Prevalence Statistics of Anaplasmosis in Cows of District Charsadda, KP, Pakistan

Shumaila[*], Wajeeha[†], Inayat Ullah[‡], Talha Khan[§], Nasir Shuaib[**], Muhammad Fawad[††], Sana Gohar[‡‡]

Abstract

The anaplasmosis-causing agent, Anaplasma spp., is mostly transmitted to susceptible hosts when infected ticks feed on blood. These diseases specifically harm animal herds, causing enormous financial losses. In various regions of Pakistan, the epidemiology of Anaplasma species in small ruminants is as follows: Using a commercially available blood smear microscope during the months of January through June 2023, the prevalence of anaplasma in sick cows from the three tehsils of Pakistan's Charsadda District is examined. A total of 60 blood samples from small farms are collected, and each location also received a typical report from farmers outlining the specifics of their livestock management practices. Microscopic analysis of blood smears revealed that anaplasmosis in cows is present in the district of Charsadda for 20% (12/60) of the time. The months of May and June (30%) showed the highest occurrence (%). High prevalence is seen for Tangi (10%) and Shabqadar (15%). According to the data, prevalence is higher in younger cows (23.8%) than in older ones (18%). Cholistani (6.66%) and Sahiwal (13.33%) are more susceptible to anaplasmosis than the other two breeds among the examined breeds. This study measures the seroprevalence of Anaplasma spp. infection in cows in the Charsadda, Anaplasma infection is a significant public health concern in Pakistan. A better understanding of disease-related factors is required in order to design creative management strategies for anaplasmosis in cattle.

Keywords: *Anaplasmosis, Prevalence, Blood Smear Screening, Cattles, Charsadda.*

[*]Department of Zoology, Bacha Khan University Charsadda, Charsadda 24420, Pakistan, shumailakha437@gmail.com

[†]Department of Zoology, Bacha Khan University Charsadda, Charsadda 24420, Pakistan, haroonwajeeha163@gmail.com

[‡]Corresponding Author: Department of Chemical & Life Sciences, Qurtuba University of Science & Technology, Peshawar, KP 25000, Pakistan, inayatullahafridi2018@gmail.com

[§]Department of Zoology, Quaid I Azam University Islamabad, Islamabad 45320, Pakistan, talhakhan@bs.qau.edu.pk

[**]Department of Chemical & Life Sciences, Qurtuba University of Science & Technology, Peshawar, KP 25000, Pakistan, nasirshuaib123@gmail.com

[††]Department of Zoology, Islamia College University Peshawar, Peshawar 25120, Pakistan, drfawad860@gmail.com

[‡‡]Department of Zoology, Bacha Khan University Charsadda, Charsadda 24420, Pakistan, sk0136613@gmail.com

Introduction

The obligatory intraerythrocytic rickettsiae of the genus Anaplasma (Rickettsiales: Anaplasmatacea), which cause anaplasmosis in domestic and wild ruminants, infect the host's red blood cells (Gebremikael, 2018). The clinical condition of anaplasmosis is widespread in the world's tropical and subtropical areas. Bovine anaplasmosis is typically caused by *A. marginale*, and this disease is not frequently associated with *A. centrale* (Ashraf et al., 2013). Twenty tick species have been shown to transmit anaplasmosis experimentally; the two most important tick genera are Hyalomma and Rhipicephalus species. Clinical manifestations of the disease can range from asymptomatic to fatal, contingent on the host's susceptibility, concurrent infections, and the severity of the Anaplasma species or strain (Karim et al., 2017). The most common agent infecting cattle is *Anaplasma marginale*, can be disseminated via the use of blood-contaminated materials as well as by tick or tabanid fly bites that belong to the genus Dermacentor. Therefore, the peak tick and fly seasons are when transmission is most active.

There may be severe disease outbreaks when native animals are introduced into an endemic region or carrier animals are introduced into a herd in a nonendemic location (Kocan et al., 2010). Such herds may lose up to 50% of their animals to death. Mature cattle are more likely to experience severe clinical signs of the illness, whereas calves under six months of age often exhibit no symptoms (Aubry & Geale, 2011).

