EMERGING STATUS OF ANAPLASMOSIS IN CATTLE IN SIRAJGANJ DISTRICT WITH THERAPEUTIC EVALUATION OF TRADITIONAL TREATMENTS

A THESIS

BY

MD. SHARIFUL ISLAM

REGISTRATION NO.: 1505044

SEMESTER: JANUARY-JUNE, 2017

SESSION: 2015-2016

MASTER OF SCIENCE (M.S.)

IN

MEDICINE

DEPARTMENT OF MEDICINE, SURGERY AND OBSTETRICS

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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JUNE, 2017

DEDICATED
TO MY
BELOVED PARENTS

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The Author

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ABBREVIATIONS

PSAC: President's Science Advisory Committee

PCR: Polymerase Chain Reaction

MSPs: Membrane Surface Proteins

TBDs: Tick-Borne Diseases

WHO : World Health Organization

DLS: Department of Livestock Services

et al. : And his associates

Fig. : Figure

gm: Gram

Ltd.: Limited

mg: Milligram

ml: Milliliter

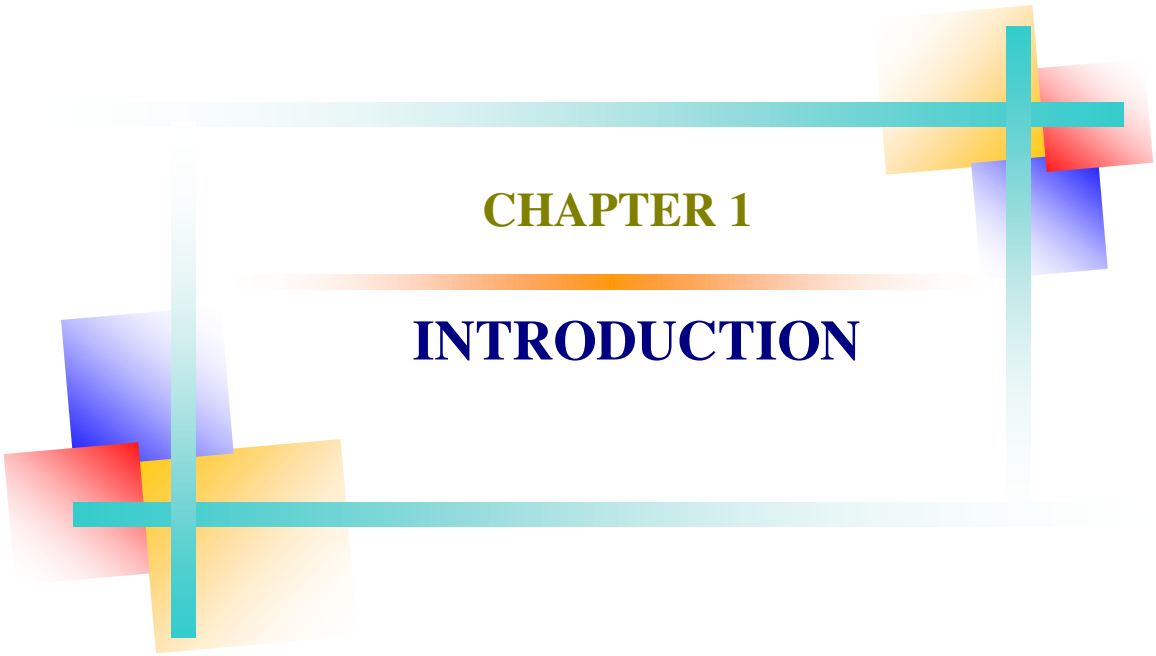
%: Percent

@: At the rate of

® : Registered

ABSTRACT

The present study was carried out to detect the prevalence of anaplasmosis in cattle at Rayganj and Ullahpara upazila in Sirajganj district of Bangladesh and to evaluate the therapeutic efficacy of traditional drugs like Oxetetracycline (Renamycine®), Imidocarb diproprionate (Babenil®) and Diminazine aceturate (Berenil®) against anaplasmosis in cattle. The study was conducted from July 2016 to December 2016. During six months of study period a total of 150 blood samples were collected from clinically ill and suspected cattle, among which 28 samples were positive for anaplasmosis by Geimsa stained blood smear method. It was observed that the overall prevalence of anaplasmosis in cattle was 18.67%, where 16.25% was Geimsa stained blood smear at Rayganj and 21.43% at Ullapara upazila respectively and the variation was not statistically significant ($p>0.05$). In respect of age the prevalence of anaplasmosis was significantly ($p< 0.05$) higher (31.7%) in 2-3 years of age group cattle than above 3 years (16.67%) and 6 months to 2 years age (10.20%) group. On the basis of sex, it was observed that the variation in prevalence in male (15.94%) and female (20.99%) was not statistically significant ($p>0.05$). Breed-wise prevalence was higher in crossbred cattle (19.5%) than local cattle (17.8%), was not statistically significant ($p>0.05$). It was observed that among three drugs used in this study, the best effectiveness of drugs was seen by Oxytetracycline (Renamycine LA®) @ 10 mg/kg body weight followed by Imidocarb dipropionate (Babenil®) @ 3.5 mg/kg body weight moderately and less by Diminazine aceturate (Berenil®) @ 3.5 mg/kg body weight. From the study it was evident that cattle were infected with the organisms and caused a heavy economic loss which recommended to take necessary preventive measurements.



CHAPTER 1

INTRODUCTION

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INTRODUCTION

Animal diseases are perhaps the most important limitation to animal production in developing countries. According to FAO estimates, there are 50 percent production losses due to diseases (PSAC, 1967). Major epizootic diseases of livestock that threaten production have either been eradicated or are under control in the developed world. Among these, the parasitic diseases have gained special significance due to adverse effects on production (Drummond *et al.,* 1978). Anaplasmosis occupy major place because of its fatality and adverse effects on animal productivity. The incidence and prevalence of this disease and its morbidity and mortality is very high in developing countries (PSAC, 1967). In Bangladesh anaplasmosis in cattle is an economically important disease and which produce jaundice in cattle without haemoglobinemia and haemoglobinuria, decreased milk production, weight loss, abortion, hyperexcitability due to cerebral anoxia and sudden death. This disease is caused by the members of genus Anaplasma (Rickettsiales: Anaplasmataceae) *Anaplasma marginale* and *Anaplasma centrale.* This disease is endemic in the tropics and subtropics. It is common in tropical and subtropical country and sporadic in temperate region. Bangladesh is usually hot and humid except in winter and the climatic condition of Bangladesh is very conducive to a wide variety of parasites as well as ticks (Razzak and Shaikh, 1969) which have been recognized as the notorious threat due to severe irritation, allergy and toxicosis (Niyonzema and Kiltz, 1986). Tick-borne diseases cause substantial losses to the livestock industry throughout the world (Ananda *et al.,* 2009; Kakarsulemankhel, 2011) as these have got a serious economic impact due to obvious reason of death, decreased productivity, lowered working efficiency (Uilenberg, 1995), increased cost for control measures (Makala *et al.,* 2003) and limited introduction of genetically improved cattle in an area (Radostits *et al.,* 2000). There are many Anaplasma species but, Anaplasma marginale is the most important one (Kumar *et al.,* 2010). Prevalence of blood rickettsia like *Anaplasma marginale, Anaplasma centrale* have been reported in animals of Bangladesh (Ahmed, 1976; Samad and Gautam, 1984). Anaplasmosis are the more prevalent in different areas of Bangladesh where Chowdhury *et al.* (2006) recorded 70% anaplasmosis in Sirajganj district, Belal *et al.* (2015) recorded the overall prevalence of anaplasmosis in cattle was as 25.82% in Sirajganj district. Talukdar and Karim (2001) also documented higher prevalence (33%) of anaplasmosis in Baghabari Milk Shed

Area, Sirajganj. Kamani (2010) and Kocan *et al.* (2003) was observed that the prevalence of anaplasmosis was higher in adult than young cattle. Breed and seasonal variation on anaplasma infection was reported by Ananda *et al.* (2009) and Radostits *et al.* (2000). Prevalence of anaplasmosis has been reported in cattle or however, reports are available from temperate regions buffaloes or from both worldwide including Costa Rica too. Transmission of Anaplasma is possible in Italy (50%), Iran (50%), China (32.35%) biologically through genera of *Boophilus*, *Rhipicephalus* and in Morocco (21.9%) by using optical and *Hyalomma* vectors and mechanically through microscopy, PCR or ELISA as diagnostic technique, while contaminated needles and biting flies of the family from Pakistan only few studies have been reported from Tabanidae. Sirajganj is the most important dairy belt area of Bangladesh and the density of cattle population of this district is high. The climatic condition and geographical location of the areas might favor the growth and multiplication of ticks which act as natural vectors of anaplasmosis and that causes one of the major veterinary problems affecting livestock industries.

There are some antirickettsiale and antiprotozoal drugs available in the local market and are being used by the field veterinarians. Tetracyclines (Chlortetracycline, Tetracycline and Oxytetracycline) are used for the treatment of anaplasmosis. Other compounds such as Imidocarb eliminate parasites from carrier animals (Urquhart *et al,* 1988).

Oxyteracycline (Renamycine®), Iimidocarb dipropionate (Babenil®) and Diminazine aceturate (Berenil®) have been marketed locally for animal use in Bangladesh.

In continuation with the existing knowledge, the present study was planned with the following objectives.

- To study the overall prevalence of Anaplasmosis in cattle at Rayganj and Ullahpara upazila of Sirajganj district.
- To know the age, sex and breed-wise prevalence of Anaplasmosis in cattle at Rayganj and Ullahpara upazilla under the district of Sirajgonj.
- To evaluate the therapeutic efficacy of different commercially available drugs against anaplasmosis in cattle.

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

2.1. Anaplasma marginale

2.1.1. Historical background

Anaplasma marginale was first described in the early 1900s by Sir Arnold Theiler who observed "marginal points" in erythrocytes of cattle suffering from gallsickness (galsiekte) (Theiler, 1910, 1911, 1912). Although two decades earlier the microorganism had already been discovered by other investigators, it had been erroneously considered as a part of the *Babesia bigemina* life cycle (Smith and Kilborne, 1893).

Yet, Theiler demonstrated that babesiosis and anaplasmosis can often co-exist in the same animal; he then succeeded in separating the two agents and produced a "pure infection" with only *A. marginale*. He indicated that the "marginal points" differ from any known blood parasite and named the new pathogen *Anaplasma marginale*.

The scientific name proposed by Theiler was based on the microscopic observation of the pathogen in infected blood smears. "Anaplasma" stands for the absence of a stained cytoplasm, and "marginale" for its marginal localization in infected erythrocytes. Furthermore, Theiler (1911) also described a subspecies of *A. marginale*: located in the centre of erythrocytes. *Anaplasma centrale*, is less pathogenic than *A. marginale* and causes only a slight attack of the disease. An *A. centrale*-based, blood-derived, live vaccine was exported from South Africa to other parts of the world, i.e. Australia, Israel and Latin America, where it has been in use for over a century (Kocan *et al.,* 2010b).

