Research Article

Prevalence and risk factors analysis associated with anaplasmosis in symptomatic cattle under field conditions in southern Khyber Pakhtoonkhwa, Pakistan

Naimat Ullah Khan[1*], Mian Saeed Sarwar[1], Sultan Ayaz[1], Hazrat Ali[1], Anwar Ali[2], Ikramullah Khan[3],Mumtaz Ali Khan[3], Azmat Ullah Khan[3], Munib Hussain[4], Mehboob Ali[5] and Ghazunfer Rashid[5]

1. College of veterinary sciences and animal Husbandry, Abdul Wali Khan University Mardan, KPK-Pakistan
2. Veterinary Research Institute, Peshawar, KPK-Pakistan
3. Department of Livestock and Dairy Development, Peshawar, KPK-Pakistan
4. Animal Health Programme, NARC, Park road Islamabad-Pakistan
5. Department of livestock and Dairy Development, Quetta, Balochistan-Pakistan
Corresponding author's email:naimatullahkhan19@yahoo.com , naimatullahkhan75@awkum.edu.pk

Abstract
The aim of the current study was to assess the prevalence of anaplasmosis and potential risk factors (age, sex, breed, months, seasons, application of acaricides) associated with occurrence of anaplasmosis in cattle in southern area of Khyber Pakhtunkhwa. *Anaplasma* is an obligate intraerythrocytic microorganism affecting a wide range of ruminants and responsible for huge economical losses in form of high mortality, weight loss and dropped milk. Overall three hundred (n=300) animals showing clinical signs under field conditions were randomly selected from district LakkiMarwat for a period of six months (January, 2018 to June 2018). As a result the overall prevalence was 19.66% where the highest prevalence (24.85%) was recorded in young cattle at the age of (≤5 years) than adults (≥5 years) where lower prevalence (13.13%) was reported and statistically highly significant (p<0.001) association was observed. The highest sex wise prevalence was recorded in female animals (22.07%) than male (11.59%) where significant (p<0.002) association was recorded. The highest breed wise prevalence was recorded in Cross Holstein Friesian cattle (28.10%) than Indigenous pure breed cattle (6.08%) and found significant association (p<0.0000). The highest month wise prevalence was recorded in the June (38%) followed by May (34%), March (10%), January (8%) while the lowest in the month of February (6%) and recorded significant (p<0.003)) association. The highest season wise prevalence was

recorded in the summer (36%) followed by spring (16%) while the lowest in the winter (7%) and found highly significant (p<0.0000) association. The highest prevalence (23.55%) was recorded where proper acaricides were not used whereas lowest prevalence (10.86%) was recorded in those areas where acaricides were used and observed significant association (p<0.0002).For future prospective the current study was conducted to reduce the economic losses by application of technical and scientific practices and control of predisposing factors.

Keywords: Anaplasma; *Anaplasma marginale*; Prevalence; Risk factors; *Rickettsiales*

Introduction

Anaplasma is an obligate intraerythrocytic microorganism belonging to family: *Anaplasmataceae*, order: *Rickettsiales*, species: *Anaplasma marginale* and *Anaplasma centrale*. The disease caused by *Anaplasma* species is known as anaplasmosis. Anaplasmosis is also known as gall sickness. Anaplasmosisis commonly occur in cattle, buffalo, sheep, goat and wild ruminants. It is tick- born rickettsial microorganism causing a huge economic losses in form of weight loss, decrease in milk production, severe anemia and high mortality. Cattle are more prone to *anaplasma* infection as compared to buffaloes [1]. There are 20 different species of ticks responsible for transmission of anaplasmosis [2, 3] including *Rhipicephalus* spp., *Hyalomma* spp., *Boophilus demacentor* spp., *Ixodes* spp., [4] though *Boophilus microplus*is the major disease transmitting tick [5].Mechanical transmission also occurs by using contaminated needles or surgical blades. Anaplasmosis is clinically characterized by development of hemolytic anemia, fever, abortions, and decrease in milk production, jaundice, nervous disorder and rapid death occur in some cases [6, 7]. Anaplasmosis mainly occur in hot, rainy and humid weather linked with abundance of ticks [8].After biting of tick, anaplasma enters in erythrocyte and start replication. As a result busting of RBCs occur known as hemolysis [9]. Cattle show clinical signs after 20 days of post bite with 15% infectivity of RBCs. [6]. Anaplasmosis is worldwide in distribution and has been reported in both cattle and buffaloes in different countries such as Italy (50%) [10]. (De La Fuente, 2005), China (32.35%), Costa Rica (59.6%) [11]. Morocco (21.9%) and Iran (50%). In Pakistan limited information's are available from few areas such as Islamabad (66.66%) [12], Khushab (31.43%), Sargodha (37.14%), Attock (75.92%) and Rawalpindi (24.57 %) [13].

