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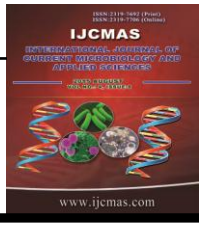

Review Article

Tropical Theileriosis and East Coast Fever in Cattle: Present, Past and Future Perspective

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Keywords

Tropical theileriosis, East coast fever, *Theileria annulata, Theileria parva, Hyalomma, Rhipicephalus appendiculatus*, Pakistan.

A B S T R A C T

Theileriosis is a burning veterinary problem of the rural livestock oriented communities. It has a profound effect on hematological values and causes huge morbidity and mortality in cattle population, which reflect economic losses and elevates the poverty level. Critical review of the literature was carried out about the prevalence, diagnostic techniques as well as prophylactic measures against the Tropical theileriosis and East coast fever in cattle population. Emphasis was made on the review of publications from databases including Google Scholar, Science Direct and PubMed using standardized keywords such as Tropical theileriosis, East coast fever, *Theileria annulata, Theileria parva, Hyalomma, Rhipicephalus appendiculatus* and Pakistan. Relevant articles were thoroughly studied to update knowledge, gain new insights and raise novel questions regarding the disease. This review ascertains the correlation of the potential occurrence of tropical theileriosis and East coast fever with seasonality, tick abundance and cattle susceptibility. The management system and cattle breed are significant predictors of infection. PCR is a most effective molecular tool for identification of the etiological agent. Tick eradication and immunization are beneficial in reducing the impact of disease. Tropical theileriosis and East coast fever are major constrain to the livestock industry, therefore, it is an essential prerequisite to develop sensitive and specific tests for parasite detection in the sample. The future policies should focus controlled crossbreeding, thoroughly monitored medication programs and implementation of appropriate tick control strategies to reduce the prevalence of disease. Research should be extended to target new metabolic enzymes for drug designing and evaluate efficacy and safety issues of available vaccines to boost productivity of the livestock industry.

Introduction

Tropical theileriosis and East coast fever are devastating diseases caused by obligate hemoprotozoan parasites belonging to genus *Theileria*. The parasites belonging to this genus are distinguished on the basis of a distinct group of unique organelles called apical complex (Bishop *et al.,* 2004). The secretory organelles of apical complex contain secretory granules needed for motility, attachment to the host and invasion of mammalian and arthropod cells (Striepen *et al.,* 2007). Globally, *Theileria annulata* and *Theileria parva* are the most important tick-transmitted pathogenic species causing bovine theileriosis (Kohli *et al.,* 2014).

Tropical theileriosis, also known as Mediterranean coast fever, is an extremely fatal and debilitating tick-transmitted disease infecting cattle (Santos *et al.,* 2013). This hemoparasitic infection is caused by *Theileria annulata* and is responsible for substantial production losses (Gharbi *et al.,* 2011). About 250 million cattle are at risk to Tropical theileriosis worldwide (Erdemir *et al.,* 2012). Another commercially important parasitic disease is East coast fever caused by *Theileria parva* (Gachohi *et al.,* 2012). This infection causes mortality in about one million cattle annually in central, eastern and southern Africa. It threatens almost twenty five million cattle in Africa and also limits the introduction of improved breeds (Salih *et al.,* 2007). Theileriosis is a widespread cattle disease in Pakistan. The climatic condition of Pakistan is favorable for growth and development of tick species. The situation has further deteriorated due to the lack of proper management practices. This intracellular infection inflicts economic burden on cattle breeders in terms of mortality and morbidity as well as expenses spent on prophylactic measures against disease and treatment (Durrani *et al.,* 2008).

Keeping in view the significance of the disease, the following study was conducted to comprehensively review the prevalence of the disease globally. The review intended to compare efficacy of diagnostic techniques, i.e. microscopy, IFAT and PCR for the detection of the etiological agent in the sample. The rationale of the study was also directed towards the evaluation of the potential impact of different prophylactic measures against the disease. The successes and challenges of available drugs and vaccines were also reviewed.

Historical background

Theileria annulata was described in Transcaucasian cattle in 1904 and was first named *Piroplasma annulatum*. It was reclassified as *T. annulata* after identification of schizont stage in its life-cycle (Weir, 2006). *Theileria parva* was first recognized in Southern Rhodesia (now Zimbabwe) in 1901/02. It was introduced to the region by the import of cattle from Tanzania and Kenya for restocking and devastated the cattle population of southern Africa within three years due to their high susceptibility. According to an estimate, it caused mortality in 1.25 million cattle out of 4 million in affected area by 1914. It also appeared in Zambia, Malawi and Mozambique (Tete Province) between 1912 and 1922. The infection is still prevalent in these countries and inflicts economical losses to livestock oriented community (Yusufmia *et al.,* 2010).

Geographical distribution

Tropical theileriosis is transmitted by ticks belonging to genus *Rhiphicephalus* and is prevalent in tropical and subtropical regions of the world including Portugal, Spain, Greece and Italy. It extends to Turkey, middle East, southern Russia, China and

Asia (Ali and Radwan, 2012). It has also been reported for the first time in cattle in Ethiopia (Gebrekidana *et al.,* 2014). East coast fever is also a vector-borne disease and is prevalent in eleven countries in Southern, Central and Eastern Africa. The affected countries are Kenya, Sudan, Burundi, Tanzania, Malawi, Rwanda, Zaire, Mozambique, Zambia, Uganda and Zimbabwe (Gachohi *et al.,* 2012).

