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# An overview of bovine theileriosis

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**Abstract**
Theileriosis is predominantly a disease of ruminants (domestic and wild) in tropical and subtropical countries of the world. Amongst several species infecting ruminants only few species are associated with severe clinical signs, notably *T. parva* and *T. annulata* in cattle and *Theileria lestoquardi* in sheep. *Theileria parva* is transmitted by *Rhipicephalus appendiculatus* and causes East Coast Fever (ECF) in susceptible cattle, while as *T. annulata* transmitted by different species of *Hyalomma* causes tropical theileriosis in cattle. The pathogenicity is mainly due to parasite development and multiplication in leukocytes. The infected host leucocytes are induced and activated by the schizonts, which associate with the mitotic spindle of the host cell during cell division. *Theileria* parasites are the only intracellular eukaryotic parasites capable of reversibly transforming host cells. The annual economic losses associated with ECF about USD 300 million and cattle loss in terms of mortality include approximately one million cattle per year. Tropical theileriosis also has serious economic impact in view of mortality, reduced milk yield, weight losses, abortions, and control costs associated with the disease, hence prevention is the best mean for effective and sustainable control.

**Keywords:** Apical complex, schizont, theileriosis, livestock, buffalo

## Introduction
*Theileria* spp. are the obligate intracellular protozoan parasites belonging to phylum Apicomplexa, which include several other important intracellular pathogens such as *Babesia, Plasmodium, Eimeria* and *Toxoplasma* species [1]. These are phylogenetically most closely related to members of the Babesia genus [2]. The parasites belonging to this phylum are characterized by the presence of a distinct group of unique organelles called apical complex [3] which helps in the invasion and establishment of the parasite in the host cells [1]. Theileriosis is predominantly a disease of ruminants (domestic and wild) in tropical and subtropical countries of the world. Amongst several species infecting ruminants only few species are associated with severe clinical signs, notably *T. parva* and *T. annulata* in cattle and *Theileria lestoquardi* in sheep [4]. *Theileria parva* is transmitted by *Rhipicephalus appendiculatus* and causes East Coacst Fever in susceptible cattle, while as *T. annulata* transmitted by different species of *Hyalomma* causes tropical theileriosis in cattle. *Theileria lestoquardi*, is closely related to *T. annulata*, transmitted by *Hyalomma* ticks is responsible for malignant the leriosis in sheep and goats [1]. Other species of *Theileria* cannot transform host mononuclear cells (*T. orientalis, T. mutans, T. velifera*) and are therefore recognized as nonpathogenic or benign species. Such species even if associated with disease, it is mainly due to multiplication of the parasites within red blood cells [5] and not due to host cell transformation. However, recently, *T. orientalis* complex, (transmitted mainly by *Haemaphysalis* spp.) caused significant morbidity, economic losses and mortality in cattle in the Asia-Pacific region [6]. Newer findings show mixed infection of benign and transforming *Theileria* species in the host cells as well as ticks increasing further the importance of understanding the epidemiology of the disease under the rapidly changing global climatic conditions, for better and effective control measures against the disease.
*Theileria parva* and *T. annulata* also infect the African buffalo (*Syncerus caffer*) and the Asian buffalo (*Bubalus bubalus*) respectively. The latter sometimes suffer mild clinical disease whereas *T. parva* is nonpathogenic in the African buffalo, but infected buffalo remains an important wildlife reservoir for infection to cattle [7].

*T. parva* most likely co-evolved with African buffalo as its vertebrate primary host, which displays no symptoms of disease when infected [7] and has under-gone a "host jump" to cattle, where it causes severe disease named as East Coast fever (ECF). *T. annulata* parasite undergoes sequential development in leukocytes and erythrocytes of the mammalian host and causes an acute, often fatal disease (tropical theileriosis) that occurs from North Africa and Southern Europe, through the Middle East and across Southern Asia while as ECF occurs across sub-Saharan Africa and ranks first among the tick-borne diseases of cattle in the region [8].