2.2. Epidemiology

A. marginale is one of the most prevalent tick-borne pathogens of cattle in tropical and subtropical areas worldwide (~40° N to ~32° S) (Aubry and Geale, 2011). It is endemic in the New World, Central and South America, Australia and some regions of Asia and Africa (Kocan *et al.,* 2010b).

In the USA anaplasmosis is enzootic throughout the southern states, but due to the movement of cattle, anaplasmosis has now been reported in almost every state (Kocan *et al.,* 2010a).

In some countries it is considered to be a foreign animal disease e.g. in Canada, where an outbreak of anaplasmosis resulted from mechanical transmission of the organism from imported cattle (Boulanger *et al.,* 1971).

In Europe, it is found mainly in Mediterranean countries like Italy (de la Fuente *et al.,* 2005e; de la Fuente *et al.,* 2005f; Torina *et al.,* 2008) and Spain (de la Fuente *et al.,* 2005d), although few isolated cases have also been reported in Hungary (Hornok *et al.,* 2012) and Austria (Baumgartner *et al.,* 1992).

The wider distribution and increase in outbreaks of the disease result from transport of asymptomatic carrier animals, which are reservoirs for subsequent mechanical or biological transmission to susceptible cattle in non-endemic areas. Wildlife may be possible reservoir hosts, and represent a source of infection for free-ranging cattle (Kocan *et al.,* 2010a; Kocan *et al.,* 2010b).

Furthermore, factors such as climate, host abundance, tick-host diversity and topography have been all shown to have an impact on the epidemiology of *A. marginale* (Estrada-Pena *et al.,* 2008). Changes in climate influence the distribution, physiology and behavior of many different arthropod vectors (Jonsson and Reid, 2000). The possible introduction of new tick species into areas where they did not exist before may complicate the control and prevention of tick-borne diseases.

2.3. Classification

In Dumler *et al.,* 2001, proposed a reclassification of Rickettsiales based upon genetic analysis of 16S rRNA and groESL genes. Organisms of this taxon were then assigned to one of the two families: Rickettsiaceae and Anaplasmataceae. All bacteria classified within these families are intracellular pathogens. However, unlike the Rickettsiaceae, which grow freely within the host cytoplasm or nucleus, members of Anaplasmataceae are found exclusively within membrane-bound vacuoles in the cytoplasm of the host cells. Moreover, almost all organisms assigned to the family Anaplasmataceae multiply in both vertebrate and invertebrate hosts (Kocan *et al.,* 2010a).

Following the phylogenetic analysis, four genera were formed within the Anaplasmataceae family, namely: *Ehrlichia*, *Neorickettsia, Anaplasma* and *Wolbachia.* All are gram-negative bacteria, demonstrating two morphological structures: large reticulate forms, or smaller dense forms with condensed protoplasm. Anaplasmataceae

infect canids, humans, ruminants and rodents. Formerly the genus *Anaplasma* consisted of *A. ovis*, *A. marginale* and a less pathogenic subspecies of *A. marginale*, *A. centrale* (*A. marginale* ss. *centrale*). Following the reclassification, *A. bovis* (formerly *Ehrlichia bovis*), *A. phagocytophilum* (formerly *Ehrlichia phagocytophila*, *E. equi* and the human granulocytic ehrlichiosis (HGE) agent), *A. platys* (formerly *Ehrlichia platys*) and *Aegyptianella* (genus *incertae sedis* due to the lack of sequence information) have also been included into the *Anaplasma* genus (Dumler *et al.,* 2001). *Anaplasma marginale* classification according to Dumler *et al.* (2001).

The level of *A. marginale* organisms in adult male *Dermacentor andersoni* ticks can reach approximately 105 organisms per salivary gland (Kocan *et al.,* 1992a) regardless of the rickettsemia level in the blood during acquisition feeding (Eriks *et al.,* 1993). During subsequent feeding rickettsiae are transmitted via the salivary glands of the tick to vertebrate hosts (Kocan *et al.,* 1992a).

At each infection site within the tick, *A. marginale* develops within membrane-bound vacuoles, forming colonies. The first form seen within *A. marginale* colonies is the reticulated (vegetative) form, which divides by binary fission and results in the formation of large colonies containing hundreds of organisms. The reticulated forms are then transformed into dense forms (0.5-0.8 μm) which are the infective forms. They can survive for a short time outside cells (Kocan *et al.,* 2008).

Cattle become infected when the dense form is transmitted during tick feeding via the salivary glands (Kocan *et al.,* 2004). The tick cell culture model has been used for studying entry and exit mechanisms of the rickettsia from IDE8 cells (Blouin and Kocan, 1998). Host cell invasion is initiated by the adhesion of the dense form to the tick cell membrane, and the rickettsia subsequently enters into cells by endocytosis.

While leaving the cell, colony and cell membranes fuse, allowing the rickettsia exit without host cell injury. Blouin and Kocan (1998) suggested that the same mechanism occurs within naturally infected tick cells, which may facilitate high infection rates without pathological changes in ticks.

2.4. Transmission

Transmission of *A. marginale* to vertebrate hosts occurs in two main ways: biologically by ticks, and mechanically by biting flies or by blood contaminated fomites.

Transplacental transmission to the calf fetus has also been reported (Grau *et al.,* 2013; Maldonado *et al.,* 2012; Rey Valeiron *et al.,* 2003; Zaugg, 1985).

Various tick species have been reported to be vectors of *A. marginale* in different regions of the world (Kocan *et al.,* 2004). *A. marginale* DNA has been identified in many tick species or in ticks which transmitted the disease experimentally. However, this does not necessarily imply that they are able to transmit the organisms under natural conditions (Shkap *et al.,* 2009; Zivkovic *et al.,* 2007). In addition, recent analysis suggests that in some regions tick species which have not previously been considered as vectors may also transmit *A. marginale (*de la Fuente *et al.,* 2005d; Fyumagwa *et al.,* 2009; Zahang *et al.,* 2013). Above all, *Rhipicephalus* (*Boophilus)* spp. are the most prevalent vectors of anaplasmosis in most tropical and subtropical countries. In the United States, however, *Dermacentor* spp., including *D. variabilis*, *D. andersoni* and *D. albipictus*, are the major vectors of anaplasmosis (de la Fuente *et al.,* 2001c; Kocan *et al.,* 1981), probably because a compulsory acaricide-treatment program in the 1940s (Stiller *et al.,* 1989) led to the eradication of the *R.* (*B.) microplus* tick.

Transmission occurs by one stage (intrastadial) or from stage to stage (inter- or transstadial). Intrastadial transmission is effectuated mainly by male ticks (Kocan *et al.,* 2010a). Serial transmission by male *D. andersoni* ticks to five consecutive cattle has been demonstrated (Kocan *et al.,* 1992a). However, it has been shown that *A. marginale* was not transmitted from infected to uninfected adult *Dermacentor spp*. ticks during co-feeding on the same cattle (Kocan and de la Fuente, 2003). Interstadial transmission e.g. ingestion by nymphs and inoculation by adults has been demonstrated by *R.(B.) annulatus,* a single-host tick (Shkap *et al.,* 2009), and by *D. andersoni,* a three-host tick (Kocan *et al.,* 1981).

The occurrence of transovarial transmission of few tick-borne pathogens e.g. *Babesia* spp. by single-host *R. Boophilus spp.* is well known (Howell *et al.,* 2007). Yet, transmission of *A. marginale* from one tick generation to the other remains controversial, although Theiler (1912) and few other authors have suggested that this type of transmission does occur (Anthony and Roby, 1962; Rees and Avery, 1939; Stich *et al.,* 1989). Interestingly, multiplication of *A. marginale* within the tissues of engorged *R.(B.) microplus* females has been confirmed (Ribeiro and Lima, 1996). Moreover, in eggs and larvae derived from *R. B. microplus* ticks collected from infected cattle, *A. marginale*

specific DNA fragments have been amplified. Yet, the transmission of *A. marginale* by these larvae to animals has never been proven (Moura *et al.,* 2003). Some authors suggested that *Ehrlichia* spp. and *Anaplasma spp.* are not transmitted transovarially due to the lack of the aldolase/adducing domain protein (Dunning Hotopp *et al.,* 2006).

Mechanical transmission of the pathogen occurs when infected blood is transferred to susceptible animals by contaminated fomites: needles, dehorning saws, nose tongs, tattooing instruments, ear tagging devices and castration instruments (Kocan *et al.,* 2004). Additionally, different species of hematophagous diptera e.g. *Tabanus* spp. flies (Hawkins *et al.,* 1982), *Stomoxys calcitrans* (stable fly) (Potgieter *et al.,* 1981) or mosquitoes have been demonstrated to have the ability to disseminate *A. marginale*. Although biological transmission has been shown to be more efficient (Scoles *et al.,* 2008), mechanical transmission is the major route of infection in areas where the strains are not tick-transmissible or appropriate tick vectors do not occur (de la Fuente *et al.,* 2001c).

2.5. Pathogenesis

A. marginale is very host specific and under natural conditions infects only ruminants. Although clinical anaplasmosis occurs most often in cattle, other ruminants like water buffalo (*Bubalus bubalis*), American bison (*Bison bison*), white-tailed deer (*Odocoileus virginianus*), black-tailed deer (*Odocoileus hemionus columbianus*), Rocky Mountain elk (*Cervus elaphus nelsoni*), black wildebeest (*Connochaetes gnou*), blesbuck (*Damaliscus pygargus phillipsi*) and duiker (*Sylvicapra grimmia*) can also become infected (Aubry and Geale, 2011; Kocan *et al.,* 2010b; Kuttler, 1984).

The only known site of *A. marginale* development in cattle is within erythrocytes. Interestingly, because bacteria can be propagated in a bovine endothelial cell line (Munderloh *et al.,* 2004), it has been suggested that endothelial cells may serve as a site of initial replication after tick attachment, or as a reservoir for *A. marginale* during persistent infection. After experimental infection of calves with the *A. marginale* St. Maries strain, Carreno *et al.* (2007) observed the infection of endothelial cells by dual fluorescence microscopy. In contrast, Wamsley *et al.* (2011) did not detect *A. marginale* within endothelial cells after tick-feeding transmission to immunocompetent cattle either in dermal samples of tick attachment sites or in post- mortem tissues. In addition, they also did not observe seroconversion or clinical anaplasmosis in calves, when *A.*

marginale grown in the endothelial cell line (RF/6A) was used for the experiments. At the moment in vivo infection of endothelial cells remains controversial.