Clinical manifestation

The clinical signs for ECF include fever, immune depression, anorexia, lymphadenopathy and secondary bacterial respiratory infection. Lacrimation, corneal opacity, nasal discharge and diarrhea are also observed. It can lead to mortality in cattle if proper treatment is not given (Muhanguzi *et al.,* 2014). Cattle may also develop an extremely fatal condition referred to as "turning sickness". In this disease, capillaries of central nervous system are blocked by infected cells and leads to neurologic symptoms (Rocchi *et al.,* 2006).

T. annulata infection is characterized by high fever, weakness, weight loss, inappropriate appetite, conjunctival petechia, enlarged lymph nodes, and anemia. Lateral recumbency, diarrhea and dysentery are also associated with later stages of infection (Radostits *et al.,* 2007).

Genome

The genome of *Theileria annulata* was fully sequenced by the Sanger Institute in a collaborative project with University of Glasgow and Moredun Research Institute. At the same time, Institute for Genomic Research sequenced genome of *Theileria parva* in Maryland (Gardner *et al.,* 2005). *T. annulata* has a haploid nuclear genome and is estimated to be 8.35 Mb. The genome is arranged in 4 chromosomes within which coding regions are predicted to be 3,792 (Pain *et al.,* 2005). *T. parva* also has a haploid genome and is almost 8.31 Mb (Gardner *et al.,* 2005).

Pathogenesis

The pathological damage is induced in cattle by schizont stage of *T. annulata* and *T. parva* (Bishop *et al.,* 2004). The cells infected by schizonts induce massive and uncontrolled proliferation of both specific and nonspecific T lymphocyte resulting in enlarged lymph nodes (Schneider *et al.,* 2007). Affected lymph nodes show reactive follicular hyperplasia, reticulo-endothelial hyperplasia, enlarged germinal centers and slight increase of interfollicular lymphoid tissue within the paracortical and cortical regions (Hassan *et al.,* 2000).

Pulmonary congestion, edema, hemorrhage and emphysema of variable extents are also observed in clinically infected cattle. These lesions are characterized by the occurrence of proteinacious fluid in alveolar spaces, enlargement of pulmonary blood vessels with erythrocytes, presence of emphysematous areas (interstitial and alveolar emphysema) and infiltration of inflammatory cells within the lung's interstitial tissue (Hassan *et al.,* 2000). These changes are attributed to T lymphocyte proliferation that produces IFN-γ and many pro-inflammatory cytokines TNF-α, IL-1α, IL-1β and IL-6 ultimately forming pathological lesions (Omer *et al.,* 2002).

Moreover, pale white areas of variable size are distributed within the parenchyma and over the external surfaces of the kidney due to the infiltration of renal interstitial fluid with mononuclear inflammatory cells (Hassan *et al.,* 2000).

Tropical theileriosis is characterized by hemolytic anemia (Omer *et al.,* 2002). Hemolytic anemia is caused by immune mediated hemolysis. Though, many attempts have been made to describe the mechanism of anemia, the underlying mechanism is not yet fully understood (Shiono *et al.,* 2004). One of the plausible reasons may be the oxidative damage to RBCs (Rezai and Dalir-Naghadeh, 2006). The infected erythrocytes show morphological disorders which may be attributed to the presence of *Theileria* schizonts, immune-mediated processes and intravascular thrombi (Singh *et al.,* 2001).

The cattle that have recovered from acute infection have low parasite level and sustain microscopically undetectable subclinical infection (Hoghooghi-Rad *et al.,* 2011). Such cattle harbor piroplasm in latent form and act as reservoir for perpetuating infection to ticks and cattle herds (Thompson *et al.,* 2008).

Prevalence

The prevalence of theileriosis depends upon geographical region and several other factors like tick density, climatic conditions, age, gender, management practices and immunity, either passive or active (Magona *et al.,* 2011). The incidence rate is high during monsoon season due to the warmth and humidity which favors tick growth and subsequently parasite transmission (Vahora *et al.,* 2012). Prevalence is also influenced by cattle breed as cattle usually differ in tick resistance and innate susceptibility to infection (Muhammad *et al.,* 2008). A survey was conducted in eastern Turkey by collecting blood samples from apparently healthy cattle and 39% prevalence of *Theileria annulata* was established by PCR (Aktas *et al.,* 2006). Studies conducted in the Kayseri province (Turkey) indicated 9.3% prevalence of theileriosis (Ica *et al.,* 2007). Aysul *et al.* (2008) reported that Tropical theileriosis is the most prevalent disease transmitted by the ticks in the Aydin region of Turkey (Aysul *et al.,* 2008). The prevalence in southwest Iran was reported to be 28.11% (Dehkordi *et al.,* 2012). A reverse line blotting assay was carried out in Portugal and the prevalence was found to be 21.3% for *Theileria annulata* (Gomes *et al.,* 2013). Muhanguzi *et al.* (2014) reported 5.3 % prevalence of *T. parva* in Tororo District of Eastern Uganda using PCR (Muhanguzi *et al.,* 2014).

