PREVALENCE OF ANAPLASMOSIS IN DIFFERENT ANIMAL SPECIES IN SAVAR REGION OF DHAKA CITY, BANGLADESH

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PREVALENCE OF ANAPLASMOSIS IN DIFFERENT ANIMAL SPECIES IN SAVAR REGION OF DHAKA CITY, BANGLADESH

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This is to certify that the thesis entitled ***"Prevalence Of Anaplasmosis In Different Animal Species In Savar Region Of Dhaka City, Bangladesh"*** *has been submitted to the Department of Pathology, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University (SAU), Dhaka in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE (MS. in PATHOLOYG,*** *embodies the results of a piece of bona fide research work carried out by* ***DODUL MAHAMUD,*** *Registration No.* ***21010013*** *under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma in any other institution.*

I further certify that any help or sources of information received during the course of this investigation have duly been acknowledged.

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DEDICATION

This thesis is dedicated to my beloved parents, wife& sons, respected teachers who offered meunconditional sacrifices, blessings, love and supports throughout my study periods

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ABSTRACT

Currently, the livestock and dairy industries are facing a number of difficulties in the form of different diseases, which ultimately have an impact on the national economy. Among them, anaplasmosis is one of the most important parasitic diseases. An important step in managing parasite disease to enhance both the health of animals and the monetary well-being of the farmers is figuring out the prevalence and control strategies of anaplasmosis in different host species. A total of 232 animal species including 102 cattle, 18 buffalo, 62 horses and 30 dogs, were studied to determine the prevalence of anaplasmosis in the Savar region of Dhaka district. The clinical findings of affected animals were noted and blood samples were collected to make the diagnosis by using Giemsa staining technique and hematology analysis. In the results, hematology revealed leukocytosis, anemia and remarkable thrombocytopenia. The overall prevalence of anaplasmosis was highest (88.9%) in buffalos, followed by dogs (60.0%), cattle (37.3%) and horses (8.1%). Breed variation significantly affected the infection rate in both cattle and horses ($p < 0.05$), although gender variation only significantly affected the infection rate in cattle ($p < 0.05$). Nearly, all species of dead animals were found to have splenomegaly with hemorrhagic lungs and spleens during necropsy. The results demonstrated in this study indicated that the presence of anaplasmosis in different host species at Savar area in Bangladesh. The farmer's awareness about anaplasmosis and tick management practices will help to implement successful control and prevention strategies locally and nationally as well.

Keywords: Anaplasmosis, cattle, buffalos, horses, dogs, Savar, Dhaka

CHAPTER I
INTRODUCTION

Animals are susceptible to a variety of infectious diseases brought on by the infection of different organisms as well as non-infectious illnesses brought on by nutrient shortages, chemical toxins, and other unidentified causes. The greatest threat to the health of animals is infectious disease. They are brought on by fungi, rickettsia, parasites, viruses, and bacteria. There are numerous ways that animal diseases can spread, including direct contact, aerosol inhalation, ingestion, indirect transfer via insects, and vector transmission.

However, the livestock and dairy industries are dealing with a number of difficulties in the form of different diseases, which ultimately have an impact on the economies of all nations (Carroll, 2008). Animal parasite illnesses over the world result in production losses and a number of serious problems (Mehmood et al., 2017; Zaman et al., 2017).

Except for the winter, Bangladesh is typically hot and humid, which is the ideal for a wide range of parasites and ticks. Ticks are known to pose a serious threat due to their ability to cause severe itchiness, allergy and toxicosis. The livestock industry suffers significant losses due to tick-borne diseases all over the world (Ananda et al., 2009) as these diseases have a significant financial impact due to the obvious cause of death, decreased productivity, decreased working efficiency (Uilenberg, 1995), increased cost of control measures (Makala et al., 2003) and limited introduction of genetically improved cattle in an area.

The obligate intra erythrocytic ricketsial microbe *Anaplasma marginale* (*A. marginale*), of the order Rickettsiales, family *anaplasmataceae*, is the cause of *Anaplasmosis*, the tick-borne disease (Sharma et al., 2013). Besides, *A. phagocytophilum* is the causative agent of diseases such as canine, feline and equine granulocytic anaplasmosis and of tick-borne fever in ruminants (Kohn et al., 2008). Anaplasmosis also known as gall sickness or yellow bag fever, is a hemo-protozaon disease of animal (Kocan et al., 2010).

One of the most significant pathogens is *Anaplasma*, which is transmitted by at least 20 ticks species of different species, but Rhipicephalus (Boophilus) microplus is the main vector of transmission (Aubry & Geale, 2011). Additionally, it can be spread mechanically by fomites that have been exposed to blood or by flies that bite (Sharma et al., 2013). Due to the absence of a conventional cell wall and inability to synthesis lipopolysaccharide and peptidoglycan, this rickettsial organism is more closely related to protozoa (Brayton et al., 2005).

Animals may exhibit signs such as fever (4–10 days), anemia, anorexia, significant weight loss, cough, lethargy, abortion, decreased milk supply, and elevated pulse and respiration rate (Coetzee & Apley, 2006). Clinical anaplasmosis accounts for 80% of fatalities in enzootic environments (Shaukat et al., 2019). According to an acceptable estimate, the US alone experiences an annual loss from anaplasmosis of $100 million, which includes 50000–100 000 cow deaths (Sharma et al., 2013).

The erythrocytic *Anaplasma* can infect cattle, sheep, goats, buffalo, and certain other wild ruminants, but it has been discovered that cattle are more vulnerable to infection than buffaloes (Rajput et al., 2005). Equine granulocytic anaplasmosis (EGA) in horses, which is brought on by *A. phagocytophilum*, is characterized by thrombocytopenia (Seo et al., 2018). Dog can be infected by the causative agent of canine infectious cyclic thrombocytopenia, which is *Anaplasma platys* (formerly *Ehrlichia platys*), an essential intracellular bacterium of platelets (Huang et al., 2005).

Clinical sign observation, blood smear and staining, hematological testing, and additional laboratory tests for confirmation are used to identify and diagnose anaplasmosis. In case of dead animals, observation of post mortem signs is also included. The primary techniques for diagnosing anaplasmosis include microscopic examination with Giemsa stain, thin blood smear (GSTBS) and serological testing. While conventional parasitological methods, such as microscopic examination of GSTBS, are usually the gold standard test for the diagnosis of anaplasmosis in clinically suspected animals, GSTBS is not applicable to subclinical (act as reservoirs) or chronic infections (Sharma et al., 2013). Hematological parameters in complete blood count shows significant decrease in hematocrit, hemoglobin level (Ashraf et al., 2021) and also leukopenia, thrombocytopenia in parasite positive animals.

The use of medications is the most efficient way to treat anaplasmosis without endangering the host. Many medications including certain dyes, arsenic compounds, antimalarials, and antimony derivatives, were used to treat acute anaplasmosis before the development of the antibiotic (tetracycline). However, these substances have limited therapeutic benefits and cannot reduce anaplasmosis-related mortality (Collett et al., 1994). On the other hand, oxytetracycline, enrofloxacin, and imidocarb are the medications that have been shown to be most effective in prior studies for the treatment of carrier or clinical states of anaplasmosis (Atif, 2016).

From the conversation about above, it is notable that, it is crucial to keep animal *Anaplasma* free to avoid health and economical losses related to the disease. For this goal, proper studies are needed to be done related to the epidemiology, prevalence, clinical sign diagnostic tests and treatment of anaplasmosis.

To the best of our knowledge, no epidemiological research on anaplasmosis had been undertaken in the Savar region. The epidemiology and associated risk factors and chemo Sterilization of anaplasmosis in naturally infected carrier host like cattle, buffalo, dog, and horse in Savar region, Dhaka, have not also yet been thoroughly studied. Hence, the current study was designed to determine the prevalence of anaplasmosis in cattle, buffalo, horses and dogs in the Savar region of Dhaka district.

Objectives:

1.1 Considering the above importance, the current research was carried out with the following objectives:

1. Assessment of the prevalence of anaplasmosis in different host animal species in the Savar area of Dhaka district.
2. Analysis of the associated risk factors with anaplasmosis in the different host species in the study region.

CHAPTER II
REVIEW OF LITERATURE

2.1 Background study of anaplasmosis:

Anaplasmosis is a notorious blood protozoal disease that causes huge production loss and decreases performance of animal that ultimately causes huge monetary loss. It is a mild to severe hemolytic disease that results in considerable economic reduction of the farmers. Prevalence of anaplasmosis is high in the areas wherein the availability of ticks is more and the environment is more suitable to survivability of ticks such as tropical and sub-tropical countries. As Bangladesh is a tropical country, anaplasmosis are more prevalent in different areas of Bangladesh due to the climate factors. Anaplasmosis not only affect farm animals but also can infect drought animals and pets like dog, cat, horse, donkey etc. A complete set of history, clinical sign, physical examination, laboratory diagnosis can help the accurate diagnosis of anaplasmosis in animals which is a must to determine the actual prevalence of the disease.

