Research Article

Prevalence of *Theileria Parva* in Large Ruminants through Conventional and Molecular Techniques in District Lakki Marwat and Peshawar (Pakistan)

Rafiullah[1]*, Abdur Rahman[2], Khalid Khan[1], Anwar Ali[1], Arifullah Khan[2], Abdul Sajid[3] and Naimatullah Khan[3]

[1]*Department of Livestock and Dairy Development (Research Wing) Khyber Pakhtunkhwa Peshawar, Pakistan;* [2]*The University of Agriculture Khyber Pakhtunkhwa Peshawar, Pakistan;* [3]*Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan.*

Abstract | During the present study two animal species (cattle and buffalo) were selected. A total of 800 samples were collected from different areas of District Lakki Marwat and District Peshawar. All the samples were processed through different diagnostic techniques, including Microscopy, ELISA and Real-time PCR for diagnosis of *Theileria parva* at Veterinary Research Institute Peshawar. The overall prevalence for Theileriosis was 27.75% through Real-time PCR, followed by Indirect ELISA (24.62%) and Microscopy (11.37%). Transect wise high prevalence for *Theileria parva* was observed in Lakki Marwat with 33.5% for Real-time PCR, significantly different at P<0.05 from Peshawar. Cattle showed 100% more positive results as compared to Buffalo. All the techniques showed highest prevalence of *Theileria parva* for female population as compared to male population. Age wise prevalence was not significantly different for both the species. Prevalence was high in animals < 1-year age. It was found that PCR is more sensitive and specific diagnostic tool for diagnosis of *Theileria parva*.

Introduction

Livestock contributed approximately 55.4 % to the agricultural value added and 11.9 % to national GDP during the year 2012-13, against 55.3 % and 11.9 % during the same period last year. Gross value addition of the livestock has increased from Rs: 735 billion to Rs: 756 billion showing an increase of 2.9 percent when we compare it with previous year (PES, 2012-13). The increase in Cattle production was not followed by improvement of sanitary conditions of breeding animals, which resulted in the occurrence of many diseases. Among those diseases,

the most common are the ones transmitted by ticks, such as Anaplasmosis, Babesiosis, Ehrlichiosis and Theileriosis (Simuunza et al., 2011).

Ticks are the source of considerable losses in Cattle production, in terms of diseases, reduced milk yield, low fertility and deaths and are the most important ecto-parasites of Cattle (Rajput et al., 2006). Furthermore, they decrease body weight gains and milk yield in addition to creating sites for secondary invasion by pathogenic organisms (Kaufman et al., 2006).

One of the most important tick borne diseases is

Theileriosis. This disease is common in cattle, goats, sheep, and other animals (Radostits et al., 2008). Theileriosis is highly prevalent all over the marshy areas in Africa, but in the horn of Africa it is absent (Norval et al., 1991). Theileriosis is extremely critical disease of Cattle in Africa (Carlson, 2009; Lawrence et al., 2004). At least six species of Theileria have been identified which cause disease in cattle; the two most important and more pathogenic are *Theileria annulata* and *T. Parva* (Durrani et al., 2010).

Theileria parva transmitted mainly by *Rhipicephalus appendiculatus* is causing a disease of Cattle and Buffalo and is the most pathogenic of all Theileriidae. The incubation period of *Theileria parva* is approximately 7-21 days (Radostits et al., 2008). The clinical signs include swelling of the regional superficial lymph nodes, loss of appetite, decrease in milk production, ceased rumination, increased heart beat and petechial hemorrhages on the tongue and vulva (Taylor et al., 2007). Cattle that recover from primary infection become long lasting carrier of the parasite and cannot be differentiated from uninfected Cattle (Odongo et al., 2008). There are several factors which determine incidence of the TBDs, like sex, tick density breed, age, geographical area, season, and management factors (Magona et al., 2010).

Laboratory diagnosis of *T. parva* infection in acutely infected Cattle is based on the direct microscopy of the parasite in Giemsa- staining technique of lymph node and blood smears (Uilenberg, 1982). For diagnosis of *T. parva* ELISA and IFAT are most commonly used. The iELISA is more accurate than IFAT (Taylor et al., 2007). Up till now the diagnosis of Theileria species infection is based on preparation of blood smears and then finding of merozoites in the smears. Clinical manifestation of the disease is observed in later stages (Ahmed et al., 2002).

The tick, *Rhipicephalus appendiculatus* is found in abundance in Lakki Marwat and District Peshawar. During hot and humid environmental conditions these ticks starts breeding and attack the susceptible hosts. The tick borne diseases cause mortalities and heavy losses to the farmers of the area. Therefore, this study was aimed to document the prevalence of *Theileria parva* and to identify the managemental factors predisposing the animals to this parasite. The prevalence of *Theileria* has been investigated through traditional Giemsa stained microscopy. It was aimed in this study to investigate the prevalence of *Theileria parva* using different techniques including Microscopy, indirect ELISA and Real-time PCR.

