Global Scientific JOURNALS

GSJ: Volume 9, Issue 4, April 2021, Online: ISSN 2320-9186
www.globalscientificjournal.com

Molecular evidence of vertical transmission of *Theileria parva* from cows to offspring in Morogoro, Eastern Tanzania

Madoshi, PB[1*], Mbassa, KG[1,2] and Komba, EVG[2]

[1]ST. Francis University College of Health and Allied Sciences, P. O. Box 175, Ifakara, Tanzania
[2]College of Veterinary Medicine and Biomedical Sciences, Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, P.O. Box 3021, Morogoro, Tanzania

KeyWords: Theileria parva; Cattle; vaccine strains; Muguga strains; vertical transmission; Tanzania

ABSTRACT

Introduction: The Apicomplexan protozoa *Theileria parva* causes the fatal disease East Coast fever in cattle in eastern and southern Africa, the transmitting vector being the brown ear three host tick Rhipicephalus appendiculatus. The high mortality rates in young calves caused by the disease in endemic places have led to suspicious of other means of transmission. The disease lacks the information that describe vertical transmission in new-borne calves; thus this study intended to describe the molecular evidence of maternal calf transmission of Theileria parva

Methodology: Blood samples were collected from 21 clinically examined five day old calves and their respective dams; and from 18 aborted foetuses. These were subjected to genomic DNA extraction using DNA extraction kit. Analysis of the extracted DNA was made by spectrophotometer and Agarose gel electrophoresis.

Results: Four out of 21 (19.0%) samples from adult cows, six out of 21 (24.0%) samples from calves and 15 out of 18 samples (83.3%) from foetuses of different gestation lengths fluoresced. Five samples showed bands (700-1000 bp) after p104 amplification, which is 20% of the samples with genomic DNA. The PCR product of one (1) sample had bands (300- 400bp) that resembled the T. parva sporozoites stabilate vaccine strains (Muguga), two (2) samples had bands (250- 350bp) that resembled the T. parva Marikebuni, and another two samples had bands (100-500bp) which had mixed band patterns which do not resemble any of the vaccine strains, indicating that they are unique to Tanzania.

Conclusion: Since *T. parva* DNA was observed in foetuses, *T. parva* is transmitted vertically through the placenta and (2) there are more *T. parva* stocks in Tanzania other than Muguga, Kiambu 5, Serengeti transformed and the earlier isolated *T. parva Melela*. Control strategies for the parasite should therefore consider means of transmission other than by the vector ticks. Vaccine development for ECF control should be preceded by determining the local *T. parva* complex circulating in the country.

Introduction

The genus *Theileria* comprises tick-transmitted sporozoan protozoa that are the causative agents of a variety of disease syndromes in domestic and wild ruminants [1]. Theileria are unique among protozoa in that certain species are capable of immortalizing either mammalian lymphocytes or cells of the monocyte/ macrophage lineage that they infect. *Theileria parva* the causative agent of lymphoproliferative disease, East coast fever (ECF) in cattle, is an important tick-borne blood parasite of cattle and wild ruminants in Eastern, Central and Southern Africa [2,3,4] (. The disease is mainly manifested by lympadenopathy and pulmonary oedema, signs being enlargement of the superficial lymphnodes, anorexia, pyrexia (41.5-42ºC), dyspnoea, petechiations on the heart and kidneys, froth in the lungs, trachea and nostrils, corneal opacity and occasionally nervous involvement [5,6]. Formerly, this protozoon was thought to have three distinct subspecies which were described basing either on disease form caused or the location of diseases occurrence. *T. parva parva* causing classical ECF which occurred mostly in East Africa, *T. parva lawrencei* responsible for Corridor disease transmitted from buffalo to cattle [2,6] and *T. parva bovis*, the causative agent of Zimbabwe theileriosis, a more benign form also known as "January disease" [6,8].