It is known that three biting insects—horse flies, stable flies, and mosquitoes—transmit anaplasmosis mechanically by transferring RBCs infected with *A. marginale* from sick calves to susceptible cattle (Gill, 1994). The tools used to handle the cattle can spread anaplasmosis organisms from one animal to another. Four to six weeks after the cattle have been processed, a major portion of the herd will exhibit symptoms of anaplasmosis when the disease is spread by infected tools. Without any prior clinical cases being noticed, this type of outbreak arises out of nowhere (Krinsky, 1976). Decreasing milk production, pyrexia, depression, anorexia, jaundice, progressive anemia, and animal abortion and mortality are the prominent symptoms of disease, especially in exotic breeds (Jabbar et al., 2015).

Antibiotics are administered as needed to animals exhibiting symptoms of anaplasmosis (Aubry & Geale, 2011). In endemic areas, the sickness can be controlled by daily low-level antibiotic medicine administered in the feed during the vector season. Controlling ticks and flies, properly cleaning and disinfecting dehorning tools and supplies, labelling, and injectable supplies can all work together to slow the development of this illness. A few vaccines are available to prevent

anaplasmosis, but because of the risk of neonatal isoerythrolysis in calves born to cows who got the vaccine, these should be given in the latter stages of pregnancy (Wormser, 2006).

Literature Review

In Pakistan, a developing nation with few resources for animal health, vaccination of cattle against anaplasmosis is not a common practice. Because of this, it is unknown how common this parasite is among the cattle in the area. Additionally, relatively little research from all over the world has been referenced in the literature about the microscopic detection of anaplasma in cows.

Given the aforementioned information, the current study's objective is to use traditional (blood smear screening) methods to determine the prevalence of anaplasmosis in four breeds of cows from three Tehsils in the District of Charsadda.

A bacterial disease spread by ticks, bovine anaplasmosis causes significant financial losses for the livestock industry in several nations, we first go over the components of the bovine immune response that enable us to reduce clinical symptoms, eradicate most blood forms, and create an immunologic memory that prevents sickness in the event of subsequent encounters with the pathogen (Salinas et al., 2022).

Due to stress associated with pregnancy and disrupted hormone levels, aged cows have a higher parasite frequency and decreased immunity (Belal et al., 2015). The fact that older cows are more likely to come into contact with contaminated needles used to inject hormones for milk letdown is another factor contributing to their greater parasite prevalence (Vieira et al., 2019).

It is believed that *A. marginale* infections are spread mechanically by biting flies, biologically by ticks, and by fomites, which are infected needles and surgical devices. Regarding the significance of mechanical transmission via biting flies, there are differing views. Since the studies do not record the existence of tabanids, associations between seroprevalence of anaplasmosis and their presence do not imply a cause-and-effect link.

The first attempts to experimentally spread *A. marginale* by biting flies only worked when the "donor" animal have acute infection and blood smears revealed microscopically detectable levels of parasitemia (Villar et al., 2023).

The proposed study provides awareness to anaplasmosis prevalence in selected district Charsadda. Further studies in the future could apply molecular approaches for a more comprehensive evaluation of factors that determine susceptibility based on age and breed.

Materials and Methods

Study Area

The current study is conducted in the Charsadda Khyber Pakhtunkhwa District of Pakistan, which is located between 34°.03' and 34°.38' N latitude and 71°.28' and 71°53' E longitude. The district is comprised of three tehsils, Shabqadar, Tangi, and Charsadda, which are situated in Khyber Pakhtunkhwa's western area and have a combined area of 996 km^2. The district's Charsadda, Shabqadar, and Tangi tehsils are the study's primary target areas.

Sample and Data Collection

Apparently healthy cows (N =60) are enrolled from various dairy farms in the district during January–June 2023. Enrolled cows included Achai (N = 15), Sahiwal (N = 15), Cholistani (N = 15), and Red Sindhi (N = 15). Animals are examined after the livestock owners gave their informed agreement, and a blood sample (about 2 ml) is taken from the jugular vein and placed in EDTA-containing tubes. For the microscopic detection of infectious cells, blood samples are employed.