Vectors

In order to study the epidemiology of theileriosis, it is crucial to have knowledge about tick vectors, their intensity and abundance (Aktas *et al.,* 2004). Ticks were considered ectoparasites of animals even in 400 B.C. Aristotle stated in his text "Historia Animalium" that ticks are grass generated disgusting parasites (Durrani *et al.,* 2009). Ticks are voracious blood sucking obligate ectoparasites of cattle (Bishop *et al.,* 2004). Loss of blood by heavy tick infestation impoverishes the hosts and cattle may remain weak and stunted. Ticks are regarded as notorious threat because these can cause stress, hypersensitivity, depreciation of skin value, immunodepression, weight loss and toxicosis in cattle (Lorusso *et al.,* 2013). Almost 80% of the cattle are exposed to tick infestation worldwide (Anim *et al.,* 2013). Warm and moist climate is conducive for rapid growth and development of ticks (Kohli *et al.,* 2014). Ticks are mostly found in the inguinal/groin region and external genitals as these body parts are richly supplied with blood. The thinner and short hair skin is usually preferred by tick for infestation because mouth parts can easily penetrate the vascular region for feeding (Sajid, 2007).

Tick species also act as a vector for disease transmission including theileriosis, babesiosis and anaplasmosis (Irshad *et al.,* 2010). Tick-borne diseases cause economic loss of almost US$ 13.9 to US$ 18.7 billion globally (Atif *et al.,* 2012). *Theileria parva* is transmitted by ticks belonging to genus *Rhiphicephalus*. These are three host ticks because nymph, larvae and adult may not necessarily feed on the same host. The nymph and larval instars of tick acquire infection through blood meal and leave the host before molting to the next stage. Both nymph and larvae are responsible for further transmission of infection by attaching to the new host. Spatial distribution of *Rhiphicephalus appendiculatus* determines the distribution and prevalence of *T. parva*. *Theileria annulata* is transmitted by two host ticks belonging to genus *Hyalomma*. These are two host ticks because the larva molt to nymph on the same cattle. The nymph detaches and drops off of the ground to molt into an adult and seeks a new host (Zajac *et al.,* 2006).

The gender of tick has been reported to play a significant role in the transmission, prevalence as well as intensity of infection (Sayin *et al.,* 2003). Male ticks of genus *Hyalomma* have a limited number of type III acini in salivary gland as compared to the female. Thus, female ticks have more potential of disease transmission than male (Aktas *et al.,* 2001). Moreover, female ticks have two histamine binding proteins to counteract host response to tick attachment (Anim *et al.,* 2013).

Life cycle

The life cycle of *Theileria* parasite is complex, involving morphologically distinct phases in two hosts. Sporogony and merogony take place in the bovine host while zygote and kinete are formed in ticks (Shaw, 2003).

Ticks acquire infection by ingesting piroplasm-infected erythrocytes during feeding and undergo an obligate sexual cycle. There is no clear evidence of sexual differentiation of *T. parva* within erythrocytes. Piroplasm differentiates to macro- and micro-gametes within lumen of tick's gut by gametogenesis. Gametes are morphologically similar and undergo syngamy to form a spherical diploid zygote. Subsequently, the zygote undergoes meiotic division, differentiates in epithelial cells of tick gut and ultimately forms motile uni-nucleate kinetes that lie free in cytoplasm. Kinetes cross the basal membrane as well as the lamina of gut cells to specifically enter salivary gland and are not found in any other tick organ (Henson *et al.,* 2012).

The salivary gland of ixodid salivary gland can be differentiated into type I, II and III. Type IV is present in male ticks only. Probably, kinete invades E-cells of Type III acinus due to its carbohydrate composition. Sporogony occurs in salivary gland and almost 30,000 to 50,000 sporozoites are produced in each infected acinar cell. The number of sporozoites in female tick is found to be higher than the male tick. Nymphal or adult ticks transmit non-motile sporozoites along with saliva into the bovine host during feeding. Sporozoites invade lymphocytes and forms multinucleate schizonts. Schizonts immortalize lymphocytes and divides in synchronization with infected lymphocytes to ensure transmission of the parasite to daughter cells (Bishop *et al.,* 2004). Merozoites are subsequently formed by differentiation of schizonts in lymphocytes by merogony and are released by cell lysis. The merozoites enter erythrocytes. Little multiplication is observed in the erythrocyte. Multiplication occurs entirely in lymphocytes. Within erythrocytes, merozoites develop into piroplasm and are ingested by ticks when they feed on cattle (Muleya *et al.,* 2012).

By contrast, kinete remains diploid and piroplasm undergoes multiple rounds of intra-erythrocytic multiplication in *Theileria annulata*. Sporozoites of *Theileria annulata* are formed in tick salivary glands and are released within 3 to 7 days after feeding (Bishop *et al.*, 2004). Sporozoites form uninucleate trophozoites. Trophozoites multiply within the host cell to form multinucleate macroschizonts. Macroschizonts bind to the mitotic spindle and synchronize their proliferation with the infected cells (Weir, 2006). Macroschizonts further develop into microschizonts and eventually form uninucleate merozoites by merogony that are liberated into the bloodstream. Further growth and proliferation of merozoites occurs in red blood cells forming piroplasms. This stage is ineffective to tick and is responsible for causing the infection (Qayyum *et al.*, 2010).

