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Prevalence of Anaplasmosis in Cows and Buffaloes of District Charsadda, Khyber Pakhtunkhwa, Pakistan

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Abstract: This research work was designed to study the prevalence of anaplasmosis among cows and buffaloes in Tehsil Shabqadar, Tehsil Charsadda and Tehsil Tangi of district Charsadda of the Khyber Pakhtunkhwa province, Pakistan. A total of 360 blood samples were collected from Tehsils Shabqadar, Charsadda and Tangi. Microscopic examination of the Giemsa stained blood smears revealed an overall prevalence of blood parasites as 6.11% and 4.44% in cows and buffaloes, respectively. The overall seroprevalence of anaplasmosis in cows using cELISA was 30.55%. Females were found to be more susceptible 41/128(32.02%) than males 14/52 (26.92%). The prevalence was significantly higher 40/92 (43.47%) in adult cows than the younger ones15/88 (17.04%) ($P<0.05$). The seroprevalence in cows was significantly ($P<0.01$) higher in summer season in the district. The overall seroprevalence of anaplasmosisin buffaloes using cELISA was 21.11% (38/180). Females have more prevalence 32/149 (21.47%) than males 6/31 (19.35%). The prevalence was higher 25/107 (23.36%) in above two year buffaloes than the younger ones13/73 (17.80%). The seroprevalence in buffaloes was significantly ($P<0.001$, $P<0.01$and $P<0.05$) higher in summer, spring and autumn seasons in the district, respectively. It was concluded that anaplasmosis is widely distributed in district Charsadda, Khyber Pakhtunkhwa, Pakistan.

Key words: Anaplasmosis · Cows · Buffaloes · Charsadda

INTRODUCTION

Livestock plays a vital role in the economy of Pakistan because it is the main source of income and livelihood for 73 million people in rural areas [1, 2]. Tick-borne diseases of livestock are usually widespread and produced heavy economic losses in tropical region and subtropical regions worldwide. It is estimated that tick- borne diseases around the globe produce losses at US$ 13.9 to US$ 18.7 billion per year due to which world's 80% cattle become atrisk [3]. Anaplasmosis is basically one of the most widespread worldwide scattered tick-borne diseases of cattle with prodigious economic effect [4]. Anaplasmosis in cows and buffaloesis usually caused by obligate intra-erythrocytic rickettsia of the order Rickettsials, family Anaplasmatacea and genus *Anaplasma* [5].

Anaplasmamarginale basically caused bovine Anaplasmosis whereas *Anaplasmacentrale* usually caused minor disease [6]. Sir Arnold Theiler described *Anaplasmamarginale* for the first time as marginal dots in red blood cells of cattle. The most important species of the genus *Anaplasma* are *Anaplasma marginale* and *Anaplasma central* [7].

Symptoms of anaplasmosis are severe anaemia, fever, jaundice, weakness, brownish urine, weight loss, pale mucous membranes, abortion, decreased milk production, hyperexitibility and death without haemoglobinemia and haemoglobinuria during critical stage of disease [8]. Clinical anaplasmosis most rottenly arises in cows [9] and death rate exceeds to 80% in enzootic zone [10]. Haemolyticanaemia is the main clinical manifestation of *Anaplasma* infection in cows and buffaloes [11].

Clinically infected animals become life-long carriers if even recovered from infection [12]. The infected animals become reservoir by resistingsubsequent clinical disease for upkeep and further spread of disease [13]. Immunosuppression in persistently infected cattle during pregnancy may lead to acute infection Acute infection may arise in pregnancy due to immunosuppression in

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cows and buffaloes where infection is persistent [14]. Exotic, high producing and well-nourished animals produce acute disease [15].

Many species of tick vectors are responsible for the transmission of Anaplasmosis. About 20 tick species of 6 the genera (*Dermacentor, Rhipicephalus, Ixodes, Argas, Hyalomma* and *Ornithodoros*) that are technically verified to transmit Anaplasmosis [16].

Very little data is available from northern Punjab on the serological study of anaplasmosis in cattle by using ELISA technique. Microscopic study of blood smears was reported mostly from Pakistan [17].