A. marginale enters erythrocytes by endocytosis and resides within small membrane-bound inclusions, referred to as initial bodies, where it divides by binary fission (Kocan *et al.,* 1978b). The membrane-bound vacuole derives from the erythrocyte membrane and can contain four to eight organisms. In acute anaplasmosis multiple infections of single erythrocytes are observed. *A. marginale* has rarely been observed free of erythrocytes. Interestingly treatment of cells with a calcium ionophore induced bacteria exit, suggesting a mechanism that is dependent on the mobilization of calcium (Brown *et al.,* 2006).

Clinical disease in cattle is directly related to the number of infected erythrocytes. During the initial infection, there is a geometric increase phase when the number of infected red blood cells doubles nearly every 24 h (Miller, 1956). In the acute phase up to 70 % of erythrocytes can be infected (Kieser *et al.,* 1990; Kocan *et al.,* 2010a), although the first symptoms can occur as soon as only 15 % of erythrocytes are infected. The incubation period varies with the number of organisms in the infective dose and ranges from 7 to 60 days (Kocan and de la Fuente, 2003).

During the course of infection, erythrocytes become chemically altered by the bacteria. Subsequently, marked erythrocytes are recognized by reticuloendothelial cells and removed from the circulation, which results in anemia and icterus (Kocan *et al.,* 2003).

The acute phase of the disease includes symptoms such as fever, weight loss, icterus, abortion, lowered milk production and even death (Kuttler, 1984). Differences in virulence between *Anaplasma* strains and the level and duration of the rickettsemia play a role in the severity of clinical manifestations. Although cattle of all ages can become infected with *A. marginale*, the severity of disease is age dependent. Calves under 6 months of age are much more resistant to disease (although not infection) than older cattle. In older calves mild or acute, but rarely fatal disease develops, while in cattle over 2 years of age, the disease often is fatal (Kocan *et al.,* 2003). Cattle that recover from anaplasmosis remain lifelong carriers serving as a reservoir of the rickettsia (Kieser *et al.,* 1990). During the carrier state, the rickettsemic cycles occur at approximately seven weeks intervals, with peaks of 107 rickettsia per ml of blood (Eriks *et al.,* 1993; French *et al.,* 1998; Kocan *et al.,* 2010b). The chronically infected cattle are generally immune

to further clinical disease; however, they can relapse to anaplasmosis, for example when infected with other pathogens.

2.6. Differences within strains

Initially a small number of *A. marginale* strains was recognized on the basis of morphological characteristics, geographical origin, whether they were cross- protective in cattle or infectious and transmissible by ticks. Presently strains are characterized not only on the basis of the above characteristics, but additionally by either level of virulence or variation in membrane surface proteins (MSPs).

2.6.1. Morphology

Two morphological forms of *A. marginale* are known, one with and one without an inclusion appendage. Inclusion appendages, are also called "tails", "bands" or "filaments". They usually occur in the form of a tapering tail, a loop, a disk or a ring, and can only be visualized through immunological or ultrastructural techniques. With traditional staining, only the “head portion” of the tailed *Anaplasma* is visible (Carson *et al.,* 1974).

The inclusion appendages observed under the electron microscope are not directly attached to the bacterium and are not surrounded by an inclusion membrane (Kocan *et al.,* 1984). In cattle erythrocytes, the tailed strain appears as a spherical marginal body, or as a “comet – shaped” organism, which contains a head, body and tail. In *D. andersoni* nymphs the inclusion appendages were observed in midgut tissues till 10 days after repletion from infected cows. Following day 15, appendages were found free in the midgut lumen or attached to the cell membrane of midgut epithelial cells (Kocan *et al.,* 1984).

The tails are composed of polymerized F-actin filaments with a diameter of 7-10 nm and contain no parasite DNA (Stich *et al.,* 1997). Ferritin-conjugated anti-*A. marginale* sera react with bacterial-specific antigens, indicating that appendages are recognized by the host’s immune system (Kocan *et al.,* 1978a; Kocan *et al.,* 1978b). Stich *et al.* (1997) have also shown that the inclusion appendage contains host actin filaments. Interestingly, unlike the classic pattern in which actin is assembled on the bacterial surface, the *A. marginale*-associated appendage assembles on the external vacuolarsss surface and does not have to be secreted across the bacterial membrane and the membrane surrounding the

parasitophorous vacuole. A new polymorphic appendage-associated protein has been identified, designated as *A. marginale* appendage associated protein (AAAP), however, its role in invasion or replication within the host cell is still unknown (Stich *et al.,* 2004).

The function of the *A. marginale* appendage in the infection of ticks and erythrocytes is unclear. At first, Kocan *et al.* (1984) suggested that the appendage may play a role in infection of tick gut cells, as they observed that only the tailed Virginia isolate, and not the Florida isolate (without appendage), infected *D. andersoni* ticks. Two years later, Smith *et al.* (1986) verified that an inclusion appendage is not responsible for infectivity, as only one of the two tailed *Anaplasma* strains tested, was readily transmitted by *D. variabilis* ticks. In some viruses and bacteria, the appendage has been shown to influence motility, which improve their propagation and enhance the spread of infection (Cossart and Lecuit, 1998). Stich *et al.* (1997) suggested that appendage increase *A. marginale* motility enhancing contact with the tick gut epithelium or bovine erythrocytes.

Subtropical Interestingly, *A. marginale* with an appendage was initially named *Anaplasma caudatum* (*caudatum* - tailed) (Boone *et al.,* 2005; Kreier and Ristic, 1963). Moreover, in 1974 the creation of a new genus *Paranaplasma* has been proposed, due to serological and immunological differences between isolates with and without an appendage (Kreier and Ristic, 1974). Nonetheless, according to the work of Smith *et al.* (1986) this classification has been abandoned and presently *A. marginale* with an inclusion is not considered a separate species.

2.7 Prevalence of Anaplasmosis

Belal *et al.* (2015), discussed an epidemiological investigation was conducted to ascertain the prevalence of anaplasmosis in cattle in Sirajganj district of Bangladesh, during the period of December 2013 to November 2014, it was observed that the overall prevalence of anaplasmosis in cattle was recorded as 25.82% and the prevalence was higher in female (28.88%) than male (19.20%). All crossbred cattle was showed higher prevalence than local cattle. The prevalence of anaplasmosis infection was noticed as the highest in the rainy season (30.68%) in relation to summer (27.50%) and winter (15.15%) season.

Sajid *et al.* (2014), conducted A cross-sectional survey in 836 cattle {Bos *(B.) indicus* and *B. taurus* × *B. indicus*} and 700 buffaloes *(B. bubalis bubulus)* (n=1536) of district

Khanewal, Punjab, Pakistan, in order to determine the epizootiology of anaplasmosis through conventional optical microscopy of Giemsa's stained blood films. With an overall prevalence of 4.17%; the distribution of anaplasmosis was higher in buffaloes than cattle, calves than adults, females than males.

Babesiosis and anaplasmosis are important tick borne diseases (TBDs) of farm animals. Babesiosis is a haemoprotozoan and anaplasmosis is a haemobacterial infection of cattle (Dumler *et al.,* 2001). Both diseases have a serious economic impact due to obvious reason of morbidity and mortality, decreased production and lowered working efficiency, and have been reported in Bangladesh (Chowdhury *et al.,* 2006; Karim *et al.,* 2012; Siddiki *et al.,* 2010; Talukdar and Karim, 2001).

The agro-ecological and geo-climatic conditions of Bangladesh are highly favorable for growth and multiplication of ticks which act as natural vectors of babesiosis and anaplasmosis. Though several species of Babesia and Anaplasma are involved in the occurrence of babesiosis and anaplasmosis, commonly bovine babesiosis is caused by the hemoprotozoa *Babesia bovis* and *Babesia bigemina* and anaplasmosis is caused by the *Anaplasma marginale* and *Anaplasma central* (Dumler *et al.,* 2001; Lucimar *et al.,* 2014). The most important biological vector for these four agents is the tick Rhipicephalus (Boophilus)

Rahman, *et al.* (2015), conducted that, A cross-sectional survey was conducted in randomly selected 400 cattle at two upazilas of Rangpur district in Bangladesh, to estimate the prevalence and identify the risk factors of Babesia and Anaplasma infections. The overall prevalence of Babesia and Anaplasma infections were 1.5% and 3.5%, respectively. The prevalence of Babesia infections recorded in Gangachara and Pirgacha upazilas were 1.3% and 1.7%, respectively while it was 3.8% and 3.3%, respectively for Anaplasma infection. Insignificantly higher prevalence of both infections was recorded in crossbred cattle than those of indigenous cattle. Female cattle had insignificantly higher infection (3.8%) with Anaplasma than the male cattle (2.3%) while no infection with Babesia was found in any male cattle. None of the calves (≤1 yr) had infection with either organism. However, infection with both organisms was more prevalent in young cattle (>1-2.5 yr) than those of adult cattle (>2.5 yr).

Alim *et al.* (2012) studied that, one year (2009-10) prevalence study on hemoprotozoan diseases was conducted in crossbred and indigenous cattle, Chittagong, Bangladesh.

Blood samples were collected randomly from 216 crossbred and 432 indigenous cattle of four representative areas in three consecutive seasons. Samples were examined by Giemsa's stained blood smear method. The overall prevalence of hemoprotozoan diseases was 16.18 and 12.02% in crossbred and indigenous cattle, respectively where babesiosis and anaplasmosis were predominant. Babesiosis was found to be consistent in all the four different areas but highest prevalence (9.25%) was found in hilly area.

The justification for this study was the paucity of epidemiological data on TBD pathogens in the country. The results presented in this chapter show that all the TBDs investigated are prevalent in the three provinces. *T. mutans* and *Anaplasma spp* are the most prevalent tick-borne pathogens of cattle in Central and Eastern Zambia, with *Ba. bigemina* being the least prevalent. *T. parva*, *Ba. Bovis*., *Ba. bigemina, Anaplasma marginale* and *E. ruminantium* are known to cause diseases of economic importance in cattle in Zambia and most of sub-Saharan Africa (Makala *et al.* 2003; Minjauw & McLeod 2003).

Kamani *et al.* 2010, conducted the prevalence and significance of hemoparasites of cattle from north-central Nigeria was determined using diagnostic records from Parasitology Division, National Veterinary Research Institute (NVRI) Vom, from May 2006 to April 2008. A total of 637 blood samples from cattle from four states (Plateau, Bauchi, Nasarawa and Kaduna) of Nigeria in anticoagulant were submitted to the laboratory for parasitological diagnosis. An overall prevalence of 25.7% was recorded for all samples examined. Babesia bigemina and B.bovis accounted for 16.0%, followed by *Theileria mutans* (3.1%*), Trypanosoma spp (T.vivax and T. congolense)* (2.8%), *Anaplasma marginale* (1.9%), Microfilaria (1.4%).