Materials and Methods

The current study was conducted to investigate the prevalence of *Theileria parva* infection in cattle and buffaloes of district Lakki Marwat and district Peshawar, Khyber Pakhtunkhwa, Pakistan. The collected samples from both the species and transects were screened through Giemsa, Elisa and PCR techniques. Besides documenting the active infection, Managemental factors which have relationship with the tick borne diseases were also studied. The present study was focused to investigate the effects of environment on the occurrence of *Theileria parva*. Close observation was taken about the nature of management practices such as grazing system; either it is pastured or confined, Farm/Shed premises for animals is congested or sufficient, shed is Cemented or Muddy (Kacha or Pakka), condition of walls and crevices in the walls were closely observed which usually work as shelter for ticks. Roof condition of the shed, space available, feeding and watering systems for animals was examined. The effect of Age and Sex on the occurrence of *Theileria parva* was also documented. The information about managemental factors, Morbidity and Mortality were collected by filling a questionnaire through observations and interviewing the owner/farmer during blood sample collection.

During the present study a total of 800 blood samples were randomly collected from Cattle and Buffaloes of the two districts. A total of 400 samples were collected from district Lakki Marwat and 400 from district Peshawar. District Peshawar was divided into four areas and 100 samples were collected from each animal species of target region. District Lakki Marwat was divided into two areas, irrigated and rain fed none irrigated. A total of 200 blood samples were collected from each animal species. Animals selected for sampling were divided into four age groups as < 1year, >1-2 year, >2-4 year and >4 years.

Sample collection

A 5 ml blood sample was collected in aseptic conditions from the jugular vein of the cattle and buffaloes. Approximately 3 ml of blood was immediately preserved in 0.5 M EDTA containing tubes for DNA extraction and the remaining blood was taken in a
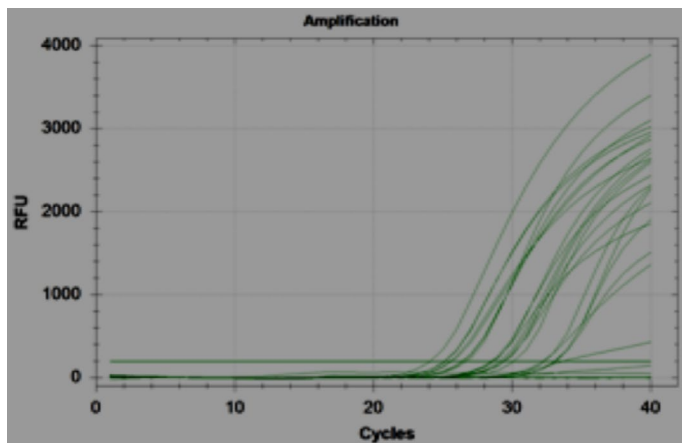
Figure 1: *Plate showing PCR result. The positive control quantified at 22 cycle. The graph represents the quantification cycles of samples with respect to positive and negative control. The earlier the quanti–fication cycle comes the more is the infection.*

serum gel tube for serological detection of *T. parva* and to prepare slide for Microscopic examination.

Blood smear preparation for microscopy

A properly prepared thin blood smear was fixed with absolute methanol and stained with Giemsa (1:10 ratio) for 25 to 30 min. The smears were rinsed 3 to 4 times with tap water to remove extra stain and air dried. The stained slides were examined under oil immersion objective (100 x) of light Microscope. The Smears were studied through Giemsa Staining for the presence of *Theileria* protozoan according to the method described by Benjamin (1978).

Indirect enzyme linked immunosorbent assay (Indirect ELISA)

Serum isolated from the blood samples was subjected to indirect ELISA for the detection of antibodies against *T. parva*. The samples were processed according to the manufacturer (Svanova®) instructions.

Detection of Theileria parva by real-time PCR

DNA extraction: Total DNA was extracted from collected blood using DNA extraction Kit in accordance with the instructions of the manufacturer (DNeasy Blood and Tissue kit of Qiagen)

Real-time PCR: The extracted DNA was then subjected to PCR amplification using *T. parva* specific primers as described by Oosthuizen et al. (2009). The reaction mixture (25 ul) containing 15ul of Ssofast mastermix (1X), 2.5ul of each of the forward and reverse primers and 5ul of template DNA was used. Each PCR run consisted of field samples, together with a positive (*T. parva* positive

sample) and a negative control. Amplification was carried out with the help of Thermal Cycler CFX96 Real- time system, Bio-Rad (Appendix III). Real time PCR results were visualized in an amplification plot. On y-axis fluorescence was represented whereas on x-axis the number of PCR cycles was plotted. The amplification curve was generated. The intersection point between the amplification curve and base line is called ct (threshold cycle). This point indicated the cycle in which fluorescence reaches the threshold value. The higher the initial amount of DNA, the lesser number of cycles needed (low ct value) to reach threshold (Figure 1).

Results and Discussion

Theileriosis results from infection with protozoa belonging to the genus *Theileria, which* are obligate intracellular parasites.

Over all prevalence

Overall technique wise prevalence of *Theileria parva* in both the species is summarized in the Table 1.

Transect wise prevalence of Theileria parva

The prevalence of *Theileria parva* was significantly higher in District Lakki Marwat as compared to Peshawar (Table 2).