It is expected that enzyme linked immunosorbent assay, will significantly improve the detection of infected animals, thus accurate epidemiological surveys of *Anaplasma* species would be possible which is important for conducting anaplasmosis control efforts in Pakistan. Further, haematological changes in cows and buffaloes naturally infected with *Anaplasma* spp. as compared to healthy animals will be analyzed to arrive at the conclusion regarding the pathogenicity induced by *Anaplasma* spp.

This research work is design to fulfill the following objectives.

- To determine the prevalence of anaplasmosis in cows and buffaloes of District Charsadda, Khyber Pakhtunkhwa, Pakistan.
- To analyze the haematological alterations in infected animals to arrive at the conclusion regarding the pathogenicity induced by *Anaplasma spp.* in latent and patent infection.

MATERIALS AND METHODS

Study Area: The district Charsadda situated in the west of the Khyber Pakhtunkhwa. It lies between 34-03' and 34-38' north latitudes and 71-28' and 71-53' east longitudes. Charsadda is surrounded by Mardan district to the east, Malakand District to the north, Nowshera and Peshawar districts to the south and the Mohmand Agency of the FATA (Federally Administered Tribal Areas) to the west. The total area of the district is 996km^2.

The district Charsadda is divided into three Tehsils (Tehsil Charsadda, Tehsil Tangi and Tehsil Shabqadar). The district has 4 seasons, winter (November to February), spring (March to April), summer (May to August) and autumn (September to October). The climatic conditions of the district are severe that is summer is very hot and winter is very cold.

Sample Collection: A total of 360 blood samples from different areas of district Charsadda were collected in gel-added tubes from jugular vein of cows and buffaloes directly with the help of sterile syringe. Animals of different age and sex were selected for sample collection and sampling was done in different areas of three tehsils randomly. At the time of collection locality age and sex of each sample was noted down and thin blood smears for microscopy were also prepared at the spot. The samples were kept in vaccutainer and then placed in ice jar. For further analysis the samples were brought to the laboratory of Zoology Department AWKUM.

Staining Procedure: The blood smears were prepared and then fixed by using methanol. Giemsa stain (1:10) ratio diluted were added for 25-30 minutes. To remove excess stain these smears were rinsed three to four times using tap water and then air-dried. These slides were then observed at 100X magnification under oil immersion lens of microscope. To detect Anaplasma species 20 microscopic fields were carefully observed

Serum Separation: Collected blood samples were centrifuged for 5 minutes at 3500 rpm. With the help of pasture pipette the supernatant was aspirated into a sterile plastic dropper. Then the serum was stored at –20°C until analysis.

Enzyme Linked Immuno-Sorbent Assay (ELISA)

Principle of Kit: The serum Anaplasma antibodies in positive serum sample inhibit the binding of horseradish peroxidase labeled monoclonal antibody to Anaplasma coatedmicro-titer plate wells. The inhibition or lack of horseradish peroxidase labeled monoclonal antibody conjugate is detected by the addition of enzyme substrate. The development of strong color indicates negative test result. On the contrary, the weak or no color reveal the presence of Anaplasma antibodies.

Preparation of Reagents and Plates: The serum samples, plates and reagents before the start of the test were brought to room temperature. Plates (Antigen coated and adsorption) were accordingly labeled. By adding antibody peroxidase conjugate with conjugate diluting buffer in 1:99 ratio the conjugate was prepared. To prepare the wash solution the wash solution and deionized water were added in 1:9 ratio.

Hematological Studies: A total of 10 blood samples were selectedfrom infected cows and 10 blood samples from non-infected cows. Similarly, 10 samples were taken from infected and non-infected buffaloes. The hematological alterations were analyzed including RBCs count, WBCs count, PCV (packed cell volume), Hb(hemoglobin), MCH(mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration) by using hematology analyzer.

RESULTS

During January 2014 to December 2014 a survey of anaplasmosis in cows and buffaloes was carried out in three tehsils (Charsadda, Tangi and Shabqadar) of district Charsadda. Total 360 blood samples were collected from cows and buffaloes and prevalence (%) of anaplasmosis was noted through microscopy and ELISA technique in each group of animals.

Anaplasmosis in Cows of District Charsadda by Microscopy

Overall Prevalence (%): An overall prevalence (%) of anaplasmosis in cows by using microscopic examination of blood smears was 6.11% (11/180) in district Charsadda (Table 1).