Atif, *et al.* 2012, conducted this study was to investigate the prevalence and distribution of tick-borne diseases in cattle in Sargodha district, Pakistan. Microscopic examination of the Giemsa stained blood smears revealed an overall prevalence of haemoparasites as 26.86%. Anaplasma marginale was the most prevalent (9.71%) hemoparasite of cattle followed by Theileria annulata (6.86%) and Babesia bigemina (6.57%), respectively. Crossbred cattle (29.1%) were more susceptible to tick-borne diseases (TBDs) as compared to the indigenous cattle (17.7%). Sex wise prevalence indicated that female animals (26%) were more prone to TBDs than males (17%). The prevalence of tick-

transmitted diseases was higher in small holders (31.3%) than large livestock farms (17.5%).

Alonso *et al*. 1992, studied the vector for the whole region is the common cattle tick, *Boophilus microplus*, though in Mexico it shares this role with *Boophilus annulatus. B. microplus* is also able to transmit *A. marginale*. However, in some regions of Latin America, the geographical distribution of anaplasmosis is more widespread than presence of Boophilus, indicating the existence of other vectors. A great variety of haematophagus Diptera, ticks other than *B. microplus* and rural farming practices were all factors involved in the transmission of *A. marginale*, but information relevant to these vectors is scarce for some zones in the region.

Siddiki1*, et al*. 2010, conducted that many crossbred cattle are available throughout Bangladesh, there are few original varieties of cattle localized in some areas of the country. The study showed that crossbred cattle and the RCC animals were infected at similar level by parasitic infestations. However, in the absence of proper management, RC cattle were generally resistant to some parasitic infections which were common in indigenous/crossbred cattle. It was also notable that in some areas the parasite prevalence was very low, indicating possible geographic factors responsible for parasite survival. This study is the first of its type to understand the prevalence of parasitic diseases in RCC breed of cattle which is thought to be the only indigenous cattle breed available in Bangladesh.

Jassem and Agaar, 2015, was designed to investigate the prevalence of bovine anaplasmosis among cattle from various areas in Wassit governorate; the investigation was performed on 184 blood samples collected from suspected cattle suffering from fever (41°C), severe anemia, pale mucus membrane, progressive emaciation and drop in milk yield, including 85 male and 99 female cattle, aged from < 1 year to > 2 years, the samples were collected during the period of October 2012 - April 2013 The study revealed that females were given higher percent of infection 14.14% than males 11.7%, there is no significant differences under $p > 0.05$ according to age groups and sex.

2.8 Therapeutic efficacy against Anaplasmosis

Nath *et al.* (2013), investigated the epidemiology and prevalence of common blood parasites of cattle, blood sample were collected three samples for each animal, from

clinically suspected febrile, anorectic, especially non responsive to antibiotic therapy, cattle at various areas of Sylhet district.

Kumar *et al.* (2015), described that, total of 168 cattle presented to Teaching Veterinary Clinical Complex, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar during the period of 3 months (July-September, 2014) with history of fever, anorexia, reduced milk yield and tick infestation were analyzed for prevalence of hemoprotozoan diseases using classical giemsa stained thin blood smear parasitological method. The most marked and common clinical signs reported in all the cases were severe anemia (hemoglobin=3-6 g/dl) and history of fever, followed by normal body temperature. Following treatment with oxytetracycline parenterally along with supportive therapy out of seven cases six got recovered without any side-effects.

Tilak Chandra Nath *et al.* (2013), investigated the epidemiology and prevalence of common blood parasites of cattle, blood sample were collected three samples for each animal, from clinically suspected febrile, anorectic, especially non responsive to antibiotic therapy, cattle at various areas of Sylhet district.

Tarun Kumar *et al.* (2015), Described that, total of 168 cattle presented to Teaching Veterinary Clinical Complex, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar during the period of 3 months (July-September, 2014) with history of fever, anorexia, reduced milk yield and tick infestation were analyzed for prevalence of hemoprotozoan diseases using classical giemsa stained thin blood smear parasitological method. The most marked and common clinical signs reported in all the cases were severe anemia (hemoglobin=3-6 g/dl) and history of fever, followed by normal body temperature. Following treatment with oxytetracycline parenterally along with supportive therapy out of seven cases six got recovered without any side-effects.

Tarek R. Abou-Elnaga *et al.* 2009, conducted the prevalence of bovine Anaplasmosis caused by *Anaplasma marginale* was studied in 360 cattle and buffaloes maintained at small holder's farmers at Matrouh governorate between months of June 2006 to July 2007. The incidence of this parasite by using microscopical examination was found to be 74 (37%) and 95 (59.3%) of the examined cattle and buffaloes, respectively. The naturally diseased cases subjected to chemotherapeutic treatment using L.A oxytetracycline (200 mg/kg b. wt) showed rapid recovery with an undetectable

parasitemia as measured by 28 days post-treatment. In conclusion, Anaplasmosis is a prevalent disease of bovine farms in the examined area.

Kuttler KL (1980), described that the treatment of anaplasmosis and babesiosis in cattle is practical and effective when given early in the course of infection, before the onset of severe anemia or neurologic disorders. The tetracyclines (usually oxytetracycline or chlortetracycline) are the only effective specific compounds approved for use against anaplasmosis in the United States. Oxytetracycline, at the rate of 6.6 to 11 mg/kg of body weight given one to three times IM or IV, effectively moderates the course of infection. Chlortetracycline administered orally in dosages as small a 1.1 mg/kg will prevent infection and in dosages ranging from 2.2 to 11 mg/kg for 30 to 90 days will eliminate carrier infection. Babesiosis in cattle is effectively treated by a large number of babesiacidal compounds, but in practice, diminazene aceturate (3 to 5 mg/kg, IM), amicarbalide (5 to 10 mg/kg, IM), and imidocarb (1 to 3 mg/kg, IM) are most often used.

CHAPTER 3

MATERIALS AND METHOD

CHAPTER 3

MATERIALS AND METHODS

3.1. Study area

On the basis of animal population, Rayganj and Ullahpara upazila under the district of Sirajganj was selected for study. Location of research site was shown in map (Fig. 1, 2 and 3). This study was carried out to detect the prevalence of anaplasmosis in cattle. The samples were collected from the study area and brought at parasitology laboratory of Bangladesh Livestock Reserch Institute (BLRI), Savar, Dhaka for laboratory diagnosis.

3.2. Study period

The study was carried out for a period of six month starting from July 2016 to December 2016.

3.3 Study design:

The study design was based under laboratory examination.

3.4. Sampling techniques and sample size

During the study period, a total of 150 blood samples were collected from clinically ill and suspected cattle.

To ensure better condition during sample collection the following precautions were taken.

- Collected fresh blood sample directly from jugular vein
- Kept in vial containing EDTA.
- Stored in ice box
- Preserved in 4-8^0 C

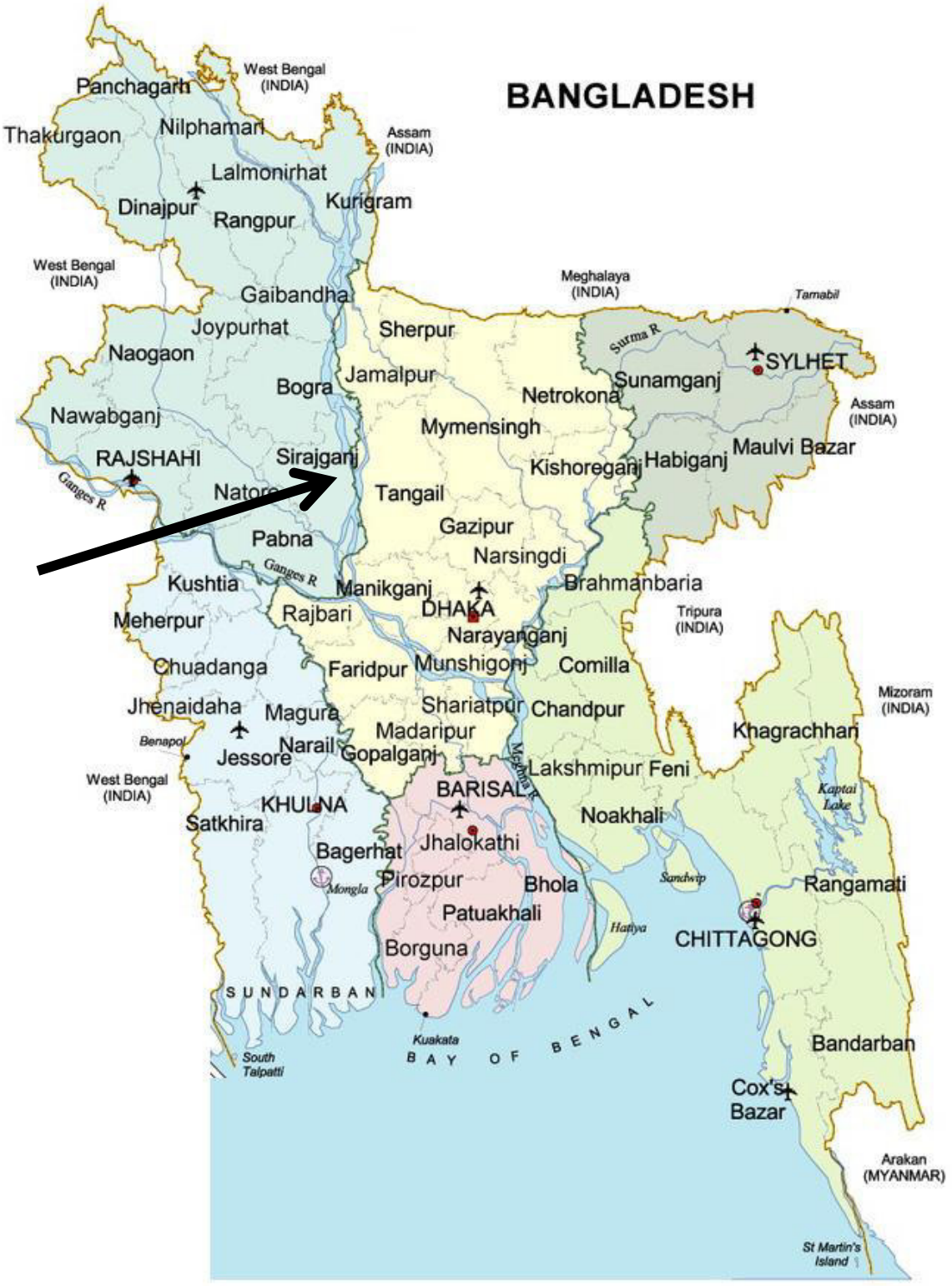

Fig. 1: Map of Bangladesh showing the location of Sirajgonj district (arrow marking).