**Life Cycle**

The causative agent (*T. parva)* of ECF in South Africa was first recognized by Dr. Arnold Theiler [9]. Disease was first identified with cattle imported to South Africa from East Africa, where rinderpest had caused huge mortality in cattle and South Africa became natural refuge to the susceptible cattle. However, in South Africa ECF caused high levels of morbidity and mortality which was identified and distinguished from Red water for the first time by Dr. Arnold Theiler. He identified the principal tick vector that transmits *T. parva* as *R.appendiculatus.*

The life cycle of *Theileria* parasite is complex, involving morphologically distinct phases in two hosts. Sporogony and merogony takes place in the bovine host while zygote and ookinete are formed in ticks [3]. Theileria sporozoites enter their bovine host during tick feeding along with tick saliva and they rapidly invade mononuclear leukocytes, where they mature into microschizonts and induce proliferation in host cells. Host cells undergo transformation resulting in clonal expansion of schizont infected cells. Microschizonts gradually develop into macroschizonts. Schizonts undergo merogony to form merozoites, which are released from leukocytes. These merozoites invade erythrocytes and develop into piroplasms which forms the infective stage for ticks. During the next feeding cycle, larvae or nymphs ingest the piroplasm infected erythrocytes and the released parasites undergo syngamy in the tick gut, forming a zygote. The zygote divides into motile kinetes that infect the tick gut epithelial cells and migrate to the haemolymph and subsequently infect the salivary glands. After moulting and commencement of feeding by the tick, sporogony results in the multiplication of sporozoites in the salivary gland acini before injection into the feeding site by nymphs or adult ticks respectively exhibiting trans-stadial transmission [10].

For tick to become infective, it must be attached for 48–72 hours on the animal; however, if environmental temperatures are high, infective sporozoites can develop in ticks on the ground and may enter the host within hours of attachment [11]. Transovarial transmission does not occur with either of the species [5] however ticks can remain infected on the pasture for up to 2 years depending on the climatic conditions.

**Economic losses**

ECF still remains an acute and usually lethal disease in countries including Rwanda, South Sudan, Kenya, Zimbabwe, Tanzania, Uganda, Burundi, Democratic Republic of Congo, Mozambique, Zambia and Malawi, [12]. The annual economic losses associated with ECF about USD 300 million and cattle loss in terms of mortality include approximately one million cattle per year [13].

About 250 million cattle are at risk to Tropical theileriosis worldwide [14]. Tropical theileriosis also has serious economic impact in view of mortality, reduced milk yield, weight losses, abortions, and control costs associated with the disease, hence prevention is the best mean for effective and sustainable control [15].

**Pathogenesis and cell tropism**

Theileriosis is an acute lymphoproliferative disease characterized by fever, lymphnode enlargement, jaundice, hepatomegaly, spenomegaly and punched out abomasal ulcers (post-mortem examination), with high levels of morbidity and mortality in susceptible populations of animals. The morbidity and mortality varies with the host's susceptibility, strain and the dose of the parasite [16]. Cattle exhibiting mild clinical symptoms are considered resistant to disease, while those showing severe clinical reactions are recognized as susceptible [16]. The endemic stability to ECF is found in indigenous cattle in regions where there is continual transmission of the parasite [17]. Spontaneously recovered animals from ECF, after a mild or moderate reaction are found to be solidly immune to reinfection [5]. The case fatality rate for untreated ECF can be as high as 100% in taurine, zebu or sanga cattle from non–endemic areas. In contrast, the morbidity rate approaches 100% among indigenous cattle, but the mortality rate is usually low. Similarly, tropical theileriosis is more severe in introduced breeds, with a mortality rate of 40-90%, while the mortality rate in indigenous cattle can be as low as 3%. Breeds of cattle that are relatively resistant to experimental infection with *T. annulata* include the Sahiwal breed of *Bos indicus* and the Kenana breed of *Bos taurus*. Infections with *Theileria spp*. other than *T. parva* and *T. annulata* are rarely fatal in cattle.

The serosal surfaces of internal organs show petechial and ecchymotic hemorrhages, and the body cavities usually contain serous fluid. Acutely infected animals exhibit lymph node enlargement which may be haemorrhagic and ecchymotic while as, in chronically infected animals, these may be shrunken. Splenomegaly and hepatomegaly are also seen with occasionally white foci of lymphoid infiltration on liver and kidney. Small intestine and abomasums shows signs of hemorrhagic enteritis. Pulmonary edema is the main cause of death in East Coast fever.

