

A REVIEW OF THEILERIA INCIDENCE IN CATTLE POPULATION, ITS IMPACT ON HEMATOLOGY OF THE INFECTED ANIMALS AND THERAPEUTIC APPROACH TOWARDS THE INFECTION

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ABSTRACT

Theileria is an obligate intracellular parasite infecting both domestic as well as wild bovines and small ruminants. In cattle, the most important species responsible for the infection are *T. annulata* and *T. parva*. *Theileria parva* is prevalent in 13 Sub-Saharan countries of Africa and causes East Coast Fever (ECF) while *T. annulata* is prevalent in Asia, North Africa, and Southern Europe and causes Mediterranean or tropical theileriosis. The disease is highly incident in exotic breeds and crossbred cattle irrespective of age groups in tropical areas. Transmission occurs through ixodid ticks associated with genera *Hyalomma*, *Rhipicephalus*, *Amblyomma*, and *Haemaphysalis*. Ticks have been classified into three families, out of which two families are of veterinary importance viz *Argasidae* and *Ixodidae*, commonly known as soft ticks and hard ticks, respectively. Biochemical and clinical variations, seen in the disease show relationship to the degree of parasitemia, degree of anemia, and the severity of hypoxia. Hemoglobin level is adversely affected in positive cases and reduced up to 8mg/dl. In severely infected cases hemoglobin level is reduced to 3mg/dl. Packed cell volume percentage decreases to 9% and the total erythrocytic count is reduced to 2.3 million per cm². Marked clinical signs of theileriosis include lymph nodes enlargement, anorexia, depression, listlessness, dullness, dyspnoea, pyrexia up to 107°F, suppressed cough, ocular and nasal discharges. In severe cases, diarrhea or dysentery and systemic changes are seen. Control can be achieved using different means including chemotherapeutic control, biological control, genetic control, and vaccines. The most authentic and effective control is the use of parasitocidal drugs harmless to the host. But, due to the emergence of resistance, in some cases the efficacy becomes marginal. However, buparvaquone is found to be the most effective drug against theileriosis.

Incidence of Theileria in cattle population

The distribution and prevalence of tropical theileriosis in bovines from different sites in Eastern Turkey utilizing serological, microscopic, and molecular techniques were investigated. A sum of 1483 blood smears, 1505 serum, and 1561 whole blood samples were collected from cows of different ages and breeds. From blood samples of cattle, the DNA of *T. annulata* parasite was extracted and amplified via PCR with specie specific primers. Using an indirect fluorescence antibody test, serum antibodies were also investigated against *T. annulata*. Moreover, the microscopic examination of the blood smears was carried out for detection of *Theileria* piroplasms. Using molecular technique PCR, DNA extracted from the blood samples were examined and thus the amplicon with 721bp size was obtained in 590/1561 samples showing a 37.8% prevalence of tropical theileriosis using the molecular method. Microscopic examination of the smears revealed 19.7% and serum antibodies against *Theileria* revealed 34.9% prevalence. A sum of 179 ticks including 136 females and 43 males were also collected from cows and were identified as *Hyalomma anatolicum anatolicum* based on their morphological features (Dumanli et al., 2005).

The distribution of ticks in different agroecological zones, factors affecting the distribution of ticks, and estimation of the prevalence of *T. parva* in ticks in Tanzania were studied. Both nymphs and adult stage ticks were collected from 29 sites using white cloth and blanket dragging techniques. Tick species recorded in increasing order were *Amblyomma variegatum*, *Boophilus* species, and *Rhipicephalus appendiculatus*. Based on PCR technique, 2.7% (22/826) prevalence of *T. parva* was determined in the *R. appendiculatus* producing 405bp at PCR. Furthermore, implications of the study were described based on temporal and spatial arrangements for accurate detection of tick-borne parasites, the endemic status of the ticks, infected ticks as vectors and strategies for treatment and control of tick-borne diseases (Swai et al., 2006).

The prevalence of theileriosis in buffaloes in 21 villages around Lahore was determined for May, June, July, and August. Animals were screened through a thin blood smear staining technique and were confirmed by PCR. Through blood smear, the prevalence was recorded as 47, 44, 33, and 36% in May, June, July, and August, respectively. Analysis through PCR showed 53.6, 58.33, 52.4, and 48.8% during May, June, July, and August, respectively. The relative efficacy of PCR was 53.3%, while the efficacy of microscopic examination was 39.9% (Durrani et al., 2008).

A surveillance study comprised ticks, playing a role as a vector in disease transmission, in three districts of Punjab (Rawalpindi, Lahore, and Multan). For this purpose, 300 specimens of ectoparasites were randomly collected from cows from each of the districts. Results revealed the highest prevalence of ticks (66.7%) in bovines in district Lahore. Similarly, in district Rawalpindi and Multan, the prevalence of lice was highest (36.3%) and mites (4%), respectively. In cattle, *Hyalomma* ticks were found to be the most prevalent (12%) while *Rhipicephalus* were found least prevalent (3.1%). The study revealed that the period of mean pre-oviposition was highest in spring and lowest in autumn. Similarly, the period of mean oviposition was also high in the spring. Species wise occurrence of *Theileria* in tick guts was confirmed via PCR technique showing the highest prevalence for *H. a. anatolicum* (86.6%) and lowest (20.8%) for *H. m. marginatum* (Durrani et al., 2008).

The clinical and therapeutic approach towards bovine theileriosis in Egypt estimated the endemic situation of theileriosis in various locations in Egypt. For this purpose, 120 animals were selected, showing the signs and symptoms of enlarged lymph nodes, fever, pale mucous membrane, corneal opacity, diarrhea, nasal and ocular discharges. Initially, all the animals were clinically examined, then blood samples were collected from each of the animals from the jugular vein for the detection of the parasite. Moreover, from the swollen lymph nodes, the lymph samples were collected, and directly lymph smears were also prepared for microscopic examination. The results obtained revealed that 25.8% (31/120) cows were positive for *T. annulata* by blood film. The infection was more common in summer 27.5% and lower in winter (22.5%). Additionally, the positive animals were treated with oxytetracycline and diminazene aceturate, and all the animals were recovered with the treatment (Rady et al., 2008).

The occurrence of *T. parva* infections was determined in cattle on different farms of district Ladysmith, South Africa. An outbreak was reported in district Ladysmith which was not declared an endemic area for Corridor disease infection. Therefore, 2000 Red Brangus cattle of all ages and all farms were surveyed. The infection was also transmitted experimentally from sick to healthy animals via tick stabilize injections and tick bites. For hematological and serological studies, 5ml blood samples were collected from each animal from jugular and caudal veins in EDTA coated vacutainers and serum tubes, respectively. Then DNA was extracted from 200µL whole blood for RT-PCR analysis. For detection of antibodies against *T. parva*, serum samples were processed on IFAT. It was indicated in the survey that a huge number of animals had antibody titers against *T. parva* while there were six animals affected based on RLB and PCR analysis. The results revealed that out of 170 cattle 6 were found positive for *T. parva* by RT-PCR, 2 were positive by RLB while 19 cattle were detected as positive using IFAT technique (Thompson et al., 2008).

The incidence of blood protozoa was determined in Sahiwal and Holstein Friesian cattle, during autumn, winter, spring, and summer season, in three districts of Punjab including Multan, Rawalpindi, and Lahore. For this purpose, 500 blood samples were collected from each breed of cattle. During the summer season prevalence of blood, protozoa were quite high in Holstein Friesian i.e. 97.6% compared to Sahiwal's 44.8%. In Holstein Friesian versus Sahiwal, winter prevalence was 17.6 versus 20.8% and in autumn 12.8 versus 9.6%, respectively. In spring, the prevalence of *Theileria* in Sahiwal cattle was 20%, while it was 30.4% in Friesian cattle. In total, out of 500 blood samples, the prevalence of *Theileria* in Holstein Friesian cattle was 7% and 5% in the case of Sahiwal cattle. Results suggested that the Sahiwal breed exhibited maximum resistance to ticks and disease is transmitted through ticks. The case fatality rate of theileriosis calculated was 14% at Government farms, Punjab. Giemsa staining of blood smears revealed comma and signet ring *Theileria* piroplasms with a diameter of 0.5-1.5µm. Blood parameters revealed increased mean corpuscular hemoglobin, mean corpuscular volume values, and slightly reduced mean corpuscular hemoglobin concentration. These were the indications of macrocytic hypochromic anemia. The increased leukocytic count revealed a high inflammatory response in the animals infected with the *Theileria* parasite.

Moreover, comparing the blood parameters of infected Sahiwal and Holstein Friesian cattle, it was evident that total leukocytic count, total erythrocytic count, and packed cell volume in Holstein Friesian was significantly reduced (Durrani et al., 2010).