Epidemiological Parameter

At the sample site, with the help of livestock owners, sixty questionnaires are completed in order to gather data on each cow and compare it with the anaplasmosis prevalence. A questionnaire is used to gather data about the cows' breed, age, tick burden, environmental factors, herd or single management, and grazing arrangement.

Blood Smear Screening

Blood smears are produced, air dried, and fixed in ethanol at the sample site for each enrolled cow. Geimsa is used later to stain the slides (Takihi & Sandes, 2013). and checked for the presence of Anaplasma spp.

Statistical Analysis

SPSS, (IBM statistics 26) software is used to conduct a detailed statistical analysis of the data. Using SPSS allowed the application of various statistical methods and tests to examine patterns, relationships, and significance within dataset.

Results

During January to June 2023, a survey of anaplasmosis in cows is carried out in three Tehsils (Charsadda, Tangi and Shabqadar) of district

Charsadda. Total 60 blood samples are collected from cows and prevalence (%) of anaplasmosis is find out through microscopy technique.

Anaplasmosis in Cows of District Charsadda by Microscopy

Overall Prevalence (%): An overall prevalence (%) of anaplasmosis in cows by using microscopic examination of blood smears is 20% (12/60) in district Charsadda (Table:1).

Month-wise Prevalence (%): The months of May and June have the highest percentages of prevalence (%), while January and February have the lowest percentages. (Table:1, Figure: 1).

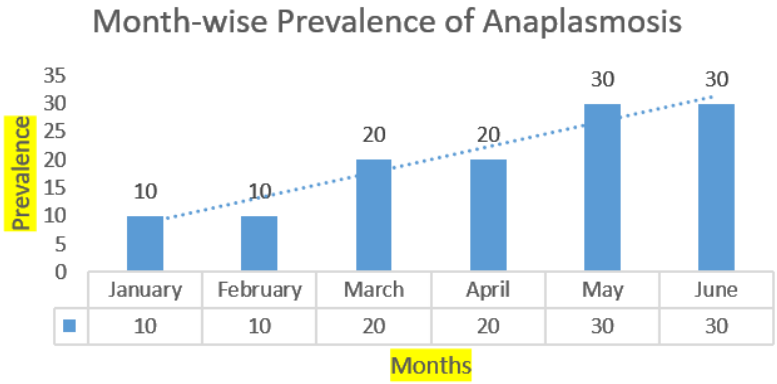

Figure 1: Month-wise Prevalence.

Tehsil-wise Prevalence (%): High prevalence is seen for Tangi (10%) and Shabqadar (15%). The least prevalent strain is Charsadda (5%) A statistical analysis shows no difference that is significant (P > 0.05) (Table 1, Figure 2).

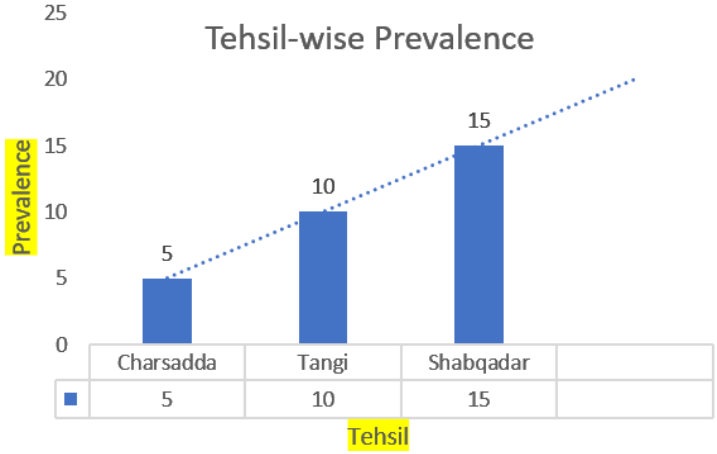

Figure 2: Tehsil-wise Prevalence.

Age-wise Prevalence (%): According to the data, prevalence is higher in younger cows (23.8%) than in older ones (18%). However, there is no statistically significant difference between the prevalence of the two age groups (P > 0.05) (Table 1, Figure 3).