2.1.1 Aetiology:

Comprising Gram-negative obligate intracellular alphaproteobacteria, the genus *Anaplasma* (family *Anaplasmataceae,* order *Rickettsiales*) is transmitted to vertebrate hosts by Ixodidae ticks. The members of this genus are microscopic pleomorphic bacteria found in the cytoplasm of eukaryotic cells in membrane-bound vacuoles called morulae. Diverse blood cells such as erythrocytes, granulocytes, monocytes and platelets can be infected by various species in vertebrate hosts (Atif, 2016; Dumler et al., 2001). Discovered by Sir Arnold Theiler in 1910, the genus *Anaplasma* is comprised of six species and is part of the family A*naplasmataceae* in the order *Rickettsiales*: *Anaplasma marginale, Anaplasma centrale, Anaplasmaovis, Anaplasma phagocytophilum, Anaplasma bovis and Anaplasma platys*. Since 2001, when Dumler and associates substantially rearranged the order *Rickettsiales* based on phylogenetic analysis of the groESL gene and 16S ribosomal RNA (16S rRNA), this taxonomy has been in use (Dumler et al., 2001; Theiler, 1910). The family *Anaplasma taceae* replaced the previous family *Ehrlichia ceae*as a result of this reorganisation, and bacteria that had previously been assigned to the *Ehrlichia* genus were added to the genus *Anaplasma*, which had only included *A. marginale, A. centrale,* and *A. ovis. Ehrlichia phagocytophila, Ehrlichia equi,* and the agent of human granulocytic ehrlichiosis were combined as the species *A. phagocytophilum* because

of their genetic closeness, whilst *Ehrlichiabovis* and *Ehrlichiaplatys* were reclassified as *A. bovis* and *A. platys*, respectively. *Aegyptinaella pullorum* is closely linked to *Anaplasma spp.*, according to later research based on the same gene analysis; nevertheless, it has not yet been formally reallocated as a member of the Anaplasma genus (Kocan et al., 2010; Lin & Rikihisa, 2003). The current classification of Anaplasma species can be considered, as shown below adapted from (Brayton et al., 2005), with seven formally recognized species and two others that have not yet been formally described.

Super kingdom Bacteria

Phylum Proteobacteria

Class Alpha-proteobacteria

Order Rickettsiales

Family *Anaplasmataceae*

Genus *Anaplasma*

Species *Anaplasma marginale*

Anaplasma bovis

Anaplasma caudatum

Anaplasma centrale

Anaplasma ovis

Anaplasma phagocytophilum

Anaplasma platys

2.1.2 Transmission

Three methods are available for the transmission of *Anaplasma* spp.: (i) Biological: infected erythrocytes are consumed by ticks, which then spread the infection to uninfected ruminants through saliva from the tick. (ii) Mechanical: infected erythrocytes are spread from infected to susceptible cattle by biting flies or blood-contaminated objects, such as needles or surgical instruments, without amplifying the infection. (iii) Transplacental: infected erythrocytes travel from infected cows to their progeny through the placenta in the uterus, also without amplifying the infection (Aubry & Geale, 2011). Worldwide transmission of *A. marginale* has been linked to at least 20 distinct tick species (Kocan et al., 2004). *Boophilus* species, certain *Dermacentor*

species, *Ixodesricinus* and *Rhipicephalus* species are generally the tick vectors of *A. marginale*, although *Ambly-omma* species do not seem to transmit *A.marginale (Kocan et al., 2004)*. Only *Rhipicephalus simus* has demonstrated effective trans-stadial transmission of the *A. centrale* vaccine strain (Potgieter & Van Rensburg, 1987). Since *R. simus* does not infect cattle in its juvenile stages, it's role in the natural transmission of *A. centrale* is dubious. According to a previous study of *A. marginale* and *A. centrale's* transmissible capacities, susceptible splenectomized calves can contract *A. marginale* but not *A. centrale* from infected *R. Sanguineus* and *Hyalomma* excavatum (Shkap et al., 2009). Therefore, even though it is highly likely that *A. centrale* strains spread among wild animals through a natural tick-borne cycle, the exact function of ticks remains unknown. TheIxodes genus of ticks, specifically *I. ricinus* in Europe, *I. scapularis* in the Eastern United States, *I. pacificus* and *I. spinipalpis* in the Western United States and *I. persulcatus* in Asia and Russia, are recognized arthropod vectors of *A. phagocytophilum (Burkot et al., 2001; Des Vignes et al., 1999; MacLeod & Gordon, 1933; Stuen et al., 2013; Telford 3rd et al., 1996; Zeidner et al., 2000)*. Numerous other tick genera and species have also been shown to have *A. phagocytophilum* DNA, although these species' ability to act as vectors has not yet been established (Stuen et al., 2013). A few additional *Ixodes* species, such *I. hexagonus* (Silaghi et al., 2012) and *I. trianguliceps* (Bown et al., 2003), seem to be involved in epidemiological cycles that are different from those involving *I. ricinus*. Because *A. platys* DNA has been detected in *R. sanguineus* ticks all over the world and because dogs affected with *A. platys* commonly also have *E. canis* infection, it is thought that *R. sanguineus* ticks are the vector of *A. platys*. Nevertheless, the experimental transmission of *A. platys* via *R. sanguineus* ticks was unsuccessful in a single try. Other tick species, including *Dermacentor auratus* ticks from Thailand, *Rhipicephalus turanicus* ticks from Israel, *Haemaphysalis* spp., and *Ixodes nipponensis* ticks from Korea, have been discovered to carry *A. platys* DNA (Sykes & Foley, 2014). Previously, experiments have demonstrated that at least 12 species of biting flies, including stable flies (*Stomoxys cacilitrans*), eight tabanid species (*Tabanidae*, also known as horse or deer flies) and three species of midges (*Culicidae*), possess the ability to mechanically transmit *A. marginale (Ewing, 1981; Hawkins et al., 1982; Potgieter et al., 1981)*. Within herds, but not between herds, iatrogenic dissemination via blood-contaminated fomites from infected to non-infected animals can be a significant mode of *A. marginale* transmission. Needles, dehorning tools, nose tongs, tattooing equipment, ear-tagging devices, and castration or spaying tools are examples of blood-contaminated objects that act as mechanical transmitters (Kocan et al., 2000). One of two cows exposed in the second or third trimester but not in the first has demonstrated

experimentally the vertical transmission of a Virginia strain of *A. marginale* (Zaugg, 1985). Additionally, 32 out of 37 calves delivered to cows who had clinical anaplasmosis within the last two months of gestation diagnosis validated by a laboratory-have been documented to have transplacental transmission. After receiving 22 mg/kg of doxycycline for five days, all of the cows were recovered (Salabarria & Pino, 1988).

2.1.3 Life cycle in vector and host:

As ticks feed on carrier hosts, they pick up rickettsia. The organism enters ticks through blood feeding, whereupon it infects the tick's intestinal cells and completes a portion of its life cycle. After infecting midgut cells in the tick, the rickettsia undergoes it's initial cycle of reproduction before dense forms spread to other tissues. Dense forms reach the salivary glands after many replication rounds, where they spread to a fresh mammalian host (Rodríguez et al., 2009). Through it's saliva, a tick that feeds on cattle spreads the bacterium. Ticks can carry the organism to numerous animals in their herd as well as herds adjacent through their sporadic feeding and chronic infections (Reinbold et al., 2010; Scoles et al., 2008). Depending on the infectious dosage, an incubation period of 7–60 days occurs after cattle are exposed to *A. marginale*. *A. marginale* is a microorganism that only lives inside erythrocytes. When an erythrocyte becomes infected, it produces an initial body, a membrane-bound inclusion that holds four to eight rickettsias per (Kocan, 2003). The rickettsia reproduces by binary fission within the erythrocyte to produce 8–12 initial bodies, and it leaves the erythrocyte without destroying the host cell. The original bodies invade new erythrocytes in an eternal cycle after they are released from the host cell (Abdisa, 2019). After completing a cycle of replication in the erythrocytes, *A. marginale* is phagocytized by the reticuloendothelial system, which allows it to infect more erythrocytes.Upto 109 erythrocytes per millilitre of blood, or 70% of all erythrocytes, can become infected during this acute phase (Kocan & Blouin, 2008). Erythrocyte phagocytosis causes icterus without hemoglobinemia or hemoglobinuria, as well as moderate to severe hemolytic anaemia (Aubry & Geale, 2011; Kocan et al., 2010).