Month-Wise Prevalence (%): The highest prevalence (%) was observed in June (13%) and July (13.33%), on the other hand November, December and January (0%) showed the lowest prevalence. All months showed non-significant difference (P>0.05) as compared to prevalence (%) of November, December and January (Table 1).

Tehsil-Wise Prevalence (%): Tangi (6.67%) and Shabqadar (6.67%) showed high prevalence. Charsadda (5%) showed lowest prevalence. Statistical analysis indicates non-significant difference (P>0.05) (Table 1).

Season-Wise Prevalence (%): The highest prevalence in three tehsils of district Charsadda was noted during summer (10 %) followed by autumn and spring 6.67% while the lowest prevalence was observed during winter 1.67%. No statistical difference was recorded in all seasons (Table1).

Age-Wise Prevalence: The data showed that prevalence was higher in cows of over 2 years old (6.52%) than in younger ones (5.68%). However, no statistical difference was observed (P>0.05) between the prevalence of two age groups (Table 1).

Gender Wise Prevalence: Females (6.25%) were more vulnerable to anaplasmosis than males (5.77%). The difference was not significant statistically between the female and male (Table 1).

Anaplasmosis in Cows of District Charsadda Using ELISA

Overall Prevalence: An overall prevalence (%) of Anaplasmosis in cows by using ELISA technique was 30.55 % (55/180) in district Charsadda from January 2014 – December 2014 (Table 2).

Month Wise Prevalence (%): The highest prevalence was recorded in June (53.33%), while the lowest prevalence (13.33%) was recorded in December. The prevalence was significantly high (P<0.05) in May, June and July as compared to the lowest prevalence in January and December (Table 2).

Season Wise Prevalence (%): The data reflects that highest prevalence was recorded in summer (45%) and the lowest (16.6%)in winter. When analyzed statistically significantly higher prevalence was noted in summer and spring as compared to winter (Table 2).

Area Wise Prevalence (%): The highest degree of prevalence was recorded in Shabqadar (33.33%) followed by Tangi (31.67%) and lowest prevalence was recorded in Charsadda (26.67%). The difference in all the areas was non-significant (P>0.05) when analyzed statistically (Table 2).

Age Wise Prevalence (%): Data showed that prevalence was higher in adult cows (43.47%) than young cows (0-2 year) (17.04%). Statistically the difference was significant between the two age groups (Table 2).

Gender Wise Prevalence (%): Females (32.03%) showed high prevalence than males (26.92%). No statistical difference was noted between the analyzed females and males (Table 2).

Anaplasmosis in Buffaloes Using Microscopy Technique

Overall Prevalence (%): An overall prevalence (%) of anaplasmosis in buffaloes using microscopy technique was found to be 4.44% (8/180) in district Charsadda from January 2014 – December 2014, (Table 3).

Table 1: Prevalence of Anaplasmosis in Cows of District Charsadda using Microscopy Technique during January 2014-December 2014

Factors		Total No. of observed samples	Total No. of infected samples	Prevalence (%±S.E)
Months	January	15	0	0±0.00
	February	15	1	6.67±6.67
	March	15	1	6.67±6.67
	April	15	1	6.67±6.67
	May	15	1	6.67±6.67
	June	15	2	13.33±6.67
	July	15	2	13.33±6.67
	August	15	1	6.67±6.67
	September	15	1	6.67±6.67
	October	15	1	6.67±6.67
	November	15	0	0±0.00
	December	15	0	0±0.00
Seasons	Winter	60	1	1.67±1.67
	Spring	30	2	6.67±3.33
	Summer	60	6	10±2.89
	Autumn	30	2	6.67±1.67
Gender	Male	52	3	5.77±0.43
	Female	128	8	6.25±0.90
Age	0-2 year	88	5	5.68±0.79
	>2 year	92	6	6.52±0.44
Areas	Charsadda	60	3	5±3.75
	Tangi	60	4	6.67±2.39
	Shabqadar	60	4	6.67±2.04
Total		180	11	6.11±0.56

Table 2: Prevalence of Anaplasmosis in Cows of District Charsadda using cELISA Technique during January 2014-December 2014