The development of scientific techniques on the diagnosis and nomenclature of different protozoan species has described the scenario beyond doubt that: the classical cause of ECF is *T. parva* [2]. It is transmitted *in* the field is trans-stadially by a three host, brown ear tick *Rhipicephalus appendiculatus*. ECF has to a large extent limited the introduction of productive exotic cattle breeds (*Bos taurus*) in most parts of East, Central and Southern Africa. Despite the well-known economic losses caused by this protozoan diseases [7,15], the only method available against ECF control is the infection and treatment method (ITM) using the tick derived live infective *T. parva* sporozoites and simultaneous treatment with 20% long acting Oxtetracycline [8].

Transplacental transmission has been demonstrated in a number of haematotropic parasites such as *Plasmodium falciparum* in human [8], *Anaplasma* spp. and *Theileria sergenti* in cattle [9]. Prenatal *T. sergenti* in cattle showed adverse reaction in infected calves when exposed to stress or concurrent infection with other parasites or viruses; they experienced severe clinical signs which were associated with high morbidity but low mortality [9,10]. Mbassa et al. [10] demonstrated *T. parva* schizonts in prescapular and parotid lymphnode lymphocytes of newborn calves and reported possibility of the parasites being transmitted vertically from pregnant cows to calves. Henjewele [11] has described histological evidence on possibilities of vertical transmission of *T. parva*. Therefore the current investigation intended to provide molecular evidence of vertical transmission of *T. parva* in cattle.

Materials and methods

Study area

The study was carried out in Kambala and Melela villages in Mvomero district (Fig. 3: Map of the Melela and Kambala Villages) and Morogoro municipal abattoir. The two villages in Mvomero district are inhabited by pastoralists who keep cattle for their subsistence under extensive system. In addition the climatic condition of the area favours the survival of the vector. Due to presence of the brown ear tick, ECF has been endemic especially in the calves where significant mortalities are reported to the nearby livestock field officers by the livestock owners. On the other hand the Morogoro municipal was chosen for the purpose of sampling foetuses that were from dams which come from different geographical location in Tanzania.

Sample collection in the field

Whole blood was collected from 21 neonatal calves of not more than 5 days old and their respective dams. The pregnant dams at their last trimester were clinically examined basing on clinical manifestation of ECF; these included the enlargement of parotid and prescapular lymphnodes (more than 4cm), rough hair coat, lacrymation and pyrexia (39.9°C and above). The age of the calves was determined through the owner's information, records, umbilical stump and hair pattern.

At least 3ml of blood was collected from ECF suspected dams before parturition by jugular vein puncture using sterile needles in vacuum tubes (vaccuette® Greiner bio-one GmbH, Austria) coated with potassium ethylene diamine-tetraacetic acid (K_3EDTA) as an anticoagulant (1mg/ml). On the other hand the samples from the calves were collected after birth within five days of life or else if the animal aborted

blood was collected by severing the jugular vein or the umbilical artery. The samples were labelled in the field, transported in a cool box and stored at -20°C till time deoxyribonucleic acid (DNA) extraction. The procedures were adopted from (Abdel-rady [22]. Under this sampling category there were a total of 42 samples; 21 from cows and 21 samples neonates.

Sample collection at the abattoir

Pregnancy diagnosis was done to cows brought to the abattoir during the ante mortem inspection to determine the age of the pregnancy. Pregnant animals were sampled after slaughter whereas blood from foetuses was collected following severing of the jugular vein or the umbilical cord and the blood samples were collected from a total of eighteen (18) foetuses. The ages of recovered foetuses were determined using the Crown (top of the head) to the rump length measurement. The foetuses were grouped into trimesters using estimated gestation period for humans. Under this sample category there were a total of 36 blood samples eighteen from each the slaughtered pregnant cows and their respective foetuses.

