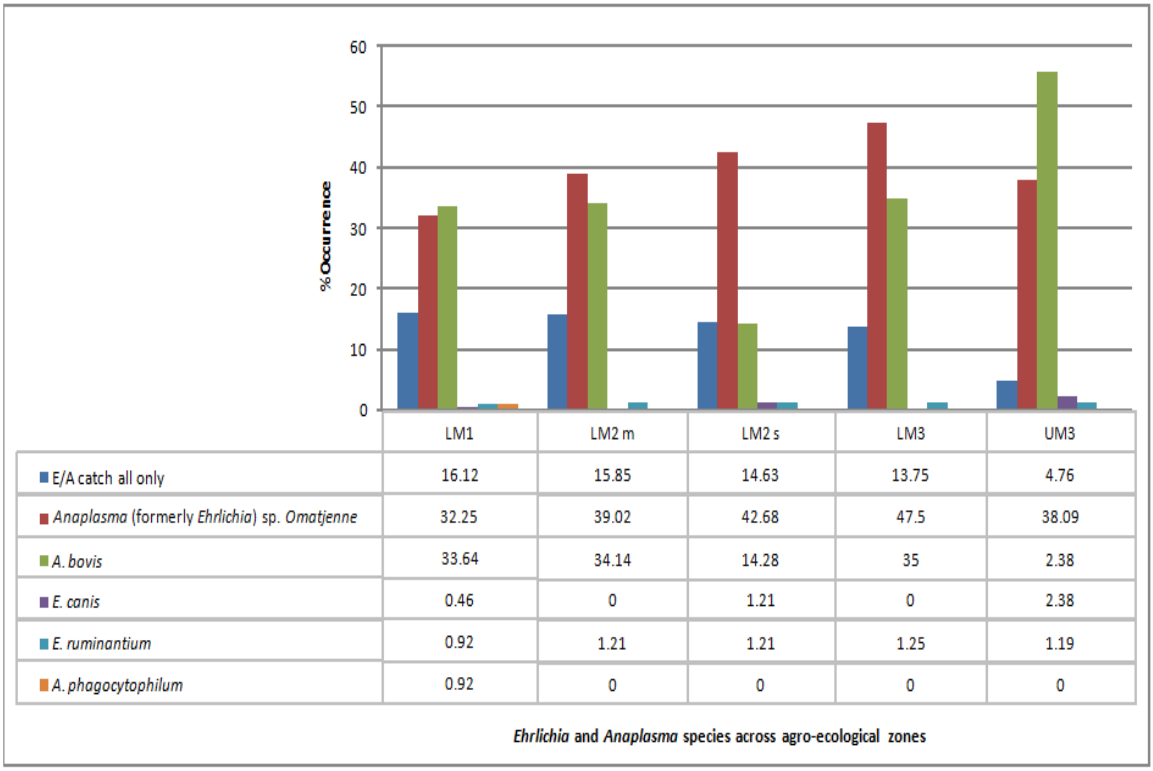


Figure 2: Reverse line blot hybridization of *Theileria*, *Babesia*, *Anaplasma* and *Ehrlichia* PCR products amplified from genomic DNA extracted from infected blood samples. Probes were loaded in horizontal lanes and samples were loaded in vertical lanes. E/A catch-all: *Ehrlichia/Anaplasma* group-specific probe; T/B catch-all: *Theileria/Babesia* group-specific probe; T catch-all: *Theileria* genus-specific probe; B catch-all: *Babesia* genus-specific probe.

The occurrence of *Ehrlichia* and *Anaplasma* species in cattle samples in the different AEZs, as determined by the RLB hybridization assay, is shown in Figure 3. *Anaplasma* (formerly *Ehrlichia*) sp. Omatjenne was detected in 207 of the samples tested, as was *A. bovis*, representing 37.98% of the samples tested. Only six of the samples, representing 1.10% of the calves sampled, tested positive for *E. ruminantium*. Two *E. ruminantium* positive samples were identified in LM1 while only one *E. ruminantium* positive sample was identified from each of the other AEZs. Other species identified included *A. phagocytophilum* and *E. canis* which occurred in 0.36% and 0.73% of the samples, respectively. Seventy-five samples (13.76 %) showed a signal on the *Ehrlichia/Anaplasma* group-specific probe but did not hybridize to any of the species-specific probes. *Anaplasma phagocytophilum* occurred in samples from LM1 only, while *E. canis* was identified in samples from all AEZs except LM2m and LM3. *Ehrlichia ruminantium*, *Anaplasma* (formerly *Ehrlichia*) sp. Omatjenne and *A. bovis* were identified in samples from all the AEZs. Mixed infections with *Anaplasma* (formerly *Ehrlichia*) sp. Omatjenne and *A. bovis* were found in samples from all five AEZs.



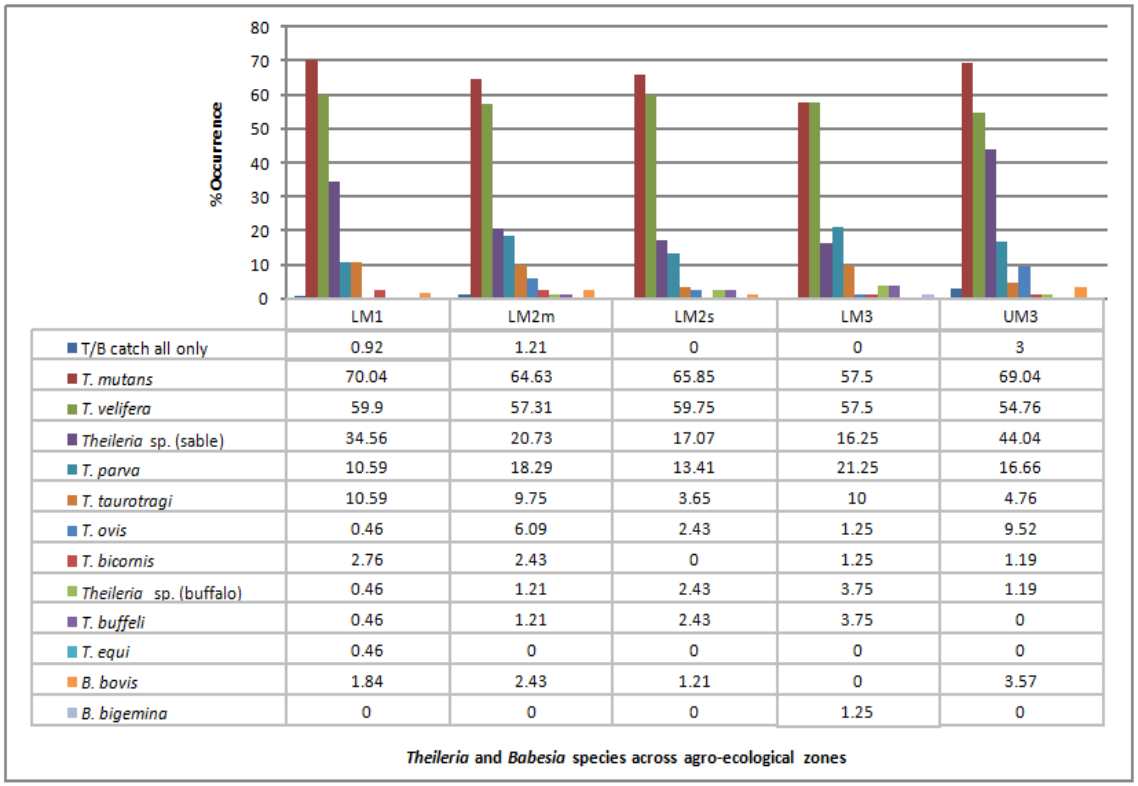
	LM1	LM2 m	LM2 s	LM3	UM3
E/A catch all only	16.12	15.85	14.63	13.75	4.76
Anaplasma (formerly *Ehrlichia*) sp. *Omatjenne*	32.25	39.02	42.68	47.5	38.09
A. bovis	33.64	34.14	14.28	35	2.38
E. canis	0.46	0	1.21	0	2.38
E. ruminantium	0.92	1.21	1.21	1.25	1.19
A. phagocytophilum	0.92	0	0	0	0

Ehrlichia and *Anaplasma* species across agro-ecological zones

Figure 3: The occurrence of *Ehrlichia* and *Anaplasma* species in cattle samples across agro-ecological zones as determined by the reverse line blot hybridization assay. E/A catch-all: *Ehrlichia/Anaplasma* group-specific probe.

The occurrence of *Theileria* and *Babesia* species in cattle samples, as determined by the RLB hybridization assay, is shown in Figure 4. Two *Babesia* species were identified and ten *Theileria* species. In decreasing order of occurrence, the *Theileria* piroplasms identified were *T. mutans* (66.61%), *T. velifera* (58.35%), *Theileria* sp. (sable) (28.62%), *T. parva* (14.68%), *T. taurotragi* (8.44%), *T. ovis* (3.12%), *T. bicornis* (1.83%), *Theileria* sp. (buffalo) (1.46%) and *T. buffeli* (1.28%). One sample was positive for *T. equi*. Relatively few samples were positive for *B. bovis* (1.65%) and *B. bigemina* (0.18%). Six samples (1.10%) were positive for the *Theileria*/*Babesia* group-specific probe but did not hybridize to any of the species-specific probes.