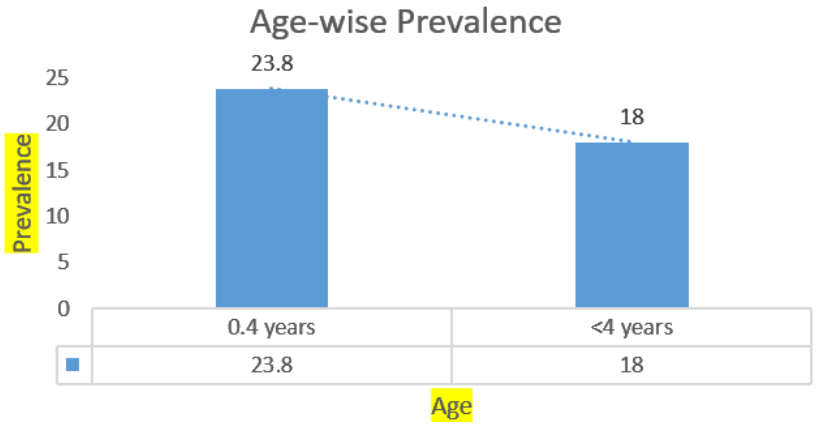

Figure 3: Age-wise Prevalence.

Breed-wise Prevalence (%): In comparison to the other two breeds, the Red Sindhi (6.66%) and Achai (13.33%), the Cholistani (6.66%) and Sahiwal (13.33%) are more susceptible to anaplasmosis. The statistical difference between the four breeds is not very large (Table 1, Figure 4).

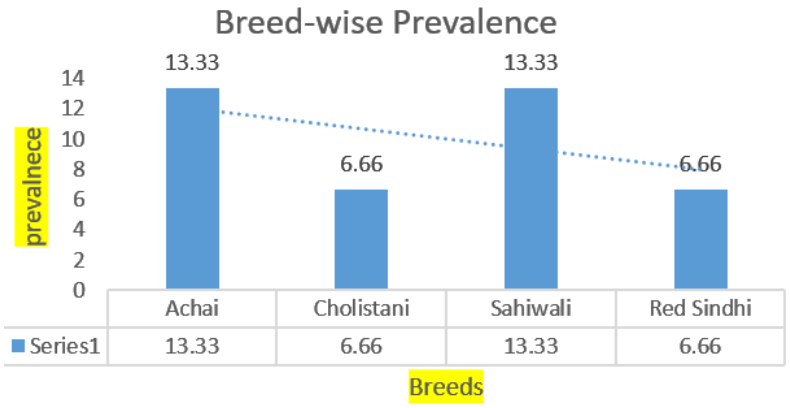

Figure 4: Breed-wise Prevalence

Table 1: Prevalence of Anaplasmosis in Cows of District Charsadda by using Microscopy technique during January 2023-June 2023.

	Factors	Total No. of observed samples	Total No. of infected samples	Prevalence (%)
Months	January	10	1	10
	February	10	1	10
	March	10	2	20
	April	10	2	20
	May	10	3	30
	June	10	3	30
Breed	Achai	15	2	13.33
	Cholistani	15	1	6.66
	Sahiwal	15	2	13.33
	Red Sindhi	15	1	6.66
Age	0-4 year	21	5	23.8
	< 4 years	39	7	18
Area	Charsadda	20	1	5
	Tangi	20	2	10
	Shabqadar	20	3	15
Total		60	12	20

Discussion

Discussion in the three tehsils of Pakistan's Charsadda district, the desire to reduce both direct infestation-related losses (such as weight loss, hide damage, and anemia-related deaths) as well as losses from tick-borne diseases drives the control of cattle ticks (Rhipicephalus, *Boophilus microplus*) (Jonsson, 2006). Tick-borne infections are more common in tropical nations because of the higher temperatures and climatic circumstances that are ideal for tick growth (Woldehiwet, 2010). *A. marginale* can be transmitted to a range of vertebrate hosts by Rhipicephalus species and Haemaphysalis species (Ashraf et al., 2020). given that we collected *A. marginale* ticks from the enrolled cows throughout the current investigation, our findings support the conclusion. We employed a traditional technique (blood smear screening) in the current investigation to find *A. marginale* in cow's blood. Since the parasite can be found if it infects 106 erythrocytes per mL during acute infections, traditional blood smear microscopy is regarded as a reliable approach. According to reports, animals that have recovered from acute anaplasmosis continue to carry the illness, which is visible as 102-107 infected erythrocytes. Although microscopic examination cannot reveal this level of rickettsiemia (Selçuk et al., 2015), a sensitive and accurate method for finding parasites carried by ticks in blood is blood smear screening.