Dignostic techniques

Theileriosis can be diagnosed from its clinical symptoms, however, various methods have been developed to detect haemoparasite in the sample.

Microscopic examination

Giemsa staining technique is the traditional method that involves microscopic examination of piroplasm in blood smear as well as in lymph node smears and is differentiated from other parasites by morphological properties (Aktas *et al.*, 2006). This method is frequently used for detection as it is comparatively inexpensive. However, this method is insensitive and not suitable for carrier animals because the pathogen level is usually low in the blood stream making it an unreliable technique for accurate results. Morphological differentiation of *T. annulata* and *T. parva* is also difficult, but both species are geographically separated (Hoghooghi-Rad *et al.*, 2011).

Serological tests

Sub clinical infections can be diagnosed using serological tests such as conglutination, IFAT (immunofluorescent antibody test), CA (capillary tube agglutination), IHA (indirect hemagglutination assay) and ELISA (enzyme-linked immunosorbent assay) in epidemiological studies. Serological methods involve determination of antibodies that are developed against the foreign invader causing infection. IFAT has been used to diagnose infection in serological surveys for decades. Comparatively, schizont IFAT, is more sensitive than piroplasm IFAT (Molad *et al.*, 2006).

Initially, ELISA was developed to antibodies that are generated from piroplasm antigens. Later on, recombinant proteins were used based on surface molecules TaMS1 (Gubbels et la., 2000). However, these methods are also not reliable due to their limitations. There are chances of cross reactivity, and may confront false positive and false negative results. These tests are impractical for the processing of a large number of samples. The results are also questionable due to the weaken immune response and insufficient antibody level during extended carrier phase (Molad *et al.*, 2006). *Theileria* piroplasm may occasionally be present in the erythrocytes of long-term carriers whereas antibodies have a tendency to disappear. The animals may still be infected despite of negative serological test (Ali and Radwan, 2012). Precise identification of carrier cattle is of crucial importance as they are capable of transmitting infection to non-endemic regions (Magona *et al.*, 2011).

Polymerase Chain Reaction

Polymerase Chain Reaction has largely superseded other methods and is widely used specie-specific molecular diagnostic assay in veterinary parasitology to determine piroplasm-carrier animals. These are highly sensitive tools employed for diagnosis of pathogens in carrier animals as compared to conventional techniques. However, contamination can lead to false positive results. Mixed infections are also not always detected by PCR (Yusufmia *et al.,* 2010).

The sensitivity of PCR is further improved by coupling it with hybridization method. The variable region of parasite's 18S ribosomal RNA gene is amplified and then hybridized with radioactively labeled specie-specific oligonucleotide probe (Georges *et al.,* 2001). This method is not only used to discriminate closely related species but also detects piroplasms of distinct species. It also indicates previously unrecognized species or new genotypes possibly present in sample (Nijhof *et al.,* 2005). However, these methods are laborious, expensive, require specialized equipment and technical skills (Renneker *et al.,* 2008).

Dumanli *et al.* (2005) used PCR, IFAT and smear method for diagnosis of *Theileria* parasite and the efficacy was found to be 37.4%, 34.9% and 19.7% respectively showing that PCR is a goldstandard method for parasite detection (Dumanli *et al.,* 2005). Similarly, clinically healthy cattle were selected for blood sample collection in Eastern Turkey and were examined by microscopy as well as PCR. The prevalence rate was higher (45%) in cattle using PCR than microscopic examination (16%) (Aktas *et al.,* 2006).

Studies were conducted in Golestan province of Iran for detection of *Theileria specie* in blood samples and the efficacy of PCR was compared with smear method. PCR revealed 7.5% and smear method indicated 3.75% positive results out of collecting samples. The obtained results demonstrated that PCR is more sensitive for detection of parasite in carrier cattle rather than smear method (Georges *et al.,* 2001). Research conducted in Southwest Iran also reported that the efficacy of PCR is higher (75%) than blood smear examination (22%) (Dehkordi *et al.,* 2012). Kohli *et al.* (2014) reported 27.2% prevalence of theileriosis by blood smear examination while using PCR, prevalence was reported to be 32.5 % (Kohli *et al.,* 2014).

Blood samples were collected from three districts of Punjab province (Pakistan) to examine parasites in cattle. PCR (41.2%) was found to be more specific diagnostic tool in determining *Theileria* parasite than IFA (23.5%) and microscopy (6.8%) (Durrani *et al.,* 2010).

Prophylactic measures

It is important to design and implement control strategies to prevent outbreaks in endemic and non-endemic regions on a priority basis (Simuunza *et al.,* 2011). Various cost effective prophylactic measures are used to control theileriosis and minimize economic losses to dairy farms globally, however, all of these need to be integrated in such a manner that they meet the specific requirements of livestock holders in different situations. It is also important to disseminate information to cattle holders regarding new technologies so that they can develop appropriate strategies according to their own requirement (Minjauw and McLeod, 2003).