B. bovis was identified in samples from all AEZs except LM3, while *B. bigemina* was identified in samples from LM3 but not from any of the other AEZs. *Theileria bicornis* was present in samples from all the AEZs except LM2s while *T. buffeli* occurred in samples from all AEZs except for UM3. LM3 recorded the lowest percentage of *T. mutans* (51.25%) and *Theileria.* sp. (sable) (17.07%) positive samples, and the highest percentage of *T. parva* positive samples (20%). LM1 had the lowest percentage of *T. parva* positive samples (9.67%) and the highest percentage of *T. mutans* positive samples (69.58%). *Theileria* sp. (sable) occurred most frequently in samples from UM3 (44.04%).



	LM1	LM2m	LM2s	LM3	UM3
T/B catch all only	0.92	1.21	0	0	3
T. mutans	70.04	64.63	65.85	57.5	69.04
T. velifera	59.9	57.31	59.75	57.5	54.76
Theileria sp. (sable)	34.56	20.73	17.07	16.25	44.04
T. parva	10.59	18.29	13.41	21.25	16.66
T. taurotragi	10.59	9.75	3.65	10	4.76
T. ovis	0.46	6.09	2.43	1.25	9.52
T. bicornis	2.76	2.43	0	1.25	1.19
Theileria sp. (buffalo)	0.46	1.21	2.43	3.75	1.19
T. buffeli	0.46	1.21	2.43	3.75	0
T. equi	0.46	0	0	0	0
B. bovis	1.84	2.43	1.21	0	3.57
B. bigemina	0	0	0	1.25	0

Theileria and *Babesia* species across agro-ecological zones

Figure 4: The occurrence of *Theileria* and *Babesia* species in cattle samples across agro-ecological zones as determined by the reverse line blot hybridization assay. T/B catch-all: *Theileria/Babesia* group-specific probe.

Many of the samples examined showed mixed populations of the different *Anaplasma* and *Ehrlichia* (Table 3) and *Theileria* and *Babesia* species (Table 4). Samples from LM2s had the highest percentage of single *Ehrlichia* and *Anaplasma* species (48.78%) while samples from LM3 had the lowest (43.75%). Samples from UM3 had the highest percentage of mixed *Ehrlichia* and *Anaplasma* species infections (25%), while those from LM1 had the least (10.59%).

There were more mixed *Theileria* and *Babesia* infections (68.44%) than single infections (12.66%). No single infections of *B. bigemina*, *Theileria* sp. (sable), *T. bicornis*, *Theileria* sp. (buffalo), *T. buffeli* and *T. equi* were observed. Samples from LM2s had the highest percentage of single *Theileria* and *Babesia* species infections (19.51%) while samples from LM1 had the lowest (8.75%). LM1 also recorded the highest percentage of mixed infections (76.49%) while LM2m and LM2s had the least (60.97%). Mixed infections were common in all the AEZs.

Table 3: The occurrence of *Ehrlichia* and *Anaplasma* species infections in cattle blood samples from five agro-ecological zones of western Kenya as determined by the reverse line blot hybridization assay.

	LM1 (n=217)	**LM2m (n=82)**	**LM2s (n=82)**	**LM3 (n=80)**	**UM3 (n=84)**	**Total (n=545)**
Single infections	**101 (46.54%)**	**39 (47.56%)**	**40 (48.78%)**	**35 (43.75%)**	**39 (46.42%)**	**254 (46.60%)**
E. ruminantium (*Welgevonden*)	1 (0.46%)	1 (1.21%)	0	1 (1.25%)	0	**3 (0.55%)**
Anaplasma (formerly *Ehrlichia*) sp. Omatjenne	49 (22.58%)	21 (21.60%)	22 (26.82%)	22 (27.50%)	12 (14.8%)	**126 (23.11%)**
E. canis	0	0	1 (1.21%)	0	1 (1.19%)	**2 (0.36%)**
A. phagocytophilum	0	0	0	0	0	**0**
A. bovis	51 (23.50%)	17 (20.73%)	17 (20.73%)	12 (15%)	26 (30.95%)	**123 (22.56%)**
Mixed *Ehrlichia* and *Anaplasma* infections	**23 (10.59%)**	**11 (13.41%)**	**14 (17.07%)**	**16 (20%)**	**21 (25%)**	**85 (15.59%)**
E. ruminantium (*Welgevonden*)	1 (0.46%)	0	1 (1.21%)	0	1 (1.19%)	**3 (0.55%)**
Anaplasma (formerly *Ehrlichia*) sp. Omatjenne	21 (9.67%)	11 (13.41%)	13 (15.85%)	16 (20%)	20 (23.80%)	**81 (14.86%)**
E. canis	1 (0.46%)	0	0	0	1 (1.19%)	**2 (0.36%)**
A. phagocytophilum	2 (0.92%)	0	0	0	0	**2 (0.36%)**
A. bovis	22 (10.13%)	11 (13.41%)	14 (17.07%)	16 (20%)	21 (25%)	**84 (15.41%)**
***Ehrlichia*/*Anaplasma* group-specific only**	35 (16.12%)	13 (15.85%)	12 (14.63%)	11 (13.75%)	4 (4.76%)	**75 (13.76%)**
Negative / below detection limit	58 (26.72%)	19 (23.17%)	16 (19.51%)	18 (22.50%)	20 (23.80%)	**131 (24.03%)**

Table 4: The occurrence of *Theileria* and *Babesia* species infections in cattle blood samples from five agro-ecological zones of western Kenya as determined by the reverse line blot hybridization assay.

	LM1 (n=217)	**LM2m (n=82)**	**LM2s (n=82)**	**LM3 (n=80)**	**UM3 (n=84)**	**Total (n=545)**
Single infections	**19 (8.75%)**	**14 (17.07%)**	**16 (19.51%)**	**11 (13.75%)**	**9 (10.71%)**	**69 (12.66%)**
B. bigemina	0	0	0	0	0	**0**
B. bovis	1 (0.46%)	0	0	0	0	**1 (0.18%)**
Theileria sp. (sable)	0	0	0	0	0	**0**
T. mutans	1 (0.46%)	6 (7.31%)	10 (12.19%)	5 (6.25%)	7 (8.33%)	**29 (5.32%)**
T. parva	2 (0.92%)	1 (1.21%)	2 (2.43%)	1 (1.25%)	1 (1.19%)	**7 (1.28%)**
T. taurotragi	1 (0.46%)	0	0	0	0	**1 (0.18%)**
T. velifera	14 (6.45%)	7 (8.53%)	4 (4.87%)	5 (6.25%)	1 (1.19%)	**31 (5.68%)**
T. ovis	0	0	0	0	0	**0**
T. bicornis	0	0	0	0	0	**0**
Theileria sp. (buffalo)	0	0	0	0	0	**0**
T. buffeli	0	0	0	0	0	**0**
T. equi	0	0	0	0	0	**0**
Mixed *Theileria* and *Babesia* infections	**166 (76.49%)**	**50 (60.97%)**	**50 (60.97%)**	**51 (63.75%)**	**56 (66.67%)**	**373 (68.44%)**
B. bigemina	0	0	0	1 (1.25%)	0	**1 (0.18%)**
B. bovis	3 (1.38%)	2 (2.43%)	1 (1.21%)	0	3 (3.57%)	**9 (1.65%)**
Theileria sp. (sable)	75 (34.56%)	17 (20.73%)	14 (17.07%)	13 (16.25%)	37 (44.04%)	**156 (28.62%)**
T. mutans	151 (69.58%)	47 (57.31%)	44 (53.65%)	41 (51.25%)	51 (60.71%)	**334 (61.28%)**
T. parva	21 (9.67%)	14 (17.07%)	9 (10.97%)	16 (20.0%)	13 (15.47%)	**73 (13.39%)**

T. taurotragi	22 (10.13%)	8 (9.75%)	3 (3.65%)	8 (10.0%)	4 (4.76%)	**45 (8.25%)**
T. velifera	115 (52.99)	40 (48.78%)	45 (54.87%)	41 (51.25%)	45 (53.57%)	**286 (52.47%)**
T. ovis	1 (0.46%)	5 (6.09%)	2 (2.43%)	1 (1.25%)	8 (9.52%)	**17 (3.11%)**
T. bicornis	6 (2.76%)	2 (2.43%)	0	1 (1.25%)	1 (1.19%)	**10 (1.83%)**
Theileria sp. (buffalo)	1 (0.46%)	1 (1.21%)	2 (2.43%)	3 (3.75%)	1 (1.19%)	**8 (1.46%)**
T. buffeli	1 (0.46%)	1 (1.21%)	2 (2.43%)	3 (3.75%)	0	**7 (1.28%)**
T. equi	1 (0.46%)	0	0	0	0	**1 (0.18%)**
***Theileria*/*Babesia* group-specific only**	**2 (0.92%)**	**1 (1.21%)**	**0**	**0**	**3 (3.57%)**	**6 (1.10%)**
Negative / below detection limit	**30 (13.82%)**	**17 (20.73%)**	**16 (19.51%)**	**18 (21.42%)**	**16 (19.04%)**	**97 (17.79%)**

3.2 pCS20 qPCR for specific detection of *E. ruminantium*

The pCS20 qPCR detected five *E. ruminantium* positive samples (representative results shown in Figure 5), representing 0.92% of the calves sampled. Although the RLB detected six positive samples, only three samples were positive by both tests. The remaining three RLB positive samples were negative by pCS20 qPCR.