A survey was reported in Punjab (India), for the determination of the prevalence of Theileria parasites in ticks which were collected from healthy animals for the assessment of natural infection level of Theileria species in the field circumstances. For this purpose, a sum of 110 semi engorged female and 156 male ticks belonging to *Hyalomma anatolicum anatolicum* were taken from buffaloes and cattle. The prevalence, abundance, and intensity of *T. annulata* were 15.45, 32.88, and 5.08%, respectively, which were significantly higher than male ticks that are 8.97, 18.86, and 1.69%, respectively. Similarly, ticks taken from cows showed significantly higher prevalence, intensity, and abundance of *T. annulata* infections 15.15, 35.53, and 5.38%, respectively compared to buffaloes having 9.58, 18.13, and 1.74%, respectively. Moreover, dry and hot climates favored ticks development and transmission of theileriosis in cattle and buffaloes (Haque et al., 2010).

The epidemiology survey of tick infestation and theileriosis in small ruminants (including sheep and goat) at the National Agriculture Research Centre (NARC) Islamabad in the winter season was conducted. Total samples collected were 662 (443 from goats and 219 from sheep) and screened for theileriosis and presence of ticks on animals. Out of these, 43.37% sheep and 41.53% goats were infested with diverse species of ticks among which *Rhipicephalus* ticks were most abundant. In the case of theileriosis, 7.36% of sheep and 3.8% goats were affected with theileriosis. When compared to different months of study, the incidence of theileriosis and ticks infestation was highest in summer (May-July). Maximum ticks were found in the ear (78.50%), then the underside of the tail (13.98%), while the least number of ticks was seen on hind limbs (4.65%) and udder (2.87%). While experiment, ticks collected belonged to 4 different genera; *Ambylomma*, *Haemaphysalis*, *Ixodes*, and *Rhipicephalus* (Irshad et al., 2010).

Theileria buffeli and *T. orientalis* were reported as widespread globally causing subclinical infections, however, can infect cattle based on epidemiological factors like health status, previous exposure to theileria, stress factors, and variable species pathogenicity of theileria as reported in New Zealand and Australia. *Theileria lestoquardi* and *T. ovis* were the species infecting small ruminants in North Africa, Asia, and the Mediterranean basin, transmitted through *Hyalomma* ticks. *Theileria luwenshuni* and *T. uilenbergi*, both are ovine piroplasms reported in north-west China. Similar sequences were reported in sheep in Turkey and northern Spain, but with low pathogenicity. *Theileria mutans* and *T. taurotragi* caused mild disease or no disease while *T. velifera* was a nonpathogenic species (Jeong et al., 2010).

The significance and prevalence of hemeprotzoan infections were studied in cattle in Northcentral Nigeria. For this purpose, a total of 637 blood samples were collected from cattle from various sites in Nigeria. Blood samples were collected and stored in anticoagulant-containing containers. Thin blood smears were prepared and screened using the Giemsa staining technique. For each of the positive samples, PCV was also determined to evaluate the effect of parasitism on blood profile while for detection of motile parasites, the hematocrit centrifuge technique was utilized. The overall prevalence of hemeprotzoans was calculated as 25.7% (164/637). The most prevalent parasite detected was *Babesia bigemina* and *B. bovis* accounting for the prevalence of 16% then *Theileria mutans* exhibiting 3.1% prevalence, *Trypanosoma vivax* and *T. congolens* jointly showed 2.8% prevalence while *Anaplasma marginale* was found least prevalent (1.9%). Moreover, evaluation of blood samples for PCV revealed a significant effect of hemeprotzoans on animal's health status by adversely affecting hematocrit values. Hemoparasitic infections in young age animals and during dry seasons significantly reduced the hematocrit values. It was concluded from the study that hemeprotzoans were endemic in bovines in the region and cause serious diseases in the animals when the animals are prone to stressful conditions (Kamani et al., 2010).

A study in dairy cows in District Sahiwal towards the prevalence, clinico-therapeutic response, and prophylactic measures for bovine theileriosis was conducted. For this purpose, a total of 300 exotic dairy cows were screened from different livestock farms of district Sahiwal to analyze the prevalence, their effects on the blood parameters, the succession of the disease, treatment response, and prophylactic aspects of *T. annulata* infections. Animals were further subjected to two groups viz Group A comprising 185 animals as control and Group B comprising 115 animals affected with theileriosis diagnosed on the clinical basis, which was further divided into two groups viz B1 (n=75) of adult animals and B2 (n=40) of calves of age less than 1 year. Group B animals were subjected to the treatment using buparvaquone @2.5mg/kg once and oxytetracycline (long-acting) @20mg/kg via intramuscular route and repeated after 48 hours. The same treatment dose was administered to Group A (healthy animals) for prophylactic measures as a single dose. Overall bimonthly prevalence of theileriosis was recorded as April- May (31.0%), June-July (59.0%), and August-September (25.0%). Among adult and young animals of Group B1 and B2, respectively, the highest incidence of theileriosis was reported in B1 (61.54%) during the months of June-July. Overall, an 18.26% case fatality rate was observed which was significantly higher in the months of June-

July (22.0%). Hematological parameters were also recorded revealing a significant reduction in the mean values of PCV, TEC, TLS, and hemoglobin levels in the affected animals compared to control group animals. The therapeutic and prophylactic efficacy of both drugs was estimated and showed that buparvaquone was 81.73% effective while oxytetracycline showed 100% efficacy. It was concluded that a combination of buparvaquone and oxytetracycline would be more effective against theileriosis infections in cattle for therapeutic and prophylactic measures (Qayyum et al., 2010).

In the Chittagong division of Bangladesh, the prevalence of hemeprotzoans in indigenous and crossbred bovine populations was determined. A total of 216 and 432 blood samples were collected randomly from crossbred and indigenous cows, respectively, and were examined via Giemsa stained smears. On the whole, the prevalence of hemeprotzoans recorded was 16.18% in crossbred cattle and 12.02% in indigenous cattle. The highest prevalence of hemeprotzoans was found in summer (12.5%) followed by rainy and winter weather. The highest prevalence of babesiosis was found that is 9.25% in crossbred and 7.17% in indigenous cattle while the lowest incidence of 0.70% of theileriosis was reported in the winter season. The prevalence of hemeprotzoans in terms of age revealed that the prevalence of hemeprotzoans was higher in adults as compared to young age animals. Similarly, female cattle were more susceptible to hemeprotzoans infections than males (Alim et al., 2012).

To determine the incidence of *T. annulata* in large ruminants in six districts of southern Punjab including Bhakar, Bahawalnagar, Layyah, Multan, Muzaffar Garh, and Vehari. For this purpose, 144 blood samples were collected from large ruminants comprising 39 buffaloes and 105 cows. It was found that major risk factors in the spread of parasites were the presence of ticks on animals, the age of animals, and the existence of ticks on dogs present with herds. For identification of parasites, two techniques were applied viz PCR amplification and Giemsa stained slides examination. PCR was found more sensitive in parasite detection (19%), while smear screening was less sensitive (3%). Twenty-eight blood samples of ruminants out of 144 were found positive by using PCR amplification (19%) at 721-bp fragment which was specific for *Theileria annulata*, while microscopic evaluation of Giemsa stained slides detected 4 samples positive out of 144 exhibiting 3% sensitivity. The parasite was highly prevalent in Bhakkar (41%) and the lowest prevalence was recorded in Layyah (10%). Parasites were found in five districts out of six, while no parasite was found in District Vehari. It was concluded that various modern and conventional techniques are applied for the diagnosis of theileriosis but Giemsa staining is the traditional one. It is economical, easy, time-saving, and sufficient for the identification of acute infections. However, the carrier stage of animals having low parasitemia cannot be detected via this technique (Shahnawaz et al., 2011).

For estimation of the prevalence of theileriosis in small ruminants, the study was carried out in Lahore comprising 529 animals, whose blood samples were collected (273 from sheep and 256 from goats). These samples were stained and subjected to microscopic analysis for detection of intra-erythrocytic piroplasms and thus prevalence of theileriosis in goats was found 8.2 and 13.9% in sheep. Age and sex did not affect theileriosis in goats but sheep were affected by age and sex. However, the prevalence of *Theileria* species varied throughout the year with different seasons. Prevalence was low in winter and high in summer because in summer tick infestation was high attributed to elevated environmental temperature, relative humidity, and rainfall (Naz et al., 2012).

The prevalence of hard ticks of cattle and buffalo infected with theileria, in the Jhang, Khanewal, and Faisalabad districts of Punjab, Pakistan was studied. Ticks from infected animals were procured in July and August, irrespective of sex, weight, and age of animals. A total of 6263 ticks were collected from 320, 710 buffaloes and cattle respectively. Ticks were preserved in the laboratory in 70% ethyl alcohol and ticks were analyzed microscopically. Using the phenol-chloroform method the DNA was extracted from the ticks and these DNA samples were stored at -40°C, and then utilized for PCR analysis. Ticks belonging to *Hyalomma* species showed significantly higher prevalence (61%) than ticks of other species. In cattle, ticks' infestation rate was quite higher (70%) compared to buffaloes (34%). Moreover, female *Hyalomma* species were exhibited the highest prevalence of (85%) and then *Amblyomma* (81%), *Boophilus*, and *Haemophysalis* showed 70% prevalence in ticks. But the PCR results revealed that 50% of the *Hyalomma anatolicum* ticks and 40% *H. dromedary* ticks were infected with theileria. *Boophilus annulatus*, *Hyalomma marginatum* and *Amblyomma varigatum* ticks were found negative for theileria (Ali et al., 2013).

