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Prevalence, Haematological Alterations and Chemotherapy of Bovine Anaplasmosis in Sahiwal and Crossbred Cattle of District Faisalabad, Punjab, Pakistan

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AS, AM and IS conceived and designed the experiments, performed experiments, interpreted the data and wrote the article. All other authors helped in preparation of the manuscript. AS wrote the manuscript.

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Bovine anaplsmosis, *Anaplasma marginale*, Haematological study, Prevalence, chemotherapy.

ABSTRACT

In the current study, prevalence related associated risk factors and hematological changes (due to bovine anaplasmosis) were studied in Sahiwal cattle and crossbred cattle of the district Faisalabad of Punjab province, Pakistan. Therapeutic efficiency of Oxytetracycline-Imidocarb dipropionate combination was compared with Enrofloxacin-Imidocarb dipropionate against *Anaplasma marginale* (*A. marginale*). For this study, the blood samples of three hundred and sixty nine cattle were collected. Giemsa stain of blood smears showed 10.84% infection of cattle with *Anaplasma. A. marginale* positive cases were further confirmed through PCR. Among risk factors, age (χ^2=19.35, P value=0.001), breed (χ^2=29.08, P value=0.000), frequency of acaricidal treatment (χ^2=18.56, P value=0.001), number of cleaning times (χ^2=16.11, P value=0.002), feeding system (χ^2=23.41, P value=0.001), floor pattern (χ^2=17.98, P value=0.000) and hygienic measures (χ^2=25.79, P value=0.001) significantly influenced the incidence of disease in the cattle of district Faisalabad. *A. marginale* induced statistically significant reduction was observed in RBC count, Hb, MCV, PCV and MCHC in infected cattle compared to healthy animals (P<0.05). Anaplasmosis infected animals more effectively treated with combination of Oxytetracycline (22mg/kg, I/V, once/day for 5 days) and Imidocarb dipropionate (5mg/kg, I/M, twice 7 days apart) compared to Enrofloxacin (12.5 mg/kg, I/V, once/day for 5 days) and Imidocarb dipropionate (5mg/kg, I/M, twice 7 days apart) combination.

INTRODUCTION

Livestock and Dairy industry is facing a lot of challenges in the form of various diseases which ultimately affect the economy of any country (Carroll, 2008). Parasitic diseases in animals cause production losses and several critical issues across the globe (Mehmood *et al.,* 2017; Zaman *et al.,* 2017 and Ijaz *et al.,* 2018). Anaplasmosis also known as gall sickness or yellow bag fever is a haemo-protozaon disease of cattle, which is caused by an obligatory intra-erythrocytic gram negative bacterium *Anaplasma marginale* (Kocan *et al*., 2010). The disease has been placed recently in B type infection category by Office of International des Epizooties, Terrestrial Animal Health Code (OIE, 2012). An annual estimated loss of $100 million in USA which includes 50,000 to 100,000 deaths of cattle (Ashuma *et al*., 2013) was due to Bovine anaplasmosis. Anaplasma is transmitted through biological vectors (ticks) i.e. *Rhipicephalus (Boophilus) microplus* (Aubry and Gaele, 2010). Besides, it can also be transmitted mechanically by blood contaminated fomites and biting flies (Ashuma *et al*., 2013)

Cattle have more susceptibility to Anaplasma than buffalo (Rajput *et al*., 2005). Cattle infected with *A. marginale* may act as carrier or reservoir for transmission by ticks (Kocan *et al*., 2003) *A. marginale* attack on RBCs and cause anemia and extravascular haemolysis of

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varying degree. As the infection progress, cattle may show symptoms of fever (4-10 days), anemia, anorexia, great weight loss, cough, lethargy, abortion, decreased milk yield, increased pulse and respiratory rate (Coetzeea *et al*., 2005). Clinical anaplasmosis leads about 80% of mortality in enzootic areas (Soulsby, 2006) and occurs mostly in cattle (Zaugg *et al*., 1996; Ashuma *et al*., 2013).

Giemsa stained thin blood smear (GSTBS) microscopic examination and serological test, are the main methods used for diagnosis of anaplasmosis. For the detection of clinically suspected animals' conventional parasitological techniques like microscopic examination of GSTBS are always a gold standard test for the diagnosis of anaplasmosis while in subclinical (serve as reservoirs) and in chronic infections GSTBS is not applicable (Eriks *et al*., 1989; Ashuma *et al*., 2013). For confirmatory diagnosis, PCR is used for detection even in case of low level of parasitemia (Ge *et al*., 1995; Torina *et al*., 2008).