Tick eradication

Tick eradication is one of the widely used methods to prevent outbreaks. For this

purpose, tick proof houses may be built particularly for crossbred and purebred exotic cattle. These sheds should have no crevices and cracks because ticks usually breed there. Improvement of cattle accommodation greatly reduces risk of parasite transmission (Gharbi *et al.,* 2011). Stacks of bricks and dung cakes should be regularly removed as these also serve as tick's breeding places (Vahora *et al.,* 2012).

Burning of pastures is also being used to annihilate tick's shelter, however, this practice may be hazardous to the ecosystem and may cause soil erosion (Vahora *et al.,* 2012).

Newly purchased cattle may first be properly examined before mixing with the existing stock. If the number of ticks or tick infested cattle is small, manual removal of tick is a common practice. Forefingers are used to grasp ticks and twisted counter-clock wise. The removed ticks are, then, put on the smoldering dung cake to kill them (Vahora *et al.,* 2012). It is preferable to remove ticks with forceps, not with fingers as they can transmit pathogens to humans e.g. CCHF virus (Crimean-Congo Hemorrhagic Fever). CCHF virus is associated with *Hyalomma* ticks and several outbreaks are reported from Pakistan (Jamil *et al.,* 2005).

Cattle are also treated with acaricides to limit contact between tick and cattle. Acaricides may be applied to kill ticks in both free living as well as parasitic stages. Acaricides are applied by spraying, injections, spot-on or dipping (Vahora *et al.,* 2012).

Dipping was initially introduced during colonial times and numerous government-sponsored schemes were started to protect crossbred and exotic livestock that were additionally backed up by legislations making dipping treatment compulsory for cattle owners (Pegram *et al.,* 1993). Dipping is a costly method but is desirable for large number of cattle to combat tick infestation. Dipping tanks are usually covered with a roof to avoid dilution by rain or evaporation. It is important to carefully adjust dip concentration according to the recommendation. Poor or incorrect application of even highly effective acaricide gives unsatisfactory results and develops acaricidal resistance. Dipping of cattle less than 3 months is not recommended. Wounds of cattle must be thoroughly checked before dipping, otherwise, it can cause discomfort and toxicity. The heads of cattle must be dipped once or twice in the solution. Cattle that are thirsty or fatigued shouldn't be dipped (Vahora *et al.,* 2012). Disruption of dipping treatments can cause serious outbreaks. Huge mortality was reported due to breakdown in dipping regimes during war in late 1970s in Zimbabwe (Cook, 1991).

Acaricides can also be applied with hand spray. Hand spraying is usually used by small-scale owners who cannot afford dip tanks. For effective control, it is important to moisten the hair as well as skin with spray. This practice is environmental friendly, easy to operate and economical but is suitable for small herds only (Minjauw and McLeod, 2003).

There are certain body parts of cattle that escape treatment by spraying and dipping. Such predilection sites include inner fringes of ear, under part of tail and legs and require special attention. Selective application of acaricides to these sites is called hand dressing and is done as a supplement to usual dipping (Vahora *et al.,* 2012).

Human safety is of utmost importance in acaricide application. Prolonged and repeated contacts with skin should be avoided. Hands and face should be properly

washed before eating (Vahora *et al.,* 2012).

Tick free or acaricide treated cattle have better productivity as compared to tick infested cattle (Sajid, 2007). However, acaricides application has many discrepancies including high cost of purchase and infrastructure maintenance, development of natural resistance to acaricides by ticks and ecological concerns (Mbyuzi *et al.,* 2013).

Selection of tick resistant cattle breeds

Selection of cattle breeds with enhanced tick resistance is proposed as a sustainable approach for controlling infection in developing world (Naik *et al.,* 2010). Generally, crossbred, purebred and exotic cattle are more vulnerable to infection than indigenous cattle. High incidence of theileriosis is reported in cross bred cattle by Annand and Ross (2001) and Malmquist *et al.* (2003) suggesting their high susceptibility to infection (Annand and Ross, 2001; Malmquist *et al.,* 2003).

Low prevalence of parasite is reported in Sahiwal cattle than European breeds suggesting that Sahiwal cattle are more resistant to tick infestation and tick borne diseases (Sajid *et al.,* 2009). Rearing disease-resistant breeds play significant role in controlling theileriosis. A national policy was developed in India to reduce proportion of exotic cattle in national herds which led to great decline in prevalence of theileriosis (Omer *et al.,* 2002).

Chemotherapy

T. annulata and *T. parva* show similar disease symptoms. These symptoms include immune-depression and secondary bacterial infection e.g. pneumonia and enteritis. Antibiotic treatment is usually recommended to limit such secondary infections (Minjauw and McLeod, 2003). Tetracycline antibiotic was probably the first chemotherapeutic compound used against ECF in 1953. This antibiotic is effective only at the early stages and can't be used at later stages of infection. In 1970s, parvaquone and buparvaquone (naphthoquinone compound) were discovered which are more effective with a wide therapeutic index (Gachohi *et al.,* 2012). These naphthoquinone compounds are not only effective for curing theileriosis but can also be used as a remarkable prophylactic measure against the disease (Qayyum *et al.,* 2010). These *Theileria* cidal drugs specifically target the etiological agent, but don't affect edema directly. Furosemide, a loop diuretic, can be used to reduce cardiovascular and pulmonary edema as well as renal and hepatic dysfunction (Musoke *et al.,* 2004).