A survey for detecting the prevalence of piroplasms infections, in three different locales of Northern Ethiopia, was carried out. For this purpose, overall 525 domestic animals were surveyed including 265 goats, 160 sheep, and 100 cattle. The blood samples were collected from the jugular vein from these animals, during October and November. Using PCR technique followed by DNA sequencing 80 (80%) cows, 150 (93.8%) sheep, and 5 (1.9%) goats were found positive for *Theileria* species. It was found that the incidence rate of theileriosis was higher in sheep than cattle and goats whilst the infection rate of theileriosis was higher in cattle as compared to goats. Gender was not significantly associated with the rate of infection. In Northern Ethiopia, the widespread distribution of the disease was caused by virulent *T. annulata* and mild pathogenic parasites including *T. orientalis* and *T.*

mutans. In sheep higher infection rates were associated with mostly subclinical forms of *T. ovis* referring to the wide exposure to the ticks (Gebrekidan et al., 2013).

A study was carried out in five metropolises of Punjab (Lahore, Gujranwala, Multan, Bahawalpur, and Faisalabad), to determine the seroprevalence of equine piroplasmosis caused due to *Theileria equi* and *Babesia caballi*, the intraerythrocytic parasites. For obtaining insights into the incidence of the disease, 430 equids were screened from five metropolises of Punjab. These equids included 33, 65, and 332, mules, horses, and donkeys, respectively, of either sex, age ranging from 5-10 years. Using competitive ELISA, these were tested serologically for detection of antibodies against *T. equi* and *B. caballi* and it was found that overall 52.6% (226/430) serum samples of equids were positive for equine piroplasmosis. The overall seroprevalence of *T. equi* was recorded as 41.2% while for *B. caballi*, seroprevalence was 21.6%. In metropolises of Lahore, the seroprevalence of *T. equi* was significantly higher in equines (66.7%) and 56.9% horses (Hussain et al., 2014).

The incidence of theileriosis was assessed in crossbred cattle at district Dehradun, India. For this purpose, 301 blood samples were screened during 10 months from crossbred cows, from different locations of district Dehradun. The collected samples were screened using Giemsa stain and PCR technique. For detection of *Theileria* piroplasms, the blood samples were collected from ear veins while 3ml of the blood samples were collected from the jugular vein in EDTA coated vacutainers and were stored at -20°C for extraction of DNA. Overall 27.2% (82/301) prevalence of theileriosis was revealed through microscopic examination of the smears whereby the highest prevalence was recorded in rainy seasons with the rate of 45.4%. Using a specific PCR test, 32.5% of the prevalence was found in which 98 blood samples out of 301 were found positive for *Theileria* parasites. Using PCR technique 16 other samples were also found positive for *Theileria* besides 82 samples detected through microscopic examination and these samples were termed as “carrier cows” (Kohli et al., 2014).

Quantitative and qualitative analysis of *T. annulata* in buffaloes and cattle was done using the technique of PCR in India. This technique was followed to detect both carriers as well as infected stages of the parasite *T. annulata* utilizing the quantitative PCR method in which the gene was targeted in which major merozoites piroplasms surface antigen TAM. In total 116 samples were collected from sick and healthy buffaloes and cattle. Out of these 63.79% (74/116) samples were found positive for *T. annulata* using real-time PCR, also it included 15 positive samples of *T. annulata* detected through Giemsa staining. The parasitic load was found to be ranging from 1.39×10^6 to 2.83×10^7 parasites per milliliter of the blood using qPCR. It was, therefore, concluded that real time PCR can be utilized for the detection of *T. annulata* parasite in the blood samples of buffaloes and cattle and the load of the parasite can be quantified (Kundave et al., 2014).

The epidemiological analysis of theileriosis in the dairy cattle was designed whereby a total of 117 cattle were screened for the detection of parasite; *T. annulata* microscopically as well as the hematological and biochemical analysis was conducted in the affected animals. Twenty-eight animals were found positive for theileriosis when the Giemsa stained blood smears were examined microscopically (Modi and Bhadesiya, 2014).

In Faisalabad (Punjab) from April to July, the clinico-epidemiological analysis of theileriosis in cattle and its comparative therapeutic response to Peganum harmala and buparvaquone was investigated. For this purpose, 260 cattle were analyzed for theileriosis by making lymph smears and thin blood smears stained with Giemsa, out of which 28 were found positive. Thus, the incidence rate of disease was 10.76%, whereby 8.93% in females and 10.71% in males. Bi-monthly incidence of April-May and June-July was also calculated, showing 28.57 and 71.42%, respectively. Twenty-eight positive animals were divided into two equal groups. Group A animals were subjected to treatment by Peganum harmala @ 7mg/kg body weight via intramuscular route and group B animals were treated by buparvaquone. The efficacy of *P. harmala* and buparvaquone was found as 85.71 and 92.85%, respectively (Saleem et al., 2014).

In Tamil Nadu (India), the cattle were assessed for the influence of age, breed, and season on the prevalence of hemeprotzoans. For this purpose, 2637 blood samples were collected from cattle in 2003-2012 for detection of hemeprotzoans, including *Theileria* and *Babesia*. Thin blood smears were prepared, stained with Giemsa, and examined under oil immersion. It was found that theileriosis showed a high prevalence of 13%, while babesiosis was 1% prevalent. Prevalence was significantly elevated during summer (14.4%) and monsoon (13.8%), while in winter prevalence was very low. When breeds were screened for prevalence, the study revealed that theileriosis was highly prevalent in Jersey cross and Holstein Friesian compared to indigenous breeds of cattle. Age-wise data revealed that prevalence was high in below 2 years of age in indigenous and among 2-7 years age groups in the cross of Jersey and Holstein Friesian. From the study, it was concluded that Tamil Nadu, particularly its Western part, was endemic for theileriosis and disease occurrence was at its peak during summer (Velusamy et al., 2014).

The prevalence of *T. annulata* antibodies and Ixodidae ticks was determined in White Nile, Sudan. The cattle that had never received any acaricidal treatment during the research were selected. Samples were collected

from five different cattle farms comprising of eight cattle in each, at random. Ticks were collected from all the sites of the body using blunt forceps to prevent any damage to the mouthparts of the ticks. The ticks were identified using a dissecting microscope. Blood samples were collected from 105 cows and smears were prepared and were examined after staining with Giemsa. Moreover, a 5ml blood sample was collected from 82 cattle in EDTA coated vacutainers for the determination of the hematological parameters and serological examination was also performed to evaluate the serum chemistry of the affected animals. *T. annulata* antibodies were detected in the serum based on the ELISA technique. Results reported three genera of ticks mainly responsible for the transmission of *T. annulata* i.e. *Hyalomma*, *Amblyomma*, and *Rhipicephalus*. Seven cattle out of 105 were found positive for *T. annulata* (6.7% prevalence) piroplasms while 61% (50/82) were detected as seropositive for *T. annulata* using the ELISA method. The highest prevalence of *T. annulata* is the marker of the endemic status of tropical theileriosis (Guma et al., 2015).

The seasonal incidence and prevalence of bovine theileriosis in district Jalandhar, Punjab, India was estimated. For this rationale, a sum of 620 cattle was selected with a history of pyrexia and their blood samples were collected in vacutainers having EDTA anticoagulant. Thin blood smears stained with Giemsa and were microscopically examined under an oil immersion lens for the detection of the parasite. Seasonal incidence revealed 4.39, 13.5, 9.85, and 2.4% of cows were positive for theileriosis in spring, summer, rainy, and winter seasons, respectively. The study revealed the highest incidence of theileriosis in summer, especially in June and July. The overall prevalence of the *Theileria* parasite measured was 9.35% (Kumar et al., 2015).

The incidence of *T. lestoquardi* in sheep and goats in two districts of Khyber Pakhtunkhwa (Peshawar and Kohat), was determined using conventional PCR. Hence, 165 blood samples were collected randomly, from the small ruminant flocks including 44 sheep and 121 goats. From each animal, 10ml blood samples were collected in Eppendorf tubes. Using standard procedures, DNA from the samples was extracted for PCR. Out of 165 samples obtained, 3% (5/165) samples showed DNA fragments of 730bp through PCR amplification of rRNA gene, specific for *T. lestoquardi*. All the samples obtained from Peshawar were found negative while positive samples were collected only from Kohat. It was observed that the presence of dogs with flocks and ticks on the animals were significant risk factors responsible for ovine theileriosis. Similarly, it was also observed that mixed flocks including both sheep and goat populations, were more sensitive to *T. lestoquardi* infections (Saeed et al., 2015).