To be best of our knowledge, no epidemiological studies had been conducted for *A. marginale* in district Faisalabad. The most effective method to overcome anaplasmosis without harming the host is the use of the drugs. Before the development of antimicrobial (tetracycline), many drugs such as some dyes and arsenic compounds, antimalarial and antimony derivatives were used to overcome acute anaplasmosis. But these compounds that have little therapeutic effects cannot control mortality due to anaplasmosis (Potgieter and Stollsz, 1994). The most effective drugs that have been used in earlier studies for the control of carrier or clinical conditions of anaplasmosis are oxytetracycline, enrofloxacin and imidocarb (Swift and Thomas., 1983; Atif *et al*., 2012). Combination of Oxytetracycline and Buparvaquone exhibited 93% efficacy that is reported by Muhammad *et al*. (1999) in Faisalabad. Combination of these drugs against anaplasmosis may prove to be more effective.

Moreover, no detailed research work has yet been done on the epidemiology, associated risk factors and chemosterilization of bovine anaplasmosis in naturally infected carrier cattle (*Bos indicus* and *Bos taurus*) in district Faisalabad, Punjab, Pakistan. Hence, the present research was planned to determine epidemiological aspects and hematological study of anaplasmosis in Sahiwal and crossbred cattle and also to evaluate combine effect of Oxytetracycline-Imidocarb dipropionate and Enrofloxacin-Imidocarb dipropionate.

MATERIALS AND METHODS

Study area

Faisalabad district is situated between the latitude of 31°25′0″N and longitude of 73°5′28 E with an altitude of 184 meters (605 ft) above sea level in the northeast of Punjab province. The district is spread on an area of about 1,230 km^2. It is bounded by the cities of Chiniot, Gojra, Jhang, Sheikhupura, Lahore, Okara and Sahiwal.

Sample collection

A total of 369 blood samples of cattle (*Bos indicus* and *Bos taurus*) of any sex or age were collected from randomly selected private livestock farms in district Faisalabad, Pakistan. Blood samples (10 ml) were drawn from jugular vein in EDTA container as described by (Ullah *et al*., 2018).

Epidemiological study

A questionnaire was designed (Supplementary Fig. 1) for the interview of livestock attendants, managers and owners of livestock sector. Information related to the risk factors such as breed, gender, farming system, age, type of herd size, housing, feeding system, floor pattern, frequency of acaricides used, cleansing time, and hygienic measures were collected.

Giemsa staining technique

Thin blood smears stained by Giemsa stain were prepared as described by Solusby (2006) and Khan and Rehan (2018). Dried blood smears were fixed in methyl alcohol by dipping for about 10 minutes and washed under tap water. Fixed slides were then stained in Giemsa's stain dilutions (1:10) for half hour. The slides were gently rinsed under tap water and then dried. Smear was observed under oil immersion lens for the detection of *A. marginale*.

Molecular diagnosis through PCR

DNA from collected blood samples was extracted by using DNA extraction kit. Polymerase Chain Reaction (PCR) for the diagnosis of *A. marginale* as per procedure of Carelli *et al*. (2007) and Ashuma *et al*. (2013). Genomic DNA (gDNA) of parasite was isolated from blood samples of animals using DNA extraction kit. Blood (300 µl) was pipetted in properly labeled autoclaved tube placed on ice. Then, RBC lysis solution (1µl) was added and the tubes were incubated in shaking incubator at room temperature for 5-10 minutes. Supernatant was removed after centrifugation for 5 minutes at 12000 rpm and 300 µl cell lysis solutions was added to pellet. Tubes were incubated in shaking incubator for 5 minutes at room temperature. 100µl of protein precipitation solution was added and incubated for 9 minutes. Tubes were then centrifuge at 13000 rpm for 5 minutes. Supernatant was removed and equal quantity of isopropanol was added. Tube containing DNA pellets was placed in concentrator

at about 45°C for 2 minutes and isopropanol was removed. Autoclaved distilled water (30µl) was added in each tube.