A statistical comparison of the RLB hybridisation assay and the qPCR test for detection of *E. ruminantium* resulted in two cells (50.0%) with expected count less than 5 (Table 5). Therefore, the Fisher's exact test was chosen for comparison of these tests. The Fisher's exact test (Table 5) indicated that the tests were not significantly different in the detection of *E. ruminantium* at $P<0.05$ and 1 degree of freedom.

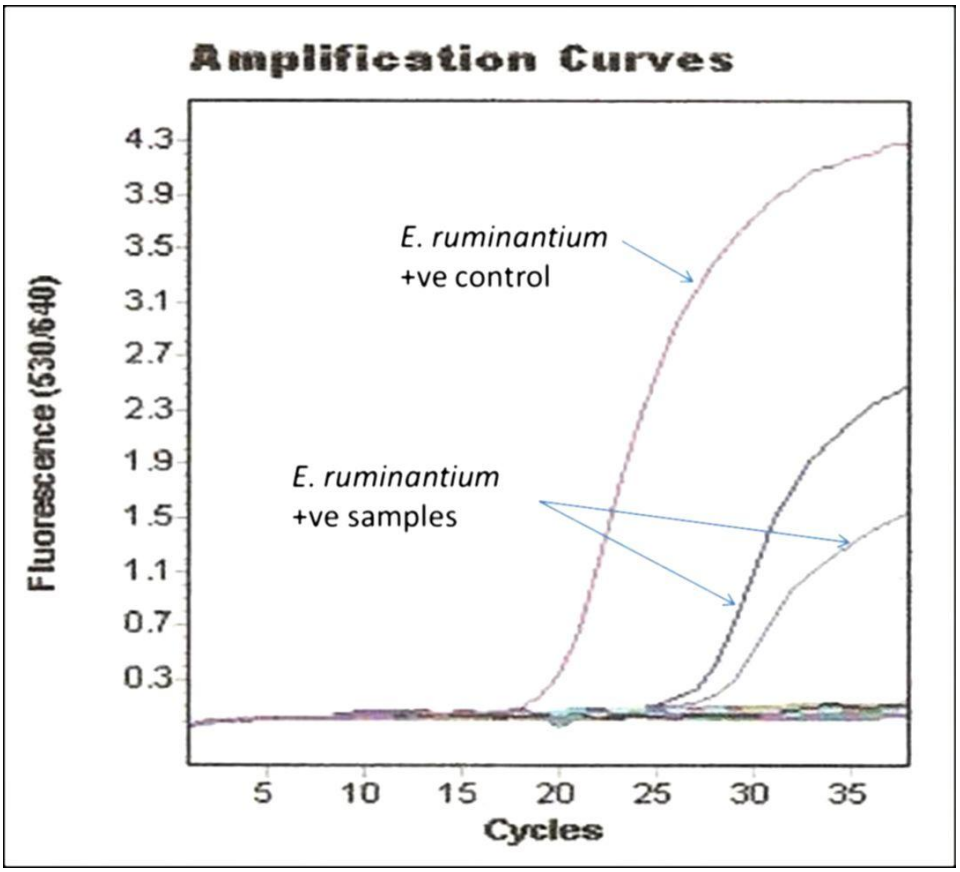


Figure 5: Diagram showing an example of the pCS20 qPCR results. (+ve: positive).

Table 5: The reverse line blot hybridization assay and qPCR cross tabulation results from the Fisher's exact test in detection of *E. ruminantium*.

RLB * qPCR Cross tabulation

		qPCR		Total
		.00 [a]	1.00 [b]	
RLB	.00 [a]	537	2	539
	1.00 [b]	3	3	6
Total		540	5	545

[a] .00 = Negative
[b] 1.00 = Positive

Chi-Squared Tests

	Value	Degrees of freedom	Asymptotic significance (2-sided)	Exact significance (2-sided)
Pearson Chi-Square	160.784[a]	1	.000	.000
Fisher's Exact Test				.000
N of Valid Cases	545			

[a] 2 cells (50.0%) have expected count less than 5. The minimum expected count is .06.

3.3 Nested p104 PCR for specific detection of *T. parva*

The *T. parva*-specific nested p104 PCR assay was used to test 86 of the samples from animals which had died before they attained one year. Twenty-eight samples tested positive for *T. parva* using the nested p104 PCR assay (Figure 6). Although 24 samples tested positive for *T. parva* using the RLB, only 17 of these were positive by both tests. Fifty-one samples were negative and /or below the detection limit of both tests.

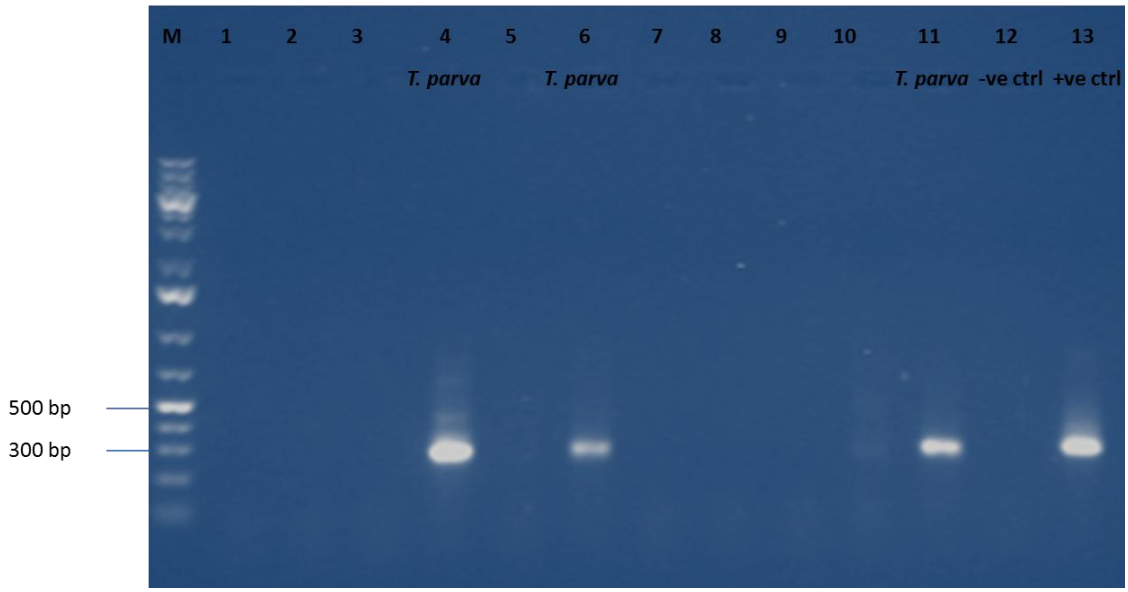


Figure 6: Diagram showing an example of a nested p104 PCR gel result with *T. parva* positive samples in lanes 4, 6 and 11. MWM (1000 bp) is in lane M, a negative control containing only PCR is in lane 12 and a *T. parva* positive control is in lane 13.

A statistical comparison of the reverse blot hybridisation assay and the nested p104 PCR test for detection of *T. parva* resulted in large expected count values (Table 6). Therefore, the Pearson's chi-squared test was chosen for comparison of these tests. The Pearson's chi-squared test (Table 6) indicated that the tests were not significantly different in the detection of *T. parva* at P<0.05 and 1 degree of freedom.

Table 6: The reverse line blot hybridization assay and nested p104 PCR cross tabulation results from the Pearson's chi-squared test in detection of *T. parva*.

p104 * RLB Cross tabulation

		RLB		Total
		.00 [a]	**1.00 [b]**	
p104	**.00 [a]**	51	7	58
	1.00 [b]	11	17	28
Total		62	24	86

[a] .00 = Negative
[b] 1.00 = Positive

Chi-Squared Tests

	Value	Degrees of freedom	Asymptotic significance (2-sided)	Exact significance (2-sided)
Pearson Chi-Square	22.211[a]	1	.000	.000
Fisher's Exact Test				.000
N of Valid Cases	86			

[a] 0 cells (.0%) have expected count less than 5. The minimum expected count is 7.81.