The *Hyalomma anatolicum* ticks containing *Theileria annulata* were collected from cross-bred cows in Ludhiana, Punjab to analyze the level of natural infections of *Theileria* in the field. For this purpose, a total of 60 *H. a. anatolicum* (semi-engorged) ticks were gathered from the cows, salivary glands of the ticks were also dissected. Salivary glands were divided into two portions, one portion was utilized for the DNA extraction for assessment at a molecular level through PCR technique and the other portion was stained using methyl green pyronin stain. The results remarkably revealed that *T. annulata* infection was 8.3, 20, and 60%, prevalent using MGP stain and PCR. Furthermore, it was recorded that the prevalence of *T. annulata* ticks was comparatively higher in females (8.8%) than males (6.6%). It was also demonstrated from the recorded results that both of the techniques viz PCR, as well as MGP staining, are reliable and valid techniques for the detection of the parasite, *T. annulata* in *H. a. anatolicum* ticks (Tiwari et al., 2015).

In Tanzania, the tick load and immunity acquisition to *T. parva* by Tarime and Sukuma cattle was evaluated. The study was based on tick burden and for the determination of the immunological parameters and norms responsible for resistance against *T. parva* infections (East Coast fever) in Sukuma and Tarime cows. Ticks burden, *T. parva* antibodies tests, PCV, prevalence, and incidence of *T. parva* protozoan were studied to season, strains, and dipping regimen. The study included fifty experimental cattle. Tick burden was calculated using whole body counts, using polymorphic and ELISA technique, antibodies positivity percentage was calculated and prevalence of *T. parva* was calculated using nested PCR technique based on P₁₀₄ gene. No significant difference was observed in the dipping of both strains of the cows on tick burden in wet and dry seasons. The values of PCV lied in the normal ranges yet, PCV values of Tarime cows remained lower than Sukuma. All the cows were found seropositive, irrespective of the cattle strains however, Tarime cattle had a higher percent positivity (15%) compared to the Sukuma cattle. The prevalence of *T. parva* was found higher in the Sukuma cattle (38.5%) than Tarime cattle (38%) with no statistical difference. During the study, 5 out of 25 (20%) Sukuma cattle contracted theileriosis infection while none of the Tarime cattle was affected with the clinical form of the disease. It was concluded that a lower incidence of parasites and higher antibodies titer was present in the Tarime cattle compared to the Sukuma cows. Tarime cattle did not contract the infection of East coast fever, however, served as carriers for the *T. parva* parasite (Laisser et al., 2016).

Hematological profile of *Theileria* infected animals

Alterations of blood biochemical values in the crossbred calves, experimentally infected with *Theileria annulata* were investigated. For this purpose, 06 male crossbred (Sahiwal x Holstein Friesian) calves were selected

of 53 days age. Detailed biochemical and hematological analysis was carried out from the serological studies before introducing the infection. *Hyalomma anatolicum anatolicum* ticks were collected from affected cows and raised on the rabbits in the laboratories. Then nymphs of these ticks were allowed to feed on the *T. annulata* infected calves. These infected ticks were pooled and stabilization was prepared. The GUTS were titrated and used in introducing the infections experimentally in the calves. The GUTS contained 0.75 ticks/ml of it. The calves were administered 1ml GUTS via a subcutaneous route to produce desired *Theileria* infections in calves. From the jugular veins of the animals, 5ml blood samples were collected from each of the animals for analysis of biochemical and hematological parameters before and after introducing the infections. And it was found that hemoglobin content and PCV percentage were decreased progressively associated with prominent reticulocytosis. While serological investigations revealed reduced cholesterol, calcium, and triglycerides levels with enhanced blood urea nitrogen levels. The marked decrease was found in the serum proteins including immunoglobulin, albumin, and their ratio. Coagulopathies were associated with thrombocytopenia and increased prothrombin time with increased activated thromboplastin and bleeding time during the disease. Moreover, the osmotic fragility of the RBCs was also increased along with morphological changes in the erythrocytes due to parasitemia (Singh et al., 2001).

Theileria positive animals were subjected to estimation of the total erythrocytic count, packed cell volume, and hemoglobin concentration. For this purpose, 600 buffaloes were selected based on clinical findings viz pyrexia, sunken eyes, anemia, emaciation, difficulty in walking, eczema, and presence of ticks on the body. Out of these, 107 (17.8%) were detected positive for theileriosis via blood examination. The incidence of the parasite was 15.5, 20.5, and 17.5% in July, August, and September, respectively. Overall average values of hematological parameters of these infected animals showed that PCV, hemoglobin and TEC (total erythrocytic count) were 0.15 l/l, 64.5 g/l and $3 \times 10^{12}/l$, respectively. These observations showed a significant decline in PCV, Hemoglobin and TEC concerning normal parameters owing to existing parasitic stages in red blood cells (Durrani et al., 2006).

In Turkey, 46 Holstein cows including 27 females and 19 males, naturally affected with *T. annulata* were selected and the blood samples were collected from all of the animals. For the control group, a total of 46 clinically healthy animals were selected including 21 females and 25 males, from different tick free cattle farms. Blood smears were prepared and were Giemsa stained and examined under an oil immersion lens of a light microscope. Specific hematological parameters of the infected cows were measured. The results of the hematological analysis revealed that the number of erythrocytes, hemoglobin amount, hematocrit value, mean corpuscular hemoglobin concentration, monocytes, lymphocytes, white blood cells, neutrophils, basophils and eosinophils counts were reduced significantly. On the other hand, mean corpuscular volume was increased significantly in *T. annulata* positive animals with marked reticulocytosis compared to the control group cattle, uninfected group. In terms of coagulation reports, prothrombin time and activated thromboplastin time were delayed significantly. Similarly, the number of platelets was also significantly less in the infected group cattle compared to the control group. In contrast, the concentration of fibrinogen was mildly higher in the affected group animals than in the controls. Thus, it was concluded from these observations that infections caused by *T. annulata* were of significant importance in changing the hematological parameters and coagulation factors of the affected animals (Ramazan and Uslu, 2006).

In a study, cattle of 2-4 years of age, hematology and other biochemical parameters were examined in *Theileria* and *Babesia* infected animals. For this purpose, 43 field cases of both sexes of cattle were investigated through clinical and laboratory examination, out of which 15 animals were found free from both internal as well as external parasites. Laboratory examination revealed that 20 animals were suffering from theileriosis and 8 were suffering from babesiosis. Lymph smears from lymph nodes and blood smears from ear veins were prepared. Lymph smears were prepared for identification of Koch's blue bodies. For the hematological picture, two blood samples were collected from the jugular vein from both healthy and infected animals. The hematological picture revealed that in cattle affected with theileriosis, normocytic hypochromic anemia was seen, while biochemical analysis revealed lowered serum level of total proteins and albumin and elevated serum globulin. Gamma-glutamyl transferase (GGT) level of serum was increased significantly and showed alteration and impairment in liver functions. Marked anemia, poikilocytosis, leucopenia, and anisocytosis were observed in the affected cattle (Hussein et al., 2007).

The clinico-hematological and chemotherapeutic picture on theileriosis affected water buffaloes in Egypt was studied. For this purpose, 30 water buffaloes naturally affected with tropical theileriosis (*T. annulata*) and 10 healthy buffaloes, free from *Theileria* parasite as negative control were selected for the determination of potential clinico-hematological and chemotherapeutic impact of theileriosis in buffaloes in Egypt. Significant reduction in the total erythrocytic count, total leukocytic count, and hemoglobin concentration was recorded in the *T. annulata* infected buffaloes compared to the control group animals. Early treated buffaloes with buparvaquone showed 100% recovery from the protozoan parasite, lymph nodes also reduced to normal size, and improvement in the clinical state was reported in the treated water buffaloes (Osman and Al-Gaabary, 2007).

Clinical and biochemical research was conducted in Egyptian buffaloes infected with *T. annulata* particularly emphasized the correlation between ketosis and oxidative stress. A total of 68 naturally infected buffaloes with *T. annulata* along with healthy buffaloes free from *Theileria* parasite as negative control were selected, in the herds at Gharbya and Dakahlia, Egypt. The study was aimed to interpret the clinical picture of *Theileria* infected buffaloes emphasized the correlation between ketosis and oxidative stress. Buffaloes were screened based on clinical signs including enlargement of lymph nodes, fever, corneal opacity, nasal discharges, drop in the milk yield, anorexia, pale mucosa and skin lesions. Hematological analysis revealed a significant reduction in the RBCs count and hemoglobin concentration of the infected animal compared to the control group. Serological investigations revealed a significant increase in the Malondialdehyde levels (MDA), nonesterified free fatty acids (NEFA), and β -hydroxybutyric acid (BHBA). In contrast, the levels of glucose, glutathione, catalase, superoxide dismutase, nitric oxide, total antioxidant capacity and G-6-Phosphate dehydrogenase were significantly increased in *Theileria* infected buffaloes. Thus, it was concluded that *Theileria* infections played an important role in the incidence, occurrence and severity of anemia, ketosis and oxidative stress in Egyptian buffaloes (Deeb and Younis, 2009).

