Bovine Theileriosis: Prevalence, Estimation of Hematological Profile and Chemotherapy in Cattle in Dera Ismail Khan, Khyber Pakhtunkhwa Province, Pakistan

Arsalan Khan[b*], Khurram Ashfaq[b], Israr ud Din[c], Rizwan ul Haq[d], Muhammad Jamil[e], Bait Ullah[f], Sibghat Ullah[g], Hanif ur Rehman[h], Farman Ullah[i]

[a,b]*Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad-38040-Pakistan*

[c]*Livestock Research & Development, Khyber Pakhtunkhwa, Peshawar-25000-Pakistan*

[d,e]*Department of Parasitology, University of Agriculture, Faisalabad-38040-Pakistan*

[f,g,h]*Foot and Mouth Disease Research Centre, Veterinary Research Institute, Peshawar-25000-Pakistan*

[i]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Education Ministry of China, College of Animal Sciences and Technology, Huazhong Agricultural University, Wuhan-430070- China*

Abstract

Theileriosis in cattle is one of the major constraints to the development of livestock enterprise in Pakistan and in most parts of the world. The disease causing agent is transmitted by Ixodid ticks having complicated life cycle. Prominent signs exhibited by cattle are; anorexia, fever, enlarged lymph nodes, occulonasal discharges and diarrhoea. The current study was planned to investigate the prevalence of theileriosis in cattle in and around district Dera Ismail Khan, Khyber Pakhtunkhwa province, Pakistan. For the determination of prevalence of theileriosis, a total of 384 animals, as calculated through statistical method, of different age and sex groups were screened. For detection of *Theileria* parasite, blood samples were collected from peripheral veins and microscopic examination of Giemsa stained blood smears revealed 14.32% (55/384) prevalence of *Theileria* parasite in cattle.

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* Corresponding author.

For hematological investigations, blood samples were collected from jugular veins of 55 affected animals in ethylene diamine tetra acetic acid (EDTA) coated vacutainers and complete blood count (CBC) was estimated through standard procedures. The hematology revealed significant (p<0.05) reduction in the RBCs count, haemoglobin level and packed cell volume i.e. $3.28x10^6/\mu L$, 5.32 g/dL and 20.98%, respectively. For chemotherapy, positive animals were treated with the combination of buparvaquone @2.5mg/Kg and oxytetracycline @10mg/kg body weight. The efficacy of the treatment was estimated in terms of number of positive animals 5 days post treatment showing 92.72% (51/55) treatment efficacy.

Keywords: Prevalence; Theileriosis; Hematology; Complete blood count.

1. Introduction

Livestock sector has a key role in the economy of Pakistan that contributes 53.2% to gross domestic product (GDP) in agriculture, while, in national GDP its share is 11.4% according to the economic survey of Pakistan, 2012-2013 [1]. But livestock industry is prone to economic losses by ticks infestation and protozoal infections [2]. Pakistan is endemic for tropical theileriosis. The climate of tropical and subtropical regions favours incidence, growth and multiplication of ticks predisposing the animals to parasitic ailments and consequently declining dairy production [3]. Mortality rate in exotic breeds of cattle may increase upto 90%, while, in indigenous breeds, it ranges upto 5% or less [4,5].

Theileria is obligate intracellular parasite infecting both domestic as well as wild bovines and small ruminants, all over the world. In cattle, the most important species responsible for the disease are: *T. annulata* and *T. parva.* *Theileria parva* is basically prevalent in 13 Sub-Saharan countries of Africa and cause East Coast Fever (ECF), while, *T. annulata* is prevalent in Asia, North Africa and Southern Europe and cause Mediterranean or tropical theileriosis. There is no overlapping of *T. parva* and *T. annulata* in the endemic regions [6,7].