4 Discussion

Both pathogenic and non-pathogenic haemoparasites were identified in cattle tested in this study. The high prevalence of the haemoparasites identified was not unexpected in view of the relatively high tick burdens that the calves were exposed to (Dr. Amy Jennings, personal communication).

4.1 *Ehrlichia* and *Anaplasma* species

The pCS20 qPCR was evaluated for the detection of *E. ruminantium* in field samples and compared to an alternative detection system, the RLB hybridization assay. *Ehrlichia ruminantium* was identified in approximately 1% of the calves tested by both qPCR and RLB, even though *Amblyomma variegatum* ticks were identified on several occasions at the study sites (Dr. Amy Jennings, personal communication). *Amblyomma gemma* and *A. lepidum* ticks, which have also been reported to be involved in the transmission of heartwater in East Africa (Wesonga et al., 1993), were not identified. *Ehrlichia ruminantium* has previously been isolated in eight districts across Kenya suggesting that this organism is widely distributed across the country and is present in ticks infesting healthy cattle, goats and sheep (Ngumi et al., 1997). However, attempts to isolate the organisms by sub-inoculation of blood were not successful and this was attributed to the likelihood of rickettsaemias being low in the blood of carrier animals as well as the reduced infectivity in ticks (Ngumi et al., 1997). The apparently low prevalence of *E. ruminantium* in the IDEAL calves may therefore have been due to the inability of the molecular tests to detect *E.*

ruminantium in carrier animals, since it is likely that the rickettsaemias are low in the blood of carrier animals (Ngumi et al., 1997).

The heartwater endemic area includes almost the whole of sub-Saharan Africa with the exception of the dry South West and the islands of Madagascar, Mauritius, Sao Tome and Reunion. Ticks in the field in heartwater endemic areas exhibit surprisingly low *E. ruminantium* infection rates, with infection rates of 1.2 -13.3% having been reported in Senegal (Gueye et al., 1993), while higher infection rates of 19.1% have been reported in Maria Galante (Molia et al., 2008). According to Uilenberg (1983), the effectiveness of *Amblyomma* ticks as vectors of heartwater depends on the vector efficiency, their adaptation to hosts, their distribution, activity and abundance with the tick population in an area being influenced by temperature and humidity. DNA probe and PCR analysis of *A. variegatum* and *A. hebraeum* ticks fed on carrier animals in Zimbabwe demonstrated that these ticks develop lower levels of infection than those fed on febrile animals (Peter et al., 1995). Only five of the IDEAL calves were confirmed to have died of heartwater (Dr. Amy Jennings, personal communication). It is therefore likely that there were relatively few ticks with high *E. ruminantium* infection rates in the study areas. This is another possible explanation for the low detection levels observed in this study.

It is possible that there may be sequence variation in the pCS20 region in Kenyan *E. ruminantium* strains which could lead to false negative results, another possible explanation for the apparently low prevalence of *E. ruminantium* in the IDEAL calves. The pCS20 sequence, which is the target for the *E. ruminantium*-specific qPCR test, has been well characterized in isolates from South Africa (Van Heerden et

al., 2004), but there are limited data on pCS20 sequences from Kenyan *E. ruminantium* strains. The pCS20 region should be sequenced from a number of Kenyan *E. ruminantium* isolates to ensure that it is also conserved, especially in the qPCR primer and probe regions.

Colostrum from dams living in a heartwater endemic area has been found to influence calfhood immunity to *E. ruminantium*. An age dependent resistance has been demonstrated with young calves (6-8 weeks old) having an innate resistance to infection and recovering spontaneously from natural or induced infections (Deem et al., 1996). In addition, indigenous breeds have been found to have a certain degree of resistance to heartwater (Ilemobade, 1977) which could also have influenced the low prevalence of the disease, since the tick vector was present on over half the calves at one year (Dr. Amy Jennings, personal communication). Animals that have recovered from infection are typically immune to reinfection for six months to four years but may be carriers of the disease for eight months or longer (Bell-Sakyi et al., 2004).

There was not much difference in the occurrence of *E. ruminantium* across the AEZs. All but LM1 had one case each, implying that *E. ruminantium* is present in all the study sites in western Kenya.

In addition to *E. ruminantium*, the RLB identified other *Ehrlichia* and *Anaplasma* species, namely *A. bovis*, *Anaplasma* (formerly *Ehrlichia*) sp. Omatjenne, *A. phagocytophilum* and *E. canis*. *Anaplasma bovis* is the causative agent of benign bovine rickettsiosis and is transmitted by the tick *Rhipicephalus appendiculatus.* It has been shown to cause serological cross reaction with *E. ruminantium* (Dumler et al.,

2001). *Anaplasma bovis* is usually associated with subclinical infection; however, it can cause fever, lymphadenopathy, depression and loss of conditioning (Wanduragala, 1993). Cattle infected with *A. bovis* have been reported mainly in African countries and little is known about the epidemiology of this agent (Woldehiwet, 2010). In our study, it was identified in 33.63% of all the cattle tested even though it has not been identified as a problem in Kenya.

Anaplasma (formerly *Ehrlichia*) sp. Omatjenne was initially isolated from *Hyalomma truncatum* and is generally thought to be apathogenic (Du Plessis, 1990). However, sheep were found to develop severe signs of disease similar to heartwater after several passages of this parasite in *Amblyomma* ticks (Du Plessis, 1990). Its main importance has been the serological cross reactivity it causes with *E. ruminantium* which could confuse serological test results (Allsopp et al., 1997).

Anaplasma phagocytophilum, the causative agent of tick-borne fever in sheep and pasture fever in cattle, was identified in 0.36% of the IDEAL calves. It was initially reported in 1999 in the Free State Province, South Africa (Pretorius et al., 1999) as a possible cause of human ehrlichiosis. Cases of *A. phagocytophilum* infection or human granulocytic ehrlichiosis have not been reported in Kenya. The RLB hybridization assay showed a strong signal for *A. phagocytophilum* in 10 out of 21 positive samples in a study done on cattle in neighboring Uganda (Muhanguzi et al., 2010b). More recently this parasite has been reported as the most widespread tick-borne infection in animals in Europe. It has unique epidemiological features including the ability to be transmitted by a number of tick and mite species, possible spread by migratory birds and an ability to infect all mammals and rodents (Stuen, 2007). In this

study, it was identified in one agro-ecological zone (LM1) which may have been influenced by the distribution of the tick vector. In North America, it is transmitted by a tick species (*Ixodes ricinus*) that is sensitive to environmental extremes and is maintained in a zoonotic cycle in which white tailed deer are the primary hosts but additional bird and mammal species are required to serve as host for the larval and nymph stages (Wilson, 1998; Wimberly et al., 2008). Its lower prevalence and spatially variable distribution likely arose from greater sensitivity to environmental factors influencing vector populations and host communities (Wimberly et al., 2008). A similar situation in Kenya may be responsible for the occurrence of *A. phagocytophilum* only in LM1, which has an altitude ranging from 1,300 to 1,500 m above sea level and a moderate climate with annual mean temperatures ranging from 20.8 to 22.0°C and rainfall from 1,800 to 2,000 mm.

Ehrlichia canis is transmitted by *Rhipicephalus sanguineus* and is the causative agent of canine monocytic ehrlichiosis (Neer et al., 2002; Siarkou et al., 2007). It is known as a parasite of dogs and was not expected in cattle. The positive IDEAL calf samples identified were probably accidentally infected by these parasites, resulting in incidental infections. An incidental host is an intermediate host that does not allow transmission to the definitive host, thereby preventing the parasite from completing its development. Recently, *E. canis* was detected in 2.7% of cattle samples tested in western Uganda (Muhanguzi et al., 2010a). It has also been reported to cause disease in humans with clinical signs consistent with those of human monocytic ehrlichiosis (Perez et al., 2006). While it is reported as an incidental infection here, its establishment in cattle could be an important epidemiological feature as far as the

spread of canine monocytic ehrlichiosis and the human form of the same is concerned (Muhanguzi et al., 2010a).

The most important tick-borne diseases in Kenya have been reported to include anaplasmosis (Wesonga et al., 2010). However, no *A. marginale* positive samples were detected in our study, although 30% of the calves were seropositive for *A. marginale* at 51 weeks (Dr. Phillip Toye, personal communication). No *A. marginale* positive controls were used on the RLB blots, but the cloned plasmid control that is periodically used in the RLB laboratory at DVTD indicated that the *A. marginale* probe was working and the same blots have been used on several occasions to test cattle samples from South Africa which tested positive for *A. marginale* (Milana Troskie, personal communication). It is therefore unlikely that there was something wrong with the RLB probes and blots. This suggests that either there was indeed no detectable *A. marginale* in the IDEAL calves, or there are differences in the sequences of Kenyan *A. marginale* strains that prevented amplification or hybridization. The results obtained with the *Ehrlichia*/*Anaplasma* group-specific probe could support either of these hypotheses: animals showed a signal at the *Ehrlichia*/*Anaplasma* group-specific probe in 75 cases, with no signal at any of the species-specific probes. These results could indicate that as yet unknown *Anaplasma* and *Ehrlichia* parasites or variants of known parasites may be present in western Kenya (Bekker et al., 2002), and will require further investigation.