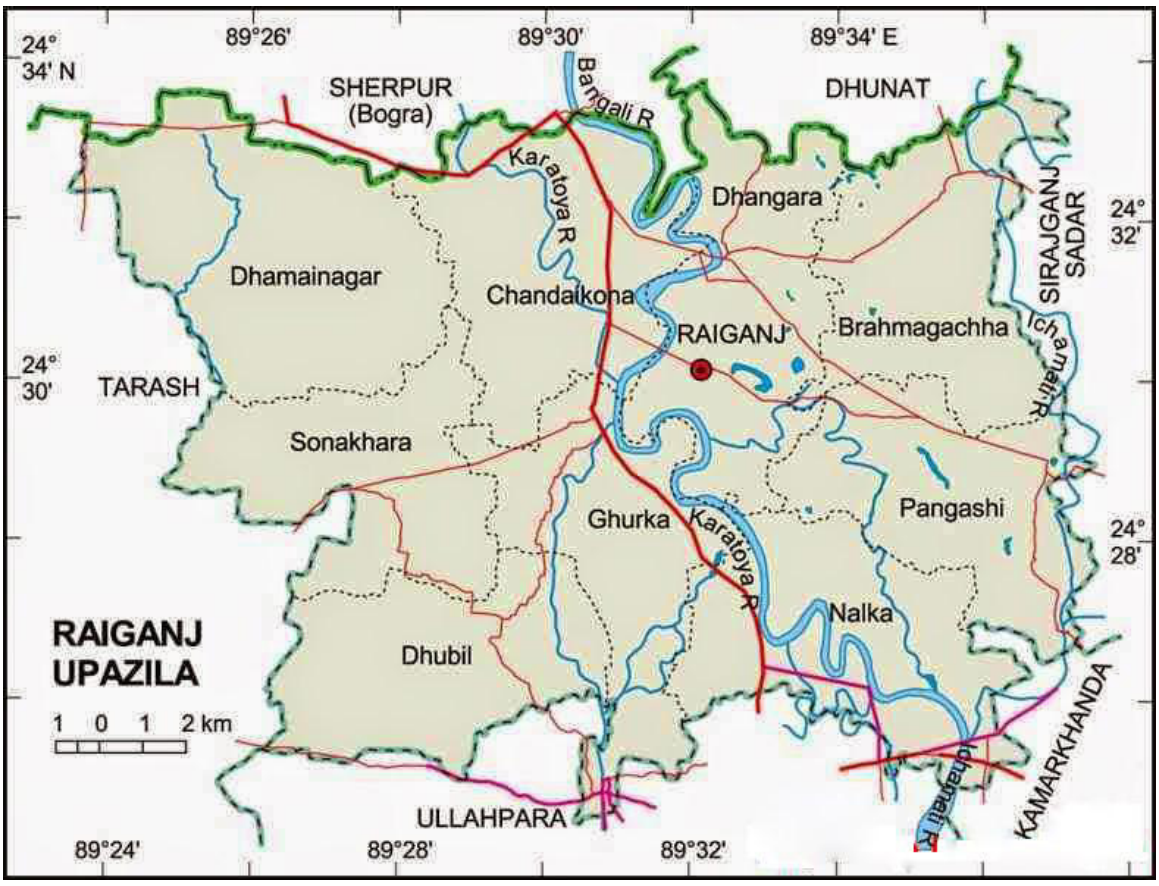

Fig. 2: Map of Sirajgonj district showing the location of Rayganj Upazila (Dot marking).

Fig. 3: Map of Sirajgonj district showing the location of Ullahpara Upazila (Dot marking).

3.5. Study layout

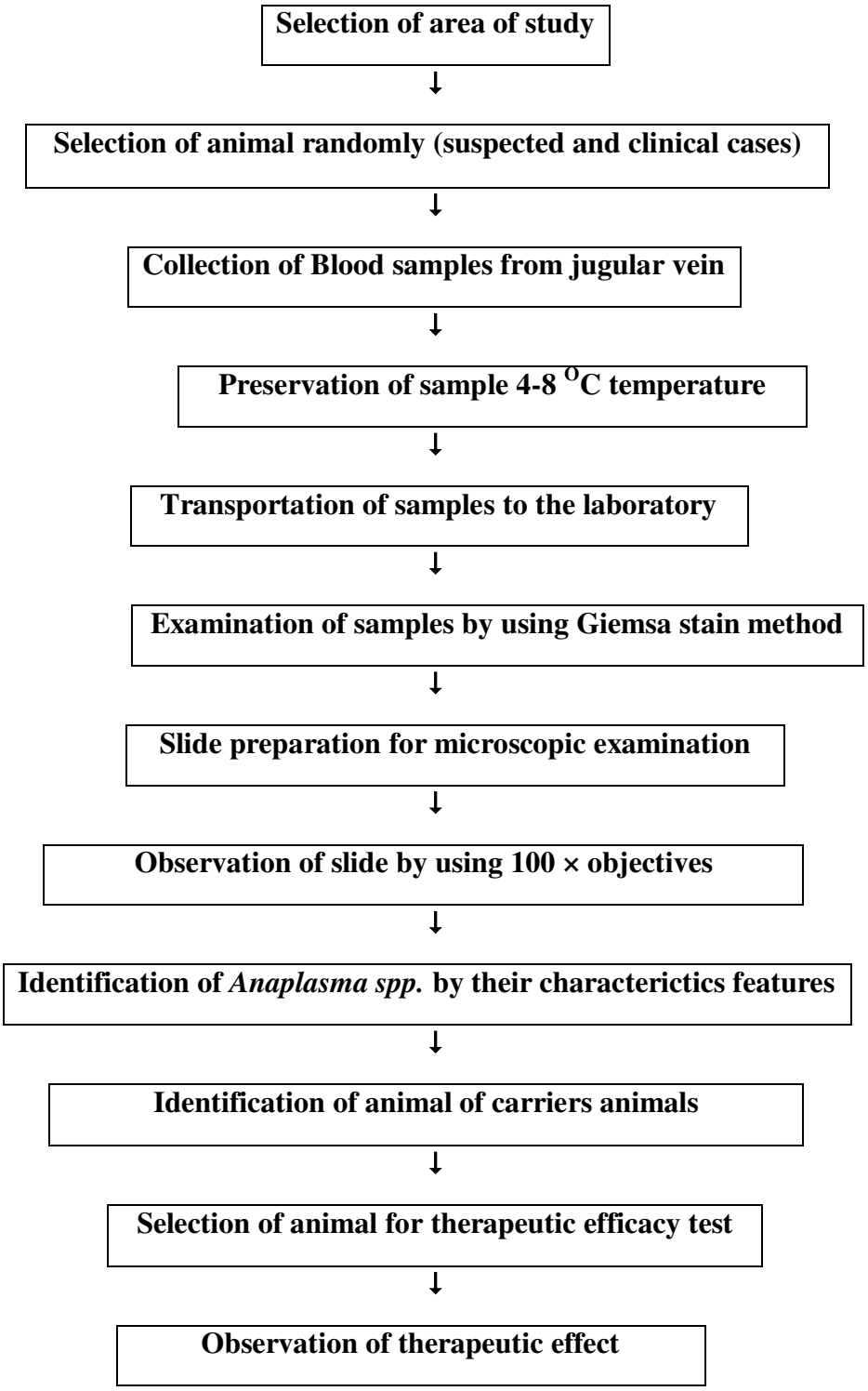

Fig. 4: Flow chart of Study layout.

3.6. Methods of diagnosis

The history and physical examination of each of the patient was carried out for diagnosis of Anaplasmosis in cattle.

3.6.1. History taking

History of patient was-

(a) Date of examination,

(b) Signalment (client and patient) identification

(c) Chief complaint,

(d) Patient illness,

(e) Past medical history was included.

In addition, the complete medical histories like

(a) Family medical history

(b) Vaccination history,

(c) Travel history,

(d) Diet history,

(f) Environmental history,

(g) Birth history

(h) Potential source of intoxication were investigated.

3.6.2. Physical examination

Physical examination was done by visual inspection, recording pulse and respiration rate and rectal temperature. Examination of the different organs and systems of the body was carried out by using the clinical methods of palpation, percussion and auscultation.

3.6.3. Sample collection and Examination

The blood samples of individual animal were collected. About 3-5 ml of samples from the jugular vein of the clinically suspected and ill animals were collected and put in a vial containing EDTA and transported to the BLRI laboratory, Savar, Dhaka-1341 in ice bags for microscopic examination following the method of Adam, Paul and Zaman (1971). Than a thin blood smear was prepared from each blood sample, air dried and fixed in methanol for 2-3 minutes. Staining was done in 10 % Giemsa's stain and rinsing was performed in two changes of distilled water buffered to pH 7.2, then examined under

microscope (100x) with immersion oil for the identification of blood parasites as described by Soulsby (1982).

Fig. 5: Vials containing collected blood samples

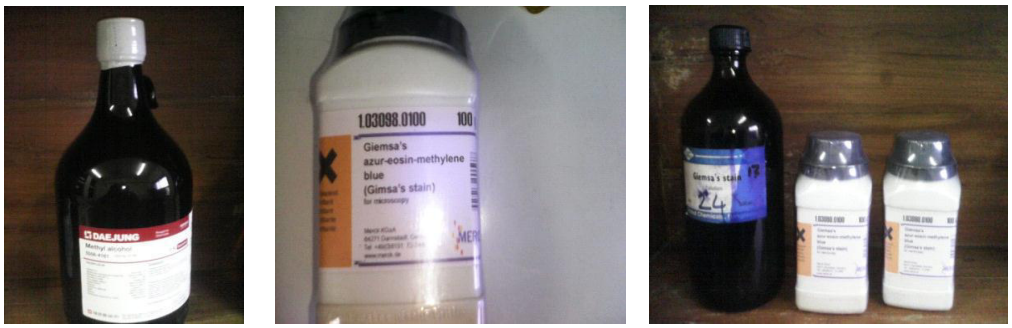

Fig. 6: Bottle Containing Giemsa's Staning Solution

3.7. Preparation of Giemsa Working Solution

3.7.1. Introduction

A freshly prepared working solution of Giemsa, made from well-prepared stock and diluted with water buffered to pH 7.2 is recommended to achieve optimal staining of protozoal blood films. Giemsa stock solution procured for national programmes was standardized to minimize frequent adjustments to SOPs on staining.

Staining with Giemsa stain was done with a rapid (10 % working solution) method or a slow (3 % working solution) method. The rapid method was used in outpatient clinics and busy laboratories where a quick diagnosis was essential for patient care. The slow method was used for staining large numbers of slides, such as those collected during cross-sectional or epidemiological surveys and field research.

Some laboratories prefer to stain slides individually, even if there are relatively large batches, as this economizes the amount of Giemsa stain required. The volume of working Giemsa stain solution that was required, particularly for staining individual slides, should be determined precisely to obviate preparation of a large volume that may be used over a long time and possible wasted.