Factors		Total No. of observed samples	Total No. of infectedsamples	Change Prevalence (%±S.E)
Months	January	15	2	13.33±6.67
	February	15	3	20±0.00
	March	15	4	26.67±6.67
	April	15	5	33.33±6.67
	May	15	6	40±0.00*
	June	15	8	53.33±6.67*
	July	15	7	46.67±6.67*
	August	15	6	40±6.67
	September	15	5	33.33±6.67
	October	15	4	26.67±6.67
	November	15	3	20±0.00
	December	15	2	13.33±6.67
Seasons	Winter	60	10	16.67±1.67
	Spring	30	9	30±0.00**
	Summer	60	27	45±2.87**
	Autumn	30	9	30±5.77
Gender	Male	52	14	26.92±0.44
	Female	128	41	32.03±2.91
Age	0-2 year	88	15	17.04±1.38
	>2 year	92	40	43.47±6.67*
Areas	Charsadda	60	16	26.67±5.54
	Tangi	60	19	31.67±5.15
	Shabqadar	60	20	33.33±7.46
Total		180	55	30.55±0.71

Table 3: Prevalence of Anaplasmosis in buffaloes of District Charsadda using Microscopy Technique during January 2014-December 2014

Factors		Total No. of observed samples	Total No. of infected samples	Change Prevalence (%±S.E)
Months	January	15	0	0±0
	February	15	0	0±0
	March	15	0	0±0
	April	15	1	6.67±6.67
	May	15	1	6.67±6.67
	June	15	1	6.67±6.67
	July	15	2	13.33±6.67
	August	15	1	6.67±6.67
	September	15	1	6.67±6.67
	October	15	1	6.67±6.67
	November	15	0	0±0
	December	15	0	0±0
Seasons	Winter	60	0	0±0
	Spring	30	1	3.33±3.33
	Summer	60	5	8.33±4.41
	Autumn	30	2	6.67±3.33
Gender	Male	31	1	3.22±3.03
	Female	149	7	4.69±0.65
Age	0-2 year	73	3	4.11±0.12
	>2 year	107	5	4.67±0.90
Areas	Charsadda	60	2	3.33±2.39
	Tangi	60	3	5±2.88
	Shabqadar	60	3	5±2.88
Total		180	8	4.44±0.16

Month-Wise Prevalence (%): The highest prevalence of anaplasma infection in buffaloes was recorded in July (13.33%), while the lowest prevalence in January, February, March, November and December (0%). The difference between months was non-significant (Table 3).

Gender-Wise Prevalence (%): Females (4.69%) were more vulnerable to Anaplasmosisthan males (3.22%). No statistical difference was observed between the female and male (Table 3).

Age-Wise Prevalence (%): Adults (4.67%) have little more prevalence than young (4.11%). The difference was statistically non-significant between the two age groups (Table 3).

Area-Wise Prevalence (%): From Shabqadar and Tangi high prevalence (5%) was recorded and low prevalence (3.33%) was recorded from Charsadda. Non-significant (P>0.05) difference was noted when analyzed statistically (Table 3).

Anaplasmosis in Buffaloes Using cELISA Technique

Overall Prevalence (%): An overall prevalence (%) of Anaplasmosis recorded in bffaloes by using ELISA technique was 21.11% (38/180) in district Charsadda from January 2014 – December 2014 (Table 4).

Month Wise Prevalence (%): The highest prevalence was recorded in July (46.47%), while the lowest prevalence was recorded in January and February (6.67%). The data showed significantly high (P<0.05) prevalence from June, July and August as compared to the lowest prevalence in January and February (Table 4).

Area Wise Prevalence (%): High prevalence was recorded from Tangi (21.67%) and Shabqadar (21.67%) and low prevalence from Charsadda (20%). The difference was non-significant (P>0.05) when analyzed statistically (Table 4).

Season Wise Prevalence (%): The highest prevalence was noted in summer in all areas while thelowest prevalence was noted in winter (Table 4).

Gender-Wise Prevalence (%): Data showed that females (21.47%) were more susceptible to anaplasmosis than males (18.57%). No statistical (P>0.05) difference was noted between the females and males analyzed (Table 4).

Age Wise Prevalence (%): Table 4.7 showed that prevalence was high in adult buffaloes (23.36%) than young ones (17.80%). Statistical analysis revealed that the difference was non-significant (Table 4).