The pathogenicity is mainly due to parasite development and multiplication in leukocytes. Parasites after entering into leucocytes, reside free within the cytosol of the host cells. This feature is not seen in most of the other apicomplexan protozoan parasites. The infected host leucocytes are induced and activated by the schizonts, which associate with the mitotic spindle of the host cell during cell division. *Theileria* parasites are the only intracellular eukaryotic parasites capable of reversibly transforming host cells [18] dividing at the same time as the host cells, ensuring that infection is retained in the daughter cells and hence the schizont stage induces host cell transformation without involving the integration of parasite DNA into the host genome [19]. Thereafter the lymphoid tissue and non-lymphoid organs such as liver, lungs, kidney are infected by day seven, brain by day twelve and heart by day fourteen after infection causing haemorrhagic lesions in these organs [20]. This process results in rapid parasite multiplication before differentiating into the erythrocyte-infective merozoite form. Infection usually results in death within 3–4 weeks. The intra-erythrocyte piroplasm stage of *T. parva* undergoes little or no multiplication, whereas there is some replication of *T. annulata* piroplasms, which is associated with higher levels of infection of erythrocytes thereby infections with *T. annulata* may result in

moderate anaemia. However, schizont stage is usually the main cause of pathogenesis and mortality in both the species. *Theileria annulata* schizonts infection results in switching to a T helper 1 phenotype, producing large quantities of IFN-γ. The abnormally high levels of IFN-γ together with excessive production of pro-inflammatory cytokines (including IL-1α, IL-1β, IL-6 and TNF-α) are probably the main causes of parasite induced pathological lesions [21, 22].

The nuclear genome of both the species *T. annulata* (8.35 Mb) and *T. parva* (8.3 Mb) is similar in size which is distributed across four chromosomes [18]. Cell tropism of the two species varies considerably; however, the net pathogenic effect on animals remains same in both parasitic infections. The sporozoites of *T. parva* infect B and all subsets of T lymphocytes *in vitro* with similar efficiency, however *T. annulata* infect predominantly macrophages and to a lesser extent B lymphocytes but not T lymphocytes [18, 23]. *T annulata* merozoites also multiply in erythrocytes to a significant level which is responsible for slight anaemia. In both infections, the parasitized cells migrate from the site of infection and become disseminated throughout the lymphoid system and there is also evidence of an early powerful nonspecific T-cell response in animals experimentally infected with a lethal dose of sporozoites, but this response appears to be ineffective in controlling the infection.

**Diagnosis**

**Clinical Signs:** In acute cases body temperature is higher 41-42 °C. On day 5 to day 10 from the clinical onset temperature will lower to a normal range (38.0-39.5 °C), from day10 to day15, there is a downfall stage, with hypothermia (37 to 38 °C), anemia, jaundice, and occassionally heart failure. Such animals rarely recover, even with intensive treatment. Lymph nodes are commonly enlarged and there may be episodes of blood from the nose, difficult breathing and weight loss. Other signs include: blood-tinged diarrhoea, bruxism (grinding of teeth) can be seen, circular raised patches of hair all over the body and haemorrhages in the ocular and vaginal mucous membranes.

**Microscopy:** Theileriosis in live animals is diagnosed by the identification of schizonts in Geimsa stained smears from blood, lymph node and spleen. Kochs blue bodies can be detected in schizont infected lymphocytes. At necropsy, schizonts may be found in impression smears from most internal organs. Piroplasms can also be found in the blood of carrier animals especially in *T. annulata* infection.

The limitations with microscopy include: inability to detect all cases of carrier [24, 25], sensitivity is lower than currently available molecular methods [26], difficult to morphologically distinguish between different *Theileria* species or to discover newer ones [24, 27, 28].

**Serological tests:** Indirect fluorescent antibody test (IFAT) [29, 30] replaced complement fixation test for diagnosis of theileriosis. It utilizes whole body antigen of either piroplasm or culture derived schizont and is defined by OIE as one of the gold standard test for parasite diagnosis [31]. It is useful to discriminate carrier animals as well as to distinguish between different *Theileria* species.

The biggest flaw with IFAT is the significant cross-reactivity between closely related species. *T. parva* antigen cross reacts with *T. taurotragi* [32] and *T. lestoquqrdi* antigen with *T. parva* and *T. annulata* [33].