Table 1: *Technique wise overall prevalence of Theileria parva in both transects.*

Transect	Microscopy (%)	ELISA (%)	PCR (%)	P-Value
Lakki	12.5	29.5	33.5	<0.001
Peshawar	10.5	19.75	22	<0.001

Table 2: *Transect wise prevalence of Theileria parva.*

Total Samples	Microscopy (%)	ELISA (%)	Real- time PCR (%)
800	91/800 (11.37)	197/800 (24.62)	222/800 (27.75)

Area wise prevalence of T. parva in cattle and buffaloes of both the transects

Samples processed through the conventional Giemsa staining technique and examined through Microscopy showed the highest prevalence of 13.5% in Lakki-2 followed by the Lakki-1 area. The lowest percentage (9%) was recorded in both Pesh-2 and Pesh-4 areas. The analysis of data via ELISA showed that the highest prevalence (32.5 %) of *Theileria parva*

occurred in Lakki-1area followed by 30 % prevalence in Lakki-2 area. The lowest prevalence (19%) was shown by both Pesh-2 and Pesh-4 areas. On the basis of Real-time PCR, the highest prevalence (37%) of *Theileria parva* was observed in Lakki-1, followed by the Lakki-2 (30%). The lowest prevalence was observed in Pesh-3 and Pesh-4 areas, where a similar prevalence rate of 21% was observed (Table 3).

Table 3: *Area wise Prevalence of T parva in Cattle and Buffaloes of both Transects.*

Area	Obser-vation	Micros-copy (%)	ELISA (%)	PCR (%)	P-Value
Lakki-1	200	23(11.5)	65(32.5)	74(37)	
Lakki-2	200	27(13.5)	53(26.5)	60(30)	
Total	400	(12.5)	(29.5)	(33.5)	<0.001
Pesh-1	100	12(12)	21(21)	23(23)	
Pesh-2	100	9(9)	19(19)	23(23)	
Pesh-3	100	11(11)	20(20)	21(21)	
Pesh-4	100	9(9)	19(19)	21(21)	
Total	400	(10.25)	(19.75)	(22)	<0.001
Total	800	91(11.37)	197(24.62)	222(27.75)	

In the 3rd, 4th and 5th column, the first value present observations and the second value presents percentage; Transect were significantly different at P <0.05 in term of prevalence of Theileria parva.

Area wise prevalence of T. parva in cattle of both the transects

Conventional Microscopy showed the highest prevalence of 18% in Pesh-1area of District Peshawar, followed by Lakki-2 area. The lowest prevalence of 10% and 12% were obtained in Pesh-4 area Pesh-2 areas respectively. The ELISA results in terms of *Theileria parva* prevalence were highest (44 %) in Lakki-1, followed by Lakki-2 area (43%). The lowest prevalence (24%) was shown by both Pesh-2 and Pesh-4 areas. On the basis of Real-time PCR, the highest prevalence (48%) of *Theileria parva* was observed in Lakki-1, followed by Lakki-2 (44%). The lowest prevalence was observed in Pesh-3 and Pesh-4 areas, showing the same value of 28% (Table 4).

Area wise prevalence of Theileria parva in buffaloes of both the transects

Samples processed through the Conventional Giemsa staining technique and examined through Microscopy showed the highest prevalence of 10% in Lakki-2 area followed by 8% prevalence in Lakki-1, Pesh-3 and Pesh-4 areas. The lowest Prevalence (6%) was obtained both in Pesh-1 and Pesh-2 areas. The

ELISA results in terms of *Theileria parva* prevalence were highest (21 %) in Lakki-1, followed by 20 % prevalence in Pesh-1 area. The lowest prevalence (10%) was recorded in Lakki-2 area. On the basis of Real-time PCR, the highest prevalence (26%) of *Theileria parva* was observed in Lakki-1, followed by Pesh-1 and Pesh-2 areas sharing the same value of 16%. The lowest prevalence was observed in Pesh-3 and Pesh-4 areas, showing the same prevalence of 14% (Table 5).

Table 4: *Area wise Prevalence of Theileria parva in Cattle of both the Transects.*

Area	Obser-vations	Microsco-py (%)	ELISA (%)	PCR (%)
Lakki-1	100	15(15)	44(44)	48(48)
Lakki-2	100	17(17)	43(43)	44(44)
Total	200	(16)	(43.5)	(46)
Pesh-1	50	9(18)	13(26)	15(30)
Pesh-2	50	6(12)	12(24)	16(32)
Pesh-3	50	7(14)	13(26)	14(28)
Pesh-4	50	5(10)	12(24)	14(28)
Total	200	(13.5)	(25)	(29.5)
G.Total	400	59(14.75)	137(34.25)	151(37.75)

In the 3rd, 4th and 5th column, the first value present observations and the second value presents percentage.