Table 4: Prevalence of Anaplasmosis in Buffaloes of District Charsadda using cELISA Technique during January 2014-December2014

Factors		Total No. of observed samples	Total No. of infected samples	Change Prevalence (%±S.E)
Months	January	15	1	6.67±6.67
	February	15	1	6.67±6.67
	March	15	2	13.33±6.67
	April	15	3	20±0.00
	May	15	3	20±0.00
	June	15	5	33.33±6.67*
	July	15	7	46.47±6.67*
	August	15	5	33.33±6.67*
	September	15	4	26.67±6.67
	October	15	3	20±0.00
	November	15	2	13.33±6.67
	December	15	2	13.33±6.67
Seasons	Winter	60	6	10±1.67
	Spring	30	5	16.68±0.00**
	Summer	60	20	33.33±1.67***
	Autumn	30	7	23.33±3.33*
Gender	Male	31	6	19.35±0.60
	Female	149	32	21.47±0.75
Age	0-2 year	73	13	17.80±2.79
	>2 year	107	25	23.36±0.49
Areas	Charsadda	60	12	20±4.08
	Tangi	60	13	21.67±5.15
	Shabqadar	60	13	21.67±6.61
Total		180	38	21.11±0.73

Student's t-test: * = P<0.05, ** = P<0.01, *** = P<0.001

Prevalence (%) by Area and Month: The result revealed that all areas had high prevalence in July Charsadda (40%), Tangi (60%) and Shabqadar (40%) while the lowest during January and February in all areas (Table 4).

Comparison of Microscopy and cELISA

Overall Comparison: Overall prevalence of anaplasmosis diagnosed through microscopical examination and ELISA technique was 6.11% and 30.55% in cow and 4.44% and 21.11% in buffaloes respectively. When analyzed statistically significant difference was noted between the two diagnostic methods ($\chi^2 = 35.92$, df = 1; P = 0.0001 in cows and ($\chi^2 = 22.43$, df = 1; P = 0.0001 in buffaloes) (Table 5 and 6).

Month Wise Comparison: In month wise data June showed highest prevalence by both microscopy (13.33%) and cELISA technique (53.33%) in cows while lowest prevalence was observed in December by microscopy and in January by cELISA (Table 5).

Buffaloes showed highest prevalence in July by both diagnostic methods (microscopy 13.33% and cELISA 46.47%) while lowest prevalence in January and February by both methods (Table 6).

Season Wise Comparison: Cows showed highest prevalence in summer by both microscopy (10%) and cELISA (45%), lowest prevalence in winter 1.67% and 16.67% respectively (Table 5).

In buffaloes summer showed highest prevalence through both microscopy and cELISA 8.33% and 33.33% respectively on the other hand lowest prevalence in winter (0%) and (10%) respectively (Table 6).

Age Wise Comparison: Adult cows showed high prevalence both by microscopic examination and cELISA 6.52% and 43.47% respectively than young ones 5.68% and 17.04% respectively (Table 5).

Adult buffaloes showed high prevalence by microscopy and ELISA 4.67% and 23.36% than young ones 4.11% and 17.80% respectively (Table 6).

Gender Wise Comparison: The data showed that Female cows were more susceptible to infection high prevalence was recorded in females by both microscopy (6.25%) and cELISA (32.03%) while males showed low prevalence both by microscopy (5.77%) and cELISA (26.92%) (Table 5).

Table 5: Comparison of Microscopy and cELISA for the Diagnosis of Anaplasmosis in Cows of District Charsadda from January 2014 to December 2014

			Microscopy		cELISA	
Factors		Total sample	Positive	Prevalence (%)	Positive	Prevalence (%)
Months	January	15	0	0	2	13.33
	February	15	1	6.67	3	20
	March	15	1	6.67	4	26.67
	April	15	1	6.67	5	33.33
	May	15	1	6.67	6	40
	June	15	2	13.33	8	53.33
	July	15	2	13.33	7	46.67
	August	15	1	6.67	6	40
	September	15	1	6.67	5	33.33
	October	15	1	6.67	4	26.67
	November	15	0	0	3	20
	December	15	0	0	2	13.33
Seasons	Winter	60	1	1.67	10	16.67
	Spring	30	2	6.67	9	30
	Summer	60	6	10	27	45
	Autumn	30	2	6.67	9	30
Gender	Male	52	3	5.77	14	26.92
	Female	128	8	6.25	41	32.03
Age	0-2 year	88	5	5.68	15	17.04
	>2 year	92	6	6.52	40	43.47
Areas	Charsadda	60	3	5	16	26.67
	Tangi	60	4	6.67	19	31.67
	Shabqadar	60	4	6.67	20	33.33
Total		180	11	6.11	55	30.55