Analysis of DNA

Analysis of DNA was carried out through agarose gel electrophoresis (AGE) by making 0.7% agarose gel. PCR was carried out by using reaction mixture of PCR buffer (5µl), $MgCl_2$ (5 or 6 µl), dNTPs mixture (5µl), forward primer (2µl), reverse primer (2µl), extracted DNA (5µl), Taq polymerase (0.5µl) and autoclaved distilled water (25.5 or 24.5µl depending upon the concentration of $MgCl_2$). All necessary details of primers are mention in Table I.

Haematological study

Fifty blood samples were taken from 40 infected cattle (crossbred n= 20 and Sahiwal n=20) and 10 samples from uninfected cattle (crossbred cattle n=5 and Sahiwal n=5). Changes in the different haematological parameters were evaluated using haematological analyzer (Sysmex, PocH 100, USA). The percentage of parasitized erythrocytes (PPE) was calculated using the formula (Coetzee and Apley, 2006; Atif *et al*., 2012):

$$PPE(\%) = \frac{\text{Number of infected cells}}{\text{Total number of cells counted}} \times 100$$

Chemotherapeutic trial

The selected animals were divided into three groups for chemotherapy, each consisting of 15 animals and designated them as OXY-IMC group I, ENRO-IMC group II, and control group III. The following treatments were applied.

OXY-IMC group I: Oxytetracycline {Rimoxyn P.D.H Laboratories (Pvt). Ltd} was given at the rate of (@) 22 mg/kg body weight intravenous (IV) once in a day for the period of 5 days and Imidocarb dipropionate (Imipro, Selmore Agencies Pvt. Ltd, ICI) was given intramuscular @ 5 mg/kg body weight twice 7 days apart.

ENRO-IMC group II: Enrofloxacin {Encure Injection 10%, Nawan Laboratories (PVT) Ltd} was given @ of 12.5 mg/kg body weight IV once daily for 5 days, Imidocarb dipropionate (Imipro, Selmore Agencies Pvt. Ltd, ICI) @ 5 mg/kg body weight intramuscular (IM) twice, 7 days apart.

Control group III: This group represented as infected untreated control group.

Statistical analysis

Repeated measure One Way Analysis of Variance (ANOVA), multiple logistic regression and Mantel–Haenszel Chi square analysis were performed to find out any association of hypothesized risk factors with the occurrence of disease. Data of haematology and chemotherapy was analysis by One Way Analysis of Variance (ANOVA).

RESULTS

GSTBS based prevalence

Giemsa stained microscopic examination of 369 blood samples revealed the prevalence of *Anaplasma* species along with some other tick-borne pathogens (Theileria and Babesia spp.). From 369 blood samples, 72 were found positive for tick-borne pathogens with an overall prevalence of 19.51%. *A. marginale* was the most prevalent haemoparasite (40/369, 10.84%) followed by *Theileria* sp. (18/369, 4.87%) and *Babesia* sp (14/369, 3.79%).

PCR based prevalence of Anaplasma

PCR based diagnosis showed that 56/369 blood samples of animals were positive for *A. marginale* with a prevalence of 15.17%. Significant higher prevalence (χ^2= 24.38, P=0.001) was noted through PCR technique as compared to GSTBS. Animals identified positive through PCR technique were considered as true positive for further epidemiological descriptions.

Table I.- Primers used to amplify 30-kDa and SSU rRNA gene sequence of *A. marginale*.

Primer set A		**Anaplasma specific**		
Primer	Sequence of Primer	Target position	Target Region	Predicted amplicon size
M60313	AGAGTTGATCCTGGCTCAG	1-20	SSU rRNA gene	781
M60313	AGCACTCATCGTTTACAGCG	781-762	SSU rRNA gene	
Primer set B		***Anaplasma marginale* specific**		
AM 100	CAGAGCATTGACGCACTACC	337-356	SSU rRNA gene	246
AM101	TTCCAGACCTTCCCTAACTA	582-563	SSU rRNA gene	