DNA extraction and PCR assay

Genomic DNA was extracted from the samples with the help of DNA extraction kit according to the manufacturer's instructions (QIA amp blood kit, Qiagen, LTD, UK). The quality and quantity of the extracted DNA was assessed using agarose gel electrophoresis and spectrophotometer respectively. The samples which had strong bands on electrophoresis and DNA quantity of 50ng and above were considered for DNA amplification. The DNA amplification was done using the semi-nested PCR with three distinct set of primers. The first round of amplification used the p104 forward and reverse primers, whereas the second round used the Normal forward and reverse primers. The primers are designed to encompass the amplification of the second locus code for a protein *p104* with polymorphic region giving 1000bp amplicons. The analysis was done using the PCR methods as it has been proven to be able to characterize and distinguish *T. parva* from multiple *Theileria* spp. infections in field samples. In general the method of amplification was adopted from (Bishop et al., 2001)

The extracted DNA was used as a template in the PCR assays: Initially single copy of genes encoding immunogenic proteins *p104* of about 1000bp region was amplified, as described Iams et al. [2]. Each PCR reaction contained buffer solution (50mM KCL, 10mM Tris-HCL pH 8.3), 2.5mM $MgCl_2$, and 2.5mM dNTP, 40pmol of each primer, 1.5U Taq polymerase and 10.5µl of PCR water, the final volume for this reaction was 25µl. The amplification program involved step 1: Initial denaturation at 94^{o}C for 5 minute for 1 cycle; Step 2; denaturing at 94^{o}C for 30 seconds; Step 3; Annealing at a temperature of 58^{o}C for 45 seconds; Step 4; Extension at a temperature of 72^{o}C for 1 minute, Steps 2, 3 and 4 were repeated for 35 cycles and final elongation was at 72^{o}C for 7 minute with one cycle. PCR products were resolved by 1.5% agarose mini gels, stained with ethidium bromide with a loading dye for detection of *p104* bands and photographed under ultraviolet illumination.

Restriction Fragment length polymorphism (RFLP) analysis

Restriction enzyme *Alu1* was used to digest the amplified PCR products generated by *p104* primer as described by Geysen et al. [23], and amplicons with 700bp to 1000 bp sizes were selected for digestion. Digestion was conducted for *Alu I* using buffer 10X (60mM Tris-HCL (pH 7.5), 500mM NaCl, 60mM $MgCl_2$ and 10mM DDT. The mixture contained 1.5µl of 1XTBE, 9.2µl of RNase free water, 0.3µl of restriction enzymes and 4.0 µl of PCR amplified product to obtain a total volume of 15µl, this digestion was performed overnight at 37°C. An aliquot of the sample was subsequently analyzed by running through a 12% polyacrylamide gel, and thereafter the gel was stained for 40 minutes using 5µl of SYBR green mixed with 50ml of 1X TBE.

Data analysis

The collected data were entered in Microsoft excel Spread sheet analysed using SPSS 20. Descriptive statistics were computed to determine proportions of samples that fluoresced and those which showed bands after *p104* amplification.

Results

The study included livestock owners who keep their animals under the extensive rearing system. This kind of cattle husbandry is typically practised in indigenous farmers who keep the Tanzania short horn zebu breeds of cattle. Samples were collected from cattle following the consent of the owner and in some families request of the owners was adhered as well. Blood samples were collected from 60 animals where 21 samples were from cows, 21 were from calves of less than five days, 8 and 10 samples were from calves of first and second trimester, respectively. Following DNA extraction and quantification, it was found that 35 samples had DNA bands which were either above or below 700bp; the samples with bands above 700bp were 25 (41.6%) of the samples (Table 1).

Table 1: Summary of the results obtained following DNA extraction from blood samples collected from cows, calves and foetuses

Age category	Blood samples for DNA extraction	Results for DNA electrophoresis (%)
Cow	21	14 (19.0)
Calves	21	13 (29.0)
Foetuses (Trimester I)	08	03 (88.0)
Foetuses (Trimester II)	10	05 (80.0)
Total	**60**	**25 (41.6)**

Basing on the above observation only 25 samples were carried for *p104* amplification, the criterion of selection was the resemblance of the bands among the samples. That is if two or more samples resembled on the band patterns, only one was taken for DNA amplification. The extracted DNA samples were amplified to obtain the amplicons using *p104* primers (Forward, Reverse and Normal Forward) in which twelve (48%) samples (Table 2) showed 700bp or more as compared to the ladder following electrophoresis analysis (Fig. 1).