Table 6: Comparison of Microscopy and ELISA for the Diagnosis of Anaplasmosis in Buffaloes of District Mardan from January 2014 to December 2014

			Microscopy		ELISA	
Factors		Total sample	Positive	Prevalence (%)	Positive	Prevalence (%)
Months	January	15	0	0	1	6.67
	February	15	0	0	1	6.67
	March	15	0	0	2	13.33
	April	15	1	6.67	3	20
	May	15	1	6.67	3	20
	June	15	1	6.67	5	33.33
	July	15	2	13.33	7	46.47
	August	15	1	6.67	5	33.33
	September	15	1	6.67	4	26.67
	October	15	1	6.67	3	20
	November	15	0	0	2	13.33
	December	15	0	0	2	13.33
Seasons	Winter	60	0	0	6	10
	Spring	30	1	3.33	5	16.68
	Summer	60	5	8.33	20	33.33
	Autumn	30	2	6.67	7	23.33
Gender	Male	31	2	6.45	6	19.35
	Female	149	6	4.03	32	21.47
Age	0-2 year	73	3	4.11	13	17.80
	>2 year	107	5	4.67	25	23.36
Areas	Charsadda	60	2	3.33	12	20
	Tangi	60	3	5	13	21.67
	Shabqadar	60	3	5	13	21.67
Total		180	8	4.44	38	21.11

On the other hand female buffaloes showed high prevalence by microscopy (4.03%) and cELISA (21.47%) while males showed low prevalence 6.45% and 19.35% by microscopy and cELISA respectively (Table 6).

Area Wise Comparison: Cows showed high prevalence in Shabqadar 6.67% and 33.33% by microscopic examination and cELISA respectively and low prevalence in Charsadda 5% and 26.67% respectively (Table 5).

In buffaloes both by microscopy and cELISA high prevalence was recorded in Tangi and Shabqadarwhile the low prevalence in Charsadda both by microscopy and cELISA (Table 6).

Sensitivity and Specificity of ELISA: Sensitivity and Specificity of ELISA was 100 % and 90% for cows while 100% and 80% for buffaloes respectively (Table 7).

Hematological Studies: Hematological parameters of infected Cows and Buffaloes showed a decrease in all blood indices except MCH which showed an increase. When analyzed statistically all blood parameters showed significant decrease except MCH which showed significant increase (Tables 8 and 9).

Table 7: Sensitivity and Specificity of ELISA

Factors	Sensitivity	Specificity
Cows	100	90
Buffaloes	100	80

Table 8: Effect of Anaplasmosis on Hematology of Cows

Parameters	Non infected	Infected
RBC (10^6/µL	6.51±0.16	4.50± 0.12
WBC (10^3/µL)	7.43±0.10	6.23± 0.13
Packed cell volume%	32.41±1.46	21.02 ± 0.94
Hemoglobin (g/dl)	10.02±0.50	7.12± 0.11
MCHC(g/dl)	33.40±0.05	31.01 ± 0.88
MCH (pg)	16.54±0.06	18.01 ± 0.38
MCV (fl)	49.50±0.18	58.31 ± 0.08

Table 9: Effect of Anaplasmosis on Hematology of Buffaloes

Parameters	Non infected	Infected
RBC (10^6/µL	10.01±1.16	6.70± 0.09
WBC (10^3/µL)	13.4±3.81	10.23± 0.06
Packed cell volume%	33.23±1.47	20.74 ± 0.89
Hemoglobin (g/dl)	13.53±1.60	7.05± 0.13
MCHC(g/dl)	38.40±1.05	34.66 ± 0.91
MCH (pg)	13.74±1.04	16.52 ± 0.29
MCV (fl)	36.12±2.45	54.31 ± 0.11

DISCUSSION

This research work was designed to study the prevalence of anaplasmosis among cows and buffaloes in Tehsil Shabqadar, Tehsil Charsadda and Tehsil Tangi of district Charsadda of the Khyber Pakhtunkhwa province, Pakistan. Moreover, differences in haematological analysis was also conducted between noninfected and *Anaplasma* infected species of cows and buffaloes.