Livestock offers a lot of advantages to the poor farmers in form of milk, meat, wool and selling of leather. There are many constraints to the rearing of cattle under field conditions. Different diseases are the major constraint to the development and production of livestock. Among all diseases ectoparasites (ticks) are the major constraint causing a huge mortality and morbidity directly or indirectly in cattle [14]. Different infectious and non-infectious diseases are responsible for heavy economic losses in livestock sector. Among all protozoal disease, anaplasmosis is the most common disease causing a huge economic losses in domestic animals [15]. Cattle are highly susceptible to tick borne diseases and result in economic losses to farmers. There are only few studies have been conducted about anaplasmosis in Pakistan. Therefore the present study was designed to explore predisposing factors responsible for prevalence of anaplasmosis in local and cross breed friesian cows in southern area of Khyber Pakhtunkhwa. The major objectives of the study were to find out the prevalence and different possible risk factors associated with anaplasmosis and give useful suggestions to the department of livestock and dairy development to make effective policy for its control.

Materials and methods
Ethical approval
This study was conducted after proper approval of University Ethical Committee. All the blood samples were collected with the proper permission of owner and the necessary veterinary services were provided free of cost.

Study area:
The study area Lakki Marwat lies at 32° 36' to N. Latitude, 70° 34' to R. Longitude and 986 feet above Sea Level. This area is mainly composed sand dunes and the weather is dry and heat. Rainfall is very occasional and generally occurs in July and August. According to the livestock census the total population of cattle, buffaloes, sheep and goats are 98550, 3827, 48697 and 291711respectively.

Selection of animals
The animals were selected by simple random sampling technique from five union councils. The selection of animals were based on the basis of clinical signs such as anemia, weakness, jaundices, high temperature (104 – 105ºF), decrease milk production and finally results were confirmed by laboratory diagnosis. From each union council, 10 animals were selected per month (n= 10×5=50 animals/ month) for a period of six months .Overall three hundred (n=300) animals were examined for anaplasmosis. The present study was continued from January, 2018 to June 2018.

Epidemiological exploration
Questionnaire
The data was collected on pretested questionnaire containing (Dichotomous or multiple choice) questions such as name of the owner with address, breed (local or cross friesian), species (cow or buffalo), sex(male or female), presence or absence of ticks, age of the animal (young ≤5 years & adult≥5 years), month of sample collection, acaricide was used or not**,** hygienic measures(good , poor and very poor) and housing (Closed, semi closed and open).

Collection of blood samples
Blood samples (3-5 ml) were collected from 300 cattle by puncturing ear or jugular vein with the help of sterile disposable syringe and butterfly needle and placed in EDTA containing vacutainer and transported to Veterinary Research Institute Peshawar in ice bags for further analysis according to the technique as reported by [16] after proper labelling.

Giemsa staining
Thin blood smears were prepared immediately after blood collection. Blood smears were labeled, air-dried, fixed with methanol, stained with Giemsa stain and examined microscopically for presence of Anaplasma spp.

Microscopic examination
All the smears were examined at 100 X magnification with compound microscope by searching at least 50 fields per slide. The parasites were identified according to the procedure as described by [17].

Statistical analysis
The collected data were analyzed by conventional percentage and average analysis and chi square analysis to achieve the objectives of the study.

Results
In the present study, the overall prevalence of anaplasmosis was 19.66% (59/300×100) in clinically symptomatic local and cross breed friesian cattle under field conditions screened by microscopic examination in district Lakki Marwat, KPK Pakistan as presented in (Table 1).

In (Table 2), the age wise prevalence of anaplasmosis is presented where the chi-square analysis has exposed a highly significant (p<0.001) association between the age of the animal and prevalence of anaplasmosis in cattle. The prevalence rate was higher in young cattle at the age of (≤5 years) than cattle above five years of age. In

young animals either having age of 5 years or less than 5 years, the highest prevalence (24.85%) was recorded than adult (≥ 5 years) where lower prevalence (13.13%) was recorded. The young animals might have weak immune system while those above five years age have strong immune system and might be immune subsequent clinical infection.

Currently, the highest prevalence of anaplasmosis was recorded in female animals (22.07%) than male (11.59%) and statistically highly significant (p<0.002) association was recorded between sex of the animals and prevalence of bovine anaplasmosis after chi-square analysis as presented in (Table 3).