2.1.4 Pathogenesis

While some members of the *Rickettsiales* order escape from the phagosome after entering the host cells and multiply in the cytoplasm before being released in the extracellular environment, bacteria belonging to the *Anaplasmataceae* family grow inside a cytoplasmic vacuole in the cytoplasm of the host cells (Pruneau et al., 2014). *Anaplasmataceae* are classified into several developmental phases based on the reorganization of their DNA from dense cored cells

(infectious form) to reticulate cells (vegetative form). Before being discharged, the reticulated forms undergo binary fission to proliferate and produce morulae, which then transform into densely cored cells. The densely cored *Anaplasmataceae* cells grow into reproducing reticulate cells within the phagosome, these cells do not merge with the lysosome to create morulae, which are the vegetative form. After that, reticulate cells develop into densely cored cells that are released either by host cell lysis (infectious form) or exocytosis (Pruneau et al., 2014).

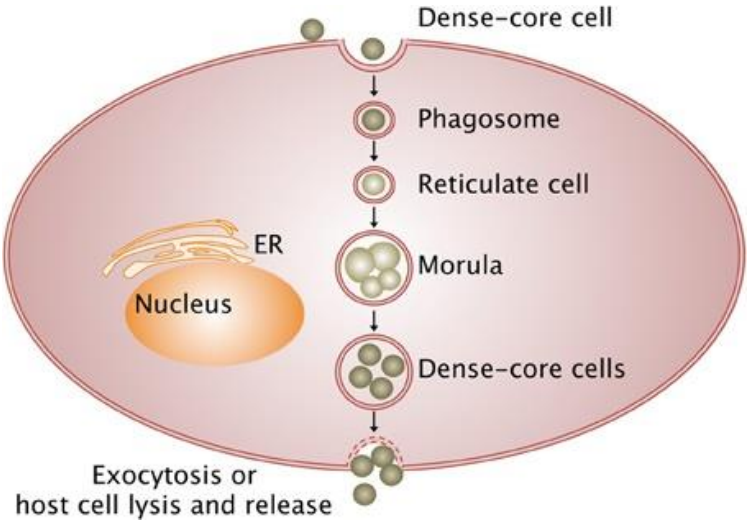


Figure-1: Intracellular life cycle of *Anaplasmataceae (Pruneau et al., 2014)*

2.2 Occurrence of anaplasmosis in different species of host animals:

2.2.1 Cattle

The agriculture sector of Bangladesh, benefits greatly from the livestock industry. Bangladesh's tropical climate is ideal for the establishment and development of tick species, which are key vectors for a number of infections that cause a wide range of diseases in cattle. A serious hemoparasitic illness of farm animals, bovine anaplasmosis spread by tick, causes substantial monetary losses. All cattle are susceptible to anaplasmosis however, it only manifests clinically in young animals. Dairy animals, particularly bovines, are a potential suitable host for haemoprotozoan parasite infections due to production stress and other disorders (Sharma et al., 2013). *A. marginale* is known to produce hemolytic anaemia in host cattle, invading more than 70% of erythrocytes under acute illness circumstances (Kocan et al., 2010). Bovine anaplasmosis is found in tropical and subtropical regions all over the world, and

it is a major constraint to cow production in many nations. In contrast to *A. phagocytophilum, A. marginale* is very host selective, infecting exclusively ruminants and causing disease primarily in cattle (Kocan et al., 2010). Cattle are more susceptible to anaplasma infection than buffaloes (Rajput et al., 2005). The principal clinical signs in *A.marginale*-infected cattle of over two-year-old include progressive anemia, fever, jaundice, loss of appetite, decreased milk production, abortion, and death (Kocan et al., 2010). The emergence of anaplasmosis in a herd can cause a 30% increasein the cull rate, 20–30% loss in body weight and death or abortions in clinically infected animals (Railey & Marsh, 2021).

2.2.2 Buffalo

Buffalo is a multipurpose ruminant that helps livestock agriculture by providing farm operations, income insurance, capital formation, and food production (Nguyen et al., 2020). Since buffalo are typically reared alongside beef cattle in rural areas, they may be exposed to the same vectors and environmental factors that cause transmissible bovine diseases. Nonetheless, when compared to cattle, buffalo rarely exhibit clinical symptoms, owing to breed resilience (Rajput et al., 2005). As a result, their ability to serve as asymptomatic reservoir hosts for tick-borne illnesses has likely been overestimated (Nguyen et al., 2020). Water buffalo have milder symptoms than cattle, including increasing weakness, anorexia, high fever, tachycardia, labored respiration, and a pale mucus membrane (Vatsya et al., 2013).

2.2.3 Horse

People primarily raise equines for draught and game reasons, and they are an important part of the farming and transportation systems in both rural and urban regions (Gebreab et al., 2004). Frequent outdoor activity of equines make them prone to tick bites and therefore, result in infection with TBPs (Butler et al., 2005) *Anaplasma phagocytophilum*, the pathogen that causes horse anaplasmosis, is an obligate intracellular gramme negative bacterium that was previously known as *Ehrlichiaequi* (Bermann et al., 2002). It is a tick-borne pathogen that primarily infects host endothelium and blood cells, mostly granulocytes and notably neutrophils, and is also zoonotic (McQuiston et al., 2003). These bacteria infect horses whenever *Ixodes spp*. the primary tick vector is found (Butler et al., 2005). While the majority of these infections are asymptomatic, it is estimated that 5-10% of infected animals will acquire clinical signs, which may vary greatly (Gribble, 1969).Equine granulocytic anaplasmosis is an acute febrile disease with a 1-2 week incubation period, after which infected horses may exhibit clinical indications such as pyrexia, depression, anorexia, unwillingness to move, limb edema, loss in body weight, icterus, petechiation, ataxia, and thrombocytopenia (Razzaq et al.,

2015).Duration of illness and age of the horse is very important for the severity of clinical signs (Thibaud et al., 2016). In younger horses, nonspecific symptoms like inappetence and aversion to movement is seen *(Madigan & Gribble, 1987). A.phagocytophilum* also infects equine neutrophils and eosinophils (Bjöersdorff et al., 2001). Mild to severe morbidity and even death have been reported in some cases of the disease (Franzén et al., 2005).

2.2.4 Dog

Dog can also be affected by anaplasmosis with *A.phagocytophilum* and *Anaplasmaplatys* through the vector like *Ixodes spp.* tick. *A. phagocytophilum* is an *Anaplasmataceae* Gram-negative obligate intracellular bacterium. It is present in cytoplasmic vacuoles, where it forms discrete inclusion bodies known as morulae (Kohn et al., 2008). *A. phagocytophilum* mostly affects neutrophilic and, in rare cases, eosinophilic granulocytes (Dumler et al., 2001). Seroconversion in dogs can occur as quickly as 2-5 days after the emergence of morulae in peripheral blood, according to experimental research (Egenvall et al., 1998). Rupture of the host cell's phagosome and cell membrane results in the release of *A. phagocytophilum,* allowing infection of adjacent cells and as a result, infection of many organs (Day, 2016). The bacterium can activate pathogenic processes that prevent it from being destroyed by immune system cells (Dumler et al., 2005). Infected phagocyte dysfunction disrupts immune defense processes, perhaps facilitating secondary infections (Day, 2016). *Anaplasmaplatys* (previously *Ehrlichia platys*) is an obligate intracellular bacterium of platelets that causes infectious cyclic thrombocytopenia in dogs. Although cyclic thrombocytopenia characterizes the acute phase of infection, infected dogs are not critically unwell and rarely show major hemorrhage (Harvey et al., 1978). In the acute phase of infection, lymph node follicular hyperplasia and plasmacytosis have been reported and some organs such as the spleen may develop hemorrhage (Baker et al., 1987). Worldwide, clinical cases of canine infectious cyclic thrombocytopenia have been reported (Huang et al., 2005). Risk factors for *A. phagocytophilum* infection in dogs include season of the year, co-infection with other tick-borne pathogens, and signalment (Poitout et al., 2005).

2.3 Diagnosis strategy of anaplasmosis:

A preliminary diagnosis of bovine anaplasmosis can be made based on geographic location, season, signalment, and the presence of clinical signs and/or necropsy findings in affected animals. Clinical outbreaks are most common during the warm, wet seasons, when vector-borne transmission is more common. Following the introduction of a carrier animals from an endemic location, naive cattle in non-endemic areas may get infected with anaplasmosis (Smith

et al., 1989). Similarly, iatrogenic *A. marginale* infection caused by contaminated surgical equipment or hypodermic needles can result in clinical instances that occur outside of the regular vector season (Kocan et al., 2010).During the acute phase of the disease, clinical symptoms, hematological abnormalities, and microscopic analysis of blood smears stained with Giemsa stains are often used to make the diagnosis of anaplasmosis (De Waal, 2000). Giemsa stains is still the best stain to employ, especially when the parasitemia is extremely low. Other fast staining techniques, such as Diff-Quick, are now available (Hart et al., 1992). Since the majority of the infected erythrocytes have been removed from circulation, blood smears from animals exhibiting prominent signs of anaplasmosis may already have very low parasitemia. Additionally, Anaplasma inclusion bodies must be distinguished from other stained artefacts in erythrocytes, basophilic stippling in reticulocytes, and Howell Jolly bodies (De Waal, 2000). It is well known that an animal's Anaplasma infection can last long beyond the disease's acute phase. The animals, which are carrier and asymptomatic stay with the herd and might spread the illness. Numerous serological and biotechnological methods have been developed to detect carrier animals, however these carriers are hard to find (De Waal, 2000).Serological tests, including the complement fixation (CF) test, capillary agglutination assay, card agglutination test (CAT), indirect fluorescence antibody (IFA) test, and several enzyme-linked immune sorbent assays (ELISA), including a cELISA, an indirect ELISA, and a dot ELISA have been widely used for epidemiological studies. The CAT and cELISA are the two serological tests that are now recommended for detecting animals that are infected (OIE, 2008). Polymerase chain reaction (PCR) techniques based on nucleic acids have also been developed to identify low-level infection in tick vectors and carrier cattle (Aubry & Geale, 2011).