The diagnostic values of proteins in indigenous cattle of Iran affected with *T. annulata* were analyzed. In this study, acute phase proteins were assessed for their relative values along with the pattern of changes. Haptoglobin, fibrinogen, ceruloplasmin, and serum amyloid were included in the acute phase proteins. Infected group of cattle (infected with *T. annulata* naturally) comprised 24 indigenous cattle of Iran of 2-3 years age groups. These infected animals were further divided into four subgroups based on their degree of parasitemia i.e. less than 1, 1, 2 and 3%. Ten infected cattle were placed in the positive control group. Using validated methods all measurements were taken from the collected blood samples. For hematological parameters, the blood samples were collected in EDTA coated vacutainers while for the analysis of proteins and other biochemical parameters the blood samples were collected in vacutainers without EDTA anticoagulant and sera were separated through centrifugation. Moreover, thin blood smears were prepared for the examination of the *Theileria* parasite. Results revealed a significant decrease in the leukocytic count, erythrocytic count, lymphocytes, hematocrit and hemoglobin concentration. Moreover, the number of neutrophils was also increased with the increase in the degree of parasitemia with no change in the concentration of monocytes in healthy and infected cattle. In contrast, the concentrations of haptoglobin, fibrinogen, ceruloplasmin and serum amyloid were markedly increased in *Theileria* infected Iranian indigenous cows compared to healthy cattle. The concentrations of haptoglobin, fibrinogen, ceruloplasmin and serum amyloid were found greater than 0.09 g/L, 1.90 g/L, 0.049 g/L and 5.68 g/L, respectively. These increased level of proteins especially serum amyloids with higher specificity and sensitivity is the indication of the inflammatory reactions in the infected cattle (Nazifi et al., 2010).

A research was conducted on hematological profile and biochemical investigation in theileriosis and babesiosis in bovine. For this purpose, 15 out of 75 cattle of age 5-7 years were selected using the Giemsa staining method, from Sharkia province, Egypt. The animals were categorized into three groups viz group A comprising healthy animals, group B with *Theileria* infected and group C comprising *Babesia* infected animals. Animals of each group were subjected to biochemical and hematological investigations. Hematological investigations of cattle suffering from theileriosis revealed a significant decline in hemoglobin content, erythrocytic count and packed cell volume. There was macrocytic hypochromic anemia due to a marked reduction in mean corpuscular volume and mean corpuscular hemoglobin concentration. In erythrogram picture, variations might be due to the damaging effect of parasites on red blood cells. Theileriosis had harmful effects on bone marrow and hindered the process of erythropoiesis, owing to its toxic metabolites. The disease evoked neutropenia, eosinopenia and leucopenia accompanied by monocytosis, basophilia and lymphocytosis. Biochemical analysis revealed a significant reduction in serum total proteins, globulin and albumin levels. Theileriosis had harmful effects on hepatocytes, resulting in impairment of hepatic functions and decreased albumin synthesis accompanied by increased albumin catabolism. Results revealed a marked increase in liver enzymes (ALT, AST and alkaline phosphatase) and total bilirubin. Cholesterol levels, triglycerides and glucose levels were reduced significantly (Alam and Nasr, 2011).

To evaluate the clinical, biochemical and hematological parameters in naturally infected cattle with *T. annulata* in province Basrah, Iraq, 447 cattle were screened out of which 114 cattle were affected naturally with *T. annulata* while the rest of the animals were healthy and served as control. A greater degree of parasitemia was revealed through blood examination and it varied from 13-53% and all the parasitic stages were observed. The erythrocytic stage of the parasite was 69.43%, the lymphocytic stage was 25.44% while the combined erythrocytic and lymphocytic stage was 5.26%. The hematological investigations revealed hypochromic macrocytic anemia along with significantly reduced PCV 29%, Hb 6.95g/L, RBCC 4.6 million/mm, WBCC 5000/mm, MCHC 24.71%, neutrophils 19% and in contrast, a marked increase was observed in the MCV 61fl and lymphocytes 69.74%. Observation of biochemical parameters revealed a significant rise in the total serum bilirubin 1.19, ALT 19.77, and

AST 67.98 while the marked reduction was prominent in the albumin 1.56 and total serum proteins 4.27 (Al-Emarah et al., 2012).

The effect of *Theileria lestoquardi* infections was analyzed based on hematological profile and biochemical parameters in desert ewes infected experimentally in Sudan. For this purpose, 10 experimentally infected Sudanese desert ewes with *T. lestoquardi* were compared to 10 healthy ewes as controls. Results obtained showed a marked reduction in the hemoglobin concentration, white blood cells count and packed cell volume compared to the controls. Reduction in the PCV percentage and hemoglobin concentration was found, 10 weeks post ticks application while the number of leukocytes was reduced at weeks 5 and 6 after the application of ticks. Serological studies revealed a significant reduction in the serum globins and total serum proteins in the infected desert ewes at week 3-7 post ticks application. Similarly, serum creatinine and serum urea levels were significantly increased in the experimentally infected ewes at week 7-8 after ticks application compared to the healthy ewes of the controls (Elsadig et al., 2013).

The biochemical and hematological changes occurring in the cows infected with *T. annulata* naturally were studied in 985 cows. These cows were found positive in laboratory examination and data was collected regarding their biochemical and hematological profile. The animals ranged from 6-9 years of age including 21% females and 79% males. Hematological investigation revealed a significant decrease in the PCV%, hemoglobin content, MCHC, RBCs, lymphocytes, basophils level in the sick cows compared to the animals of the control group. Lymphopenia, neutropenia, eosinopenia, monocytopenia was recorded along with a significant increase in the thrombocytes. *Theileria* infection was associated with the increase in the blood urea nitrogen and total urea in the infected animals than control group animals. While phosphorus, iron, calcium, chloride and sodium levels were depressed in the affected animals. Zinc, glucose and manganese levels remained unaltered in the affected cattle (Tehrani et al., 2013).

A study was reported in *Camelus dromedarius* on the alterations of clinical, parasitological, biochemical and hematological values associated with epidemics of theileriosis in Saudi Arabia. It was found that 67 out of 173 camels were suffering clinically from pyrexia, swelling of superficial lymph nodes, anorexia, lacrimation, loss of condition, infertility and abortion. Blood and fecal samples of all camels were collected and examined microscopically using the Giemsa staining technique and sedimentation floatation techniques, respectively. Sixty-seven camels were found positive for theileriosis and thus 38.7% prevalence rate of theileriosis was obtained together with a 0% case fatality rate. Camels found positive for theileriosis were further subjected to biochemical and hematological investigations and were compared to 23 clinically healthy camels serving as control. The hematological investigations revealed that total RBCs counts were significantly reduced in the affected camels, along with it, hemoglobin concentration, MCV and PCV values were also reduced significantly whereas platelets counts were significantly increased. In biochemical investigations, a highly marked reduction in the iron level was observed in positive cases. Similarly, significant increases were observed in AST, GGT, ALT, blood urea nitrogen, total serum proteins, total bilirubin and LDH levels than the camels of the control group. It was, therefore, concluded that theileriosis significantly affected the hematological and biochemical parameters of the *C. dromedarius* including muscle, kidney, and liver functions of the affected animals compared to the camels of control groups (Ismael et al., 2014).

In the Gujrat district of India, the crossbred cattle were selected for the estimation and analysis of alterations of serum biochemical profile in *Theileria* affected animals. For this reason, 117 animals were selected belonging to crossbred cows out of which 20 cows were found positive through microscopic examination of the blood smears. The cattle were organized into two groups viz group A and group B having *Theileria* positive crossbred cows and healthy cows, respectively. It was evident from the obtained results that there were significant alterations in the serum biochemistry and hematological profile of the animals affected with theileriosis. Blood urea nitrogen was increased while serum proteins were significantly decreased in the affected cattle which is the indication of renal damage (Modi and Bhadesiya, 2014).

In a study conducted in Turkey in *Theileria* infected cattle, the correlation between the level of anemia and blood gases were analyzed. It comprised 28 infected cattle with *Theileria* with a control group including 7 animals. *Theileria* infected cattle were again classified into four groups based on their hematocrit values. Group 1 included cattle with hematocrit >26 (nonanemic), Group 2 included cattle with hematocrit value of 20-26 (mildly anemic), Group 3 with a hematocrit value of 14-19 (moderately anemic), and Group 4 with hematocrit value of 10-13 (severely anemic). In the study, it was found that TCO_2 , PCO_2 and HCO_3 levels were lower in the infected cattle compared to the control group cattle. Moreover, a decrease in the concentrations of TCO_2 and HCO_3 was significant in the severely anemic group of animals. No significant change was evident in the SO_2 level of infected and healthy animals of the control group. The results markedly indicated the tendency of uncontrollable metabolic acidosis in theileriosis infected cattle with a severe degree of anemia, badly affecting the prognosis of the cases (Temiz et al., 2014).