However, these drugs are not used by cattle breeders due to their high price (Gachohi *et al.,* 2012). These drugs infiltrate the muscles and are not easily eliminated from the cattle's body (Mirzaei, 2007). The meat and milk products may be contaminated with drug residues leading to health hazards (Sonenshine *et al.,* 2006). Drug resistance is also reported in Tunisia recently. 4 out of 7 cattle died of acute tropical theileriosis in spite of buparvaquone injections (Mhadhbi *et al.,* 2010). Similarly, 7 out of 8 cattle died in southern Iran, though buparvaquone treatment was given. Resistance against anti-*Theileria* l drugs is reported due to point mutation in the parasite's cytochrome *b* gene (Sharifiyazdi *et al.,* 2012).

Investigations based on genome mining and gene characterization have focused on other metabolic enzymes as new targets for anti-*Theileria* l drug designing (Fernandez-Robledo and Vasta, 2010). Lactate dehydrogenase abbreviated as LDH is a one of such targeted glycolytic enzyme.

Recently, LDH gene has cloned from *Theileria* and has provided valuable insight into LDH structure that will be beneficial in new drug design studies (Erdemir *et al.*, 2012).

Calotropis procera (also known as "Akra" or "Ak") is a wild plant found in Asia and Africa that has multipurpose chemotherapeutic activities and can be effectively used to treat bovine theileriosis (Durrani *et al.*, 2009). *Peganum harmala* is a medicinal plant that grows in arid and semiarid conditions. Extract of *Peganum harmala* can also be used to treat bovine theileriosis without infiltration into the muscles (Mirzaei, 2007).

Immunization

Each developmental stage of *Theileria* specie elicits specific immune response. Different protocols of vaccination have been implemented in several countries with varying degree of success. The acquisition of adaptive immunity led to the concept of deliberate inoculation of parasite to cattle and simultaneous treatment of cattle with tetracycline antibiotic (Di Giulio *et al.*, 2009). Schizont-infected cells can be grown in culture indefinitely and loses virulence on prolonged cultivation which suggests antigenic variation. The culture-derived, attenuated vaccine is effective in prevention of theileriosis (Pipano and Shkap, 2000).

The attempt of immunization in cattle against tropical theileriosis was first made in Algeria in 1930s. Blood with low virulence strain was donated from infected cattle followed by mechanical passage between healthy cattle. This practice resulted in subsequent loss of parasite's ability to differentiate into merozoites with one year estimated protection in the absence of natural challenge (Weir, 2006). A similar attempt was made in Israel in the 1940s by inoculating cattle with low virulence Tunisian strain and boosting with local strain after two months to reinforce immunity (Pipano and Shkap, 2000). "Infection and treatment" method was pioneered by Neitz in early 1950s for ECF and is still widely used (Weir, 2006). In this method, live sporozoites were inoculated in cattle and the resultant infection was simultaneously treated with administration of Oxytetracycline (Marcelino *et al.*, 2012). Vaccines were also used for host immunization to control tick infestation by Humphreys and Allen in 1979 (de la Fuente *et al.*, 2007). Immunity is for prolonged duration if tick infestation continues to evoke immunity regularly. In the absence of tick infestation, immunity lasts up to three years (Gachohi *et al.*, 2012).

"Infection and treatment" method is effectively used against *T. parva and T. annulata* to induce cytotoxic T cell response against parasitic schizonts. The sporozoite stage of *T. parva* and *T. annulata* possess major surface antigen called p67 and SPAG-1 respectively. These antigens are serologically cross-reactive and have been found to induce protection against homologous strains (Hall *et al.*, 2000). Recombinant forms of surface antigens of sporozoites can be further improved by using tick peptides in multivalent vaccines (Bishop *et al.*, 2004).

As a consequence of this practice, a mild reaction appears to parasitic infection and the cattle acquire immunity to succeeding attacks. Broad spectrum vaccines may be developed using multiple antigens to target various tick species and reduce transmission of parasite (de la Fuente *et al.*, 2007). TaSp protein coded by TaSp gene of *Theileria* is demonstrated to possess polymorphism and can be helpful for manufacturing broad band vaccines (Sadr-Shirazi *et al.*, 2012).

Figure.1 Life cycle of *T. parva* (Bishop *et al.*, 2004)