2.4 Clinical signs related to anaplasmosis:

In case of cattle, clinical anaplasmosis is more common in cattle older than one year old in terms of signalment (Kocan et al., 2010). According to Jones and Brock (1966), carrier cows in advanced pregnancy and/or lactation may relapse and display indications of acute infection. Such events in cows may be related to immune suppression during the per parturient phase (Kehrli et al., 1989). Per acute anaplasmosis is most commonly seen in purebred animals and high-producing dairy cows, and is characterized by a high mortality rate within a few hours of clinical indications arising (Kocan et al., 2010). According to a previous study, cattle fed a higher energy plane had more severe anaplasmosis than animals fed a lower energy plane (Ajayi et al., 1978). There are also conflicting reports about the vulnerability of *Bosindicus* and

Bostaurus cattle to *A. marginale* infection (Otim et al., 1980). The previous studyJones and Brock (1966) provide a comprehensive assessment of the clinical symptoms of anaplasmosis. This paragraph briefly discusses the key issues of the clinical diagnosis of anaplasmosis. Pyrexia is the most common early indication of anaplasmosis and can develop before infection of 1% of the erythrocytes. Fever above 40ºC is common throughout the period of growing parasitemia, with subnormal temperatures observed prior to death in terminal cases. The most notable hallmark of clinical anaplasmosis is anemia caused by parasitized erythrocyte phagocytosis. The severity of clinical symptoms, which include pallor of the skin and mucous membranes as well as elevated heart and respiratory rates is related to the degree of anemia. Animals become feeble, anorexic, and lethargic as packed cell volume decreases in tandem with parasitemia. Lactating cows' milk output decreases, and pregnant cows may abort; bulls may have temporary sterility Cattle with advanced illness experience gastrointestinal atony, rumen stasis, and constipation, which are accompanied by dehydration and weight loss. Some animals may develop neurological impairments as a result of cerebral anoxia. Icterus frequently appears late in the course of a disease and is most common during the early stages of recovery (Kocan et al., 2010). Buffalo demonstrates a clinical history of deteriorating strength, anorexia, high temperature (105°F), tachycardia, labored breathing, pale mucus membranes, and a little tick infestation (Vatsya et al., 2013). Horses with *A. phagocytophilum* infection may have a clinical or subclinical symptomatology. The incubation period for spontaneous infections is roughly one to three weeks (Schäfer et al., 2022). Fever, lethargy, anorexia, ataxia, and icterus are the most typical clinical symptoms, while thrombocytopenia, anemia, and leukopenia are the most typical laboratory abnormalities (Franzén et al., 2005). Dog anaplasmosis has a variety of clinical symptoms, although the illness typically manifests as an acute febrile syndrome with a high body temperature, lethargy, anorexia, and a lack of will to move. The musculoskeletal system (lameness), the digestive system (diarrhea), or the central nervous system (seizures or proprioceptive impairments) may all exhibit more specific symptoms, particularly during the chronic phase of the disease (Ravnik et al., 2011).

2.5 Alteration of hematological parameters:

CBC or complete blood count is a salient feature in diagnosis of anaplasmosis. As anaplasmosis is a haemoprotozoal disease it makes changes in different hematological parameters. Using a hematology analyzer, the following parameters can be assessed: total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin (Hb), packed cell volume, mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) (Atif, 2016) to

check the changes in blood level in anaplasmosis.The ticks must have fed for at least 24 hours in order to spread the contagious germs. The organisms infect white blood cells, and 4 to 14 days later, they can be found. In addition to impacting a specific type of white blood cell, it can lower platelet counts. Although the specific mechanism of platelet damage is unknown, it can provide the veterinarian a hint as to what is wrong with the animals. A study (Bakken et al., 2001) showed an inverse association between mean white blood cell (WBC) and platelet (PLT) counts, in patients with anaplasmosis, indicating these numbers may be utilized to identify patients with current infection. Another study (Franzén et al., 2005) performed electronic analysis on total hemoglobin, red blood cell count, total and differential white blood cell, and thrombocyte counts from sample of horse. During the feverish phase of the infection, all horses had a prominent although temporary leucopenia, neutropenia, and a noticeable lymphopenia. All horses also experienced a significant thrombocytopenia that continued longer than the leucopenic phase.

2.6 Giemsa-stained thin blood smear (GSTBS):

Giemsa-stained thin blood smear (GSTBS) is a process of staining thin blood smear to visualize blood cells and parasite under the microscope. Thin blood smears stained with Giemsa, Wright Giemsa or Diff-Quick stain may make it easier to see *A. marginale* organisms in erythrocytes. Anaplasma species show up as thick, uniformly stained blue-purple inclusions 0.3–1 mcm in diameter in thin blood films stained with Giemsa. *A centrale* inclusion bodies are found more centrally than *A. marginale* inclusions, which are often seen around the edge of the infected erythrocyte. Individual rickettsiae are contained in inclusion bodies, which have 1–8 starting bodies with a diameter of 0.3–0.4 mcm. The stage and severity of the disease affect the percentage of infected erythrocytes; maximal rickettsemias of over 50% can occur with *A. marginale*. Two to six weeks after transmission, the infection becomes evidentunder a microscope. The rickettsemia can increase by doubling every day for up to 10 days over the duration of the infection before decreasing. Even if parasites cannot be seen in blood smears, severe anemia might continue for weeks (Tabor, 2022).However, this method might not be accurate in animals with severe anemia and advanced illness (Kocan et al., 2010). Additionally, it could be challenging to detect intraerythrocytic inclusions in samples taken during necropsy. Following recovery from acute infection, *A.marginale* inclusions are often not visible on blood smears taken from persistently infected calves.

2.7 Post-mortem findings

From anaplasmosis in cattle, recovery is more common in young animals, with mortality rates in adults ranging from 50 to 60%, especially when older animals are agitated. But cattle can frequently die while being handled in preparation for treatment. The clinical indicators predict necropsy findings, which include severe anemia, icterus, splenomegaly and hepatomegaly. Petechial hemorrhages are common on serosal surfaces, particularly around the heart and pericardium; the heart is typically pallid and flabby (Coetzee & Apley, 2006). The previous study (Lima et al., 2019) found a pale carcass with ascites, enlarged liver with rounded margins, a full and swollen gallbladder with thick bile, mild jaundice, and an accumulation of dry stools in the rectum during necropsy on cattle (Franzén et al., 2009) stated that, degenerative joint lesions in the horse ranged in severity from mild to moderate found in necropsy. These were distinguished by tiny articular cartilage erosions and linear grooves in the joints of the horse. The diaphragmatic surface of the liver had mild to severe fibrous scarring.

CHAPTER III
MATERIALS AND METHODS

3.1 Study area

The study was carried out in different farms located in the Savar union of Dhaka city during the time between March and September 2023. These farms were chosen for their accessibility, communication capabilities, and sound recording techniques.

3.2 Study population

A total of 232 (n=232) animals were studied in this study including cattle (n=102), Cross breed=61, Friesian=25, and Red Savar=16, buffalos (n=18), Horse (n=62), Arabian=24, Thoroughbred=38 and dogs (n=30), German shepherd=12, Labrador=10 and Lassa breed=8.

3.3 Data collection

Animals with a history of tick infestation, fever, jaundice and pale mucous membranes were subjected of data collection. The affected animals' medical histories and clinical symptoms were recorded. Age, breed, sex, the presence of ticks, prior treatment and herd size were subjected of a questionnaire to demonstrate their relationship.

3.4 Samples

Blood samples were collected from affected animals by puncturing jugular vein in case of cattle, buffalo, horse and saphenous vein in case of dog. Collected blood samples were taken into EDTA tubes for complete blood count and Giemsa staining. Following collection, all samples were transported to the laboratory processed for further testing and kept at $4^{o}C$.