Analysis of risk factors

Table II indicates the relationship of hypothesized risk factors risk factors factor with disease. Analysis of all the hypothesized risk factors revealed that breed, age, floor system, feeding system, cleaning times, hygienic measures and acaricidal treatment were the factors significantly associated (P<0.05) with prevalence on anaplasmosis in cattle of district Faisalabad. The gender, farming system, herd size (P>0.05) were found non significantly associated with the infection of *A. marginale.* The higher prevalence of *A. marginale* was recorded in young animals of crossbred cattle (23.80%) and young animals of Sahiwal cattle (18.51%), and also higher prevalence in adult animals of crossbred cattle (13.33%) and adult of animals Sahiwal cattle (10.16%), age related risk factor between Sahiwal and crossbred cattle is (χ^2=19.35, P value=0.001). Prevalence rate (P=0.000) of *A. marginale* was higher in crossbred cattle (χ2= 29.08, P value=0.000) as compared to Sahiwal cattle. The higher prevalence (27.27%) of *A. marginale* was recorded in farms where no acaricide was used as compared to those farms where acaricides were used twice (6.25%) or thrice (2.59%) per year in cattle (χ^2=18.56, P value=0.001). *A. marginale* prevalence was highest (26.05%) in farms where cleaning was done weekly (χ^2=16.11, P value=0.002) than those farms which were cleaned one or two times daily. Prevalence of *A. marginale* was significantly higher (χ^2=23.41, P value=0.001) in stallfed animal groups as compared to groups which were on grazing. Type of floor was also observed for effect on prevalence of *A. marginale* and significantly higher (χ^2=17.98, P value=0.000) prevalence was recorded in un-cemented floor cattle (24.28%) as compared to cemented floor (8.16%). Cattle kept under poor hygiene were suffering from strong anaplasmosis (χ^2=25.79, P value=0.001) as compared to those kept under good or excellent hygiene. Non-significant association (P>0.05) was observed in herd size, gender and farming system of cattle. Although prevalence rate of *A. marginale* was slightly higher in medium herd size (15.97%) cattle than large herd size cattle (14.5%) but non-significant difference (χ^2=2.070, P value=0.238) was found. A little higher prevalence of *A. marginale* was found in females than males, but difference is also statistically non-significant (χ^2=1.98, P value=0.379). There was no statistically significant difference (χ^2=3.87, P value=0.134) between different farming system and the prevalence of *A. marginale*.

Haematological analysis

A significant difference (P<0.01) was observed in haematological parameters (RBC count, PCV, Hb, MCHC) among infected and heathy cattle of Cross bred and Sahiwal breed, whereas non-significant differences (P>0.05) were observed in WBC count and MCH in both species. Haematological values (Mean±SD) are presented in Table III.

Table II.- Mantel–Haenszel chi-square analysis of hypothesized risk factors associated with *A. marginale* infection in cattle of district Faisalabad, Pakistan.

Determi-nants	Variables	Prevalence (%)	P-value
Breed/ Age/ Sex	Crossbred	18.66	29.08(0.000)
	(Young)	23.80	19.35(0.001)
	(Adult)	13.33	
	(Male)	18.18	1.98(0.379)
	(Female)	18.75	
	Sahiwal	12.78	28.03(0.000)
	(Young)	18.51	17.84(0.001)
	(Adult)	10.16	
	(Male)	12.75	2.13(0.461)
	(Female)	12.88	
Herd size	Medium	15.97	2.07(0.238)
	Large	14.5	
Cleaning times	Once a day	7.69	16.11 (0.002)
	Twice a day	12.5	
	Weekly	26.05	
Farming system	Separate	15.38	3.87(0.134)
	Mixed	15.59	
Feeding system	Grazing	12	23.41(0.001)
	Stall feeding	17.35	
Floor system	Un-cemented	24.28	17.98(0.000)
	Partially cemented	22.23	
	Cemented	8.16	
Hygienic measure	Poor	27.14	25.79(0.001)
	Good	18.48	
	Excellent	8.33	
Acaricidal treatment	Once/year	18.62	18.56(0.001)
	Twice/year	6.25	
	Thrice/year	2.59	
	None	27.27	

Chemotherapic trial

Table IV indicates the results of chemotherapeutic

trials for *A. marginale* infection sahiwal and crossbred cattle of district Faisalabad, Pakistan. In OXY-IMC group I, examination of GSTBS has shown that a cattle suffering from light and moderate intensities of anaplasmosis recovered completely at 2nd day. Whereas from four cattles having heavy intensity of anaplasmosis infection, two of them recovered moderately and two of them recovered completely at 2nd day. From these two cattle, one cattle recovered at 4th day and second recovered at 6th day completely. Cattles with heavy anaplasmosis infection recovered completely at day 6. Out of six animals that were suffering from severe infection, 5 recovered moderately at 2nd day and all recovered completely at 9th day.