3.7.2. Supplies, Materials and Equipment

For 10% Giemsa working solution:

- Giemsa stain, transferred and filtered from the stock solution into a 25 or 50 mL bottle;
- buffered water, pH 7.2;
- a beaker or tube, clean, 5–10-mL capacity;
- a Pasteur pipette and
- Whatman filter paper, grade 1.

For 3% Giemsa working solution:

- Giemsa stain, transferred and filtered from the stock solution into a 25- or 50-mL bottle;
- buffered water, pH 7.2;
- a measuring cylinder, clean, 100–500-mL capacity;
- a Pasteur pipette and
- Whatman filter paper, grade 1

3.8. Preparation of blood smear

Firstly taking of sterilized slide

↓

Then Clean with fresh cotton

↓

Taking one drop of blood on slide

↓

Finally preparation of smear by another sterilized slide

↓

Then air dried

Fig. 7: Preparation of blood smear

Fig. 8: Collection of blood from cattle

Fig. 9: Working in Biosafety Laboratory

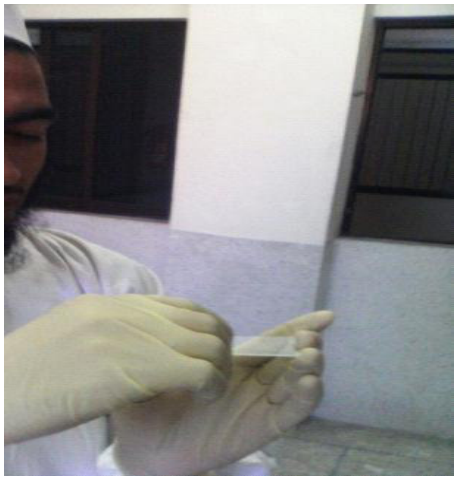

Fig. 10: Cleaning with Cotton

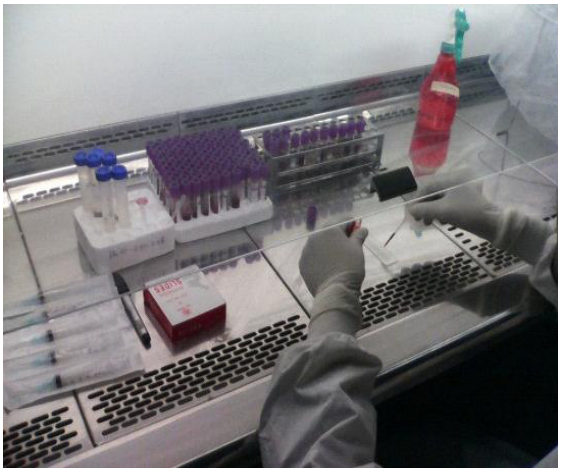

Fig. 11: Taking one drop of blood on grease free slide

Fig. 12: Preparing blood smear

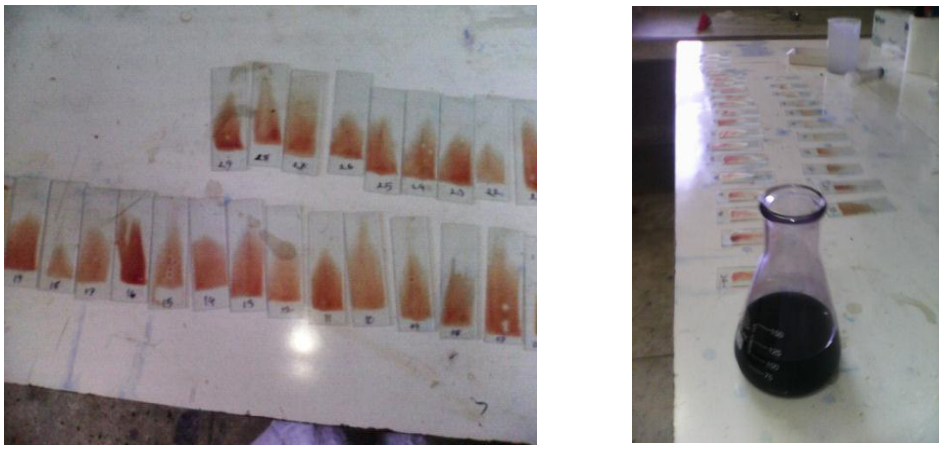

Fig. 13: Addition of methanol and air dried

Fig. 14: Addition of Giemsa stain

3.9. Procedure

Flow Chart	Description of Activity
A. Preparation of 10 mL of 10% of Giemsa working solution 1. 9 mL of buffered water into a tube was placed. 2. Then Giemsa stock solution was filtered through paper Whatman 1 and transfer to a 25 to 50 mL container. 3. Than added 1 mL of Giemsa stock solution. 4. The stain was used within 15 min of preparation, and discarded unused stain.	**A. Preparation of fresh 10% Giemsa working solution from stock solution for rapid staining of a few slides.** About 3 mL of stain were required for each slide with a blood film. • Placed 9 mL of previously prepared buffered water, pH 7.2, into a clean beaker or tube. • Filtered the Giemsa stock solution through paper Whatman #1 and transfer to a 25 to 50 mL container. • Used a clean, dry pipette, added 1 mL of Giemsa stock solution. Did not take the aliquot from the large bottle containing the Giemsa stock solution, to avoid contaminating it. • Prepared the Giemsa working solution just before staining the blood film, and used it within a maximum of 15 minutes of preparation. Discarded any unused stain.
B. Preparation of 100 mL of 3% Giemsa working solution 1. Placed 97 mL of buffered water into a measuring cylinder. 2. Filtered the Giemsa stock solution through paper Whatman 1 and transfer to a 25 to 50 mL container. 3. Added 3 mL of Giemsa stock solution. 4. Used stain within 15 min of preparation, and discard unused stain.	**B. Preparation of fresh 3% Giemsa working solution from stock solution for slow staining of a batch of 20–100 slides** • Placed 97 mL of previously prepared buffered water, pH 7.2, into a clean measuring cylinder. • Filtered the Giemsa stock solution through paper Whatman 1 and transfer to a 25 to 50 mL container. • Used measuring cylinder or a pipette, measure 3 mL of Giemsa stock solution. Did **not** take the aliquot directly from the large bottle containing the Giemsa stock solution, to avoid contaminating it. • Prepared the Giemsa working solution just before staining the blood film, and used it within a maximum of 15 minutes of preparation. Discarded any excess stain.

Fig. 15: Microscopic examination of slide.

3.10. Giemsa Staining of Blood Smears and Estimation of Parasitemia

3.10.1. Reagents:

- Giemsa stain
- 100% Methanol
- Microscope
- Immersion Oil

3.10.2. Protocol:Giemsa Staining of Blood Smeared Slide

1) The slides were fixed in 100% methanol for ~30 min.

2) Then air dried.

3) Made up a fresh solution of 10% Giemsa stain in distilled water.

4) Stained ~45 min

5) Rinsed off slide in tap water and dry thoroughly using bibulous paper to dab.

Estimation of Parasitemia

6) Air dried.

7) The slide was viewed under oil immersion with a 100x objective.

8) The parasitemia was estimated by counting the number of infected cells. A 10x10 grid square in the eyepiece of the microscope facilitated the procedure as an even blood smear yields ~100 red blood cells per 10x10 grid. Thus for example, 8 infected blood cells in a 10x10 grid is ~8% parasitemia. Several fields (~10) was counted and the average taken to obtain a representative estimate of the total parasitemia.

3.10.3. Performance Characteristics

Nuclei were varied shades of purple. Cytoplasmic staining was varied shades of blue to light pink. Fine reddish to lilac granules was present in cytoplasm of some cell types. Basophils was demonstrated dark blue black granules in the cytoplasm. Eosinophils was demonstrated a bright orange granules in the cytoplasm. Red blood cells was pink to orange.

3.11. Therapeutic procedure

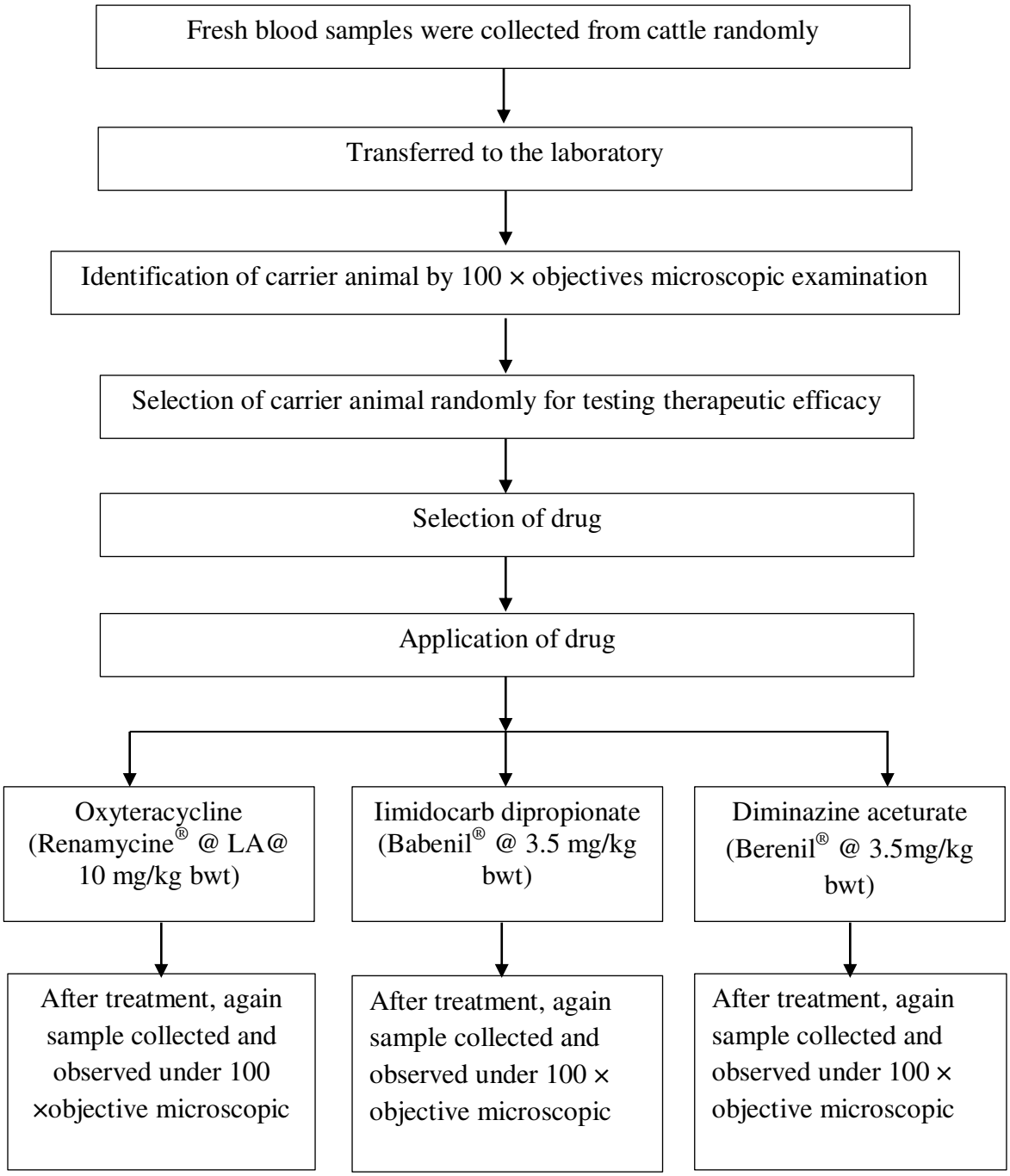

Fig. 16: Therapeutic procedure

3.12. Statistical analysis

The experimental data were recorded and analyzed statistically by using SPSS program (version 20). The Multivariate analysis was done following the strategy described by Hosmer & Lemeshow (1989). Later, a backward elimination procedure was applied and statistical significance of individual variable was tested using likelihood-ratio test (Dohoo *et al.,* 2003) with a p value ≤ 0.05. The prevalence of anaplasmosis infections in cattle was estimated through using formula denoted by Thrusfield, 1995.