Hematological parameters were studied in the naturally infected sheep with *T. ovis* and *Anaplasma* species for the clarification of pathogenic aspects of both organisms. For this purpose, 109 sheep were screened and blood samples were collected. The parasites were diagnosed via microscopic examination of thin blood smears prepared and molecular techniques (PCR) then blood samples were analyzed for hematological studies. By microscopic examination of the samples, 67.8% of sheep were found positive for *T. ovis* infections out of which 88% of sheep were positive using the PCR technique. The results revealed that hematological parameters were significantly altered in the infected animals than healthy animals showing significantly decreased Hb, RBC, MCHC, PCV and significantly increased RDW and MCV. Similarly, 86.2% of the sheep were detected positive for *A. ovis* and 53.2% were positive for mixed infections of *A. ovis* and *A. marginale* by PCR analysis. The same hematological profile was reported in *Anaplasma* infected sheep like *Theileria* revealing a decrease in Hb, RBC, MCHC, PCV and increase in RDW and MCV levels of the blood. It was also reported that mixed infection of *Theileria* and *Anaplasma* caused degenerative anemia in the sheep compared to healthy sheep (Khaki et al., 2015).

Therapeutic approach towards *Theileria* infected animals

Therapeutic and clinico-epidemiological aspects of theileriosis were determined in 112 clinical cases of cattle in district Faisalabad. In 72 cases, the response to oxytetracycline and buparvaquone was analyzed in terms of alleviation of the clinical signs of the affected animals. Clinical signs recorded were high grade fever (n=112), pronounced swelling of parotid (n=54), prescapular (n=99) and prefemoral (n=58) lymph nodes. Other signs included hemorrhagic conjunctiva and exophthalmos (n=16), diarrhea (n=38), intramuscular swelling (n=11), dyspnoea, pneumonia and tachypnoea (n=10), inappetence (n=110), anemia (n=48), nervous signs (n=4) and hemoglobinuria (n=2). As most of the animals exhibited the signs of fever and swollen lymph nodes, therefore, these may be the surrogate indications of theileriosis in the field cases where laboratory facilities are inaccessible. A huge percentage (42%) of the total cases were found in the calves of age 15-21 days, although 5 cases were also observed in the calves of age less than 7 days and 2 cases of less than 2 days aged calves. The disease had a strong association with the presence of ticks on the animal coat. Moreover, on seasonal distribution, the disease was reported in February and onwards with a peak in June and then steadily reduction up to November while no cases were recovered in December and January. As far as treatment of the sick animals was concerned, 67 out of 72 (93%) animals recovered on treatment with oxytetracycline @10mg/kg and buparvaquone @ 2.5mg/kg body weight. The body temperature of the treated animals was decreased within 2-3 days of the initiation of treatment. Five cases were expired despite treatment including 2 cases of cerebral theileriosis and 3 cases of terminal illness (Muhammad et al., 1999).

Holstein Friesian and Jersey breeds of cattle at Pattoki (District Kasur), were studied for the determination of incidence and therapeutic response to drugs in theileriosis. For this purpose, 50 cows each of Jersey and Friesian breed were selected randomly and examined through a blood smear. The overall incidence of theileriosis in Jersey was 15 and 24% in Friesian cows. In Jersey and Friesian cows, the prevalence of *Theileria* parasite was 8 and 16% in the first quarter of the study, 10 and 20% in the second quarter, 19 and 38% in the third quarter while 11 and 22% in the fourth quarter, respectively. Division of quarters was; first quarter from July-September, the second from October-December, the third January-March, and the fourth from April-June. Animals found positive were treated with injection Butalex® @ 5ml/100 Kg body weight via the intramuscular route. After three days of treatment, cows were again tested for *Theileria* parasite for assessment of efficacy against the parasite and it was concluded that Butalex® was 100% effective in both breeds of cattle against theileriosis (Zahid et al., 2005).

The treatment regimen for tropical theileriosis in cattle used the extracts of *Peganum harmala* plant in Iran. For this purpose, fifty cows were selected which were naturally infected with tropical theileriosis caused by *T. annulata*. The extracts of the plant were prepared from the seeds and the animals were administered with extracts of *Peganum harmala* @5mg/kg body weight intramuscularly, for 5 days. And 78% (39/50) of cattle responded to the treatment and recovered with the administration of extracts of *Peganum harmala* while 11 animals died of the disease (Mirzaei, 2007).

A chemotherapeutic trial comprised *Calotropis procera* plant and buparvaquone against experimental infections of *T. annulata* in crossbred cows in Okara Punjab. One hundred animals were selected for this purpose and rectal temperature and blood smears were examined on daily basis and the animals free from parasitic infections were rendered to the research. For introducing the experimental infection in the cows, 2000 ticks positive for *T. annulata*, confirmed through PCR technique, belonging to *Hyalomma* were collected from 10 crossbred cattle. Eighty animals were infected with these ticks and 20 animals were left healthy and free from parasitic infestations. On days 7, 14, and 21 after introducing the infection, the blood samples were collected in EDTA coated vacutainers and examined using Giemsa staining methods. The severity of the infection was calculated in terms of piroplasms concentration, change in the number of erythrocytes and leukocytes. Eighty animals were divided into eight groups i.e. A1, A2, B1, B2, C1, C2, D1 and D2, each comprising of 10 animals while rest of the 20 animals served as

control. Group A1, B1, C1 and D1 were treated with buparvaquone @2.5mg/kg body weight at alternative days and Group A2, B2, C2 and D2 were administered with homogenized flowers and buds of *Calotropis procera* @0.3mg/kg at alternative days. The efficacy of these used drugs was determined in terms of reduction in clinical signs, recovery, piroplasms infection and hematological profile at days 0, 7, 14 and 21. Giemsa stained smears revealed abnormalities in RBCs like poikilocytosis, anisocytosis and basophilic stippling. Results revealed that 75% (30/40) of cattle were recovered completely after the treatment by buparvaquone while the efficacy of *Calotropis procera* was reported as 92.5% (37/40) and the hematological values were also returned to the normal ranges. Moreover, at the end of the treatment with *C. procera* ticks were also eliminated from the animal coat however, at first 10-12 hours of this plant administration diarrhea was reported in the animals which was later on recovered spontaneously (Durrani et al., 2009).

In bovines, the efficacy of different antiprotozoal drugs against bovine theileriosis, anaplasmosis and babesiosis was evaluated. For this reason, 38 naturally affected buffaloes were divided into four groups, in which groups A, B, C and D comprised of 10, 10, 8 and 10 buffaloes, respectively and treated with diminazene, buparvaquone and imidocarb dipropionate. Group A comprised of 5 pure *Anaplasma* affected buffaloes and 5 buffaloes with mixed protozoal infections and was treated with imidocarb dipropionate @3mg/kg body weight. Group B animals were treated with diminazene aceturate @3.5mg/kg body weight while group C was treated with buparvaquone @2.5mg/kg body weight. The results revealed 80% drug efficacy of imidocarb dipropionate, 60% efficacy of diminazene aceturate while buparvaquone showed 55% efficacy (Akhter et al., 2010).

The comparative treatment efficacy of buparvaquone and Fruvexone® was analyzed against tropical theileriosis induced in the Friesian calves. Twelve susceptible Friesian calves were selected of age 3-6 months, for this purpose and were confirmed as serologically negative for *T. annulata* using an indirect fluorescent antibody test. Then these calves were introduced with sporozoites of *T. annulata* through the subcutaneous route of frozen tick (*H. excavatum*) stabilize and clinical theileriosis was produced in the calves. These calves were treated with buparvaquone @2.5mg/kg and Fruvexone® at the rate of 1ml/30kg (5mg parvaquone and 1.8mg frusemide). After the treatment, the calves were monitored clinically carefully and through Giemsa stained blood smear examinations for the determination of *T. annulata*. Calves treated with Fruvexone® showed no recovery and the schizonts were also present in their smears. Moreover, the reaction severity was also increased in Fruvexone® in treated calves; therefore they were administered with a single dose of buparvaquone to save their lives at days 14 and 15 post-infection. In contrast, buparvaquone proved highly effective against experimentally produced topical theileriosis because all the calves treated with buparvaquone recovered and thus it remained the drug of choice (Shkap et al., 2010).

The activity of the extracts of *Gardenia ternifolia* was studied in vitro against *T. lestoquardi*. Lymphocytes from the affected animals were isolated from heparin mixed blood and were grown on the culture media. For the detection of the parasite, an indirect fluorescence test was performed. Results revealed that the activity log of *G. ternifolia* extracts against *T. lestoquardi* was 0% at 250ppm. The efficacy of the plant extracts was increased significantly at the concentrations of 500, 5000 and 10000ppm to 13, 41 and 60%, respectively. While, 6745.28 and 177010ppm were recorded as lethal doses of the plant extracts at 50% and 99% LC₅₀ and LC₉₉, respectively. It was defined from the results that the extracts were effective and significantly reduced the number of macroschizonts. Furthermore, it was recommended to evaluate the efficacy of extracts of *G. ternifolia* against malignant ovine theileriosis in vivo (Farah et al., 2012).