3.5 Blood Smear and Microscopy Using Giemsa stain:

Thin blood smears were prepared immediately after blood samples reached to the laboratory. Blood smears were labeled, air-dried, fixed with methanol, stained with giemsa stain and examined microscopically for presence of *Anaplasma spp.* in erythrocytes. Examination of the smears was performed at 100x magnification with compound microscope and organisms were identified.

1.2 Hematological analysis in Blood (Complete Blood Count):

Hemoglobin (HGB), Red blood cell (RBC) count, Hematocrit (HCT), Total white blood cell (WBC) and Platelet counts (PLT) were analyzed using Hematological Analyzer (Elite 3 Hematological Analyzer, Germany) at laboratory.

1.3 Treatment:

The Animals found positive for anaplasmosis were treated with imidocarb dipropionate. Additionally, horse and dog were given ceftriaxone sodium and other supportive medicines. In case of cattle, tetracycline and in case of buffalo combination of procaine penicillin, benzyle penicillin and streptomycin were used besides imidocarb dipropionate.

1.4 Post mortem examination:

Post mortem examination was done systemically by taking proper precaution in case of suspected dead animals and the significant observations were recorded.

1.5 Statistical analysis:

The raw data were inserted, stored and coded accordingly using Microsoft® Excel® 2013. Statistical analysis was done by using SPSS Advanced Statistics 26.0 software (SPSS, Inc, Chicago, USA). Prevalence of anaplasmosis and mean of the hematological parameters were calculated and presented as table. Chi-square (χ^2) tests for $\alpha = 0.05$ was used to assess the relationship of gender and breed to the prevalence of anaplasmosis.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Clinical findings

Affected animals showed several types of clinical signs, those were different from species to species. In case of cattle under one-year age, did not show any kind of signs. Rest of them showed fever, anorexia, aggression and loss of coordination. Similar to the cattle, buffalos less than one year of age were symptomless. Older animals had cold and fever of 105°F. Anorexia, drowsiness and loss of coordination were also observed among the animals. Affected horses had high temperature of about 105°F. Their mucous membranes were pale and anorexia was one of the symptoms. They also had lameness due to pain in the joint. Clinical findings of affected dogs were similar to the horses such as high temperature of 105°F, anorexia, pale mucous membrane, joint pain and lameness.

4.2 Giemsa-stained thin blood smear (GSTBS) findings

Thorough examination of slide stained with giemsa revealed presence of intracellular small organisms within the RBC. *Anaplasma spp*. in the blood smear was observed as dense, rounded, intra-erythrocytic bodies situated on or near the margin of the erythrocytes. Image of some of the positive slides stained with giemsa has been Shown in **figure 1.**

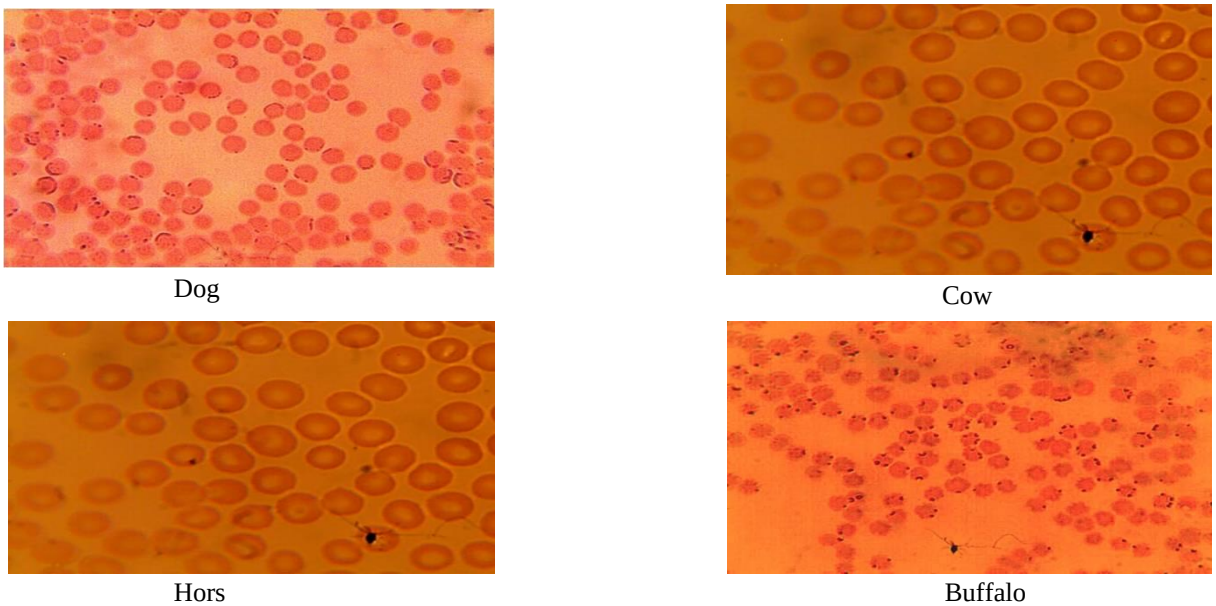


Figure 1: Presence of *Anaplasma spp.*within RBC.
(Image captured from GSTBS of horse (left) and dog (right)

4.3 Hematological results

All of cases infected with anaplasmosis showed noticeable changes in hematological values which mean values is represent in **Table 1**.

Hemoglobin estimation revealed severe anemia in all infected cases with low count of red blood cell (RBC) and hemoglobin. RBC count mean was 3.3822 ±0.27672 (mean ± SD) in case of dog and 6.240 ±0.2191 (mean ± SD) in case of horse. Similarly, hemoglobin (HGB) levels were lower than normal which mean was 11.028 ±4.363 (mean ± SD) in dog and 10.3 ±2.739(mean ± SD) in horse. The mean hematocrit (HCT) values were 20.28±4.363 (mean ± SD) and 29.20 ±1.095 (mean ± SD) in dog and horse respectively that is lower than the normal level. Unlike red blood cell, the white blood cell (WBC) counts were higher than the normal levels that is 8.2711 ± 0.23933 (mean ± SD) in dog and 8.5960 ± 0.41627 (mean ± SD) in horse after calculating the mean. Platelet count revealed significant variation from actual values that are 110.28±4.363 (mean ± SD) and 93.60±3.286 (mean ± SD) in dog and horse respectively.

Table 1: Mean and standard deviation of hematological parameters of horse and dog.

Parameters	Dog		Horse	
	Mean	**SD**	**Mean**	**SD**
RBC (× 10^6/mcL)	3.3822	0.27672	6.240	0.2191
HGB (g/dL)	11.028	4.363	10.30	2.739
HCT (%)	20.28	4.363	29.20	1.095
WBC (×10^3/µL)	8.2711	0.23933	8.5960	0.41627
PLT (×10^3/µL)	110.28	4.363	93.60	3.286

4.4 Prevalence rate in different animals:

Prevalence of hemoprotozoan anaplasma infections in different species of animals is shown in **Table-2**. Prevalence rate in cattle, buffalo, horse and dog are 37.3%, 88.9%, 8.1% and 60.0% respectively. This analysis is indicating an abrupt rise in the prevalence of *Anaplasma* spp. in buffalo and lower in horse that is represented as histogram in **Figure-2.**

Table 2: Prevalence of anaplasmosis in cattle, buffalo, horse and dog.

Species	Frequency	Percent (%)
Cattle	38 (n= 102)	37.3
Buffalo	16 (n=18)	88.9
Horse	5 (n= 62)	8.1
Dog	18 (n= 30)	60.0

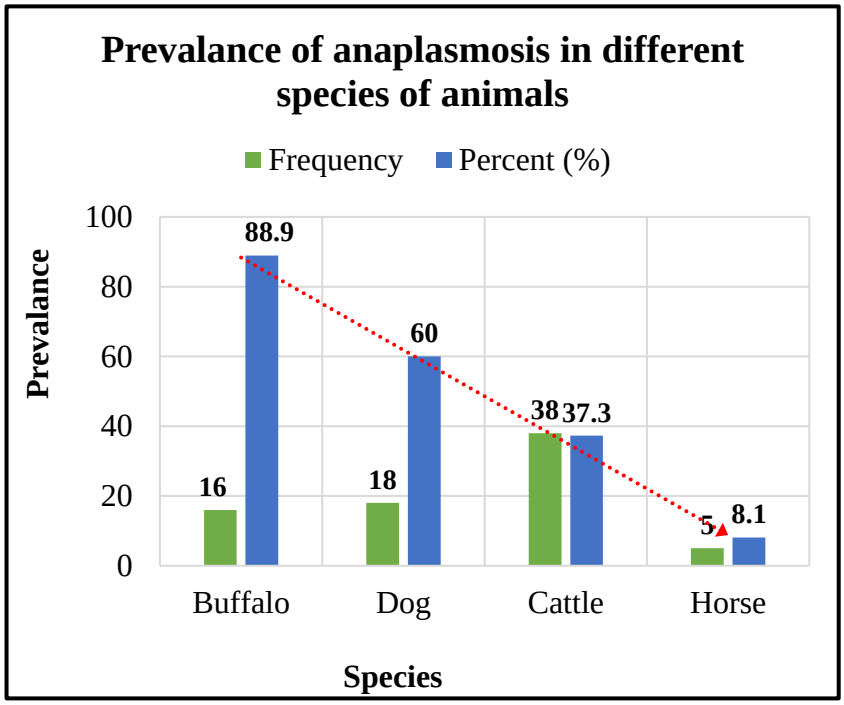


Figure 2: Comparative analysis of prevalence in different species of animals.