4.2 *Theileria* and *Babesia* species

Theileria and *Babesia* species are common parasites of cattle where the tick vectors exist. In this study, various different *Babesia* and *Theileria* species were identified in the IDEAL calves. The most prevalent was *T. mutans*, followed by *T. velifera* and *Theileria* sp. (sable). *Theileria parva* and *T. taurotragi* were also identified in the IDEAL calves, but occurred less frequently. *Theileria mutans* is generally thought to be benign although virulent strains have been reported from South Africa (Flanagan and Le Roux, 1957). Since *T. mutans* does not usually cause disease, it is likely to spread in a cattle population wherever the vector ticks, *A. hebraeum*, *A. lepidum*, *A. gemma* and *A. variegatum*, are present. *Theileria velifera* is considered apathogenic (Stoltsz, 1989). *Theileria taurotragi* is also thought to be apathogenic in cattle and is considered to be primarily a parasite of eland (Grootenhuis et al., 1979). *Theileria* sp. (sable) is a parasite of roan and sable antelope in which it causes fatal disease (Nijhof et al., 2005). It has also been identified in African shorthorn cattle using RLB (Nijhof et al., 2005).

Theileria bicornis, *Theileria* sp. (buffalo), *T. buffeli* and *T. ovis* were identified in a few calves and one calf was positive for *T. equi*. *Theileria bicornis* was originally described in South Africa in black rhinoceroses (Nijhof et al., 2003), and more recently also in cattle tested in Uganda (Muhanguzi et al., 2010b). Its pathogenicity in cattle has not been described. *Theileria* sp. (buffalo) was first recognised in an isolate from a buffalo in Kenya (Allsopp et al., 1993). It is commonly found in buffalo in southern Africa (Chaisi et al., 2011) and was identified in buffalo from the Lake Mburo National Park in Uganda (Oura et al., 2011), but has not previously been identified in cattle. *Theileria ovis* and *T. equi* are parasites of sheep and horses, and

are not expected in cattle. These positive samples probably represent incidental infections, although unexpected positive results could also arise from contamination during the RLB assay. *Theileria buffeli* is transmitted by ticks of the genus *Haemaphysalis* in Australia, Asia and Europe but the vector is still unknown in Africa and America (Uilenberg. 1995; M'ghibi et al., 2008). The parasite was identified by serological and DNA techniques in studies done in Kenya on immunization of cattle against theileriosis (Ngumi et al., 1992; Young et al., 1992). Prior to this, it was unknown in Kenya. In our study, *T. buffeli* was identified in all but UM3 suggesting that the tick vector is present in most of western Kenya.

The most economically important of the *Theileria* species is *T. parva* which was detected by the RLB hybridization assay in 14.68% of the cattle tested. Out of the 86 calves that died before 1 year of age, *T. parva* was detected in 19.76% by p104 PCR. This set of 86 animals was also tested for other diseases such as Bluetongue and Epizootic Haemorrhagic Disease by other IDEAL researchers and some were found positive (Dr. Phillip Toye, personal communication). Although post mortem examination data also are available for all of these animals, the cause of death for each animal has not yet been conclusively determined. The results obtained using the molecular tests do not correlate well with the known seroprevalence rates in western Kenya. A study done in Kisumu, western Kenya, found the prevalence of antibodies to *T. parva* to be quite high at 75.5% in calves, 80% in yearlings and 73.4% in adult cattle with no significant difference ($P>0.05$) across the age groups (Chenyambuga et al., 2010). The percentage of IDEAL calves seropositive for *T. parva* at 51 weeks was 64% (Dr. Phillip Toye, personal communication). The results of the molecular tests and the serological tests may have failed to correlate because as opposed to molecular

tests that detect the parasite genetic material, serological tests detect antibody responses which can persist for a long time after elimination of the parasites. In addition, most infections are completely cleared by an animal's innate and antigen-specific immune responses, making molecular testing not very useful for documenting past exposure.

Babesia bovis and *B. bigemina* are the cause of bovine babesiosis in cattle in Africa. *Babesia bovis* is transmitted by the tick vectors *Rhipicephalus* (*Boophilus*) *microplus* and *R. (B.) annulatus*, while *B. bigemina* is transmitted by *R.* (*B.) microplus*, *R. (B.) annulatus*, *R.* (*B.) decoloratus* and *R. evertsi* (Donatein and Lestoquard, 1930; Blood et al., 1983). The distribution of bovine babesiosis is confined strictly to the distribution of the vector ticks (Blood et al., 1983). In our study, *B. bovis* was identified in all AEZs except LM3, while *B. bigemina* was identified in LM3 but not in any of the other AEZs. This could have been influenced by the distribution of the tick vectors, although *B. bigemina* is usually more widely distributed than *B. bovis*, because it is transmitted by a greater range of vector ticks.

PCR products from six animals gave a signal on the *Theileria*/*Babesia* group-specific probe but did not hybridize to any of the species-specific probes. This could imply that as yet unknown *Theileria* and *Babesia* haemoparasites or variants of known parasites may be present in western Kenya (Gubbels et al., 1999) which will require further investigation.

Different haemoparasites species were distributed differently across the AEZs. The occurrence and importance of tick-borne diseases is a reflection of complex

interactions involving the causative organisms, the vertebrate hosts, the tick vectors and the environment (Norval et al., 1992). These interactions are influenced by a number of factors ranging from climate to soil and vegetation, to human activities including crop/livestock production systems and measures taken to control ticks and tick-borne diseases (Rubaire-Akiiki et al., 2004). *Ehrlichia ruminantium*, *A. bovis* and *Anaplasma* (formerly *Ehrlichia*) sp. Omatjenne occurred in all the AEZs, and it is therefore likely that the tick vectors for these pathogens were present in all the AEZs.

The RLB and qPCR tests did not correlate completely in the detection of *E. ruminantium*; similarly, the results of the RLB and p104 nested PCR did not completely correlate in the detection of *T. parva*. This is probably because the rickettsaemias/parasitaemias were low. Multiple tests of the same blood samples may have identified the parasites in at least one of the replicates.

5 Conclusion

There was no significant difference between the reverse line blot (RLB) hybridization assay and the PCRs in the detection of *E. ruminantium* and *T. parva*. The results obtained using both the *E. ruminantium*-specific qPCR and the RLB hybridization assay suggest that there is a low prevalence of *E. ruminantium* in western Kenya, although it is possible that the tests may have been unable to detect *E. ruminantium* DNA in blood from carrier animals. In addition, the animals examined in this study were indigenous breeds that have a significant level of resistance to tick-borne diseases (Norval et al., 1992; Perry and Young, 1995). In the case of heartwater, animals which have recovered from infection are immune to reinfection for six months to four years but may be carriers of the disease for eight months or longer (Bell-Sakyi et al., 2004). An age-dependent resistance has also been demonstrated in young calves which have an innate resistance probably due to low grade infection of *E. ruminantium* obtained in colostral cells. Therefore, although *E. ruminatium* DNA was not detected in many of the IDEAL calves, it is possible that these animals may have been exposed to *E. ruminantium* infection. It might be worthwhile to use a serological test such as the MAP1-B ELISA to determine whether the animals had sero-converted. *Theileria parva* was detected by both RLB (in 14.68% of the samples) and p104 nested PCR (in 19.76% of the 86 calves that died before 1 year of age), suggesting that this is the most common pathogenic parasite of cattle in western Kenya. The RLB hybridization assay detected a total of seventeen parasite species of which twelve were either *Theileria* or *Babesia* species. *Theileria mutans* and *T. velifera* were the most common *Theileria* species identified. *Anaplasma bovis* and

Anaplasma (formerly *Ehrlichia*) sp. Omatjenne were the most common of the *Ehrlichia* and *Anaplasma* species detected.

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Appendix 1

Table A1: Results of the RLB hybridization assay and the *E. ruminantium*-specific pCS20 qPCR for 545 IDEAL calf samples. RLB probes listed in Table 1 which gave a negative result for all samples have not been included. RLB probes for which positive results were obtained are designated 1-22. 1: *Ehrlichia/Anaplasma* group-specific probe (E/A catch-all); 2: *A. bovis*; 3: *E. ruminantium*; 4: *Anaplasma* (formerly *Ehrlichia*) sp. Omatjenne; 5: *A. phagocytophilum*; 6: *E. canis;* 7: *Theileria/Babesia* group-specific probe (T/B catch-all); 8: *Theileria* genus-specific probe (T catch-all); 9: *Babesia* genus-specific probe 1 (B catch-all 1); 10: *Babesia* genus-specific probe 2 (B catch-all 2); 11: *B. bigemina*; 12: *B. bovis*; 13: *Theileria sp*. (sable); 14: *T. mutans*; 15: *T. parva*; 16: *T. taurotragi*; 17: *T. velifera*; 18: *T. ovis*; 19: *T. bicornis*; 20: *T. sp*. (buffalo); 21: *T. buffeli*; 22: *T. equi*. The results of the *E. ruminantium*-specific pCS20 qPCR are shown in column 23.