In (Table 4), the breed wise prevalence of anaplasmosis is presented where the chi-square analysis has been exposed a highly significant (p<0.0000) association between the indigenous pure breed and Cross Holstein Friesian cattle. The prevalence rate was higher in Cross Holstein Friesian cattle (28.10%) than Indigenous pure breed cattle (6.08%). This may be due to natural resistance in local breeds to ticks and suitable adaptation to harsh environment.

In the present study, the highest prevalence was recorded in the month of June (38%) followed by May (34%), March (10%), January (8%) while the lowest in the month of February (6%) and statistically significant (p<0.003)) association was recorded in different months of the year and prevalence of anaplasmosis as presented in (Table 5).

The highest prevalence was recorded in the summer season (36%) followed by spring (16%) while the lowest in the winter season (7%). Statistical analysis showed highly significant (p<0.0000) association between prevalence of anaplasmosis and seasons (Table 6).

In the present study, the application of insecticides was also studied where the prevalence was lower (10.86%) in those areas where pesticides/ acaricides were regularly used whereas highest prevalence (23.55%) was recorded in those area where acaricides were not used. Statistically highly significant (p<0.002) association was recorded between application/non-application of pesticides and prevalence of anaplasmosis (Table 7).

Table 1. Overall prevalence of anaplasmosis in cattle

Factors (overall prevalence)	Total number of cases examined	Total number of infected cases	Overall Prevalence (%)
Anaplasmosis in cattle	300	59	19.66

Table 2. Age wise prevalence of anaplasmosis in cattle

Factor(Age)	Total examined cases	Positive cases	Prevalence (%)	P. value
Young(≤5 years)	165	41	24.85	p<0.001
Adult(≥5 years)	135	18	13.13	

*Significant difference p<0.05

Table 3.Sex wise prevalence of anaplasmosis in cattle

Factor(sex)	Total examined cases	Positive cases	Prevalence (%)	P. value
Male	69	8	11.59	p<0.002
Female	231	51	22.07	

*Significant difference p<0.05

Table 4. Breed wise prevalence of anaplasmosis in cattle

Factor(Breed)	Total examined cases	Positive cases	Prevalence (%)	P. value
Indigenous pure breed	115	7	6.08	p<0.0000
Crossbred Holstein Friesian	185	52	28.10	

*Significant difference p<0.05

Table 5. Month wise prevalence of anaplasmosis in cattle

Factor(Months)	Total examined cases	Positive cases	Prevalence (%)	P. value
January	50	4	8	
February	50	3	6	
March	50	5	10	p<0.003
April	50	11	22	
May	50	17	34	
June	50	19	38	

*Significant difference p<0.05

Table 6. Season wise prevalence of anaplasmosis in cattle

Factor(Months)	Total examined cases	Positive cases	Prevalence (%)	P. value
Winter (Jan& Feb)	100	7	7	
Spring (March& April)	100	16	16	p<0.0000
Summer (May& June)	100	36	36	

*Significant difference p<0.05

Table 7. Association between application of insecticides/ non-application of insecticides and Prevalence of anaplasmosis

Factor(Insecticides)	Total examined cases	Positive cases	Prevalence (%)	P. value
Insecticides used	92	10	10.86	P<0.002
Insecticides not used	208	49	23.55	

*Significant difference p<0.05

Discussion
In the present study, the overall prevalence of anaplasmosis was 19.66% in cattle showing clinical signs under field conditions and similar results (18.50%) were also reported by [18]. Our results are higher than [19] who reported 5.95% prevalence of subclinical anaplasmosis. On the other hand [20, 21] reported higher prevalence (33%) and (70%) of bovine anaplasmosis respectively than our results. These variations may be due to geographical location, different breeds, hygienic conditions, availability of veterinary services and seasons of sample collection. In the present study, the highest prevalence was observed in young cattle at the age of (≤5 years) followed by adult cattle (≥5 years).Similar observations were also reported by [22, 21] who reported higher prevalence in adults than young calves. In young animals having age of 5 years or less than 5 years, the highest prevalence (24.85%) was recorded than adult (13.13%) and statistically highly significant (p<0.001) association between the age of the animal and prevalence of anaplasmosis was recorded. Our results are close with the findings of [18] who reported (22.72%) and (20%) prevalence in young calves at the age of 48 months and 1-24 months respectively.