4.5 Rate of fatality

Death rate was very high in buffalo than the other animals that was 87.50%, whereas lower death rate was observed in cattle that is 7.89%. In horse and dog, the rate of fatality was 40% and 33.33% respectively. The observed fatality rates in different animals are shown in **Figure-3.**

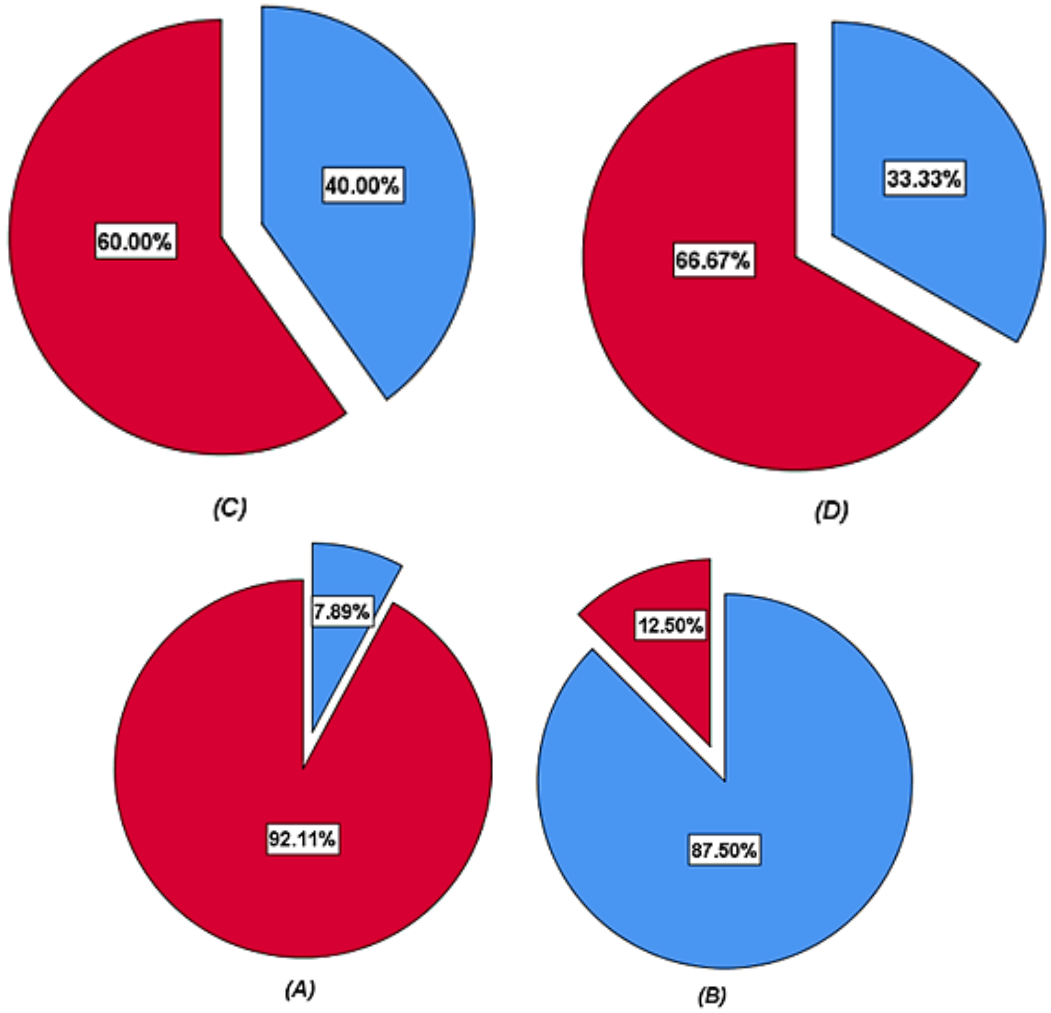


Figure 3: Rate of mortality in anaplasmosis (Blue indicates death).
(A) = Cattle, (B) = Buffalo, (C) = Horse, (D) = Dog

4.6 Variation between sexes

The chi square test was statistically significant in case of cattle, $\chi 2$ (df=1, N=102) = 6.391, p= 0.011 (p < 0.05), with Phi co-efficient of 0.250 indicating a moderate relationship. As seen in the **Figure 4**, the female was more likely to be affected than male in case of buffalo, the chi square test was statistically non-significant, $\chi 2$ (df=1, N=18) = 1.004, p = 0.316 (p > 0.05). As seen in the figure, the female was more likely to be affected than male. Relationship of sex to infection rate in horse was statistically non-significant and the chi square value was, $\chi 2$ (df=1, N=62) = 0,038, p = 0.845 (p > 0.05), with Phi co-efficient of -0.0250 indicating a negative relationship. In dog, the chi square test was statistically non-significant, $\chi 2$ (df=1, N=30) = 1.094, p = 0.296 (p > 0.05), with Phi co-efficient of 0.191.

Table 3: Relationship between sex and infection rate.

	Species			
	Cattle	**Buffalo**	**Horse**	**Dog**
Pearson Chi-Square value	6.391	1.004	0.038	1.094
Degrees of freedom (df)	1	1	1	1
Approximate significance	0.011	0.316	0.845	0.296
Phi co-efficient value	0.250	0.236	-0.025	0.191
No. of valid cases	102	18	62	30

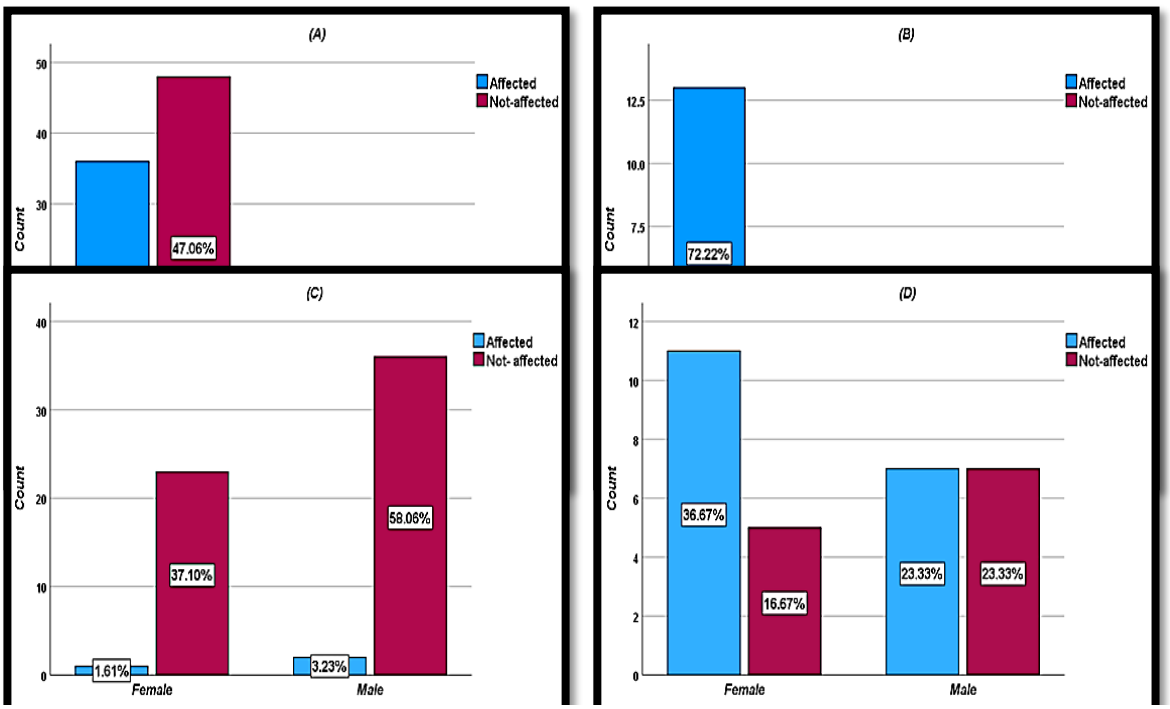


Figure 4: Effect of sex in infection rate. (A) = Cattle, (B) = Buffalo, (C) = Horse, (D) = Dog

4.7 Relationship to the breeds:

Relationship of breed to infection rate was statistically highly significant in case of cattle. The chi-square value was $\chi 2$ (df=2, N=102) = 22.262, p = 0.00 (p < 0.05), with Phi co-efficient of 0.467 indicating a very strong relationship. As seen in the **Figure 5**, the cross-breed cattle were more likely to be affected than HF and RCC cattle. In case of horse, the chi square test was statistically significant, $\chi 2$ (df=1, N=62) = 3.908, p = 0.048 (p < 0.05), with Phi co-efficient of 0.067 indicating a lesser relationship. As seen in the **Figure 5**, Arabian breed was more likely to be affected than thoroughbred.