Figure 1: Agar gel electrophoresis of PCR amplified DNA *Theileria parva DNA*

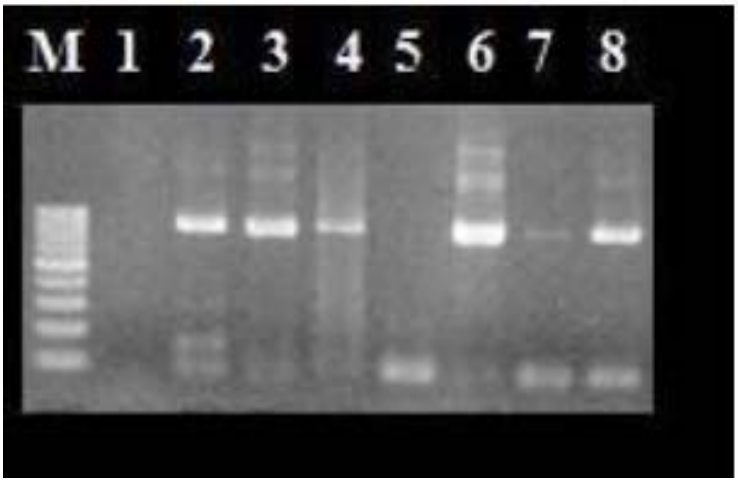


Fig 1: M is 100bp DNA marker, DNA lane 1 is negative control, Lane 2, 3, 4, 5, 6 and 7 are animal samples and Lane 8 is a positive control. The sample with lane 5 was left during digestion.

Table 2: Fluorescence after polymerization of the DNA samples with p104

Age category	DNA electrophoresis results	*p104* enzyme digestion results (%)
Cow	04	02 (50.0)
Calves	06	04 (66.7)
Foetuses (Trimester I)	07	02 (28.6)
Foetuses (Trimester II)	08	04 (50.0)
Total	**25**	**12 (48.0)**

After digestion of the PCR products (Table 3) using an enzyme *Alu* 1, followed by staining with SYBR green; it was revealed that two samples from a foetus (Lane number 2) aged 2 months (in utero) had bands (250- 400bp) which resembled the positive control which is "Muguga" strain (Lane number 6). Lane 1 (a sample from a 3 day old calf) and Lane 5 (a sample from a 5 month old foetus) resembled Marikebuni (Katete) strain, which is also a vaccine strain in the "Muguga" cocktail as previously described by Bishop et al. [25] and Geysen et al. [24]. Lane 3 and 4 show mixed bands of 100- 400bp which resembled neither the vaccine strains nor the wild *T. parva* isolates (Figure 2).

Figure 2: P104 PCR- RFLP profile (*Alu 1* enzyme).

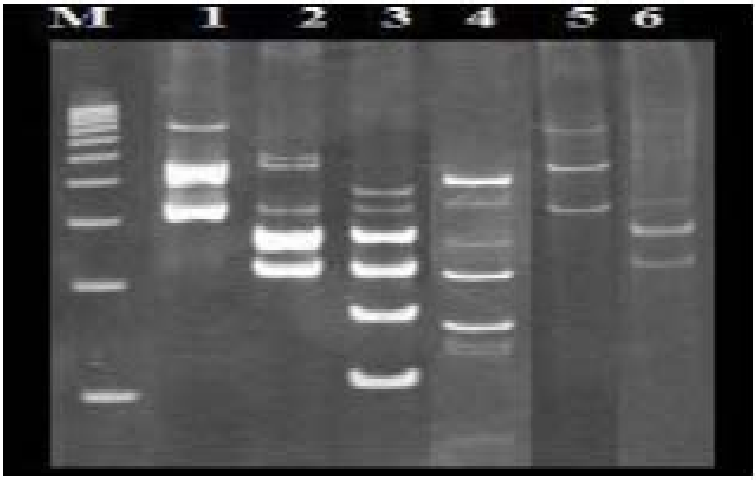


Fig. 2: Lane M is the 100bp DNA ladder, lane 1 (calf 5 days old), Lane 2, 4 and 5 are foetus of 2, 4 and 3 months old respectively and Lane 3 adult cow with *T. parva* DNA and Lane 6 is a positive control.