In ENRO-IMC group II, cattle with light infections recovered completely at 2nd day. While cattle with moderate infections recovered completely at 6th day. Animals with heavy and severe infections did not recover completely even at 9th day post treatment.

In Control group III, intensities of infections remained same except moderately infected group in which one cattle was died and one cattle got heavy infection.

Table III.- Haematological parameters (mean ± SD) of *A. marginale* infected Sahiwal and crossbred cattle of district Faisalabad, Pakistan.

Blood parameters	Sahiwal cattle		Crossbred cattle	
	Healthy	Infected	Healthy	Infected
WBC (X 10^3/µL)	7.13±0.12	6.79±0.12	7.43±0.17	7.01±0.10
RBC (X 10^3/µL)	6.40±0.14	4.43±0.82*	6.50±0.08	4.56±1.07*
PCV (%)	32.4±1.49	21.23±1.78*	32.4±1.32	24.19±0.27*
Hb (g/dL)	10.02±0.47	7.84±1.43*	10.02±0.85	7.50±0.68*
MCV (fL)	49.50±0.22	58.56±2.02*	49.50±0.18	58.22±0.46*
MCH (pg)	16.53±0.05	17.67±0.78	16.53±0.08	17.89±1.20
MCHC (g/dL)	33.41±0.051	31.09±0.68*	33.41±0.07	31.28±0.67*

Mean values with steric (*) represents a statistically significant difference (ANOVA; $P<0.05$).

Table IV.- hemotherapeutic trials for *A. marginale* infection in Sahiwal and crossbred cattle of district Faisalabad, Pakistan.

Group	Drug used	Intensity before treatment (N*)	Intensity				P-value
			Day 2 (n)	Day 4 (n)	Day 6 (n)	Day 9 (n)	
I	Oxytetracycline with Imidocarb dipropionate	+ (3)	- (0)	- (0)	- (0)	- (0)	0.002
		++ (2)	- (0)	- (0)	- (0)	- (0)	
		+++ (4)	++ (2)	+ (2)	- (0)	- (0)	
		++++ (6)	++ (5)	++ (2)	+ (2)	- (0)	
II	Enrofloxacin with Imidocarb dipropionate	+ (2)	- (0)	- (0)	- (0)	- (0)	
		++ (3)	+ (2)	+ (1)	- (0)	- (0)	
		+++ (3)	++ (3)	++ (3)	++ (1)	+(1)	
		++++ (7)	+++ (5)	+++ (3)	++ (3)	++ (2)	
III	Control- infected non-treated	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	
		++ (3)	++ (3)	++ (3)	+++ (1)	+++ (1)	
		+++ (5)	+++ (5)	+++ (5)	+++ (5)	+++ (5)	
		++++ (5)	++++ (5)	++++ (5)	++++ (5)	++++ (5)	

* N is number of animals.

DISCUSSION

Jabbar *et al*. (2015) reviewed the prevalence of *A. marginale* in cattles of different cities of Pakistan. In Karachi it was 60 %(30/50), in Hyderabad it was 11% (11/100), in Attock and Islamabad it was 17.3% (53/307), in Peshawar it was 4.2% (12/285), in Sargodha it was 9.7 % (34/350), in Khushab, Rawalpindi and Sargodha it was 5.8 % (61/1050), and in Khanewal it was 4.1 % (34/836). Till now full-scale investigation for anaplasmosis epidemiology has not been conducted based on district Faisalabad. Mostly, previous reports of Pakistan were based on GSTBS and lack of full investigation of distribution of *A. marginale* on the basis of age, breed, gender, herd size, farming system, feeding system, hygienic measures, housing, cleaning effects and floor system. This is the first time that detailed research was carried out on risk factors associated with the distribution of anaplasmosis in crossbred and Sahiwal breeds in district Faisalabad, Pakistan. Further, combine efficacy of oxytetracyclin-imidocarb dipropionate and imidocarb dipropionate-enrofloxacin has not been studied in earlier researches for the control of bovine anaplasmosis.