The chi square test was statistically non-significant in case of dog, $\chi 2$ (df=2, N=30) = 0.135, p = 0.935 (p < 0.05), with Phi co-efficient of 0.067.

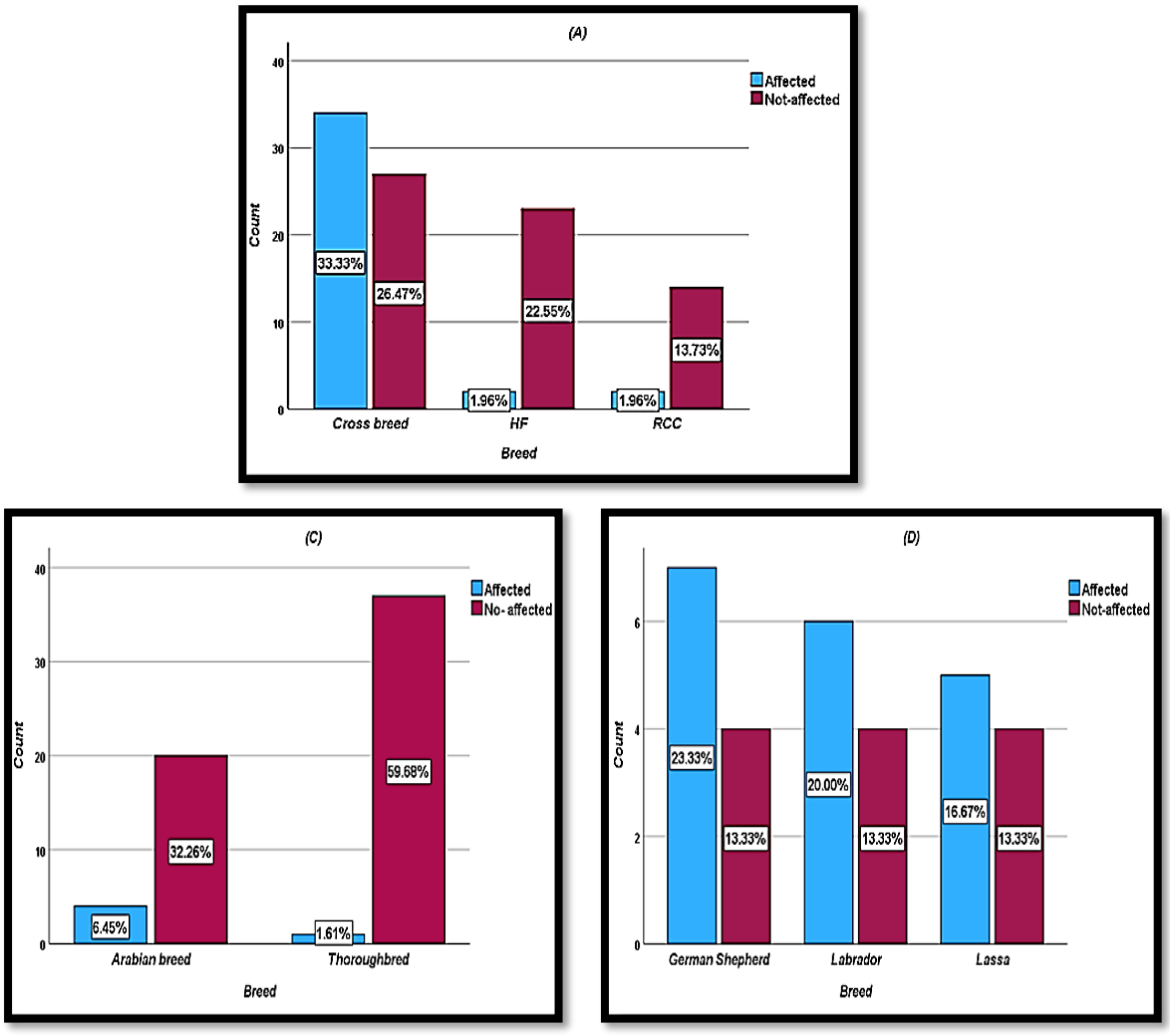


Figure 5: Effect of breed in infection rate. (A) = Cattle, (B) = Buffalo, (C) = Horse, (D) = Dog

Table 4: Relationship of breed and infection rate.

	Species		
	Cattle	**Horse**	**Dog**
Pearson Chi-Square value	22.262	3.908	0.135
Degrees of freedom	2	1	2
Approximate significance	0.00	0.048	0.935
Phi co-efficient value	0.467	0.251	0.067
No. of valid cases	102	62	30

4.8 Post mortem findings

Post mortem observations of dead animals revealed specific lesions in different animals. Cattle and buffalo had almost similar lesions in their spleen and lung. The spleens of both species were larger in size that is called splenomegaly and the spleens were blackish in color. Lungs of them were hemorrhagic.

Similarly, horse and dogs had splenomegaly and blackish spleen and hemorrhagic lung that is showed in **Figure 6** and **Figure 7.**

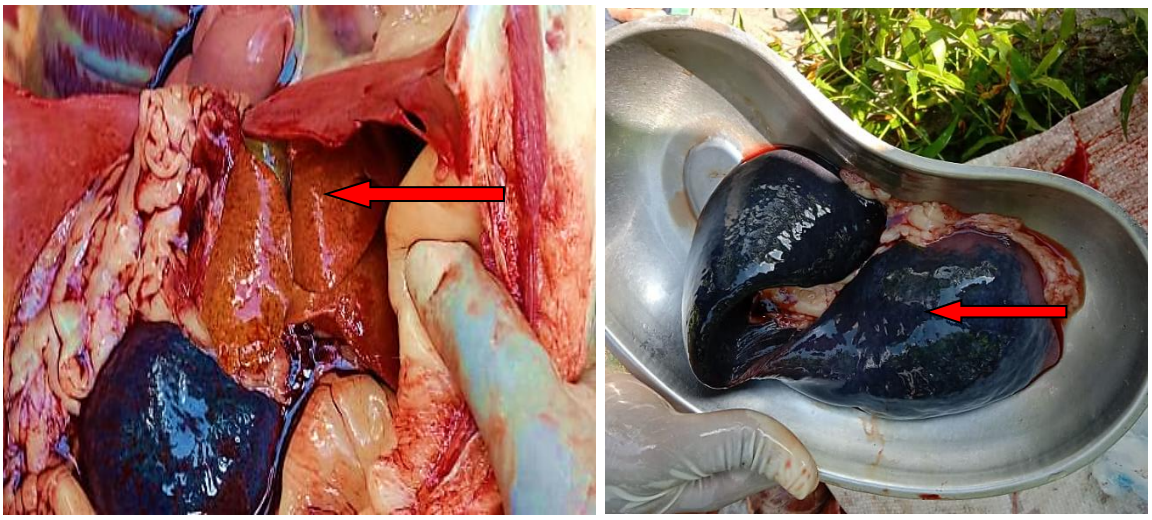


Figure 6: Hemorrhagic lung (left) and splenomegaly with blackish spleen (right) in postmortem findings of dog.



Figure 7: Splenomegaly with hemorrhage (Left) and hemorrhagic heart (Right) in post mortem analysis of horse.