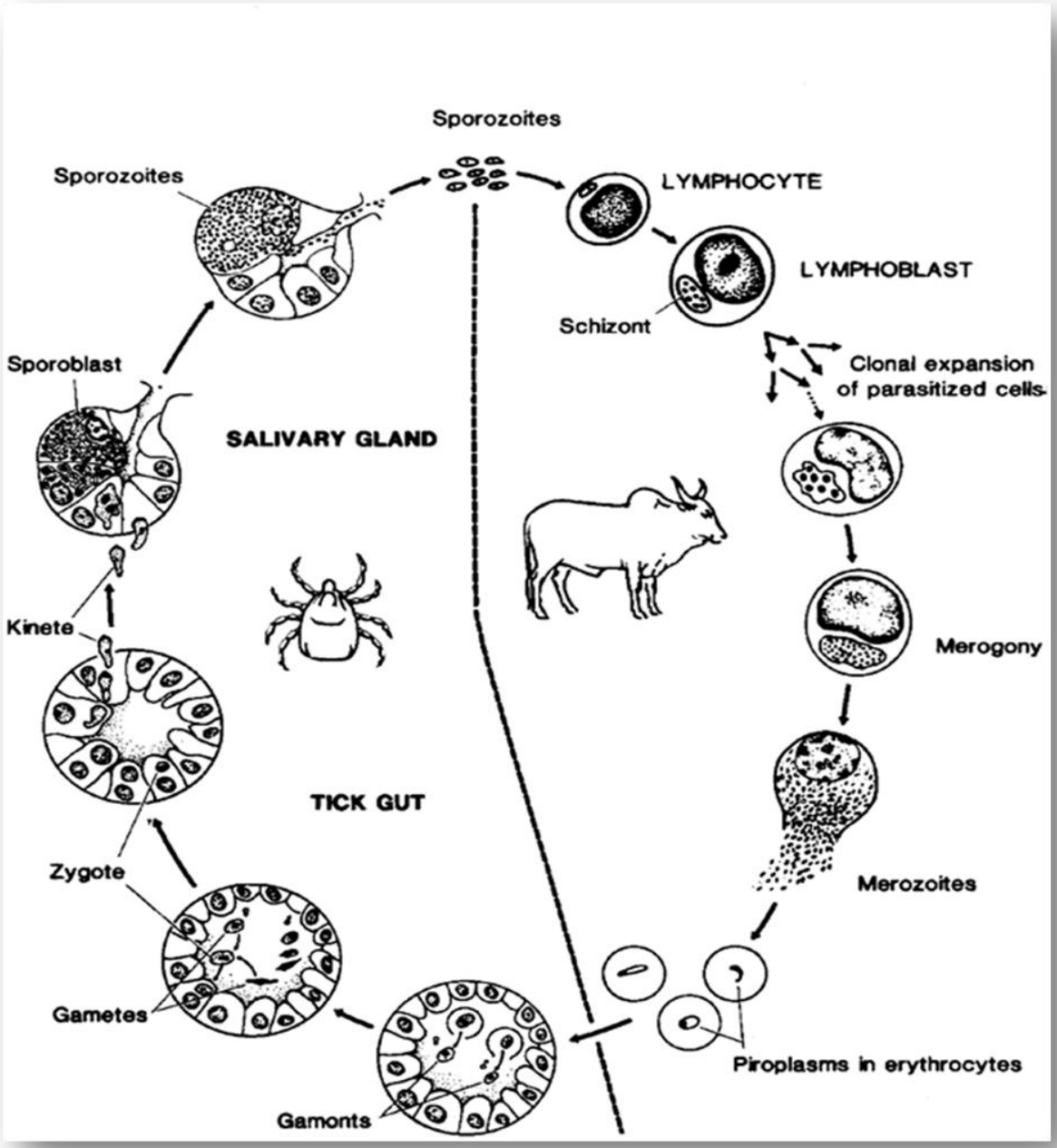
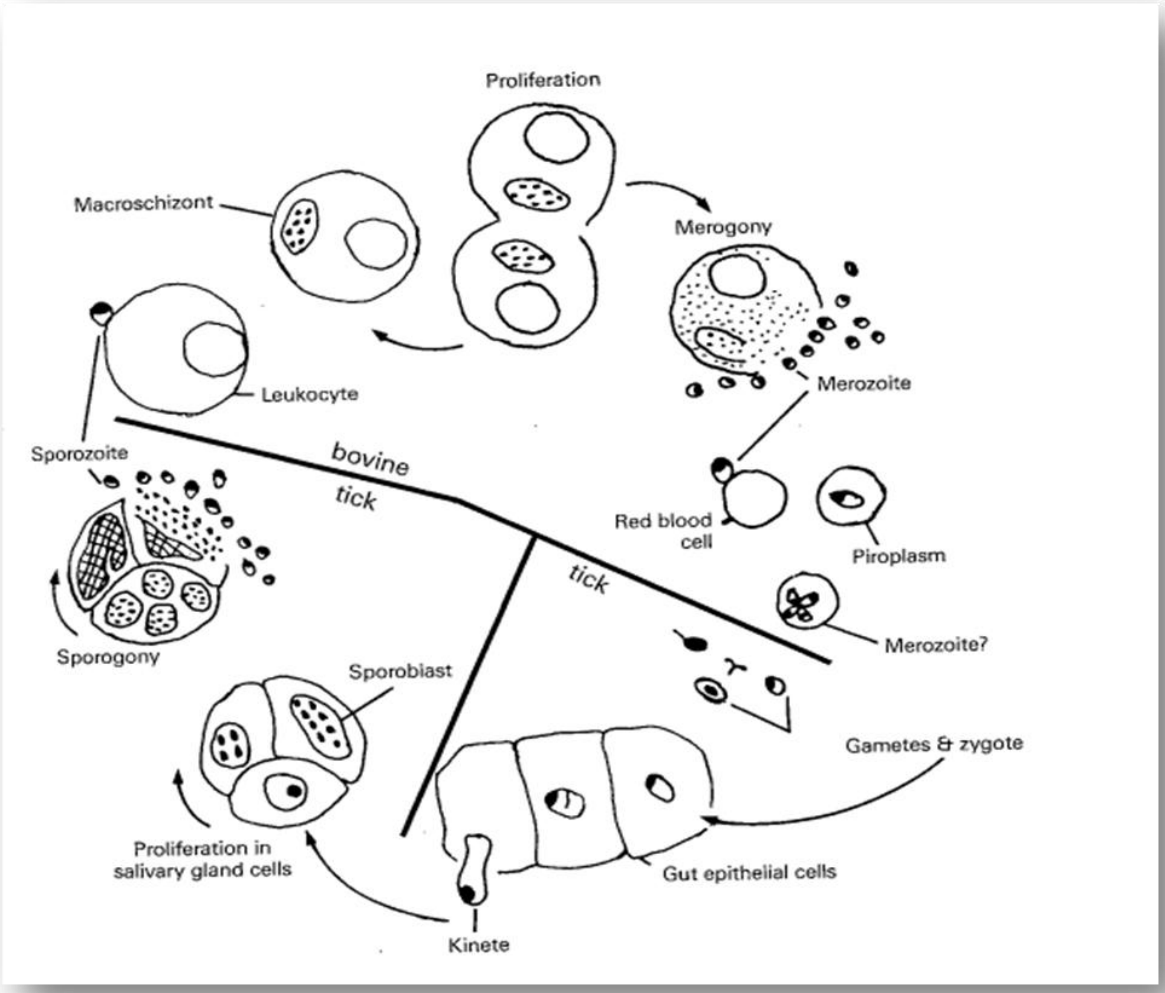