However [21] reported higher bovine prevalence (53.33%) at the age of three years or above. However [23] reported little higher prevalence(36.59%) in cattle at the age of more than 5 years while 22.95% at the age less than 3 years of cattle. The young animals might have weak immune system while those above five years age have strong immune system and might be immune consequent clinical infection.

Presently the highest prevalence of anaplasmosis was recorded in female cattle (22.07%) than male (11.59%) and statistically highly significant (p<0.002) association was recorded between sex of the animals and prevalence of bovine anaplasmosis. Our findings are also in line with the observations reported by [23] who also reported the highest prevalence in the female cattle (29.71%) than the male (12.50%). Higher prevalence of anaplasmosis in female cattle has been also reported by other researchers [24, 13]. The harshness of anaplasmosis is influenced by sex of the host [25]. The reason for higher prevalence in female animals may be due to lower immunity caused by pregnancy stress, hormonal disturbances, draught power, keeping of female cattle for long period for the purpose of breeding and milking [26, 27]. The breed wise prevalence was higher in cross Holstein friesian cattle (28.10%) than Indigenous pure breed cattle (6.08%) and statistically highly significant (p<0.0000) association was observed between breeds and prevalence of bovine anaplasmosis. Our findings are similar to the statement of [19] who also recorded the highest prevalence of blood protozoan in crossbreed cattle than indigenous breeds. Our results are also close to the findings reported by [23] who also reported the highest prevalence in crossbred Holstein-Friesian cattle (32.38 percent) while lowest in local breeds (10.64 percent). Our study was also supported by [22, 21]) who claimed that different breeds are not equally susceptible to prevalence of anaplasmosis. However some researchers have been suggested that there is no susceptibility to anaplasmosis in different breeds of cattle [28]. This may be due to natural resistance in local breeds to ticks and suitable adaptation to harsh environment. Indigenous cattle are often exposure to constant infections of anaplasmosis and as a result there is development of protection that is responsible for lower occurrence of indigenous cattle [29] Our observations are very similar to [30] who also reported that local breeds are relatively highly resistive than pure friesian breeds. Some researchers stated that crossbred cattle are managed with great attention and less chances of pre exposure to vectors. As a result there is less or no development of immunity against to anaplasmosis [29]. In the present study, the highest prevalence was recorded in the month of June (38%) followed by May (34%), March (10%), January (8%) while the lowest in the month of February (6%) and statistically significant (p<0.003)) association was recorded. Our study has been also supported by [31] who observed prevalence of anaplasmosis throughout of the year but the highest incidences were recorded in hot months of the year(May, June, July, August, September, October) because hot and humid environmental conditions are more suitable for the development and reproduction of insects and ticks. Similar observations were also documented in other parts of Pakistan very earlier by [13]. Presently the highest prevalence was recorded in the summer season (36%) followed by spring (16%) while the lowest in the winter (7%).Statistical analysis showed highly significant (p<0.0000) association between prevalence of anaplasmosis and seasons. Our results are close with the findings reported by [23] where highest prevalence was recorded in the summer season (35.23%) while lowest in winter

season (6.06%). Other researchers also reported similar findings. This protozoal infection is highly prevalent in the summer months of the year. The reason behind high prevalence is that blood sucking flies and ticks are found in abundance in summer season while less in number in winter season which act as a carrier for anaplasma species. In the present study, lower prevalence (10.86%) was determined in those areas where pesticides/ acaricides were used regular whereas highest prevalence (23.55%) was recorded in those areas where acaricides were not used. Statistically highly significant ($p<0.0000$) association was recorded between application/non-application of pesticides and prevalence of bovine anaplasmosis. Similar findings were also reported by [32] who reported the highest prevalence (33.62%) in those cattle where acaricides were not used while lower prevalence (17.07%) was observed in those animals where acaricide were used and significant association was observed.

Conclusion

In the present study it has been concluded that few factors are responsible for an outbreak of anaplasmosis such as cross breed cattle, young age animals, female animals, summer season, hot months of the year and non uses of acaricides. Ticks and other flies are responsible for parasitic transmission. Bovines need more care toward females and young calves during rearing regarding to anaplasmosis. Therefore it is recommended that proper introduction of resistive breeds, intensive care of young animals at early age of life, more care of female animals, proper preventive measures in summer season and regular use of insecticides can reduce the prevalence of anaplasmosis.

Authors' contributions

Conceived and designed the experiments: NU Khan, MS Sarwar & S Ayaz, Performed the experiments: A Ali, Analyzed the data: M Ali & G Rashid, Contributed reagents/materials/analysis tools: M Hussain, MA Khan& AU Khan, Wrote the paper: H Ali.

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