In present investigation, prevalence of *A. marginale* in Sahiwal and cross breed was found higher in age category 2-4 years that is in agreement with findings of Swai *et al*. (2005) and Urdaz-Rodriguez *et al*. (2009). Kocan *et al*. (2010) has also reported that clinical anaplasmosis was found to be more evident in those cattle that reached at the age of more than one year. Sex wise prevalence (high in females) of present study is showing similarity with the results of Rajput *et al*. (2005) and Durrani (2008) who also reported higher prevalence of anaplasmosis in female animals. But in present research, gender was found to have no effect on prevalence of bovine anaplasmosis.

Lower prevalence of anaplasmosis in Sahiwal cattle and indigenous breed are in agreement with the results of Khan *et al*. (2004). They found that there was higher percentage prevalence of haemotoparasites diseases in cross bred cattle (19.4%) as compared to local breeds (Dhanni;14% and Red Sindhi; 17%). Likewise, Chakraborti (2002) reported that cross bred animals suffered from higher infection of haemoprotozoan diseases. Swai *et al*. (2007) correlated it with the inherent resistance possessed by indigenous cattle against tick infection. Resistance trait in indigenous cattle may result in lower *A. marginale* infection. Furthermore, Velusamy *et al*. (2014) describes that high milk production in cross breeds due to the change in genetic makeup and seasonal stress could be a reason of less immunity response in these animals.

Like the findings of Sajid (2007) and Sethy (2016) it was observed higher risks were associated with stallfeeding as compared to field grazing which may be result of higher tick infestation chances in same area. But these findings are contraindicated with the results of Swai *et al*. (2005), Rodriguez *et al*. (2009), who reported that significant positive association of grazing in pasture with the prevalence of *A. marginale.* The reason of higher prevalence in stallfed animal might be due to the reason that no stage of tick in pasture was detected for pathogen (Halos *et al*., 2010).

Further in present study, cleanliness, hygienic measures, farming system, housing and herd size were also observed to have an effect on the prevalence rate of *A. marginale*. Poor management system, insufficient economic sustainability, poor practices of tick control and animal health leads to higher prevalence of anaplasmosis (Swai *et al*., 2005). The prevalence of *A. marginale* varies from host to host, region to region, depending upon management system and environmental factors (Kivaria, 2006).

In the current study anaemia was the most evident finding in haemotology analysis. Compared with the heathy animal, a significant difference was observed in haemotological parameters. It has been found that alteration in haemotological parametes depends on level of parasitemia. The haematological values recorded at different levels of parasitaemia were lower than the normal values. Significant difference (RBCs, PCV and MCH) was observed in heathy and infected animals of both species.

Haematological analysis of blood samples of affected cattle of present study showed no significant change in the numbers of the white blood cell (WBCs). These result findings correlate with the findings of Adejinmi *et al*. (2004), Ahmadi-hamedani *et al*. (2011) and Yasini *et al*. (2012). They also observed a decrease in the number of WBCs in infected animals than in non-infected animals. But Biobaku *et al*. (2011) observed different case. They reported that an increase in the number of WBCs take place during protozoan infection. Similarly reduction of RBCs and PCV was also recorded in Sahiwal and crossbred cattle that are exhibiting similarity with the findings of Jatau *et al*. (2011) and Biobaku *et al*. (2011). It happens because *A. marginale* attack on RBCs and increase the haemolysis of RBCs. Breakdown of RBCs become faster than the formation of RBCs.

Riond *et al*. (2007) carried out haematological study on Swiss cattle suffering from anaplasmosis (*A. marginale*), also infected with *A. phagocytophilum, Theileria buffeli/ sergenti/orientalis, Babesia bigemina* and *Mycoplasma* species. They observed decreased in platelets, increased number of white blood cells, increased aminotransferase, blood aspartate, blood urea nitrogen, serum bilirubin, glutamic dehydrogenase and gamma glutamyl-transferase.

The key sign of anaplasmosis infection is

extravascular haemolytic anaemia in cattle (Ajayi *et al*., 1978; Kuttler, 1984; Atif *et al*., 2012). Phagocytosis of infected erythrocytes by the bone marrow cells and spleen leads to high risk of anaemia (Jain, 1993). Usually there is a simultaneous haemolysis of intravascular and extravascular RBCs (Riond *et al*., 2007). In addition to the breakdown of parasitized RBCs, destruction of the non-parasitized RBCs also started due to immune mediated autolysis. While, antibodies that produced against *A. marginale* infected RBCs also started to destroy non-infected RBCs.