CHAPTER 4

RESULTS

CHAPTER 4

RESULTS

4.1. Overall prevalence of anaplasmosis in cattle

The prevalence of anaplasmosis in cattle at Rayganj and Ullapara upazila of Sirajganj district was shown in Table 1. The overall prevalence of anplasmosis in Sirajganj district was 18.67% and among two upazila the heighest prevalence of anaplasmosis were found in Ullahpara (21.43%) than raygonj upazila which was not statistically significant.

Table 1. Overall prevalence of anaplasmosis in 150 cattle in Sirajganj district and their proportional prevalence rate in Raygonj and Ullahpara Upazila

Upazilla	**No. of cattle tested**	**Positive case**	**Prevalence (%)**
Rayganj	80	13	16.25
Ullapara	70	15	21.43
Overall	150	28	18.67
P-value (Chi-square test)			0.417
Level of significance			NS

NS means ($p>0.05$)

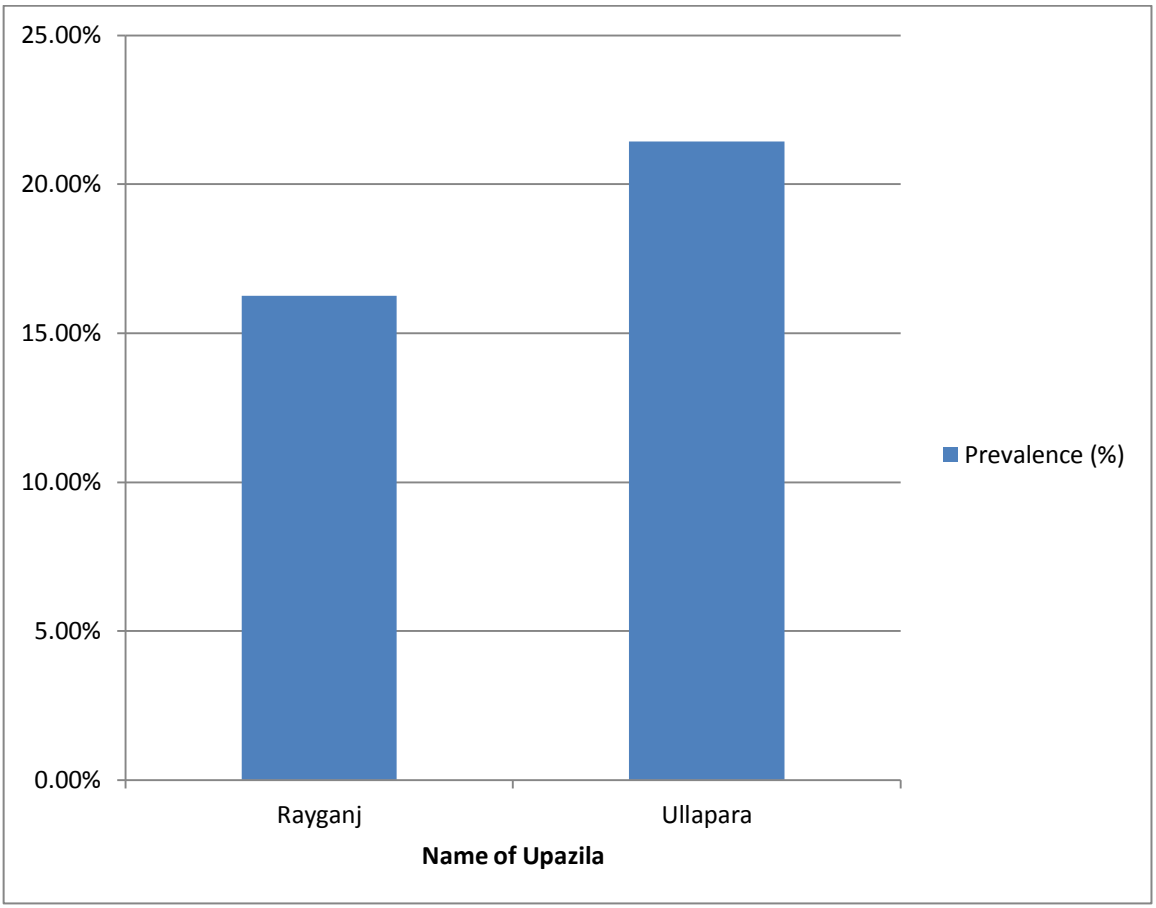

Fig. 17: Overall prevalence of anaplasmosis in cattle in Sirajganj district

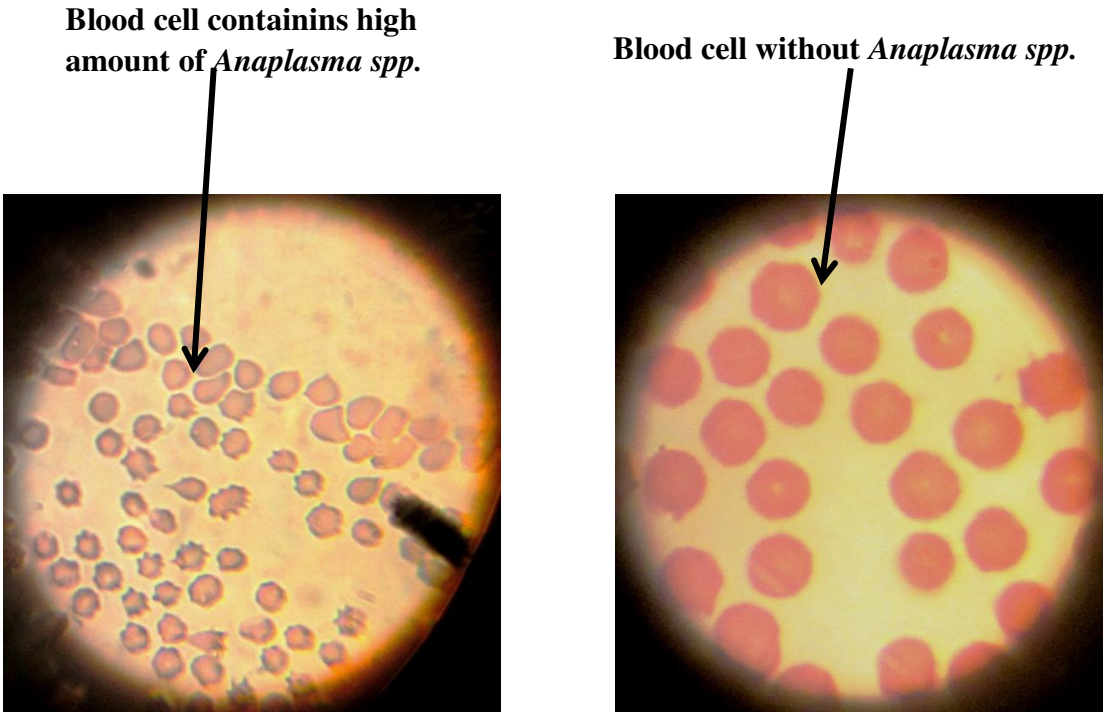

Fig. 18: Microscopic examination of blood smear containing high amount of *Anaplasma spp.* (100 × objective).

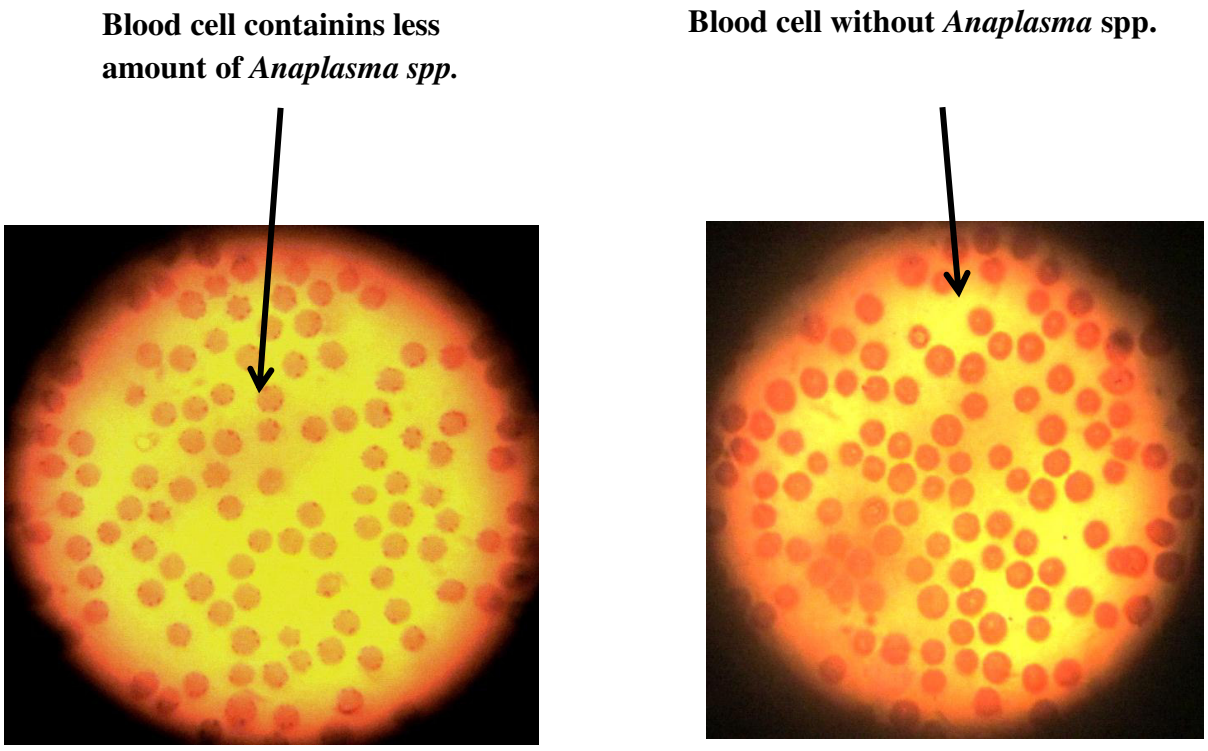

Fig. 19: Microscopic examination of blood smear containing less amount of *Anaplasma spp.* (100 × objective).