Table 2: Fluorescence after polymerization of the DNA samples with p104

Age category	DNA electrophoresis results	*p104* enzyme digestion results (%)
Cow	04	02 (50.0)
Calves	06	04 (66.7)
Foetuses (Trimester I)	07	02 (28.6)
Foetuses (Trimester II)	08	04 (50.0)
Total	**25**	**12 (48.0)**

Table 3: Summary of the results obtained after digestion of PCR products with *Alu* I enzyme

Age category	*p104* results after *Alu I* digestion	PAGE analysis (bp)	Associated *T. parva* strains
Cow	02	100-350	Lane 3 - Mixed bands
Calves	03	100-350	Lane 4 - Mixed bands
	01	300-400	Lane 1 - Marikebuni
Foetuses (Trimester I)	02	250-400	Lane 2 – Muguga
Foetuses (Trimester II)	04	300-400	Lane 5 - Marikebuni

Discussion

This study utilised *T. parva* genomic DNA extracted from the blood of foetuses (first and second trimester) and very naive calves (1-5 days old), which are presumed not to have been exposed to tick bites. These findings provide confirmatory molecular evidence that *T. parva* parasites are vertically transmitted through the syndesmochorial cotyledonary placenta. Our finding therefore supports the findings of Mbyuzi et al. [12] who provided preliminary evidence of vertical transmission of *Theileria parva* sporozoites from ECF immunized cows to offspring in southern Tanzania. Transplacental transmission during the active infection and at carrier state has been reported also in *Theileria equi* infection in foals [7]. Vertical transmission of these parasites could be beneficial in the control of the disease in that calves can be born immune due to maternal conferred immunity. This mode of transmission may also yield deleterious effects, particularly if schizonts in newborn calves received from the dam via placenta mature to merozoites and piroplasms [10,12,13].

The presence of the molecular markers of *T. parva* in foetuses is explained by the fact that acquisition of the infection is through the trigger mechanism of macroschizonts infected lymphocytes to lymphoid and non-lymphoid organs before birth across the placenta [6]. The parasites also induce transformation of the lymphocytes they infect leading to clonal extension in the placenta, which shields them from destruction, which presumably is the same as in human trans-placental malaria. This is better explained by the findings of who reported that infected lymphocytes divide into two daughter cells; therefore this automated phenomenon increases the infective stages of the parasites significantly.

During cell mitosis *T. parva* become closely associated with the spindle microtubules and the parasite ensures that each daughter cell inherits infection meanwhile preventing themselves to natural apostosis. During this process the parasites trigger lymphocyte proliferation to cross the syndesmochrionic nature of the cotyledonary placenta leading to transmission of the parasite across the placenta. Similarly Henjewele [11] observed phenotypic changes when histologically examined the lymphocytes following *T. parva* infection in the foetuses of different ages. On the other hand Carmen and Sinai [6] reported the effect of parasitized erythrocytes adherence to the glycosaminoglycan chondroitin sulphate (CSA) and therefore the parasites are not removed by the selection process of the placenta leading to accumulation of parasites which bind to CSA in the placenta. The parasite creates enabling environment to through the placenta using the apoptotic mechanism of the infected gravid uterus; a scenario which has been explained in protozoan infections [6,14,15].

Apparently the current study has been able to establish that there are several *T. parva* strains in the field (fig. 2). This finding complicates the epidemiology and the control of the disease particularly by immunization strategy. Previously Norval et al. [16] and Uilenberg [17] highlighted that problems do occur in the recognition of suitable antigenic stocks for immunization. The authors argued that any vaccination scheme can only follow after a careful assessment of the local complex of *T. parva* strains. This information could be very useful to authorities in the country during registration and approval for use of “Muguga” cocktail for immunization against ECF. Unfortunately the product is currently widespread in the country without a thorough investigation of the strains prevailing in the field [2,15,19].