Cattle affected with theileriosis, showed normocytic hypochromic anemia which may be due to their toxic metabolites which cause impairment in bone marrow and hinder erythropoeisis. Constant blood loss owing to tick infestation is accountable for anemia. Leucogram revealed significant increase in monocytes and lymphocytes whereas neutrophils and total leukocytic count was dropped off. Such alterations might also be due to Theileria toxic metabolites which affect haemopoietic organs [8]. Progressive anemia and related maladies are the primary cause of pathological changes in case of theileriosis. Anemia is developed due to oxidative damages to erythrocytes, their increased fragility, and destruction of erythrocytes by Theileria in reticuloendothelial system, intraerythrocytic piroplasms and autoimmune reactions. Biochemical and clinical variations seen in the disease showed relationship to degree of parasitemia, degree of anemia and severity of hypoxia [9-11].

Haemoglobin levels were adversely affected in positive cases and reduced upto 8mg/dl. In severely infected cases haemoglobin level was reduced to 3mg/dl. Packed cell volume percentage was decreased to 9% and total erythrocytic count was reduced to 2.3 million per cm2. This was due to destruction caused by the organism inside the RBCs during parasite multiplication [12]. Marked clinical signs of theileriosis include lymph nodes

enlargement, anorexia, depression, listlessness, dullness, dyspnoea, pyrexia upto 107oF, and suppressed cough, ocular and nasal discharges. In severe cases, diarrhoea or dysentery and systemic changes are seen [13]. Theileria can be diagnosed on the basis of clinical signs and microscopic examination of the Giemsa stained thin blood smear for estimation of the prevalence of Theileria in cattle [14].

For determination of prevalence of theileriosis in small ruminants, the study was carried out at Lahore (Punjab, Pakistan), in which 529 animals were selected and blood samples were collected (273 from sheep and 256 from goats). The smears were stained using Giemsa 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 [15].

Control of parasitic diseases 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 parasiticidal drugs harmless to the host.

2. Materials and Methods

The study was designed for the estimation of the prevalence of theileriosis in cattle in district Dera Ismail Khan and its peripheral villages, Khyber Pakhtunkhwa province of Pakistan.

Sample Size Estimation

Sample size was calculated by keeping 50% expected prevalence with 95% Confidence interval limits and 5% desired absolute precision. Sample size was reached by using the following equation [16]:

n = 1.962 Pexp (1 – Pexp) / d2

n= 1.962 x 0.50 x (1−0.50) / (0.05)2 = 384

Therefore, a total numbers of 384 cows of all ages and sexes were screened during the months of March, April and May of 2016, from the selected areas.

Collection of Blood Samples

After disinfection with methylated spirit, blood samples were collected from ear vein of 384 animals randomly, for preparation of thin smears. Three milliliter of the blood was collected from jugular vein from the affected animals for the determination of hematological profile of the animal like haemoglobin concentration, packed cell volume and complete blood cells count. Blood from jugular vein was collected in the dry, sterile and clean vacutainers containing an anticoagulant EDTA (ethylenediaminetetra acetic acid) @2mg/ml of the blood sample [17].

Smear Preparation

Thin blood smear was prepared from the unclotted blood collected aseptically from the ear vein. Pre-cleaned

slides were labeled and a drop of blood about 2-3 mm diameter was placed on the slide at a distance of 1 cm from the edge. Clean spreader slide was brought and held at a 45o angle towards the drop of the blood on the specimen slide. The blood was spread along the entire width of the spreader slide. While, holding the spreader slide at the same angle, it was pushed forward rapidly and smoothly and in this way thin blood smear was prepared [18,19].

Giemsa Staining

Smears were stained using Giemsa stain, as described by [20-22] and were examined under oil immersion lens of microscope for the identification of Theileria parasites based on the morphology of the parasite [23,24].

Hematological Profile

Urit 3010 model hematology analyzer (Boule Medical), was used for the determination of hematological parameters. The measuring principle of hematology analyzer was based on spectrophotometry.