Findings from the current study are similarly consistent with these well-established facts, since blood smear screening identified *A. marginale* in 20% more blood samples than it did in cases where Anaplasma spp. are detected using PCR (8.2%). 232 cattle from three different regions of Tunisia are included in the study. Real-time PCR and/or nested PCR have been used to find Anaplasma spp. in cattle blood samples from participants. They discovered that 3.9% of cattle have A. bovis infection, 25.4% have anaplasma spp. infection, 15.1% have *Anaplasma centrale* infection, and 34.9% have anaplasma spp. infection (Belkahia et al., 2015). The *A. centrale* prevalence reported is more similar to the 20% *A. marginale* prevalence found in the current study. 389 cow blood samples from six different provinces in Turkey are screened using the reverse line blot (RLB) hybridization technique. Anaplasma spp. infections are discovered in 9% of the cattle that are recruited. *A. marginale* (2.8%), *A. centrale* (1%), and *A. phagocytophilm* (1.0%) have the highest prevalence rates, respectively (Khumalo et al., 2016). Additionally, *A. centrale* incidence in cow samples collected from southern Italy is shown to be 13.8% (Ceci et al., 2014). Through blood smear testing on cattle from Cameroon's Vina division, it is discovered that 53.5% have an *A. centrale* prevalence (Mamoudou et al., 2017). The varying prevalence rates of *A. centralein* cattle reported from throughout the world are likely a result of the distinct geographical and environmental factors, tick control initiatives, farming management techniques, and husbandry practices in these regions (Magona et al., 2011). The Achai, Cholistani, Sahiwal, and Red Sindhi breeds are shown to be the most susceptible to *A. marginale* infection during our current analysis. There is no trend in Pakistan with regard to the immunization of cattle against *A. centrale*. This indicates that *A. marginale* is not the only parasite present in the area cattle; other parasites should also be found and treated. Due to the exotic cow breed's immunization against *A. marginale*, which gives them immunity against this parasite, the prevalence of *A. marginale* is reduced in the exotic *Holstein friesian* breed (Hove et al., 2018). Similar to how cross-breed cattle are created in Pakistan by mating exotic breeds with local cattle to give both breeds' characteristics, cross-breed cattle are likely found to be less susceptible to *A. marginale* infection than the Cholistani breed during the current study.

Findings of the proposed study are also consistent with research showing that Holstein cattle have much lower *A. marginale* infection rates than other local breeds (Zaka et al., 2016). Our results, however, are in direct opposition to those of (Rjeibi et al., 2018), who found that the Holstein Friesian breed have a much greater rate of Anaplasma spp. infection than any other breed under study. The findings of (Tay et al.,

2014), who claimed that crossbred cattle are more susceptible to anaplasmosis than native cattle, are similarly in conflict with our findings. When the age-specific prevalence data are analyzed, it became clear that younger cattle samples have greater *A. marginale* infection rates than older bovine samples. This is in line with (Rahman et al., 2015) findings, which indicated that older cattle have a higher prevalence of anaplasmosis. The incidence of Anaplasma spp. varies with the age of the host animal, according to research (Muraleedharan et al., 2005).

Conclusion

In our investigation, we observed a notable trend revealing that younger cows, specifically those under the age of 4, exhibit a higher likelihood of contracting an *A. marginale* infection. As age progresses, the infection rates display noteworthy variations, indicating a dynamic relationship between age and susceptibility. Additionally, our study shed light on breed-specific differences, demonstrating that distinct cow breeds carry varying risks of acquiring *A. marginale*.