SampleID	Sublocation	AEZ	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
431	East Siboti	UM3	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
397	Mabusi	LM2 m	1	0	0	0	0	0	1	1	0	0	0	0	1	1	1	0	1	1	0	0	0	0	0
424	Kamunoit	LM2 m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
369	Karisa	LM2 m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
385	Otimong	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
405	Bulwani	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
379	Bumala A	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

408	Bumala A	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
368	Yiro w	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
419	Magombe East	LM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
425	Kokare	UM3	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
373	Otimong	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0
415	Otimong	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
428	Bumala A	LM1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
409	Bujwanga	LM3	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1
377	Magombe East	LM3	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
389	Magombe East	LM3	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	1	0
414	Magombe East	LM3	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	1	0
435	Magombe East	LM3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
380	East Siboti	UM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
386	Kokare	UM3	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
381	Karisa	LM2 m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
365	Igero	LM1	1	0	1	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	1	0	0	1
383	Bumala A	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	1	0
384	Bumala A	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0
423	Yiro w	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	1	0	0	1	0	0	0
366	Namboboto	LM2 s	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
445	Ojwando B	LM2 s	1	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	1	0
396	Magombe East	LM3	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	1	0	0	0	0
416	Magombe East	LM3	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
440	East Siboti	UM3	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0
376	Kidera	UM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
439	Kidera	UM3	1	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
450	Kidera	UM3	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0

438	Kokare	UM3	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
427	Karisa	LM2 m	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0
391	Bulwani	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
394	Yiro w	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
426	Simur East	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
454	Ojwando B	LM2 s	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
448	Kodiere	LM2 s	1	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0
406	Luanda	LM3	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
370	Bujwanga	LM3	1	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0
436	Mabusi	LM2 m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
392	Otimong	LM1	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
413	Bumala A	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
403	Yiro w	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
404	Ojwando B	LM2 s	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
398	Kodiere	LM2 s	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
375	Kokare	UM3	1	1	1	0	0	1	1	1	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0
390	Igero	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
421	Bulwani	LM1	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
418	Ikonzo	LM1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
455	Ikonzo	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
401	Magombe East	LM3	1	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
371	East Siboti	UM3	1	1	0	1	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
407	East Siboti	UM3	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
432	Otimong	LM1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
456	Igero	LM1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
374	Magombe East	LM3	1	0	0	1	0	0	1	1	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0
400	Magombe East	LM3	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0

402	Kamunoit	LM2 m	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0
393	Bumala A	LM1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	1	1	0	0	0	0	0
430	Yiro w	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
442	Namboboto	LM2 s	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
595	Ojwando B	LM2 s	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
399	Ojwando B	LM2 s	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
437	Luanda	LM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
388	Bujwanga	LM3	1	1	0	0	0	0	1	1	0	0	0	0	1	1	1	0	1	0	0	0	0	0
453	Kidera	UM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
446	Mabusi	LM2 m	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
417	Otimong	LM1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
412	Bukati	LM1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
410	Ikonzo	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
434	Simur East	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
378	Ojwando B	LM2 s	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
429	Bujwanga	LM3	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0
447	Bujwanga	LM3	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
443	Bujwanga	LM3	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	1	1	0	0	0	0	0
444	East Siboti	UM3	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
452	East Siboti	UM3	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
387	East Siboti	UM3	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0
422	Mabusi	LM2 m	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0
449	Bulwani	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
596	Bulwani	LM1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
433	Bulwani	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
597	Ikonzo	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
395	Yiro w	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0

457	Yiro w	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
420	Namboboto	LM2 s	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
451	Ojwando B	LM2 s	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
75	Kodiere	LM2 s	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
84	Kodiere	LM2 s	1	0	0	1	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
110	East Siboti	UM3	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
122	East Siboti	UM3	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
123	East Siboti	UM3	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
145	East Siboti	UM3	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
144	East Siboti	UM3	1	0	0	0	0	0	1	1	1	0	0	1	1	1	0	0	1	0	0	0	0	0	0
184	East Siboti	UM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
200	East Siboti	UM3	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
237	East Siboti	UM3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
233	East Siboti	UM3	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
275	East Siboti	UM3	1	1	0	1	0	0	1	1	1	0	0	1	1	1	0	0	1	1	0	0	0	0	0
274	East Siboti	UM3	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
301	East Siboti	UM3	1	0	0	1	0	0	1	1	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0
360	East Siboti	UM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
543	East Siboti	UM3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
569	East Siboti	UM3	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
568	East Siboti	UM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
593	East Siboti	UM3	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
510	East Siboti	UM3	1	1	0	1	0	0	1	1	1	0	0	1	1	1	0	0	1	0	0	0	0	0	0
74	East Siboti	UM3	1	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
98	East Siboti	UM3	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
129	Kidera	UM3	1	1	0	1	0	0	1	1	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0
140	Kidera	UM3	1	1	0	1	0	0	1	1	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0

182	Kidera	UM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
183	Kidera	UM3	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
192	Kidera	UM3	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
174	Kidera	UM3	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
175	Kidera	UM3	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	1	0	0	0	0
253	Kidera	UM3	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
254	Kidera	UM3	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
269	Kidera	UM3	1	1	0	1	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0
290	Kidera	UM3	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
289	Kidera	UM3	1	1	0	0	0	0	1	1	1	0	0	0	1	1	0	0	1	0	0	0	0	0
316	Kidera	UM3	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
315	Kidera	UM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
327	Kidera	UM3	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	1	0	0	0	0
328	Kidera	UM3	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
557	Kidera	UM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
558	Kidera	UM3	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	1	0	0	0	0
583	Kidera	UM3	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
472	Kidera	UM3	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
494	Kidera	UM3	1	0	0	1	0	0	1	1	0	0	0	0	1	1	1	0	1	0	0	0	0	0
493	Kidera	UM3	1	1	0	1	0	0	1	1	1	1	0	0	1	1	0	0	1	0	0	0	0	0
69	Kidera	UM3	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
93	Kidera	UM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94	Kokare	UM3	1	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
178	Kokare	UM3	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
161	Kokare	UM3	1	1	0	0	0	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0
162	Kokare	UM3	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
47	Kokare	UM3	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0

246	Kokare	UM3	1	1	0	0	0	0	1	1	1	1	0	0	1	1	0	0	1	0	0	0	0	0	0
225	Kokare	UM3	1	0	0	1	0	0	1	1	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0
224	Kokare	UM3	0	0	0	0	0	0	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1
264	Kokare	UM3	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
285	Kokare	UM3	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
307	Kokare	UM3	1	1	0	1	0	0	1	1	1	1	0	0	1	1	0	0	1	0	0	0	0	0	0
342	Kokare	UM3	1	1	0	1	0	0	1	1	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0
343	Kokare	UM3	1	1	0	0	0	0	1	1	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0
321	Kokare	UM3	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
322	Kokare	UM3	1	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
532	Kokare	UM3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
578	Kokare	UM3	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
467	Kokare	UM3	1	0	0	1	0	0	1	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0
518	Kokare	UM3	1	1	0	1	0	0	1	1	0	0	0	0	1	1	1	0	1	1	0	0	0	0	0
520	Kokare	UM3	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0
519	Kokare	UM3	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0
521	Kokare	UM3	1	0	0	1	0	0	1	1	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0
64	Kokare	UM3	1	1	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0
31	Kokare	UM3	1	1	0	0	0	0	1	1	1	1	0	0	1	1	0	0	1	0	0	0	0	0	0
115	Mabusi	LM2 m	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
147	Mabusi	LM2 m	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	1	0	0	0	0	0
148	Mabusi	LM2 m	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0
187	Mabusi	LM2 m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
188	Mabusi	LM2 m	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
210	Mabusi	LM2 m	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0
211	Mabusi	LM2 m	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
206	Mabusi	LM2 m	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

243	Mabusi	LM2 m	1	0	0	1	0	0	1	1	1	1	0	1	1	1	0	0	1	0	0	0	0	0	0
259	Mabusi	LM2 m	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
279	Mabusi	LM2 m	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
278	Mabusi	LM2 m	1	1	0	1	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
304	Mabusi	LM2 m	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
339	Mabusi	LM2 m	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0
340	Mabusi	LM2 m	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
352	Mabusi	LM2 m	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
548	Mabusi	LM2 m	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
574	Mabusi	LM2 m	1	0	0	1	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
573	Mabusi	LM2 m	1	0	0	0	0	0	1	1	1	1	0	0	1	1	0	0	1	0	0	0	0	0	0
462	Mabusi	LM2 m	1	1	0	0	0	0	1	1	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0
482	Mabusi	LM2 m	1	1	0	1	0	0	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
514	Mabusi	LM2 m	1	1	0	0	0	0	1	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0
515	Mabusi	LM2 m	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
79	Mabusi	LM2 m	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
80	Kamunoit	LM2 m	1	0	0	1	0	0	1	1	1	1	0	1	1	1	1	0	1	1	0	0	0	0	0
102	Kamunoit	LM2 m	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0
130	Kamunoit	LM2 m	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
142	Kamunoit	LM2 m	1	0	0	1	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
155	Kamunoit	LM2 m	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
196	Kamunoit	LM2 m	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
209	Kamunoit	LM2 m	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
218	Kamunoit	LM2 m	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
219	Kamunoit	LM2 m	1	0	1	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1
16	Kamunoit	LM2 m	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
257	Kamunoit	LM2 m	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0