Therapeutic Studies

On the basis of clinical examination and blood smear analysis, the positive animals for theileriosis were treated with single dose of Butalex® (buparvaquone 50mg/ml, ICI Pakistan Ltd) intramuscular injection @2.5mg/Kg and long acting oxytetracycline at the dose rate of 20 mg/kg b.wt., intramuscularly. After treatment of the positive animals, again the blood samples were collected five days post treatment and the efficacy of the treatment was estimated in terms of number of positive animals at day five post treatment.

Data analysis

The association between presence (positive and negative blood samples) of *Theileria* and the aforesaid parameters including age, gender, absence or presence of ticks was analyzed. Hematological values of infected and non infected animals were also compared and analyzed using Analysis of Variance Test, while, Chi square method of significance was used to compare prevalence among different age and sex groups of cattle.

3. Results

Out of 384 animals' blood smears examination revealed that 55 cows harbored *Theileria* parasite, using Giemsa staining technique showing 14.32% prevalence of theileriosis in cattle in district Dera Ismail Khan (Table 3.1). The results showed non-significant ($p > 0.05$) difference of prevalence between *Theileria* in different age groups of the animals. Sex-wise incidence of theileriosis revealed that 10.25% (8/78) of the males were found positive for *Theileria* parasite, while, 15.35% (47/306) of the female cows were found positive for *Theileria* parasite (Table 3.2). Young age cattle, of less than one year showed 13.15% (5/38), cattle of age 1-5 years showed higher prevalence of 16.04% (39/243), while, cattle of age more than 5 years showed 11.33% (11/103) prevalence of *Theileria* parasite (Table 3.3). Overall incidence percentage calculated was 12.5% in the month of March 2016, 14.06% in April 2016 while 16.4% in May 2016 (Table 3.1). Significant reduction in the level of

haemoglobin, packed cell volume, number of RBCs, WBCs and platelets, was observed in *Theileria* positive animals. Mean hematological values of Hb, PCV, TEC, platelets and TLC (total leukocytic count) recorded were 5.32g/dL, 20.98%, $3.28x10^6/\mu L$, $238x10^3/\mu L$ and $5.75x10^3/\mu L$, respectively (Table 3.4). For determination of chemotherapeutic response all of the affected cows (n=55) were treated with buparvaquone @2.5mg/kg and oxytetracycline @10mg/kg body weight and 51 animals were recovered with the treatment in terms of diminished clinical signs and parasite from RBCs in blood examination and thus 92.72% efficacy of the treatment was recorded (Table 3.5).

Table 3.1: Overall and month wise prevalence of theileriosis in cattle in district Dera Ismail Khan

Month wise Prevalence	Theileria positive	Theileria negative	Total animals examined	Prevalence (%)
March	16	112	128	12.5
April	18	110	128	14.06
May	21	107	128	16.4
Overall	55	329	384	14.32

Table 3.2: Sex-wise prevalence of theileriosis in cattle sampled from district Dera Ismail Khan

Sex	No. of positive animals	Total animals examined	Prevalence %	Chi square value	p-value
Male	8	78	10.25	31.601	0.00
Female	47	306	15.35	24.01	
Total	55	384	14.32	55.611	

Table 3.3: Age-wise prevalence of theileriosis in cattle sampled from district Dera Ismail Khan

Age	No. of positive animals	No. of animals examined	Prevalence %	Chi square value	p-value
<1 year	5	38	13.15	27.15	0.694
1-5 years	39	243	16.04	23.06	0.00
>5 years	11	103	11.33	29.90	0.77
Total	55				

Table 3.4: Mean hematological values of infected and healthy cattle. Means with steric denote statistically significant difference

Parameters	Mean values of Healthy animals	Mean values of Infected animals
Hb (g/dL)	14 + 0.46	5.32 + 1.43*
PCV (%)	45 + 1.49	20.98 + 1.78*
RBC (106/μL)	4.5 + 0.14	3.28 + 0.82*
TLC (103/μL)	7.50 + 0.12	5.75 + 0.12
Platelets count (103/ μL)	250 + 3.21	238 + 12.58
Monocytes (103/ μL)	0.39 + 0.02	0.30 + 0.07
Lymphocytes (103/ μL)	3.82 + 0.03	3.27 + 0.869