Table 5: *Area wise Prevalence of T. parva in Buffaloes of both the Transects.*

Area	Obser-vations	Micros-copy (%)	ELISA (%)	PCR (%)	P-value
Lakki-1	100	8(8)	21(21)	26(26)	
Lakki-2	100	10(10)	10(10)	15(15)	
Total	200	(9)	(15.5)	(20.5)	
Pesh-1	50	3(6)	10(20)	8(16)	
Pesh-2	50	3(6)	7(14)	8(16)	
Pesh-3	50	4(8)	6(12)	7(14)	
Pesh-4	50	4(8)	6(12)	7(14)	
Total	200	(7)	(14.5)	(15)	
Total	400	32(8)	60(15)	71(17.75)	<0.001

In the 3rd, 4th and 5th column, the first value present observations and the second value presents percentage; Transect were significantly different at P <0.05 in term of prevalence of Theileria parva.

Species wise prevalence of T. parva in cattle and buffaloes of both the transects

The species wise difference was significant. Cattle were showing almost 100% more positive results as compared to Buffaloes. The Cattle of District Lakki Marwat showed the highest prevalence of 16 % in the blood smears processed through Microscopy, The

Prevalence through ELISA was higher (43.5 %) in the Cattle of Lakki Marwat as compared to District Peshawar. Molecular technique Real-time PCR was showing 46% prevalence in the Cattle population of Lakki Marwat. All the three techniques for diagnosis of *Theileria parva* showed the lowest prevalence in the Buffalo blood samples of District Peshawar (Table 6).

Table 6: *Species wise prevalence of T. parva in cattle and buffaloes of both Transects.t*

Species	Transect	Obser-vation	Micro (%)	ELISA (%)	PCR (%)	P-Value
Cattle	Lakki	200	16.5	43.5	46	
	Peshawar	200	13.5	25	29.5	
Total		400	(14.5)	(34.25)	(37.5)	
Buffalo	Lakki	200	9	15.5	20.5	
	Peshawar	200	7	14.5	15	
Total		400	(8)	(15)	(17.75)	<0.001

Species were significantly different at P <0.05 in term of prevalence of Theileria parva.

Sex wise prevalence of T. parva in cattle and buffaloes of both the transects

The overall sex wise prevalence of Theileria parva in Cattle and Buffaloes of both the Transects is shown in Table 7.

Sex wise prevalence of Theileria parva in cattle of different areas

The prevalence of *Theileria parva* was significantly different in male and female population of Cattle species. Through blood smear the highest prevalence of 21.41% was recorded in female population of Cattle species in Pesh-1 area of District Peshawar, followed by 17 % prevalence in Lakki-2 area of District Lakki Marwat, while the lowest prevalence 10.5% was documented in Pesh-4 area. In case of Male population, the prevalence was highest (16.6%)

in Lakki-2 area and lowest in Pesh-1 area where all collected samples were found negative via Microscopy. The analysis of the data via ELISA showed the highest prevalence (48.8 %) in Lakki-1 area while lowest was observed in Pesh-2 area. The analysis of the ELISA tested data for male population reflected that *Theileria parva* prevalence was highest (33.3 %) in Lakki-2 area and lowest in Pesh-4 (8.3%) The analysis of Real-time PCR data for Male and Female populations showed the highest prevalence 33.3% in Lakki-2 area and 54.6 % in Lakki-1 area respectively. All the samples of Male population of Pesh-1 area were found negative when processed through Real-time PCR (Table 8).

Table 7: *Sex wise prevalence of T. parva in Cattle and Buffaloes of both Transects.*

Technique	Obser-vations	Male	Female	P-Value
Microscopy (%)	800	13/120(10.83)	78/680(11.47)	
ELISA (%)	800	22/120(18.33)	175/680(25.73)	
PCR (%)	800	26/120(21.66)	196/680(28.82)	
Total	800	(16.94)	(22.0)	<0.001

Sex was significantly different at P <0.05 in term of prevalence of Theileria parva.

Sex wise prevalence of Theileria parva in buffalo of different areas

The highest prevalence of *Theileria parva* in female population of Buffaloes via Microscopy after Giemsa staining was 10.5% in Lakki-2 area and lowest was 4.8% in Pesh-1 area, while the highest prevalence in male population of Buffalo species was 11.1% in Pesh-1 area. The Microscopic analysis of the Male samples showed that there was no single positive

Table 8: *Sex wise prevalence of Theileria parva in Cattle of different areas.*

Areas	♂Micro (%)	♀ Micro (%)	♂ELISA (%)	♀ ELISA (%)	♂ PCR (%)	♀ PCR (%)	P–Value
Lakki-1	1/14(7.1)	14/86(16.2)	2/14(14.2)	42/86(48.8)	3/14(21)	47/86(54.6)	
Lakki-2	2/12(16.6)	15/88(17.0)	4/12(33.3)	40/88(45.4)	4/12(33.3)	41/88(46.5)	
Total	3/26(11.5)	29/174(16.6)	6/26(23.0)	82/174(47.1)	7/26(26.9)	88/174(50.5)	<0.001
Pesh-1	0/8(0)	9/42(21.4)	1/8(12.5)	12/42(28.5)	0/8(0)	15/42(35.7)	
Pesh-2	1/7(14.28)	5/43(11.62)	1/7(14.28)	11/43(25.5)	2/7(28.5)	14/4(32.5)	
Pesh-3	1/8(12.5)	5/42(11.9)	1/8(12.5)	12/42(28.5)	1/8(12.5)	13/42(30.9)	
Pesh-4	1/12(8.3)	4/38(10.5)	1/12(8.3)	11/38(28.94)	3/12(25)	11/38(28.94)	
Total	3/35(8.5)	23/16(13.9)	4/35(11.4)	46/165(27.8)	6/35(17.1)	53/165(32.1)	<0.001
G.Total	6/61(9.8)	52/339(15.3)	10/61(16.3)	128/339(37.7)	13/61(21.3)	141/339(41.5)	

In the 2nd 3rd, 4th, 5th, 6th and 7th column, the first value present observations and the second presents percentage; Sex was significantly different at P <0.05 in term of prevalence of Theileria parva.