The detection of the *T. parva* sporozoites stabilate vaccine strain (Muguga) in the analysed samples is something worth noting. This could imply more than two scenario; it is either that the strain was prevalent in the field before introduction of the cocktail, or it could imply that the strain is transmitted from vaccinated animals with “Muguga cocktail” to non-vaccinated animals, hence immunization possibly introduces the vaccine parasite strains into local tick populations [13, 19]. This is possible because vaccinated animals are known to be long-term carriers of piroplasm forms, a scenario describe by Bishop et al. [2]; Kariuki et al. [20] and Young et al. [21]. This may pose a risk of undesirable consequences in resident cattle populations due to introduction of new strains of the parasite. Resemblance of *T. parva* strains detected from aborted foetuses and neonates aged less than 6 days and those obtained from their respective dams suggests a flow of these parasites from the dams to offspring. This further indicates that there is trans-placental transmission of *T. parva*.

This study used a relatively small sample size to describe this enigma phenomenon on molecular theileriosis transmission; however the findings are worth considering for intensive research. The appearance of bands which are similar to the vaccine strains in the samples from aborted foetuses and neonates following restriction enzyme analysis using *Alu 1* is a specialty finding in the *T. parva* transmission. The study

further describes that transmission of the parasites by other portals than ticks can occur through the placenta. Conclusively these findings could be used to explain the early occurrence of classical ECF clinical signs and high mortalities in calves before even exposure to pastures and this information may be useful in the control of *T. parva* infection in calves. In summary this study shows two salient findings (1) Detection of *T. parva* DNA in foetuses and naive calves, a finding that might be used as baseline data to confirm that *T. parva* can be transmitted vertically through the placenta; and (2) there are more *T. parva* stocks in Tanzania other than Muguga, Kiambu 5, Serengeti transformed and the earlier isolated *T. parva* Melela. It is recommended that a comprehensive study to be carried out using large sample size so as to increase the validity and statistical significance of the results. The use of alternative techniques such as real time PCR (RT- PCR) should be thought of in future in order to improve the detection of *T. parva* in foetuses. Investigations on the integrity and permeability of the cattle placenta to *T. parva* are also recommended.

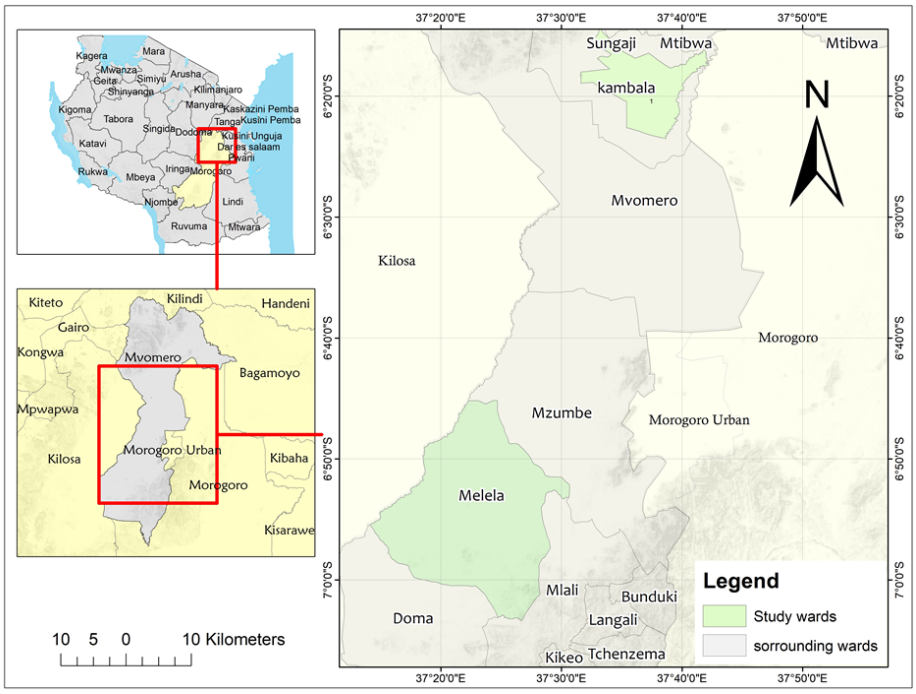
Acknowledgement

The authors would like to express their sincere appreciation to the Belgian Technical Cooperation for funding this research