The decrease of MCHC and increase of MCV was observed in infected as compared to healthy animals in both breeds. The increase in MCV is indicating that anaemia is a regenerative, hypochromic and macrocytic disease (Riond *et al*., 2007). Fast destruction of RBCs by phagocytosis leads to their increased demand, therefore bone marrow cells starts to release immature RBCs. The immature RBCs those are bigger in size than mature RBCs indicating the reason for increased MCV.

Further regarding about chemotherapy of cattle of current study, both Sahiwal and crossbred were treated with the combination of oxytetracycline-imidocarb dipropionate and imidocarb dipropionate-enrofloxacin drugs in order to study efficacy of these drugs. Splenectomized calves that were infected experimentally with *A. marginale* by Kuttler and Simpson (1978) were successfully treated with 1-3 injections of oxytetracycline (10-20 mg/kg of body weight) and doxycycline (100 mg/kg/body weight) intramuscularly. While Radostits *et al*. (2000) treated the anaplasmosis in animals by using 6-10 mg/kg body weight of oxytetracycline three times daily. Animals were completely recovered by given them a single injection of oxytetracycline (20 mg/kg body weight) intramuscularly. He further confirmed that for clinical anaplasmosis, Imidocarb at the dose rate of 3 mg/kg body weight is also an effective drug. Further, Coetzee and Apley (2006) noticed 25% clearance of anaplasmosis in carrier calves treated with imidocarb and oxytetracycline drugs. Efficacy of imidocarb dipropionate against anaplasmosis and babesiosis in naturally infected buffalo calves was further evaluated by Akhter *et al*. (2010). The *Anaplasma* infected animals were treated with single injection and carrier status was successfully eliminated with two intramuscular injections of imidocarb dipropionate at the dose rate of 3 mg/kg Coetzee *et al*. (2006) reported that oxytetracycline is more effective drug leads to complete recovering of animals than Imidocarb diapropionate. High effectiveness of oxytetracycline Imidocarb dipropionat and Enrofloxacine against anaplasmosis has also been confirmed by Atif *et al*. (2012). Further, Akhtar *et al*. (2010) demonstrated that diaminazene aceturate is also a drug used against blood protozoan infection showed more effectiveness than Imidocarb dipropionate.

Atif *et al*. (2012) examined the efficacy of Oxytetracycline, Enrofloxacine and Imidocarb against the carrier state of *A. marginale* in Sahiwal cattle. They selected 60 *A. marginale* infected cattle and made four groups of Oxytetrcycline, Enrofloxacine and Imidocarb dipropionate and a control group respectively. They found Oxytetracycline is most effective medicine. Combination of Oxytetracycline and Buparvaquone exhibited 93 % efficacy that is reported by Muhammad *et al*. (1999) in Faisalabad, this efficacy may be due to the combined utilization of Oxytetracycline and Buparvaquine. Combination of these drugs against anaplasmosis may prove to be more effective. The remarkable point regarding to chemotherapy of anaplasmosis in present research is that combination of oxytetracyclin-imidocarb dipropionate which showed 100% efficacy 6 days' post exposure in case of heavy infection and 9 days post exposure in case of severe infection. Thus, it is concluded that combination of Oxytetracycline-Imidocarb dipropionate is the most effective way of chemosterilization for bovine anaplasmosis in cattle.

CONCLUSION

The prevalence of *A. marginale* by GSTBS was 10.84%, which was enhanced by detection by PCR up to 15.17%. Significant risk factor associated with bovine anaplasmosis were age, breed, frequency of acaricidal application, feeding system, number of cleaning times, floor pattern and hygienic measures while non significant factors were sex, farming system and herd size. Haemotological study shows that significant reduction in RBC count, PCV, Hb, MCV and MCHC while nonsignificant reduction in MCH and WBC count was also observed in this study. The combination of Oxytetracycline-Imidocarb dipropionate is the most effective way of chemosterilization for bovine anaplasmosis in cattle.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjz/2019.51.6.2023.2032

Statement of conflict of interest

Authors have declares that there is no conflict.

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