272	Kamunoit	LM2 m	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
295	Kamunoit	LM2 m	1	1	0	1	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
294	Kamunoit	LM2 m	1	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
319	Kamunoit	LM2 m	1	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
355	Kamunoit	LM2 m	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
356	Kamunoit	LM2 m	1	0	0	1	0	0	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
538	Kamunoit	LM2 m	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
588	Kamunoit	LM2 m	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
587	Kamunoit	LM2 m	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
503	Kamunoit	LM2 m	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
501	Kamunoit	LM2 m	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
502	Kamunoit	LM2 m	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
530	Kamunoit	LM2 m	1	1	0	1	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
85	Kamunoit	LM2 m	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
62	Karisa	LM2 m	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
86	Karisa	LM2 m	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
111	Karisa	LM2 m	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
124	Karisa	LM2 m	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
125	Karisa	LM2 m	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
146	Karisa	LM2 m	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
185	Karisa	LM2 m	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
202	Karisa	LM2 m	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
201	Karisa	LM2 m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
238	Karisa	LM2 m	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
234	Karisa	LM2 m	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
277	Karisa	LM2 m	1	1	0	1	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
276	Karisa	LM2 m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

302	Karisa	LM2 m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
336	Karisa	LM2 m	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
349	Karisa	LM2 m	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
348	Karisa	LM2 m	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
545	Karisa	LM2 m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
570	Karisa	LM2 m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
544	Karisa	LM2 m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
594	Karisa	LM2 m	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
458	Karisa	LM2 m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
478	Karisa	LM2 m	1	0	0	1	0	0	1	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0
66	Karisa	LM2 m	1	0	0	1	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0
89	Otimong	LM1	1	1	0	1	0	0	1	1	0	0	0	0	0	1	1	1	0	0	1	0	0	0
88	Otimong	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
117	Otimong	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
116	Otimong	LM1	1	1	0	1	0	0	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0
138	Otimong	LM1	1	1	0	0	0	1	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
149	Otimong	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0
189	Otimong	LM1	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
158	Otimong	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
212	Otimong	LM1	1	1	0	1	0	0	1	1	1	1	0	1	0	1	1	1	0	0	1	0	0	0
213	Otimong	LM1	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
207	Otimong	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
260	Otimong	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
280	Otimong	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
281	Otimong	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
305	Otimong	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
363	Otimong	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0

549	Otimong	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
575	Otimong	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
463	Otimong	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
484	Otimong	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
483	Otimong	LM1	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
81	Otimong	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
82	Igero	LM1	1	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
106	Igero	LM1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
107	Igero	LM1	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
120	Igero	LM1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
157	Igero	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0
167	Igero	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0
168	Igero	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
197	Igero	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
19	Igero	LM1	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
20	Igero	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
231	Igero	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	0	0	0	0
273	Igero	LM1	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
298	Igero	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
299	Igero	LM1	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
309	Igero	LM1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0
359	Igero	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
358	Igero	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
541	Igero	LM1	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
540	Igero	LM1	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0
567	Igero	LM1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
476	Igero	LM1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0

508	Igero	LM1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
507	Igero	LM1	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	1	1	0	0	0	0	0
506	Igero	LM1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0
70	Igero	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
95	Bulwani	LM1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
179	Bulwani	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
163	Bulwani	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
164	Bulwani	LM1	1	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0
35	Bulwani	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
248	Bulwani	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
247	Bulwani	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
226	Bulwani	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
265	Bulwani	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0
266	Bulwani	LM1	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
286	Bulwani	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
310	Bulwani	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0
323	Bulwani	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
533	Bulwani	LM1	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
552	Bulwani	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
580	Bulwani	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0
579	Bulwani	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
468	Bulwani	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
489	Bulwani	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
490	Bulwani	LM1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
491	Bulwani	LM1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
522	Bulwani	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
76	Bulwani	LM1	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0

77	Bukati	LM1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
99	Bukati	LM1	1	0	1	0	1	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
131	Bukati	LM1	1	1	0	0	0	0	1	1	1	1	0	0	1	0	0	0	1	0	0	0	0	0	0
132	Bukati	LM1	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
141	Bukati	LM1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
152	Bukati	LM1	0	0	0	0	0	0	1	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0
151	Bukati	LM1	1	1	0	0	0	0	1	1	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0
193	Bukati	LM1	0	0	0	0	0	0	1	1	1	1	0	0	1	1	0	0	1	0	0	0	0	0	0
194	Bukati	LM1	1	1	0	0	0	0	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0
14	Bukati	LM1	1	0	0	0	0	0	1	1	1	0	0	1	1	1	0	0	1	0	0	0	0	0	0
216	Bukati	LM1	1	1	0	0	0	0	1	1	1	0	0	1	0	1	0	0	1	0	0	0	0	0	0
270	Bukati	LM1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
292	Bukati	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
291	Bukati	LM1	1	1	0	1	0	0	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
317	Bukati	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
57	Bukati	LM1	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
329	Bukati	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0
330	Bukati	LM1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
559	Bukati	LM1	1	1	0	1	1	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
560	Bukati	LM1	1	0	0	1	0	0	1	1	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0
584	Bukati	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
473	Bukati	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0
495	Bukati	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
496	Bukati	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0
527	Bukati	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0
103	Bukati	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
105	Bukati	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0

104	Ikonzo	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
133	Ikonzo	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
136	Ikonzo	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
143	Ikonzo	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
156	Ikonzo	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
166	Ikonzo	LM1	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
220	Ikonzo	LM1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
18	Ikonzo	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
17	Ikonzo	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
230	Ikonzo	LM1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
296	Ikonzo	LM1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0
297	Ikonzo	LM1	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
344	Ikonzo	LM1	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
345	Ikonzo	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
357	Ikonzo	LM1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
539	Ikonzo	LM1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
566	Ikonzo	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
565	Ikonzo	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
564	Ikonzo	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
589	Ikonzo	LM1	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
505	Ikonzo	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
504	Ikonzo	LM1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
63	Ikonzo	LM1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
112	Bumala A	LM1	1	0	0	1	0	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
203	Bumala A	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0
240	Bumala A	LM1	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
239	Bumala A	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0

303	Bumala A	LM1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
350	Bumala A	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
362	Bumala A	LM1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
361	Bumala A	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0
547	Bumala A	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
546	Bumala A	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
459	Bumala A	LM1	1	0	0	1	0	0	1	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
479	Bumala A	LM1	1	1	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
480	Bumala A	LM1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
512	Bumala A	LM1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
61	Bumala A	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
83	Yiro w	LM1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
108	Yiro w	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
109	Yiro w	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
121	Yiro w	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
199	Yiro w	LM1	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
198	Yiro w	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
236	Yiro w	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
235	Yiro w	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
232	Yiro w	LM1	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
300	Yiro w	LM1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
335	Yiro w	LM1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
334	Yiro w	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
346	Yiro w	LM1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
347	Yiro w	LM1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
542	Yiro w	LM1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
591	Yiro w	LM1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

590	Yiro w	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
592	Yiro w	LM1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
477	Yiro w	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
509	Yiro w	LM1	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
73	Yiro w	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
135	Simur East	LM1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
134	Simur East	LM1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
181	Simur East	LM1	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
190	Simur East	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
191	Simur East	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
173	Simur East	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
252	Simur East	LM1	1	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
251	Simur East	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
228	Simur East	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
229	Simur East	LM1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
268	Simur East	LM1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
288	Simur East	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
308	Simur East	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
314	Simur East	LM1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
313	Simur East	LM1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
326	Simur East	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
535	Simur East	LM1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
556	Simur East	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
555	Simur East	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
582	Simur East	LM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
470	Simur East	LM1	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
471	Simur East	LM1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0