Ethics Statement

The study protocol was approved by the Postgraduate Studies Committee of the College of Veterinary and Medical Sciences of Sokoine University of Agriculture Review board. The sampling was done by well qualified and registered veterinarians and livestock field officers

Fig. 3: A map representing the study area of Kambala and Melela Villages



References

[1] R, Bishop, B Sohanpal, S. Morzaria. *Theileria parva*: detection of genomic polymorphisms by polymerase chain reaction amplification of DNA using arbitrary primers. Exp. Parasitol. 77, 53–61. DOI:10.1006/expr.1993.1060

[2] Bishop, R., Musoke, A., Morzaria, S., Gardner, M., Nene, V., 2004. Theileria: Intracellular protozoan parasites of wild and domestic ruminants transmitted by ixodid ticks. Parasitology. 129 suppl, S271-283. PMID:15938515.

[3] Uilenberg, G., Perié, N.M., Lawrence, J 333 .A., de Vos, A.J., et al. 1982. Causal agents of bovine theileriosis in Southern Africa. Trop. Anim. Health Prod. 14, 127-140.

[4] Kivaria, F.M., Ruheta, M.R., Mkonyi, P.A., Malamsha, P.C., 2007. Epidemiological aspects and economic impact of bovine theileriosis (East Coast fever) and its control: a preliminary assessment with special reference to kibaha district, Tanzania. VJ. 173, 384–390. DOI:10.1016/j.tvjl.2005.08.013

[5] Radley, D.E., 1975. East Coast fever. 3. Chemoprophylactic immunization of cattle using oxytetracycline and a combination of Theilerial strains. Vet. Parasitol. 1, 51–60. https://doi.org/10.1016/0304-4017(75)90007-2

[6] Carmen, J.C., Sinai A.P., 2007. Suicide prevention: Disruption of apoptotic pathways by protozoan parasites. Mol Microbiol. 64 (4), 904-916. https://doi.org/10.1111/j.1365-2958.2007.05714.x

[7] Allsopp, M.T.E.P., Lewis, B. D. and Penzhorn, B.L., 2007. Molecular evidence for transplacental transmission of *Theileria equi* from carrier mares to their apparently healthy foals. Vet Parasitol. 148, 130-136. DOI:10.1016/j.vetpar.2007.05.017

[8] Uneke, C.J., 2007. Impact of placental *Plasmodium falciparum* Malaria on pregnancy and perinatal outcome in sub-saharan Africa. II: Effect of transplacental Malaria on perinatal outcome. Malaria and HIV. Yale J Biol Med. 80 (3), 95- 103.

[9] Baek, B.K., Soo, K.B., Kim, J.H., Hur, J., Lee, B.O., Jung, J.M., et al. 2003. Verification of Polymerase Chain Reaction of vertical transmission of *Theileria sergenti* in cows. Can J Vet Res. 64 (4), 278- 82. doi: [10.1007/s12639-011-0072-1]

[10] Mbassa, G.K., Luziga, C., Mgongo, F. O. K., Kashoma, I. P. B., Bundala, S. A. F., 2009. *Theileria parva* infection (ECF) is vertically transmitted from pregnant cow to offspring. Research initiative for improvement of Livelihood: Contribution of SUA and her partners. In: Proceedings of the fourth Annual PANTIL scientific conference held in Morogoro 19-21 Oct. 2009. 153pp.

[11] Henjewele, P.J. 2010. Investigation on vertical transmission of east coast fever (*Theileria parva* infection) from cows to calves. Research paper- MPVM, SUA, Morogoro. Tanzania.