The significance of our findings extends beyond the laboratory, as this study represents a significant advancement in our understanding of the prevalence of *A. marginale* in Pakistani cows. The acquired information serves as a valuable resource for strategic planning, offering insights crucial for developing effective measures to control the disease nationwide. By tailoring interventions based on age and breed considerations, we pave the way for a more targeted and impactful approach to safeguarding the health and well-being of Pakistani cattle. This research thus contributes not only to the scientific understanding of *A. marginale* but also holds practical implications for the improvement of livestock management practices in the country.

References

Ashraf, Q. U., Khan, A. U., Khattak, R. M., Ali, M., Shaikh, R. S., & Iqbal, F. (2013). A report on the high prevalence of Anaplasma sp. in buffaloes from two provinces in Pakistan. *Ticks and tick-borne diseases*, *4*(5), 395-398.

Ashraf, S., Parveen, A., Asif, M., Awais, M. M., Khan, A., Aktas, M., ... & Iqbal, F. (2020). A Report on the Tick Burden, Molecular Detection and Phylogenetic Analysis of Anaplasma Marginale in the Blood Samples of Cattle Collected from District Layyah in Punjab (Pakistan).

Aubry, P., & Geale, D. W. (2011). A review of bovine anaplasmosis. *Transboundary and emerging diseases*, *58*(1), 1-30.

Belal, S. S. H., Al Mahmud, M. A., & Ferdous, M. J. (2015). Prevalence of anaplasmosis in cattle in Sirajganj district of Bangladesh. *Research in Agriculture Livestock and Fisheries*, *1*(1), 97-103.

Belkahia, H., Said, M. B., Alberti, A., Abdi, K., Issaoui, Z., Hattab, D., & Messadi, L. (2015). First molecular survey and novel genetic variants' identification of Anaplasma marginale, A. centrale and A. bovis in cattle from Tunisia. *Infection, Genetics and Evolution*, *34*, 361-371.

Ceci, L., Iarussi, F., Greco, B., Lacinio, R., Fornelli, S., & Carelli, G. (2014). Retrospective study of hemoparasites in cattle in southern Italy by reverse line blot hybridization. *Journal of Veterinary Medical Science*, *76*(6), 869-875.

Gebremikael, H. G. (2018). *Exploring oriental theileriosis in bovines using advanced molecular tools* (Doctoral dissertation, The University of Melbourne, Australia).

Gill, R. J. (1994). Anaplasmosis in beef cattle. *Texas FARMER Collection*.

Hove, P., Khumalo, Z. T., Chaisi, M. E., Oosthuizen, M. C., Brayton, K. A., & Collins, N. E. (2018). Detection and Characterisation of Anaplasma marginale and A. centrale in South Africa. *Veterinary sciences*, *5*(1), 26.

Jabbar, A., Abbas, T., Sandhu, Z. U. D., Saddiqi, H. A., Qamar, M. F., & Gasser, R. B. (2015). Tick-borne diseases of bovines in Pakistan: major scope for future research and improved control. *Parasites & vectors*, *8*, 1-13.

Jonsson, N. N. (2006). The productivity effects of cattle tick (Boophilus microplus) infestation on cattle, with particular reference to Bos indicus cattle and their crosses. *Veterinary parasitology*, *137*(1-2), 1-10.

Karim, S., Budachetri, K., Mukherjee, N., Williams, J., Kausar, A., Hassan, M. J., ... & Iqbal, Z. (2017). A study of ticks and tick-borne livestock pathogens in Pakistan. *PLoS neglected tropical diseases*, *11*(6), e0005681.

Khumalo, Z. T., Catanese, H. N., Liesching, N., Hove, P., Collins, N. E., Chaisi, M. E., ... & Brayton, K. A. (2016). Characterization of Anaplasma marginale subsp. centrale strains by use of msp1aS genotyping reveals a wildlife reservoir. *Journal of Clinical Microbiology*, *54*(10), 2503-2512.

Kocan, K. M., de la Fuente, J., Blouin, E. F., Coetzee, J. F., & Ewing, S. A. (2010). The natural history of *Anaplasma marginale*. *Veterinary parasitology*, *167*(2-4), 95-107.

Krinsky, W. L. (1976). Animal disease agents transmitted by horse flies and deer flies (Diptera: Tabanidae). *Journal of medical Entomology*, *13*(3), 225-275.