Table 9: *Sex wise prevalence of Theileria parva in buffalo of different areas.*

Area	♂Micro (%)	♀ Micro (%)	♂ELISA (%)	♀ ELISA (%)	♂ PCR (%)	♀ PCR (%)
Lakki-1	1/12(8.3)	7/88(7.9)	1/12(8.3)	20/88(22.7)	2/12(16.6)	24/88(27.2)
Lakki-2	1/15(6.6)	9/85(10.5)	2/15(13.3)	9/85(10.5)	13.3(2/15)	13/85(15.2)
Total	2/27(7.4)	16/173(9.2)	3/27(11.1)	29/173(16.7)	4/27(14.8)	37/173(21.3)
Pesh-1	1/9(11.1)	2/41(4.8)	1/9(11.1)	7/41(17.0)	1/9(11.1)	8/41(19.5)
Pesh-2	0/6(0.0)	3/44(6.8)	0/6(0.0)	7/44(15.9)	0/6(0.0)	6/44(13.6)
Pesh-3	0/9(0.0)	4/41(9.7)	1/9(11.1)	5/41(12.1)	1/9(11.1)	7/41(17.0)
Pesh-4	0/8(0.0)	4/42(9.5)	1/8(12.5)	6/42(14.2)	1/8(12.5)	6/42(14.2)
Total	1/32(3.1)	13/168(7.7)	3/32(9.3)	25/168(14.8)	3/32(9.3)	27/168(16.0)
Total	3/59(5.0)	29/341(8.5)	6/59(10.1)	54/341(15.8)	7/59(11.8)	64/341(18.7)

In the 2nd, 3rd, 4th, 5th, 6th and 7th column, the first value present observations and the second presents percentage; Sex was significantly different at P <0.05 in term of prevalence of Theileria parva.

case in the rest of the areas of district Peshawar. The analysis of ELISA tested samples showed that the highest Prevalence (22.7) % in Female population of Buffalo species was recorded in Lakki-1 while lowest (10.5%) was recorded in Lakki-2 area of District Lakki Marwat. When samples of male population of Buffalo species were processed through ELISA, the highest prevalence (13.3%) was documented in Lakki-1 and no case was positive in Pesh-2 area. Real-time PCR revealed 27.2% of prevalence in the Female population of Buffalo species in Lakki-1 area. In case of male population of Buffalo, the highest prevalence 16.6% was found in the same area, while no male animal was found positive for *Theileria parva* when the samples of Pesh-2 area were processed through PCR (Table 9).

Age wise prevalence of Theileria parva in cattle of different areas

The Cattle in the study area were divided into four different age groups. The highest prevalence (20.2%) via Microscopy after Giemsa staining was found in the animals having age more than 4 years (group4), while the lowest (10.1%) was recorded in the animals having age ranging between 3-4 years (group3). The prevalence through ELISA technique was highest (38%) in the animals having age less than 1 year (group1), followed by group4, while the Cattle having age 2-3 years (group2) showed the lowest (28.4%) prevalence. The analysis of the PCR tested data revealed that the prevalence was highest (39%) in the group1 and lowest (35.2%) in the group2 (Table 10).

Age wise prevalence of Theileria parva in buffaloes of different areas

The Buffaloes in the study area were divided into

four different age groups. The highest prevalence of Theileria parva through Microscopy was 11.9 % in the animals having age more than 4 years (group4), followed by 8.4% in the animals having age less than 1 year (group1), while the lowest was 5.3% in the age group2. The prevalence via ELISA technique was highest (19 %) in the age group 4, followed by age group1 (16.9%), while the Buffaloes having age 2-3 years (group2) showed the lowest (11.5%) prevalence. The analysis of the PCR tested data revealed that the prevalence was highest (19.8%) in the age group1 and lowest (15%) in the age group2 (Table 11).

In the present study the prevalence of *T. parva* was investigated in District Lakki Marwat and Peshawar by using Microscopic, Serological and Molecular techniques. The overall prevalence of *Theileria parva* infection through Conventional Microscopy, indirect ELISA and Real-time PCR was 11.37%, 24.62% and 27.75% respectively. This analysis of the data reveals that PCR is more sensitive of all the used techniques. The present findings are supported by Bazarusanga *et al.* (2008), who reported that PCR is more sensitive technique as compared to ELISA and Microscopy for the detection of *Theileria parva*. Similarly, Durrani et al., 2010 conducted study on Theileriosis in Cattle in the year 2010 and on Buffaloes in 2008 by using Microscopy, ELISA and PCR. In both the studies he reported that PCR is more sensitive and reliable technique. In Cattle he mentioned the prevalence of 6.8 %, 23.5% and 41.2% via Microscopy, ELISA and PCR respectively which revealed that PCR is more sensitive technique. The study conducted by the Several studies reported that for those animals showing no obvious signs of Theileriosis, the Molecular technique PCR is more suitable and specific as compared to

other conventional techniques (Oliveira et al., 1995).