Table 3.5: Number of animals treated with the combination doses of buparvaquone and oxytetracycline

No. of animals treated	No. of animals recovered	No. of animals not recovered	Treatment efficacy
55	51	04	92.72

4. Discussion

In the current study, the Giemsa stained blood smears examination revealed 14.32% prevalence of *Theileria* parasite in cattle in district Dera Ismail Khan. Similar results were derived, reporting 10.76% prevalence of theileriosis in cattle in district Faisalabad [1]. Our results also coincide with a study, in which, overall 9.35% prevalence of *Theileria* was determined via examination of smears microscopically whereby peak prevalence of 13.3% was recorded in the season of summer (July and August), which shows that the prevalence of *Theileria* is higher in summer season [25]. Our study is comparable with the research in which 17.5% in Assiut and 20% Sohag, *Theileria* prevalence was recorded in Egypt [26]. Similarly, a study retrieved the matching results and reported 17.8% (107/600 positive samples) prevalence of bovine theileriosis studied from July-2003 to September-2003 in Cameroon, using Giemsa staining method [27]. On contrast to our results, 53.3% prevalence of *Theileria* in cattle was reported in the months of June and July using PCR technique [28]. This difference might be due to season because the incidence of theileriosis is highest in the months of June and July. Or the difference might be due to PCR technique, because it is extensively sensitive and can even replicate a single copy of available DNA of the parasite.

By comparing the overall means of Hb, PCV, TEC, TLC and platelets count of theileriosis infected and healthy animals, it was found that these parameters were significantly decreased (P<0.05) due to this particular infection [29,30], according to which hematological values of *Theileria* infected cattle, were declined due to severe hemorrhages, importunate parasitic stages in RBCs and abdominal ulcers. Our results are in agreement to the studies conducted [31], in which the decline in RBCs count and Hb was observed, which was attributed to erythrolysis by macrophages and monocytes. Our results are in agreement with study [32], where significant declination in WBCs count, RBCs count, hematocrit and haemoglobin was observed in *Theileria* infected Holstein Friesian cattle. Furthermore, it was reported that MCHC, number of eosinophils, basophils, monocytes, neutrophils and lymphocytes was also decreased in these studies with increased MCV and reticulocytosis. Likewise, studies were carried out [33], in which significant decrease in the number of RBCs, WBCs and platelets was seen in *Theileria* infected buffaloes in comparison to control group animals. It was attributed by [34] that theileriosis caused anemia in the cattle owing to the presence of intraerythrocytic piroplasms and erythrophagocytosis. Moreover, normocytic normochromic anemia was reported [35] in cattle as a consequence of theileriosis.

Buparvaquone @2.5mg/kg and oxytetracycline @10mg/kg was administered to 55 *Theileria* infected cattle, out of which 51 cattle recovered in terms of diminished clinical signs and parasites from the erythrocytes, 5 days post treatment. The combination of the drugs showed 92.72% treatment efficacy. Our results are comparable [36], where 100% efficacy of the treatment with buparvaquone in *Theileria* infected cows was reported. Similarly, 100% drug efficacy with buparvaquone was also claimed [26] in the treatment of cows with acute theileriosis but in chronic theileriosis, no response of the drug was seen. For the treatment of chronic theileriosis, combination of oxytetracycline and diminazine aceturate, were found effective than buparvaquone. Our findings are in line with a study [37], in which the early treatment of theileriosis was found effective, immediately after noticing the clinical signs of the disease. It was also observed that the combination of oxytetracycline and buparvaquone had 81.73% recovery rate in Sahiwal cattle against theileriosis [28]. Prophylactic efficacy of these drugs was also monitored and was found 100%.

5. Conclusion

From our studies, it was concluded that district Dera Ismail Khan is endemic for theileriosis and ticks are the main source of its transmission. It is therefore, further recommended that effective strategies should be planned and adopted in the control and prevention of this most economical and fatal disease of livestock.

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