Magona, J. W., Walubengo, J., Olaho-Mukani, W., Jonsson, N. N., Welburn, S. W., & Eisler, M. C. (2011). Spatial variation of tick abundance and seroconversion rates of indigenous cattle to Anaplasma marginale, Babesia bigemina and Theileria parva infections in Uganda. *Experimental and Applied Acarology*, *55*, 203-213.

Mamoudou, A., Nguetoum, N. C., Sevidzem, S. L., Manchang, T. K., Ebene, N. J., & Zoli, P. A. (2017). Bovine babesiosis and Anaplasmosis in Some Cattle Farms in the Vina Division, Adamaoua Plateau. *Int. J. livestock res*.

Muraleedharan, K., Ziauddin, K. S., Hussain, P. M., Pattabyatappa, B., Mallikarjun, G. B., & Seshaveri, S. J. (2005). Incidence of Anaplasma sp., Babesia sp. and Trypanosoma sp. in cattle of Karnataka. *Journal of Veterinary Parasitology*, *19*(2), 135-137.

Rahman, A. S. M. S., Sumon, S. M. M. R., Khan, M. A. H. N. A., & Islam, M. T. (2015). Current status of subclinical form of babesiosis and anaplasmosis in cattle at Rangpur district in Bangladesh. *Progressive Agriculture*, *26*(1), 51-59.

Rjeibi, M. R., Ayadi, O., Rekik, M., & Gharbi, M. (2018). Molecular survey and genetic characterization of Anaplasma centrale, A. marginale and A. bovis in cattle from Algeria. *Transboundary and emerging diseases*, *65*(2), 456-464.

Salinas-Estrella, E., Amaro-Estrada, I., Cobaxin-Cárdenas, M. E., Preciado de la Torre, J. F., & Rodríguez, S. D. (2022). Bovine Anaplasmosis: Will there ever be an almighty effective vaccine? *Frontiers in Veterinary Science*, *9*, 946545.

Selçuk, Ö., Alver, O., Çatık, S., Aydın, L., & Şenlİk, B. (2015). Determination of diagnostic value of cELISA for the diagnosis of anaplasmosis in clinically suspected ruminants.

Takihi, I. Y., & Sandes, A. F. (2013). Killers on the road: Klebsiella and Pseudomonas bacteremia detected on peripheral blood smear. *Blood, The Journal of the American Society of Hematology*, *122*(11), 1851-1851.

Tay, S. T., Koh, F. X., Kho, K. L., & Ong, B. L. (2014). Molecular survey and sequence analysis of Anaplasma spp. in cattle and ticks in a Malaysian farm.

Vieira, L. L., Canever, M. F., Cardozo, L. L., Cardoso, C. P., Herkenhoff, M. E., Neto, A. T., ... & Miletti, L. C. (2019). Prevalence of Anaplasma marginale, Babesia bovis, and Babesia bigemina in

cattle in the Campos de Lages region, Santa Catarina state, Brazil, estimated by multiplex-PCR. *Parasite epidemiology and control*, *6*, e00114.

Villar, D., Beltran, D. G., Schwartz, K., Magstadt, D., & Brewer, M. (2023). Diagnosis of Anaplasma marginale in cattle at the Iowa State University veterinary diagnostic laboratory 2003–2021. *Veterinary Parasitology: Regional Studies and Reports*, *39*, 100845.

Woldehiwet, Z. (2010). The natural history of Anaplasma phagocytophilum. *Veterinary parasitology*, *167*(2-4), 108-122.

Wormser, G. P., Dattwyler, R. J., Shapiro, E. D., Halperin, J. J., Steere, A. C., Klempner, M. S., ... & Nadelman, R. B. (2006). The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. *Clinical Infectious Diseases*, *43*(9), 1089-1134.

Zaka Saeed, Z. S., Furhan Iqbal, F. I., Mureed Hussain, M. H., Shaikh, R. S., Umer Farooq, U. F., Atif Akbar, A. A., ... & Aktas, M. (2016). Molecular prevalence and haematology of tropical theileriosis in Cholistani cattle from nomadic herds of the Cholistan desert, Pakistan.