526	Simur East	LM1	1	1	0	1	0	0	1	1	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0
525	Simur East	LM1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	Simur East	LM1	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
100	Namboboto	LM2 s	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
127	Namboboto	LM2 s	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
153	Namboboto	LM2 s	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
154	Namboboto	LM2 s	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
195	Namboboto	LM2 s	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
176	Namboboto	LM2 s	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0
15	Namboboto	LM2 s	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
601	Namboboto	LM2 s	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0
598	Namboboto	LM2 s	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
599	Namboboto	LM2 s	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
600	Namboboto	LM2 s	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0
318	Namboboto	LM2 s	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
333	Namboboto	LM2 s	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
331	Namboboto	LM2 s	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
332	Namboboto	LM2 s	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
537	Namboboto	LM2 s	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
561	Namboboto	LM2 s	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
585	Namboboto	LM2 s	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
474	Namboboto	LM2 s	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
497	Namboboto	LM2 s	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
498	Namboboto	LM2 s	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
528	Namboboto	LM2 s	1	0	0	1	0	0	1	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0
529	Namboboto	LM2 s	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
87	Namboboto	LM2 s	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

113	Ojwando B	LM2 s	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
137	Ojwando B	LM2 s	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
114	Ojwando B	LM2 s	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
186	Ojwando B	LM2 s	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
170	Ojwando B	LM2 s	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
169	Ojwando B	LM2 s	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
205	Ojwando B	LM2 s	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
204	Ojwando B	LM2 s	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
241	Ojwando B	LM2 s	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
242	Ojwando B	LM2 s	1	1	0	1	0	0	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
258	Ojwando B	LM2 s	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
338	Ojwando B	LM2 s	1	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
337	Ojwando B	LM2 s	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
351	Ojwando B	LM2 s	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
571	Ojwando B	LM2 s	1	0	0	0	0	1	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
572	Ojwando B	LM2 s	1	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
460	Ojwando B	LM2 s	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
461	Ojwando B	LM2 s	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
481	Ojwando B	LM2 s	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
513	Ojwando B	LM2 s	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
67	Ojwando B	LM2 s	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
68	Kodiere	LM2 s	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
92	Kodiere	LM2 s	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
119	Kodiere	LM2 s	1	0	0	1	0	0	1	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0
177	Kodiere	LM2 s	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
160	Kodiere	LM2 s	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
245	Kodiere	LM2 s	1	0	0	1	0	0	1	1	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0

244	Kodiere	LM2 s	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
222	Kodiere	LM2 s	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
223	Kodiere	LM2 s	1	1	0	0	0	0	1	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0
263	Kodiere	LM2 s	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
262	Kodiere	LM2 s	1	1	0	1	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
283	Kodiere	LM2 s	1	1	0	1	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
284	Kodiere	LM2 s	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
341	Kodiere	LM2 s	1	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
59	Kodiere	LM2 s	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
320	Kodiere	LM2 s	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
531	Kodiere	LM2 s	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
551	Kodiere	LM2 s	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
577	Kodiere	LM2 s	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
466	Kodiere	LM2 s	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
465	Kodiere	LM2 s	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
488	Kodiere	LM2 s	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
36	Kodiere	LM2 s	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
71	Luanda	LM3	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
96	Luanda	LM3	1	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
97	Luanda	LM3	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
128	Luanda	LM3	1	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
139	Luanda	LM3	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
180	Luanda	LM3	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
165	Luanda	LM3	1	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
172	Luanda	LM3	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
249	Luanda	LM3	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
250	Luanda	LM3	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0

227	Luanda	LM3	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
267	Luanda	LM3	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
287	Luanda	LM3	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
311	Luanda	LM3	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
312	Luanda	LM3	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
324	Luanda	LM3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
325	Luanda	LM3	1	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
534	Luanda	LM3	1	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
554	Luanda	LM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
553	Luanda	LM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
581	Luanda	LM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
469	Luanda	LM3	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
492	Luanda	LM3	1	0	0	1	0	0	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
523	Luanda	LM3	1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
524	Luanda	LM3	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
90	Luanda	LM3	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
91	Bujwanga	LM3	1	0	0	1	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
118	Bujwanga	LM3	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
150	Bujwanga	LM3	1	0	0	1	0	0	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
159	Bujwanga	LM3	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
214	Bujwanga	LM3	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
215	Bujwanga	LM3	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
208	Bujwanga	LM3	1	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
221	Bujwanga	LM3	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
261	Bujwanga	LM3	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0
282	Bujwanga	LM3	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0
306	Bujwanga	LM3	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0

364	Bujwanga	LM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
550	Bujwanga	LM3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
464	Bujwanga	LM3	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
485	Bujwanga	LM3	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
487	Bujwanga	LM3	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
486	Bujwanga	LM3	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
517	Bujwanga	LM3	1	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
516	Bujwanga	LM3	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
576	Bujwanga	LM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
101	Bujwanga	LM3	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
126	Magombe East	LM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	Magombe East	LM3	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	1	1	0	0	0	0	0
217	Magombe East	LM3	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
255	Magombe East	LM3	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
256	Magombe East	LM3	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
271	Magombe East	LM3	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
293	Magombe East	LM3	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0
353	Magombe East	LM3	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
354	Magombe East	LM3	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
562	Magombe East	LM3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
563	Magombe East	LM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
586	Magombe East	LM3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
475	Magombe East	LM3	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
499	Magombe East	LM3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
500	Magombe East	LM3	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0

Appendix 2

Table A2: Results of the nested p104 PCR assay for the specific detection of *T. parva* in cattle blood samples.

Sample ID	Sublocation	AEZ	p104
RDN 431	E. Siboti	UM3	0
RDN 397	Mabusi	LM2 m	0
RDN 424	Kamunoit	LM2 m	0
RDN 369	Karisa	LM2 m	1
RDN 385	Otimong	LM1	0
RDN 405	Bulwani	LM1	0
RDN 379	Bumala A	LM1	0
RDN 408	Bumala A	LM1	0
RDN 368	Yiro w	LM1	0
RDN 419	Magombe East	LM3	0
RDN 425	Kokare	UM3	0
RDN 373	Otimong	LM1	1
RDN 415	Otimong	LM1	0
RDN 428	Bumala A	LM1	1
RDN 409	Bujwanga	LM3	0
RDN 377	Magombe East	LM3	1
RDN 389	Magombe East	LM3	0
RDN 414	Magombe East	LM3	1
RDN 435	Magombe East	LM3	0
RDN 380	E. Siboti	UM3	0
RDN 386	Kokare	UM3	1
RDN 381	Karisa	LM2 m	0
RDN 365	Igero	LM1	1
RDN 383	Bumala A	LM1	1
RDN 384	Bumala A	LM1	1

RDN 423	Yiro w	LM1	1
RDN 366	Namboboto	LM2 s	0
RDN 445	Ojwando B	LM2 S	1
RDN 396	Magombe East	LM3	0
RDN 416	Magombe East	LM3	0
RDN 440	E. Siboti	UM3	1
RDN 376	Kidera	UM3	0
RDN 439	Kidera	UM3	0
RDN 438	Kokare	UM3	1
RDN 427	Karisa	LM2 m	1
RDN 391	Bulwani	LM1	0
RDN 394	Yiro w	LM1	0
RDN 426	Simur East	LM1	0
RDN 454	Ojwando B	LM2 s	0
RDN 448	Kodiere	LM2 s	0
RDN 406	Luanda	LM3	0
RDN 370	Bujwanga	LM3	0
RDN 436	Mabusi	LM2 m	0
RDN 392	Otimong	LM1	1
RDN 403	Yiro w	LM1	0
RDN 404	Ojwando B	LM2 s	0
RDN 398	Kodiere	LM2 s	0
RDN 375	Kokare	UM3	1
RDN 390	Igero	LM1	0
RDN 421	Bulwani	LM1	0
RDN 418	Ikonzo	LM1	0
RDN 455	Ikonzo	LM1	1
RDN 401	Magombe East	LM3	1
RDN 371	E. Siboti	UM3	1
RDN 407	E. Siboti	UM3	0
RDN 432	Otimong	LM1	0
RDN 456	Igero	LM1	1
RDN 374	Magombe East	LM3	1
RDN 400	Magombe East	LM3	0

RDN 402	Kamunoit	LM2 m	1
RDN 430	Yiro w	LM1	0
RDN 442	Namboboto	LM2 s	0
RDN 595	Ojwando B	LM2 s	0
RDN 399	Ojwando B	LM2 s	1
RDN 437	Luanda	LM3	0
RDN 388	Bujwanga	LM3	0
RDN 446	Mabusi	LM2 m	0
RDN 417	Otimong	LM1	0
RDN 412	Bukati	LM1	0
RDN 410	Ikonzo	LM1	0
RDN 434	Simur East	LM1	0
RDN 378	Ojwando B	LM2 s	0
RDN 429	Bujwanga	LM3	0
RDN 447	Bujwanga	LM3	1
RDN 443	Bujwanga	LM3	1
RDN 444	E. Siboti	UM3	0
RDN 452	E. Siboti	UM3	0
RDN 387	E. Siboti	UM3	1
RDN 449	Bulwani	LM1	0
RDN 596	Bulwani	LM1	1
RDN 433	Bulwani	LM1	1
RDN 597	Ikonzo	LM1	0
RDN 395	Yiro w	LM1	0
RDN 457	Yiro w	LM1	0
RDN 420	Namboboto	LM2 s	0
RDN 451	Ojwando B	LM2 s	0