Table 10: *Age wise prevalence of Theileria parva in Cattle of different areas.*

Transect	Age Group	Obser-vations	Micros-copy (%)	ELISA (%)	PCR (%)
Lakki	1	50	12(24)	19(38)	20(40)
Peshawar	1	50	6(12)	18(36)	19(38)
Total		100	18(18)	37(37)	39(39)
Lakki	2	50	4(8)	15(30)	19(38)
Peshawar	2	52	7(13.4)	14(28)	17(32.6)
Total		102	11(10.7)	29(28.4)	36(35.2)
Lakki	3	50	3(6)	20(40)	20(40)
Peshawar	3	49	7(14.2)	14(28.5)	18(36.7)
Total		99	10(10.1)	34(34.2)	38(38.3)
Lakki	4	50	12(26)	20(40)	20(40)
Peshawar	4	49	8(18.3)	15(30)	18(36.7)
Total		99	20(20.2)	35(35.3)	38(38.3)
G.Total		400	59(14.7)	135(33.3)	151(37.7)

In the 4th, 5th and 6th column, the first value present observations and the second value presents percentage; group1= < 1 year, group2= >1–2 years, group3= >2–4 years group4= >4 years.

Table 11: *Age wise prevalence of Theileria parva in buffaloes of different areas.*

Transect	Age group	Obser-vations	Micros-copy %	ELISA %	PCR%
Lakki	1	47	4(8.5)	9(19.1)	10(21.2)
Peshawar	1	59	5(8.4)	9(15)	11(18.6)
Total		106	9(8.4)	18(16.9)	21(19.8)
Lakki	2	63	4(6.3)	8(12.6)	8(12.6)
Peshawar	2	50	2(4)	5(10)	9(18)
Total		113	6(5.3)	13(11.5)	17(15)
Lakki	3	50	3(6)	7(14)	9(20)
Peshawar	3	47	4(8.5)	6(12.7)	8(17)
Total		97	7(7.2)	13(13,3)	17(17.5)
Lakki	4	40	6(15)	8(20)	7(17.5)
Peshawar	4	44	4(9)	8(18.1)	9(20.4)
Total		84	10(11.9)	16(19)	16(19)
Grand Total		400	32(8)	60(15.5)	71(17.7)

In the 4th, 5th and 5th column, the first value present observations and the second value presents percentage; group1= < 1 year, group2= >1–2 years, group3= >2–4 years group4= >4 year.

The prevalence of *Theileria parva* was significantly higher in Lakki Marwat (33.5 %) as compared to Peshawar (22 %). This transect difference is supported by the findings of Atif et al. (2012) who studied the prevalence of tick borne diseases in Indigenous and Crossbred Cattle of different districts of Punjab including Sargodha, Khushab and Rawalpindi. There was significant difference between the prevalence of the disease. In the present study the difference in the epidemiological status of the disease may be explained that it may be due to the difference in the immunity level of the animals, difference in the climatic conditions of the two districts, distribution of vectors, difference in management practices and awareness about the tick borne diseases in the areas.

The species wise analysis of the data reflected that Cattle were showing 100 % more positive results as compared to Buffaloes. Low level of prevalence in Buffalo suggests that this species exhibits high level of resistance to the tick borne diseases. The current study is supported by the findings of Khan et al. (2004) who conducted study on the prevalence tick borne diseases in Buffaloes and Cattle of two different Livestock Institutes N.A.R.C, Islamabad and Barani Livestock Production Research Institute

Sex wise analysis shows that the prevalence of *Theileria parva* is significantly higher in female population of Cattle and Buffaloes as compared to male. These findings are supported by the work of Khan et al. (2004) who documented distribution and prevalence of tick borne diseases in Cattle farms in Sargodha. They reported that female Cattle population was more (26 %) susceptible to Tick borne disease as compared to male.

The study of the Kabir et al. (2011) is also in agreement with this study. They mentioned that prevalence of ticks was significantly higher in female population of Cattle as compared to male. They justified that some hormonal influences may be associated with this. Lloyd (1983) reported that higher level of prolactin and progesterone hormones make the individual more susceptible to any infection. In the present study females were found more positive as compare to male population. It may be explained that in female animals the body immunity might be low due weaker body conditions. The female population of the livestock is kept longer for breeding purposes and milk production and also given insufficient feed against high demand. Female population also faces different stress conditions of immune suppression during pregnancy and lactation, while in male population the infection was very low because in most of the cases their feed demands are satisfied. Contacts of Male

population with other animals like cows, calves etc are less as compared to female population.