Competetive inhibition ELISA kit using EMA-1 have been successfully developed to diagnose *T. equi* and is forcasted to replace IFAT in near future for diagnosis of this species [31, 34]. ELISAs were also developed using polymorphic immunodominant molecule (PIM) of *Theileria parva* and the p32 antigen of *T. mutans* [35, 36] and were commercially marketed showing more sensitivity than the IFAT, but the tests were discontinued due to specificity issues [31]. However, still ELISA being a high throughput, cheap and fast method, it can be used to screen and diagnose large numbers of samples under meager infrastructure conditions.

Conventional PCR [37], nested-PCR [38] real-time PCR methods [39, 40, 33] SYBR green real-time PCR assays [33], loop-mediated isothermal amplification (LAMP) assays [40-42] and high-resolution melt analysis [43] have been used for direct confirmation of the presence of parasite genomic material. The developmental evolution from conventional PCR to more recent molecular techniques has helped improve sensitivity, quantification and speed of detection and also provides more reliable means for detection of multiple species or genotypes at the same time. LAMP assays provide possibility of application under field conditions.

**Prevention and Control**

**Prophylaxis**

**a)** Use of acaricides to kill ticks on animals and thereby indirectly control the tick borne diseases. However, this method is not sustainable because of associated environmental contamination, increasing acaricide resistance, problem of acaride residues in meat and milk and the expensive nature of drugs.

**b) Use of Chemotherapeutic agents like buparvaquone:** Although the drug is effective but treatment does not completely eradicate infections and leads to the development of carrier animals. Moreover, for effective cure drug needs to be given at early stage of the disease, otherwise several doses are required for several days to bring animal to normal state.

**C) Use of vaccines:** Currenly only live attenuated vaccines are commercially available. For *T. annulata*, schizont-infected cell lines attenuated by prolonged in-vitro culture passage are being successfully used in Asian countries and Mediterranean region. Infection and treatment method is currently used to control ECF. In this method, cattle are given subcutaneous dose of tick derived sporozoites and a simultaneous treatment with a long acting tetracycline formulation. Immunised animals demonstrate a robust immunity to homologous challenge, which varies from one year to even lifetime of an animal. To increase the spectrum of coverage against different strains, Muguga cocktail has been developed which consists of three strains.

The limitation with live attenuated vaccines include, perceived notion of introduction of foreign strain in different countries compelling nearly every country in producing its own versions of vaccine which is limiting commercialization prospects of vaccine. Moreover, the infrastructure requirement in developing vaccine is huge such as, requirement of live cattle for producing infective tick stabilates to be used in vaccination for ECF. Furthermore, the risk of reversion to virulence remains always a possibility.

**d) Recombinant Vaccines:** Experimental subunit vaccines are being developed which include sporozoite antigens recombinant p67 (67kda mol. wt) and SPAG1. In lab. trials

the results were encouraging with protection from 20-70% but under field trials the results showed varying results from lab trials. Tams-1 (*T. annulata* merozoite surface ag) is a 30-32kDa protein expressed upon merozoite surface upon differentiation from schizont to merozoite. It induces strong antibody response. In immunization trials with Tams-2ag (rec. and naked) followed by infecting *T. annulata* infected blood (30%) parasitaemia showed substantial decrease in anaemia as well as protection in challenge cattle [24].

**Conclusion**

Theileriosis is an acute lymphoproliferative disease with high levels of morbidity and mortality in susceptible cattle in tropical and subtropical regions of the world resulting in huge economic losses to the livestock industry. The pathogenicity is mainly due to parasite development and multiplication in leukocytes. Diagnosis by microscopy is useful in detecting theilerial infection but is not effective to distinguish between morphologically different parasitic strains or to detect cases of carrier states. Serological tests such as ELISA being a high throughput, cheap and fast method can be used to screen and diagnose large number of samples under meager infrastructure conditions. LAMP assays also provide a possibility of application under field conditions. Treatment by the use of buparvaqone is effective but does not completely eradicate the infection thereby may result in producing carrier animals. Currenly only live attenuated vaccines are commercially available for both tropical theileriosis and east coast fever, which restricts commercialization prospects of vaccine because of inherent limitations with the production of vaccine. Research on recombinant vaccines is currently going on which will help in sustainable control of disease in future.

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