Figure.2 Life Cycle of *Theileria annulata* (Tait and Hall, 1990)



The development of accessible and affordable vaccines is an effective measure to control tick-borne diseases. They reduce the chances of environmental contamination. However, they also have some discrepancies. Though immunization contributes to attaining and improve endemic stability of indigenous breeds having adequate genetic tolerance, the resultant carrier state may transmit infection to disease free regions if the vector is present. Blood vaccines pose unacceptable risk to the recipient. It may not only cause clinical infection, but is also demonstrated to transmit other blood-borne etiological agents (Pipano and Shkap, 2000). Live vaccines are not widely used because they require cold storage and have limited shelf life. Poor nutritional status of the recipient or incorrect administration of the drug may cause clinical signs. Adverse reactions can probably occur despite treatment and animal monitoring is recommended which depends on herd size and may be extensive (Di Giulio *et al.*, 2009). It also fails to provide protection against different strains in a locality. Moreover, attenuated organisms are at risk of reverting to pathogenic state and

may cause morbidity and mortality in cattle (Jenkins, 2011).

Early life clinical signs are also reported in calves born to vaccinated cows. Calves are imperative to the propagation of livestock and calf mortality greatly shatters livestock economy. Further investigation is required to understand the mechanism of vertical transmission to reduce calve mortality (Mbyuzi *et al.*, 2013).

Pakistan is located in Warm Climate Zones. The geographical location and climatic condition of Pakistan is conducive to growth of tick species which is correlated with potential occurrence of *Theileria*. The incident rate of ticks is high during monsoon season due to warm and humid climate. Tick infestation not only causes physical distress, but also transmits parasite to cattle and causes morbidity and mortality leading to the economic burden on cattle owners. Theileriosis is a major constraint to livestock industry all over the world, therefore, it is an essential prerequisite to develop sensitive and specific tests for parasite detection in the sample. PCR is the most beneficial molecular tool for diagnosis of infection till date than blood and lymph node smear examination and serological tests. Tick eradication particularly by using acaricides and burning pastures effectively limits the incidence of theileriosis. Investigation should be carried out to exploit the benefits of other control measures, e.g. introduction of natural predators of ticks to be used as a substitute for traditional acaricide application because of emergence of acaricide resistance in ticks. Extracts derived from *Calotropis procera* and *Peganum harmala* are therapeutically effective in treating bovine theileriosis.

Parvaquone and buparvaquone have been used effectively as anti-*Theileria* l drugs since 1970s. However, research should be extended to design new drugs having different modes of action because resistance is reported with currently available drugs. Genome mining and gene characterization can be helpful in designing new drugs by focusing essential metabolic enzymes as new targets. Immunization appears beneficial. Though, current vaccine encourages preliminary results, research should evaluate safety issues and their efficacy in field use. Immunization leads to carrier state and carrier cattle can disperse infection to non-endemic localities. The currently used prophylactic methods against theileriosis are expensive and have many limitations regarding their sustainability and efficacy. It is recommended to devise and implement cost-effective and integrated control strategies against the infection. The future policies should focus controlled crossbreeding, thoroughly monitored medication programs, implementation of appropriate tick control strategies and screening carrier cattle to reduce the prevalence of theileriosis. Properly controlled cattle movement can limit dispersion to other regions. It is also important to disseminate information to cattle holders regarding new technologies so that they can develop appropriate strategies according to their own requirement.

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