There was no significant difference between the age groups, however microscopy showed the highest prevalence in the animals having age more than 4 years (group 4), while analysis of the data through ELISA and PCR showed highest prevalence in the animals having less than one year age (group1). The lowest prevalence of *Theileria parva* was observed in animals with the age group ranging between1-2 years. Prevalence increased with the increase in age. Current study is in agreement with the findings of Bazarusanga et al. (2008). They conducted study on the epidemiology of Theileriosis in Rawanda and found the highest prevalence of infection in young animals less than one year. The study of the Qayyum et al. (2010) supports our findings. They showed high prevalence of *Theileria* in young calves as compared to adults. The highest infection in young ones in the present study is justified by the facts that after birth up to the age of six months the calves are kept at farm under observation with care in the sense to provide them ideal environment both in the summer and winter with balance diet, but on the other side, the farms in the area of the study were muddy (kacha) having the crevices between bricks and these provide shelter and breeding sites for ticks and attack the young animals easily. Mohanguzi et al. (2010) conducted study on the prevalence and characterization of Theileria and Babesia species in Cattle population. Their results revealed that in animals with age group ranging from 9-24 months, the prevalence of Theileria was highest while the calves were having the lowest prevalence. This is justified by the fact that with the passage of time the animals get exposed to multiple environmental pathogens and these factors affect the immune system of the animals and is further weakened by the malnutrition due to which the animal fail to resist and get infections.

The Overall prevalence of *Theileria parva* in Lakki Marwat and Peshawar *is* 27.75%. The prevalence of *Theileria parva* is 100 % more in cattle (37.5 %) of both transects as compared to buffaloes (18 %). The prevalence of *Theileria Parva* in cattle and buffaloes of district Lakki Marwat is significantly higher (33.5%) as compared to the cattle and buffaloes of district Peshawar (22 %). Real-time PCR is very sensitive and specific diagnostic tool for diagnosis of *Theileria Parva* as compared to the conventional Microscopy

and indirect Elisa. Immune system or genetic makeup of the buffalo may be further explored in terms of *Theileria parva* prevalence rate. All species of livestock in other transects of the province may be studied on these lines to get a real picture of the tick borne diseases. Acaricidal therapy may be used before the onset of vector breeding season to prevent tick infestation and hence parasitic transmission. All the Research Institutes may be equipped with PCR for diagnosis of tick borne diseases. Further studies are required to be carried out especially on vaccine production.

Author's Contribution

Rafiullah: Collected and processed the samples.
Abdur Rahman: Supervised the overall activities.
Khalid Khan: Helped in processing of samples.
Anwar Ali: Helped in collection of samples.
Arifullah Khan: Helped in collection and transportation of samples.
Abdul Sajid: Compiled and interpreted the results.
Naimatullah Khan: Helped in data analysis.

References

Ahmed, J.S., H. Yin, L. Schnittger and F. Jongejan. 2002. Ticks and tick borne diseases in Asia with special emphasis on China. Para. Res. 88: 51-55.

Atif, F.A., M.S. Khan, H.J. Iqbal and T. Roheen, 2012. Prevalence of tick-borne diseases in Punjab (Pakistan) and hematological profile of *Anaplasma marginale* infection in indigenous and crossbred Cattle. Pak. J. Sci. 64(1): 11-15.

Benjamin, M.M. 1978. Outline of veterinary clinical pathology, 3rd Edi. Iwoa State Univ. Press, Ames, Iowa, USA. 7-8: 29-30.

Bazarusanga, T. 2008. The epidemiology of theileriosis in Rwnda and implications for the control. PhD thesis, Dept. Vet. Med. Ghent Univ. Rwanda.

Bazarusanga, T., T. Marcotty, A.M.K.I. Ahouandjinou, N. Tshinyama, C. Katendi and D. Geysen, 2011. Estimation of the *Theileria parva* entomological inoculation rate (EIR) by means of tick burden and proportion of infected questing ticks in three different farming systems in Rwanda. Int. J. Vocat. Tech. Edu. 3(7): 99-106.

Carlson, G.P. 2009. Theileriosis. In: Smith, B.P. Large Animal Internal Medicine. Mosby,

Elsevier. St. Louis. pp. 1160.

Durrani, A.Z., M. Ahmad, M. Ashraf, M.S. Khan, J.A. Khan, N. Kamal and N. Mumtaz. 2008. Prevalence of Theileriosis in buffaloes and diagnosis through blood smear examination and PCR test in district Lahore. J. Anim. Pl. Sci. 18(2-3): 2008

Durrani, A.Z., N. Mehmood and A.R. Shakoori, 2010. Comparison of three diagnostic methods for *T. annulata* in Sahiwal and Friesian Cattle in Pakistan. P. J. Zool. 42(4): 467-472.

Houwen, H. 2000. Blood film preparation and staining procedures. Lab Hematol. 6: 1-7.

Kabir, M.H.B., M.M.H. Mondal, M. Eliyas, M.A. Mannan, M.A. Hashem, N.C. Debnath, O.F. Miazi, C. Mohiuddin, M.A. Kashem, M.R. Islam and M.F. Elahi. 2011. An epidemiological survey on investigation of tick infestation in cattle at Chittagong District, Bangladesh. Afr. J. Microbiol. Res. Vol. 5(4): pp. 346-352.