The Epidemiology of tick-transmitted diseases has not been fully studied in this region, despite the fact that they cause major constraint to livestock production in Pakistan [18]. The diagnosis of tick-borne diseases is usually based on the microscopic examination of blood smear. Previous reports have mentioned the prevalence of *Anaplasma marginale* ranging from 4.2-22% in cattle from Peshawar and Hyderabad regions [19]. The higher prevalence (75.7%) of *Anaplasma marginale* was reported by Khan *et al*.[18] at two government livestock farms located at Islamabad and Attock district, Pakistan as compared to present study conducted at Charsadda district.

The higher prevalence in females as compared to male cattle has been recorded for *Anaplasma* infection in the present study. These findings are in agreement with the results of Durrani [20] and Rajput *et al.* [21] who reported higher prevalence of *Anaplasma marginale* in female animals.

The highest prevalence of *Anaplasma* infections were recorded in summer in all study regions. The prevalence of *Anaplasma* spp.in the months of tick free season is the indication of mechanical transmission. Simuunza and coworkers has mentioned that wet season is associated with TBDs in Zambia [22].

There is no known serological survey against *Anaplasma* spp in cows and buffaloes in Khyber Pakhtunkhwa, Pakistan. Probably this is the first serological survey against *Anaplasma* spp in cows and buffaloes in Khyber Pakhtunkhwa. Earlier reports were based on microscopic examination ofblood smears. In the present study three tehsils of district Charsadda were selected. The competitive ELISA (cELIZA) was used for the detection of serum antibodies as recommended by World Animal Health Organization for the serodiagnosis of anaplasmosis in cattle [23]. Various serological tests have been used earlier for the large scale identification of persistently infected cattle including agglutination test [24]. There was the problem of sensitivity, reproducibility and interpretation associated with these tests [25].

Competitive ELISA based on major surface protein-5 has obvious advantage over other serological tests because of higher sensitivity 96% and specificity 95% for anaplasmosis [23]. The cELISA uses a 19-kDa antigen based on recombinant major surface protein (MSP5) which is highly conserved among *Anaplasma* species [26].

Overall seroprevalence of *Anaplasma* spp.in cows and buffaloes in the study area was 25.83% (93/360). There were variations in the distribution of *Anaplasma* infection in different geographical regions. Highest seroprevalence was recorded in tehsil Shabqadar 27.5% (33/120) followed by Tangi 26.67% (32/120) and then in tehsil Charsadda 23.33% (28/120). The seroprevalence was statistically non-significant among all study Areas. Seroprevalence of *Anaplasma*has been reported as 26% by Marufu *et al*. [27] in semi-arid area of South Africa using competitive inhibition ELISA.

The seroprevalence recorded in the present study was 25.83%, indicating the region is rather endemically unstable. Endemic stability most likely occurs in regions where serum antibodies prevail in 70% of animal population [28, 29].

Anaemia was the major clinical finding in haematological studies. All animals showed a significant difference among haematological parameters. It was observed that blood parameters had decreased with the increase of parasitaemia. Significant difference was revealed among RBCs, PCV and MCH haematological parameters of cows and buffaloes.

The MCV was found to be higher in infected as compared to healthy control. The decrease of MCHC and increase of MCV was noticed as compared to healthy controls. This classify the anaemia as hypochromic and macrocytic. The increase in MCV is usually the indication of regenerative anaemia [30]. After rapid destruction of RBCs by phagocytosis the immature RBCs are released from bone marrow due to increase demand. The immature RBCs are larger in size than mature red blood cells explain the reason for increased MCV. The decrease of WBCs was also recorded in the present study. This finding coincide with [31] who depicted significant decrease of WBCs in prepatent phase and non-significant decrease of WBCs in early and late stages of the diseases.

CONCLUSION

It was concluded that anaplasmosis is widely distributed in district Charsadda, Khyber Pakhtunkhwa, Pakistan. Hemolytic anemia is the major hematological finding of Anaplasmosis. The findings of the present study would help in planning prevention and control strategies for bovine anaplasmosis in Pakistan.

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