4.9 Discussion

All the documented and analyzed data in this study has shown some similarities and dissimilarities with some of the previous studies related to the anaplasmosis in several species of animals. Blood physiology parameters were beyond the normal ranges described in the Adiva 2120. Through giemsa stain the blood parasite *Anaplasma spp.* was identified as described by Soulsby (1982). In this study, prevalence of anaplasmosis in several species of animals were different from each other and highest prevalence was seen in buffalo followed by dog, cattle and horse respectively. The current study revealed 37.3% prevalence of anaplasmosis in cattle that supported by an earlier report of anaplasma infection in Bangladesh (Talukdar & Karim, 2001) wherein stated that 33% of cattle in the Baghabari (Shahjadpur) Milk Shed Area had anaplasmosis. Sex-wise prevalence of anaplasmosis in cattle documented that female had a higher prevalence (36.29%) than males (1.96%), which is highly significant (if significant then put here p value). This finding is consistent with the findings reported earlier (Sarkar et al., 2010), where they found that females were considerably more likely than males . This investigation's findings regarding the incidence of hemoprotozoan illnesses in female cattle were consistent with those in the research of (Kamani et al., 2010). Higher incidence in female cattle may be related to their being held for extended periods of time for breeding and milk production, being given insufficient feed despite having a high demand(Kamani et al., 2010), or differences in the size of sample. According to the results of the current study, crossbred cattle had a significant and high prevalence (33.33%) of Anaplasma infections than Holstein Friesian cattle and indigenous cattle Red Chittagong Cattle, which is consistent with the findings of the previous study(Radostits et al., 2000). The varying prevalence of hemoprotozoan infections in the studied sites may be attributed to variations in geo-climatic conditions, breed, exposure to vectors, and animal age (Muhanguzi et al., 2010). The reduced occurrence of infections in native cattle may be attributable to ongoing infection exposure and the development of immunity against such illnesses (Siddiki et al., 2010). A study (Richey, 1992) stated that if the animal passes away in the latter stages of acute infection, icterus may be present in post-mortem with frequently swollen and deep red-brown spleen. It's typical to have an enlarged liver and a gall bladder that is swollen with dark bile. Present study partly supports this statement with blackish larger spleen and hemorrhagic lung in dead cattle (put figure number). Under the same environmental conditions, buffalo frequently exhibit less severe clinical anaplasmosis symptoms than sick cattle, which is probably at least partially because of their breed resistance (Rajput et al., 2005). According tothe previous report (Kuttler,

1984), buffalo can develop a prolonged infection and harbor a sub-clinical condition, despite the fact that cattle are the species most commonly affected by clinical anaplasmosis. In this study, 88.9% buffalo were affected by anaplasmosis which is very high. This high prevalence of buffalo infection with rickettsial organisms was similar to those observed previously in Cuba (52%) and Mozambique (72.2%), but was higher than those reported in the Philippines (29%), Malaysia (21.8%), India (18.33%), South Africa (17.3%), Pakistan (14.73%), Columbia (13.1%), and northeast Thailand (8%) (Nguyen et al., 2020). In the current investigation, the infection rate among female buffalo was noticeably higher (77.22%) than that of male (16.67%) buffalo although it was statistically non-significant. This may be explained by the fact that different numbers of male and female buffalo were being considered for the study. This result was supported by the findings of a previous study (Nguyen et al., 2020), but the earlier findings in Pakistan, where male buffalo had a higher disease prevalence (26.25%) than female buffalo (16.62%), contradicted this conclusion (Farooqi et al., 2018). Nearly all of the horses showed symptoms of fever, anorexia, discomfort and limb lameness found in this current study; however, other studies (Franzén et al., 2005) and Gribble (1969) also found these findings along with ataxia and distal limb edema. The horses in the present investigation did not show ataxia and/or distal limb edema but exhibited clinically identifiable icterus. Present study showed leukocytosis, anemia and marked thrombocytopenia in horses compared to the normal hematological reference values listed in *Hematology (Advia 2120*) documented by Cornell University College of Veterinary Medicine (reference). A study (Schäfer et al., 2022) similarly observed anemia and thrombocytopenia in their study that is consistent with the results found in this study. Prevalence rate of anaplasmosis in horse had been estimated 8.1% in the current study that is higher than the prevalence found in the Czech Republic (5%) (Hulínská et al., 2004) and in Switzerland (4%) (Bretscher, 1990). On the other hand, results found in this study was lower than the prevalence demonstrated in the report published earlier (Schäfer et al., 2022). In Central Europe, 10% of the horses in the Netherlands had anaplasmosis (Butler et al., 2008) that exceeded the findings found in the current study. According to the report (Razzaq et al., 2015), it was showed that age, gender, and species of the animals did not significantly correlate with the presence of *Anaplasma spp.* in horse. No discernible gender differences were found in the prevalence rate observed in the current study that can be said significant, which is consistent with the study reported earlier (M'ghirbi et al., 2012). On the other hand, a considerable differences between breeds was seen in the work published previously (Razzaq et al., 2015), which is consistent with the present work. Relationship of breed to the prevalence was statistically significant while in Arabian breed it was 6.45% compare to the thoroughbred

of horse (1.61%). A previous study (Franzén et al., 2009) demonstrated that the dead horse's heart exhibited ecchymoses that were moderate in size. On the thoracic portion of the diaphragm and within the lung parenchyma, there were multifocal petechial hemorrhages as well as edema and hemorrhage in the mediastinum. The liver, spleen, and kidneys experienced a small amount of serosal petechiae within the abdominal cavity. Though present study did not reveal all of these findings, state of spleen, lungs and heart is similarly hemorrhagic in both studies. High body temperature, anorexia, pale mucous membranes, joint pain, and lameness are the important clinical signs in dogs that were revealed in the current study. Lethargy, fever, anorexia, unwillingness to move, and lameness were found to be the most prevalent clinical symptoms in the study reported earlier (Kohn et al., 2008), which also validates the findings observed in the current study. Another previous study (Kohn et al., 2008)furthermore noticed hemorrhage, lymphadenopathy, diarrhea, and coughing in case of anaplasmosis in dogs but these were absent in this study. According to the results of the current analysis, the clinical symptoms and results indicated a 60% prevalence of anaplasmosis in dog. 33% of the dogs at one kennel in North Carolina, USA, tested positive for *Anaplasma. spp*. by PCR (Kordick et al., 1999). *A.platys*-specific PCR detected 32% of stray dogs in Okinawa, Japan, as positive(Motoi et al., 2001). 50% of the canines tested in Louisiana, USA, were IFA positive, which is almost identical to the results of the current study (Hoskins et al., 1988). Hematologic data from 22 dogs reported earlier study (Egenvall et al., 1997) in which co-infections were not present compared to the findings demonstrated in the present study. Parallel to the present study, anemia and thrombocytopenia were the most prevalent abnormalities compared to the normal hematological reference values listed in Hematology (Ad via 2120) documented by Cornell University College of Veterinary Medicine. When a variety of blood protozoa infect people or other animals, thrombocytopenia frequently results. Increased platelet consumption brought on by diffuse intravascular coagulation, sequestration in an enlarged spleen, immunologically driven platelet destruction, or generation of inhibitory factors are possible explanations. The abnormalities of blood cells may be caused by intricate immunologic processes (Kohn et al., 2008).In the current study, it was shown that female dogs were more prevalent (36.67%) than the male (23.33%) dogs. This finding is consistent with those previous studies (Gadahi et al., 2008) and (Elom et al., 2015), wherein they found that female dogs had higher blood parasite infestation rates than male dogs. On the contrary, another previous study (Mehta et al., 2020) found that the male dogs have a higher infection rate than female dogs. Similar findings were also reported in the other study as well (Ombugadu et al., 2021) wherein the male dogs had a higher prevalence of blood parasites (54.0%) than the female dogs (42.9%).

However, there was no discernible difference in blood parasite infection between male and female dogs. However, more male was infected in their study even though there was no difference in the incidence of blood parasites according to sex. In this analysis, the German shepherd showing the highest prevalence of all the breeds. In similar fashion, German shepherds possessed a greater prevalence of blood parasites reported earlier (Phuyal et al., 2017). The identical findings were also reported by (Shrivastava et al., 2014) where they found that the German shepherd breed had the highest frequency. All of the dogs in a previous study (Kohn et al., 2008) had splenomegaly with severe lesions. Pathological examination of seven experimentally infected dogs revealed that all of their spleens were mild to moderate enlarged, congested and somewhat mushy in texture. The necropsy findings of the current study demonstrated identical outcomes and hemorrhagic lungs were also identified. Based on the above data demonstrated in the current study, it can be concluded that the prevalence of anaplasmosis differs from one species to another in terms of several characteristics. Notwithstanding our country's ignorance of buffalo diseases, blood protozoa can cause serious infections in this species. In practically, all the animal species, gender and breed either statistically significantly or not significantly affect the *Anaplasma* infection rate. Although there are some modest parallels between cattle and buffalo as well as horse and dog, clinical symptoms, blood parameters, necropsy findings etc. that will help in the diagnosis of anaplasmosis, which may vary from species to species.

CHAPTER V
SUMMARY AND CONCLUSION

One of the key indicators for assessing animal health and farms and/or animal management is the level of incidence of parasitic infections. Anaplasmosis, one of the major parasitic diseases, has provoked research interest recently due to its unusual pace of escalation caused by climate change and devastating negative impact on animal health as well as owner financial affairs. The findings demonstrated in the current study concluded a notable prevalence of anaplasmosis in many animal species with the apparent variation between them; especially the incidence of anaplasmosis in buffalo was particularly concerning. Though the different variables such as gender, breed, blood parameters had different prevalence, the results of this study may contribute to improving the health status of animals by aiding in the diagnosis and prevention of anaplasmosis in different host animals.

CHAPTER VI
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