Kaufman, P.E., P.G. Koehler and J.F. Butler. 2006. External parasites on beef cattle. Entomology and nematology department, Florida cooperative extension service. Inst. Food Agric. Sci. Univ. Florida.

Khattak, R.M., M. Robib, Z. Khan, M. Ishaq, H. Hameed, A. Taqddus, M. Faryal, S. Durrani, Q.U.A. Gillani, R. Allahyar, R.S. Sheikh, M.A. Khan, M. Ali and F. Iqbal. 2011. A comparison of two different techniques for the detection of Blood, *Theileria annulata* in cattle from two districts of Khyber Pakhtunkhwa, Pakistan Parasite. 2012, 19: 91-95. https://doi.org/10.1051/parasite/2012191091

Khan, M.Q., A. Zahoor, M. Jahangir and M.A. Mirza. 2004. Prevalence of blood parasites in cattle and Buffaloes, Pak. Vet. J. 24: 193-195.

Latif, A. 1992. Sustainable control methods for ticks and tick-borne diseases in Africa. Workshop on "Future of livestock industries in East and Southern Africa". Proce. Workshop held Kadoma Ranch Hotel, Zimbabwe. 20-23.

Lawrence, J.A. 2004. Theileriosis of sheep and goats in: Infectious diseases of livestock, Volume one 2th Edi. Oxf. Univ. Press. 498-499.

Lloyd, M. 1983. An experiment in the organization of a minimum complex measure against haemosporidiasis in northern Tadzhikistan. J. Vet. Res. 6: 64 - 74.

Magona, J.W., J. Walubengo, M.W. Olaho, N.N. Jonsson, S.C. Welburn and M.C. Eisler, 2010. Clinical features associated with sero conversion to Anaplasma marginale, Babesia bigemina and Theileria parva infections in African Cattle under natural tick challenge. Vet. Para. 171: 3: 207- 215.

Muhanguzi, D., E. Matovu and C. Waiswa. 2010. Prevalence and characterization of Theileria and Babesia species in cattle under different husbandry systems in western Uganda. Int. J. Anim. Vet. Adv. 2(2): 51-58, ISSN: 2041-2908.

Norval, R.A.I., B.D. Perry, F. Gebreab and P. Lessard. 1991. East coast fever: a problem of the future for the horn of Africa. Prev. Vet. Med. 10: 3: 163-172. https://doi.org/10.1016/0167-5877(91)90001-I

Oliveira, D.C., M. Vanderweide, M.A. Habela, P. Jacquietand and F. Jongejan. 1995. Detection of *Theileria annulata* in blood samples of carrier cattle by PCR. J. Clin. Microbiol. 33: 2665-2669.

Oosthuizen, M.C., B.A. Allsopp, M. Troskie, N.E. Collins and B.L. Penzhorn. 2009. Identification of novel *Babesia*and *Theileria*species in South African giraffe and roan. Vet. Para. 163: 39-46. https://doi.org/10.1016/j.vetpar.2009.03.045

Odongo D., H. Kiara, B. Perry, R. Skilton and R. Bishop. 2003. An investigation into the carrier state of *Theileria parva* by the polymerase chain reaction assay International Livestock Research Institute, Kenya. Proceedings of the 10th International Symposium on Vet. Epi. Eco.

Pakistan Economic Survey (PES). 2013-13. Ministry of Finance, III. Livestock & poultry, (a) Livestock. pp. 29.

Qayyum, A.U., Farooq, H.A. Samad and H.R. Chauhdry. 2010. Clinicotherapeutic and prophylactics studies on theileriosis in district Sahiwal(Pakistan). J. Anim. Plant Sci. 20: 266-270.

Radostits, O.M., C.C. Gay, K.W. Hinchcliff and P.D. Constable. 2008. Diseases associated with protozoa. 10th Edi. In: Vet. Med.: A textbook of diseases of cattle, horses, sheep, pigs, and goats. Saunders Elsevier: 1483-1540. ISBN 072026042-7020-2604-2.

Rajput, Z.I., S. Hu, W. Chen, A.G. Arijo and C. Xiao. 2006. Review: Importance of ticks and their chemical and immunological control in livestock. J. Zhejiang Univ. Sci. B. 7: 11: 912-921. https://doi.org/10.1631/jzus.2006.B0912

Simuunza, M. 2011. Epidemiological analysis of tick-borne diseases in Zambia, Vet. Para. 175: 3: 331-342. https://doi.org/10.1016/j.vetpar.2010.09.027

Taylor, M.A., R.L. Coop and R.L. Wall. 2007. Vet. Para. 3rd. Edi. Blackwell publishing USA, ISBN:978-1-4051-1964-1.

Uilenberg, G.N., N.M. Perie, J.A. Lawrence, A.J. de Vos, R.W. Paling and A.A. Spanjer. 1982. Causal agents of *bovine* Theileriosis in southern Africa. Trop. Anim. Health Prod. 14: 127-140. https://doi.org/10.1007/BF02242143