Epidemiology and therapy of *T. equi* infections in the horses of different age and sex groups and different months of the year, in Giza, Egypt, were studied. For this purpose, 149 horses were screened in terms of clinical signs and blood examination. Forty horses were found infected with *T. equi* and blood samples were collected from all of these horses for measurement of hemoglobin, PCV and RBC count. The overall prevalence of *T. equi* was recorded at 41.61% in horses. The prevalence was greater in males compared to females, the maximum prevalence was recorded (22.81%) in the horses of age 5-10 years and the highest prevalence of theileriosis in horses was observed in summer with a minimum incidence in winter. For chemotherapy, horses were classified into four different groups, each group comprising 10 horses and treated with different drugs. Horses of group A were administered with diminazene aceturate @3.5mg/kg, Group B with imidocarb dipropionate @1.2mg/kg, Group C with buparvaquone @50mg/20kg body weight while Group D was administered with the combination of buparvaquone @25mg/20kg and imidocarb dipropionate @1.2mg/Kg body weight intramuscularly. All of the horses were examined prior and post medication by examination of blood smears, blood chemistry and blood picture. The efficacy of anti-theileria drugs was measured in terms of elimination of the clinical signs, hematological investigations, blood chemistry and serological testing. The results revealed that all the medication tested against theileriosis in equines had the same potential of eradicating the disease from the horses, improvement in the clinical signs, hematological and biochemical picture of the blood (Salib et al., 2013).

Hematological profile and oxidative stress, before and post-treatment of theileriosis in *T. annulata* infected cattle were studied. A total of 27 cows age 1-5 years were selected for the study out of which 17 cattle were positive for theileriosis and 10 cattle were parasite-free, confirmed through clinical signs, blood smear examination and Tams 1 based PCR technique. Blood samples were collected on a routine basis, from the jugular veins in EDTA coated vacutainers for the estimation of complete blood count and antioxidant capacity was detected by utilizing commercially available kits of Biodiagnostic Company. The clinically positive animals were treated with buparvaquone at the dose rate of 2.5mg/kg b.w. intramuscularly at 48 hours intervals. Marboflaxacin was used to check secondary bacterial infections and respiratory complications. Results proved that buparvaquone was most effective in the control of theileriosis infection in cattle and in eliminating the protozoan from lymph nodes and blood. Hematological investigation revealed a significant rise of MCV, RDW and MCH values whilst a significant decrease was observed in the Hb, TEC and PCV in the diseased cows than controls. Biochemical analysis showed that malondialdehyde and nitric oxide levels were decreased by the infection and total antioxidant capacity was increased significantly. Hence, it was concluded that following the treatment by buparvaquone, the animals recovered in terms of diminished clinical signs and parasites from the blood. Additionally, oxidative stress was characterized by reduced TAC and elevated MDA and NO. Moreover, a significant decline in oxidative stress and improved hematological parameters were observed following the appropriate treatment (Amira et al., 2015).

LITERATURE CITED

- Akhter, N., C. Lal, J.A. Gadahi, K.B. Mirbahar and M.I. Memon, 2010. Efficacy of various antiprotozoal drugs on bovine babesiosis, anaplasmosis and theileriosis. *Vet. World*, 3(6): 272-274.
- Alam, T.H., and S.M. Nasr, 2011. Hematological and biochemical investigation in bovine babesiosis and theileriosis. *Benha Vet. Med. J.*, 22 (2): 118-126.
- Al-Emarah, G.Y.A., M.H. Khudor and H.R. Daham, 2012. Clinical, haematological and biochemical study to cattle naturally infected with *Theileria annulata* in North of Basrah province. *AL-Qadisiya J. Vet. Med. Sci.*, 11(1): 54-62.
- Ali, Z., A. Maqbool, K. Muhammad, M. S. Khan and M. Younis, 2013. Prevalence of *Theileria annulata* infected hard ticks of cattle and buffalo in Punjab, Pakistan. *J. Anim. Plant Sci.*, 23(1): 20-26.
- Alim, M.A., S. Das, K. Roy, M. Masuduzzaman, S. Sikder, M.M. Hassan, A.Z. Siddiki and M.A. Hossain, 2012. Prevalence of hemoprotozoan diseases in cattle population of Chittagong division, Bangladesh. *Pak. Vet. J.*, 32(2): 221-224.
- Amira, A.T., A. Hosary, H.K. Elsayed and L.S. Ahmed, 2015. Oxidative stress and hematological profile in *Theileria annulata* clinically infected cattle before and after treatment. *Assiut. Vet. Med. J.*, 61(144): 123-129.
- Deeb, W.M.E. and E.E. Younis, 2009. Clinical and biochemical studies on *Theileria annulata* in Egyptian buffaloes (*Bubalus bubalis*) with particular emphasis on oxidative stress and ketosis relationship. *Cercetări Agro. Moldova*, 3(139): 63-73.
- Dumanli, N., M. Aktas, B. Cetinkaya, A. Cakmak, E. Koroglu, C.E. Saki, Z. Erdogmus, S. Nalbantoglu, H. Ongor, S. Simsek, M. Karahan and K. Altay, 2005. Prevalence and distribution of tropical theileriosis in Eastern Turkey. *Vet. Parasitol.*, 127: 9-15.
- Durrani, A.Z., A. Maqbool, N. Mahmood, N. Kamal and A.R. Shakoory, 2009. Chemotherapeutic trials with Calotropis procera against experimental infection with *Theileria annulata* in cross bred cattle in Pakistan. *Pak. J. Zool.*, 41(5): 389-397.
- Durrani, A.Z., A.R. Shakoory and N. Kamal, 2008. Bionomics of *Hyalomma* ticks in three districts of Punjab, Pakistan. *J. Anim. Pl. Sci.*, 18(1): 17-23.
- Durrani, A.Z., M. Ahmad, M. Ashraf, M.S. Khan, J.A. Khan, N. Kamal and N. Mumtaz, 2008. Theileriosis and babesiosis in cattle: Haemogram and some biochemical parameters. *Tartu, Estonia*: 143-150.
- Durrani, A.Z., N. Kamal and M.S. Khan, 2006. Incidence of theileriosis and estimation of packed cell volume, total erythrocyte count and hemoglobin in buffaloes. *J. Anim. Pl. Sci.*, 16(3-4): 85-88.
- Durrani, A.Z., N. Mehmood and A.R. Shakoory. 2010. comparison of three diagnostic methods for *Theileria annulata* in Sahiwal and Friesian cattle in Pakistan. *Pak. J. Zool.*, 42(4): 467-472.
- Elsadig, A.A., H. Yousif, A. Elmansoury, H.M. Elbasheir, A.E. Babiker, A.A. Adam, T.O. Abdelmageed and S. Hussein, 2013. Effects of *Theileria lestoquardi* infection on haematological and biochemical parameters in experimentally infected desert ewes. *Jordan J. Bio. Sci.*, 6(4): 316-319.
- Farah, H.M., H. Tigani, E. Amin, H.S. Khalid, S.M. Hassan and A. Rahim and M.E. Hussein,