[12] Mbyuzi, A.O., Komba, E.V.G, Magwisha, H.B., Salum, M 311 .R., Kafiriti, E.M. Malamla, L.J., 2013. Preliminary evidence of vertical transmission of *Theileria parva* sporozoites from ECF immunized cows to offsprings in southern Tanzania. Res. Opin. Anim. Vet. Sci. 3(4), 92-100.

[13] McKeever, D.J., 2007. Live immunisation against Theileria parva: containing or spreading the disease? Trends Parasitol. 23, 565-568. doi.org/10.1016/j.pt.2007.09.002

[14] Dahlbäck M, Rask TS, Andersen PH, Nielsen MA, Ndam NT, et al. (2006). Epitope mapping and topographic analysis of VAR2CSA DBL3X involved in P. falciparum placental sequestration. PLoS Pathog 2(11):e124

[15] Ouédraogo, A., Tiono, AB., Diarra, A., Edith, C., Bougouma, C., Nébié, I., et al. Transplacental Transmission of Plasmodium falciparum in a Highly Malaria Endemic Area of Burkina Faso. Journal of Tropical Medicine, Vol. 2012, ID 109705, 7 pages, 2012. Doi.org/10.1155/2012/109705

[16] Norval, R.A.I., Perry, B.D., Young, A.S., 1992. The epidemiology of Theileriosis in Africa. Academic Press, London, 481 pp. OIE, 2008. Terresterial Manual "Theileriosis" pg. 789- 804.

[17] Uilenberg, G., 1999. Immunization against diseases caused by Theileria parva: a review. Trop Med Int Health. 4(9), A12-20

[18] Oura, C.A., Bishop, R., Asiimwe, B.B., Spooner, P., Lubega, G.W., Tait, A., 2007. Theileria parva live vaccination: parasite transmission, persistence and heterologous challenge in the field. Parasitology, 134, 1205-1213. doi.org/10.1017/S0031

[19] Morisson, W.I., & Mckeever D.J., 2006. Current status of vaccine development against Theileria parasites. Parasitology, 133, 169–187.

[20] Kariuki, D.P., Young, A.S., Morzaria, S.P., Lesan, A.C., Mining, S.K., et al. 1995. Theileria parva carrier state in naturally infected and artificially immunised cattle. Trop. Anim. Health Prod. 27(1), 15-25. PMID:7770947

[21] Young, A.S., Leitch, B.L., Newson, R.M., Cunningham, M.P., 1986. Maintenance of *Theileria parva* infection in an endemic area of Kenya. Parasitology, 93, 9-16.

[22] Abdel-Rady, A., Mohameda, A.M., Ahmed, L.S., Al-Hosary, A., 2010. Using polymerase chain reaction (PCR) for diagnosis of bovine theileriosis in Upper Egypt. Int. J. Agro-Vet. Med. Sci. 4, 67-74.

[23] Geysen, D., 2002. Molecular epidemiology of *Theileria parva* in the field. Trop. Med. Int. Health 4, A21–A27. PMID:10540308

[24]. Geysen, D., Bazarusanga, T., B randt, J., Dolan, T.T., 2004. An unusual mosaic structure of the PIM gene of *Theileria parva* and its relationship to allelic diversity. Mol Biochem Parasitol. 133, 163-174.

[25] Bishop, R., Geysen, D., Spooner, P., Skilton, R., Nene, V., Dolan, T., Morzaria, S., 2001. Molecular and immunological characterization of *Theileria parva* stocks which are components of Muguga cocktail‟ used for vaccination against East Coast fever in cattle. Vet Parasitol. 94, 227-37. https://doi.org/10.1016/S0304-4017(00)00404-0

[26] Iams, K.P., hall, R., Webster, P., Musoke, A.J., 1990. Identification of λgt11 clones encoding the major antigenic determinants expressed by *Theileria parva* sporozoites. Infect Immun. 58, 1828- 31. www.cabdirect.org/cabdirect/abstract/19910872300