2012. In vitro activity of the aqueous extract of *Gardenia ternifolia* fruits against *Theileria lestoquardi*. *J. Med. Plants Res.*, 6(41): 5447-5451.
- Gebrekidan, H., A. Hailub, A. Kassahun, I. Rohousovac, C. Maiac, D.T. Franka, A. Warburge and G. Baneth, 2013. *Theileria* infection in domestic ruminants in Northern Ethiopia. *Vet. Parasitol.*, 1-8.
- Guma, E.I., M.O. Hussien, D.A. Salih, B. Salim and S.M. Hassan, 2015. Prevalence of ticks (Acari: Ixodidae) and *Theileria annulata* antibodies in White Nile State, Sudan. *J. Adv. Vet. Anim. Res.*, 2(1): 69-73.
- Haque, M., N. Jyoti, K. Singh and S.S. Rath, 2010. Prevalence of *Theileria annulata* infection in *Hyalomma anatolicum anatolicum* in Punjab state, India. *J. Parasit. Dis.*, 34(1): 48–51.
- Hussein, A.H., N.A.E.S Mohammed and H.K. Mohammed, 2007. Intracellular protozoan parasites of wild and domestic ruminants transmitted by ixodid ticks. *Parasitol.*, 129: S271–S283.
- Hussain, M.H., M. Saqib, F. Raza, G. Muhammad, M. Nadeem Asi, M.K. Mansoor, M. Saleem and A. Jabbar, 2014. Seroprevalence of *Babesia caballi* and *Theileria equi* in five draught equine populated metropolises of Punjab, Pakistan. *Vet. Parasitol.*, xxx: 1-9.
- Irshad, N., M. Qayyum, M. Hussain and M.Q. Khan, 2010. Prevalence of tick infestation and theileriosis in sheep and goats. *Pak. Vet. J.*, 30(3): 178-180.
- Ismael, A.B., A.A. Swelum, A.F. Khalaf and M.A. Abouheif, 2014. Clinical, haematological and biochemical alterations associated with an outbreak of theileriosis in dromedaries (*Camelus dromedarius*) in Saudi Arabia. *Pak. Vet. J.*, 34(2): 209-213.
- Jeong, W., S.H. Yoon, D.J. An, S.H. Cho, K.K. Lee and J.Y. Kim, 2010. A molecular phylogeny of the benign *Theileria* parasites based on major piroplasm surface protein (MPSP) gene sequences. *Parasitol.*, 137: 241–249.
- Kamani, J., A. Sannusi, O.K. Egwu, G.I. Dogo, T.J. Tanko, S. Kemza, A.E. Tafarki and D.S. Gbise, 2010. Prevalence and significance of haemoparasitic infections of cattle in North- Central, Nigeria. *Vet. World*, 3(10): 445-448.
- Khaki, Z., S.M. Jalali, B. Kazemi, M.R. Jalali and S.P. Yasini, 2015. A study of hematological changes in sheep naturally infected with *Anaplasma* spp. and *Theileria ovis*: Molecular diagnosis. *Iranian J. Vet. Med.*, 19(1): 19-26.
- Kohli, S., U.K. Atheya and A. Thapliyal, 2014. Prevalence of theileriosis in cross-bred cattle: its detection through blood smear examination and polymerase chain reaction in Dehradun district, Uttarakhand, India. *Vet. World*, 7(3): 168-171.
- Kumar, V., P. Kaur, V.M. Wadhawan, H. Pal, H. Sharma and P. Kumar, 2015. Theileriosis in cattle: Prevalence and seasonal incidence in Jalandhar district of Punjab (INDIA). *Int. J. Recent Sci. Res.*, 6(3): 2998-2999.
- Kundave, V.R., A.K. Patel, P.V.I. Patel, I.J.J. Hasnani and G.C. Joshi, 2014. Qualitative and quantitative assessment of *Theileria annulata* in cattle and buffaloes polymerase chain reaction. *Tropic. Biomed.*, 31(4): 728–735.
- Laisser, E.L.K., S.W. Chenyambuga, E.D. Karimuribo, G. Msalya, M.J. Kipanyula, A.J. Mwilawa, R.H. Mdegela, L.J.M. Kusiluka and P.S. Gwakisa, 2016. Tick burden and acquisition of immunity to *Theileria parva* by Tarime cattle in comparison to Sukuma cattle under different tick control regimes in the Lake Zone of Tanzania. *J. Vet. Med. Anim. Heal.*, 8(3): 21-28.
- Mirzaei, M., 2007. Treatment of natural tropical theileriosis with the extract of the plant *Peganum harmala*. *Korean J. Parasitol.*, 45(4): 267-271.
- Modi D.V. and C.M. Bhadesiya, 2014. Tick-borne *Theileria annulata* infection in dairy cows: A short note for field vets. *Int. J. Life Sci. Res.*, 2(4): 127-129.
- Modi, D.V., C.M. Bhadesiya and G.C. Mandali, 2014. Serum biochemistry alterations in theileriosis affected crossbred cows in Banaskantha district of Gujarat. *Int. J. Novel Res. Healthcare Nursing*, 1(1): 12-14.
- Muhammad, G., M. Saqib, M. Athar, M.Z. Khan and M.N. Asi, 1999. Clinico-epidemiological and therapeutic aspects of bovine theileriosis. *Pak. Vet. J.*, 19(2): 64-71.
- Naz, S., A. Maqbool, S. Ahmed, K. Ashraf, N. Ahmed, K. Saeed, M. Latif, J. Iqbal, Z. Ali, K. Shafi and I.A. Nagra, 2012. Prevalence of theileriosis in small ruminants in Lahore-Pakistan. *J. Vet. Anim. Sci.*, 2: 16-20.
- Nazififi, S., S.M. Razavi, M. Reiszadeh, Z. Esmailnezhad and M.A. Lari, 2010. Diagnostic values of acute phase proteins in Iranian indigenous cattle infected with *Theileria annulata*. *Vet. Arhiv*, 80(2): 205-214.

- Osman S.A., H. Magdy and Al-Gaabary, 2007. Clinical, haematological and therapeutic studies on tropical theileriosis in water buffaloes (*Bubalus bubalis*) in Egypt. *Vet. Parasitol.*, 146: 337–340.
- Qayyum, A., U. Farooq, H.A. Samad and H.R. Chaudhry, 2010. Prevalence, clinicotherapeutic and prophylactic studies on theileriosis in district Sahiwal (Pakistan). *J. Anim. Plant Sci.*, 20(4): 266-270.
- Rady, A.A., S. Kot, M.R. and A. Ellah, 2008. Clinical, Diagnostic And Therapeutic Studies On Theileriasis (*Theileria annulata*) in cattle in upper Egypt. *SCVMJ*, XIII (2): 387-395.
- Ramazan, L. and U. Uslu, 2006. Haematological and coagulation profiles during severe tropical theileriosis in cattle. *Turk. J. Vet. Anim. Sci.*, 30: 577-582.
- Saeed, S., M. Jahangir, M. Fatima, R.S. Shaikh, R.M. Khattak, M. Ali and F. Iqbal, 2015. PCR based detection of *Theileria lestoquardi* in apparently healthy sheep and goats from two districts in Khyber Pukhtoon Khwa (Pakistan). *Tropic. Biomed.*, 32(2): 225–232.
- Saleem, M.I., A. Tariq, A. Shazad and S.A. Mahfooz, 2014. Clinical, epidemiological and therapeutic studies on bovine tropical theileriosis in Faisalabad, Pakistan. *Iraqi J. Vet. Sci.*, 28(2): 87-93.
- Salib, F.A., R.R. Youssef, L.G. Rizk and S.F. Said, 2013. Epidemiology, diagnosis and therapy of *Theileria equi* infection in Giza, Egypt. *Vet. World*, 6(2): 76-82.
- Shahnawaz, S., M. Ali, M.A. Aslam, R. Fatima, Z.I. Chaudhry, M.U. Hassan, M. Ali and F. Iqbal, 2011. A study on the prevalence of a tick-transmitted pathogen, *Theileria annulata* and hematological profile of cattle from Southern Punjab (Pakistan). *Parasitol. Res.*, 109: 1155–1160.
- Shkap, V., B. Leibovich, Y. Krigel, L. Fish and U. Orgad, 2010. Evaluation of the combined formulation of parvaquone and frusemide (fruvexon) in the treatment of experimental tropical theileriosis. *Int. J. Appl. Res. Vet. Med.*, 8(1): 73-77.
- Singh, A., J. Singh, A.S. Grewal and R.S. Brar, 2001. Studies on some blood parameters of cross bred calves with experimental *Theileria annulata* infections. *Vet. Res. Communications*, 25: 289-300.
- Swai, E.S., E.D. Karimuribo, E.A. Rugaimukamu and D.M. Kambarage, 2006. Factors influencing the distribution of questing ticks and the prevalence estimation of *T. parva* infection in brown ear ticks in the Tanga region, Tanzania. *J. Vector Eco.*, 31(2): 224-228.
- Tehrani, A.A., E. Hosseini and A.M. Bahrami, 2013. Biochemical, hematological studies in cattle naturally infected with *Theileria annulata*. *Bull. Env. Pharmacol. Life Sci.*, 2 (9): 7-10.
- Temiz, M., N. Altug, N. Yükses, 2014. Relationship between degree of anemia and blood gases in cattle with theileriosis. *Turk. J. Vet. Anim. Sci.*, 38: 82-87.
- Thompson, B.E., A.A. Latif, M.C. Oosthuizen, M. Troskie and B.L. Penzhorn, 2008. Occurrence of *Theileria parva* infection in cattle on a farm in the Ladysmith district, KwaZulu-Natal, South Africa. *J. S. Afr. Vet. Ass.*, 79(1): 31–35.
- Tiwari, A., N.K. Singh, H.S. Jyoti, S.A. Bhat, S.S. Rath, 2015. Prevalence of *Theileria annulata* infection in *Hyalomma anatolicum anatolicum* collected from crossbred cattle of Ludhiana, Punjab. *J. Parasit. Dis.*, 39(1): 57–61.
- Velusamy, R., N. Rani, G. Ponnudurai, T.J. Harikrishnan, T. Anna, K. Arunachalam, K. Senthilvel and P. Anbarasi, 2014. Influence of season, age and breed on prevalence of haemoprotozoan diseases in cattle of Tamil Nadu, India. *Vet. World*, 7(8): 574-578.
- Zahid, I.A., M. Latif and K.B. Baloch, 2005. Incidence and treatment of theileriasis and babesiasis. *Pak. Vet. J.*, 25(3): 137-139.