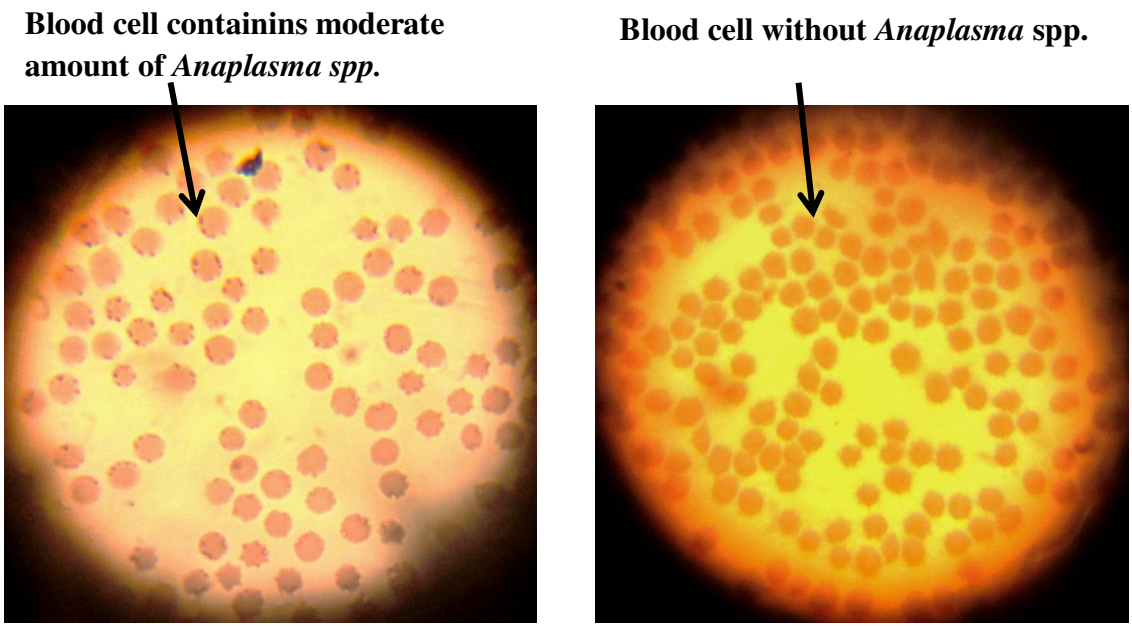

Fig. 20: Microscopic examination of blood smear containing moderate amount of *Anaplasma spp.* (100 × objective).

4.2. Age-wise prevalence of anaplasmosis in cattle

Age-wise prevalence of anaplasmosis in cattle was shown in table 2. The highest prevalence rate (31.7%) was significantly found in the 2-3 years age group followed by (16.67%) in above 3 years and lowest (10.20%) in 6 months – 2 years of age group.

Table 2. Age-wise prevalence of anaplasmosis in cattle

Age group	**No. of cattle tested**	**Positive case**	**Prevalence (%)**
6 months-2 years	49	5	10.20
2-3 years	41	13	31.7*
Above 3 years	60	10	16.67
Total	150	28	18.67
P-value (Chi-square test)			0.029
Level of significance			*

* Significant at 5% level of significance

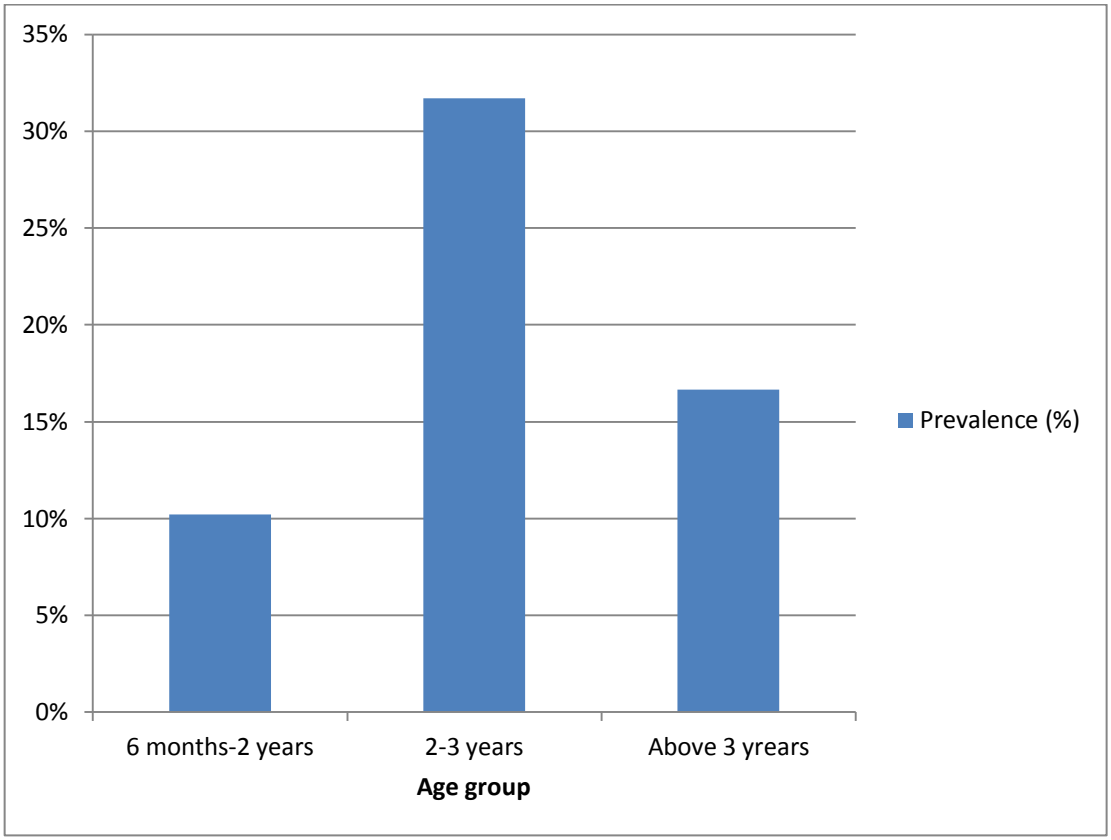

Fig. 21: Age-wise prevalence of anaplasmosis in cattle

4.3. Sex-wise prevalence of anaplasmosis in cattle

Sex-wise prevalence of anaplasmosis in cattle was shown in table 3. The heighest rate of prevalence was reported in female (20.99%) than male (15.94%) and was not statistically significant.

Table 3. Sex-wise prevalence of anaplasmosis in cattle.

Sex group	**No. of cattle tested**	**No. of positive case**	**Prevalence (%)**
Male	69	11	15.94
Female	81	17	20.99
Total	150	28	18.67
P-value (Chi-square test)			0.43
Level of significance			NS

NS means ($p>0.05$)

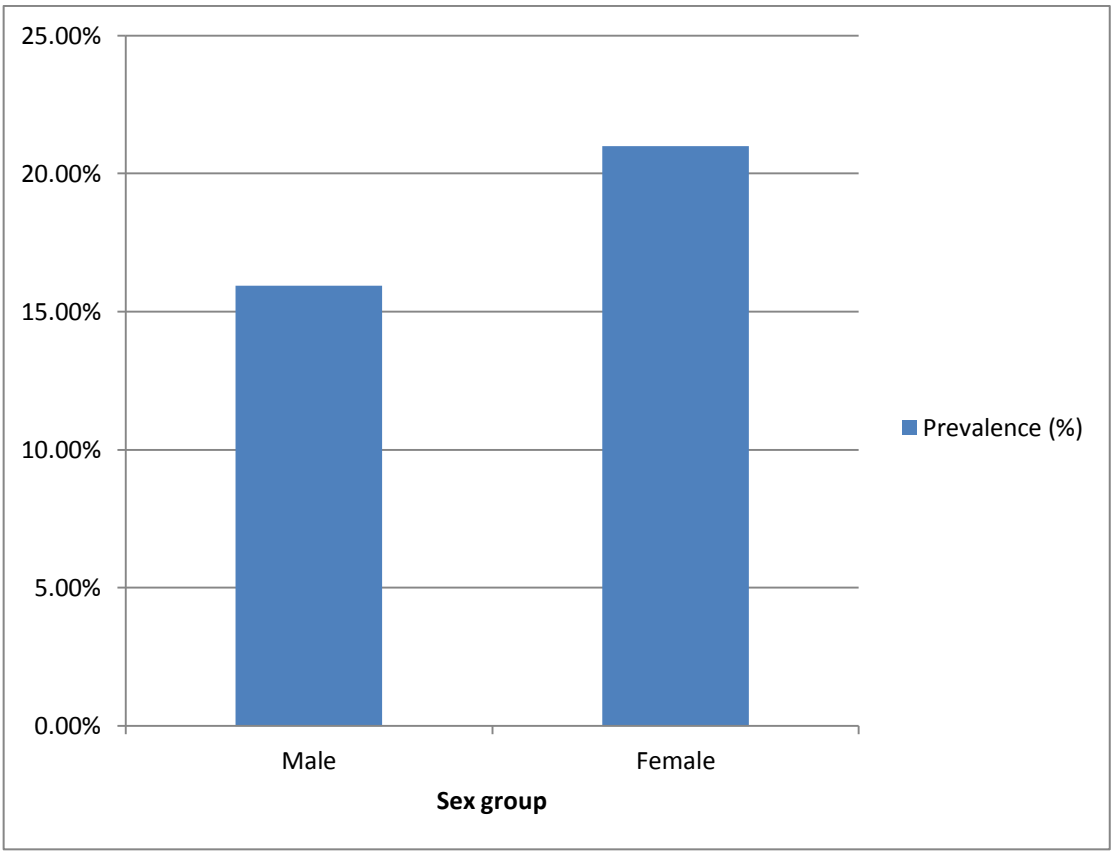

Fig. 22: Sex-wise prevalence of anaplasmosis in cattle

4.4. Breed-wise prevalence of anaplasmosis in cattle

Breed-wise prevalence of anaplasmosis in cattle was shown in table 4. The highest prevalence was recorded in cross breed (19.5%) followed in local (17.8%) and the variation was not statistically significant.

Table 4. Breed-wise prevalence of anaplasmosis in cattle

Breed	**No. of cattle tested**	**No. of positive case**	**Prevalence (%)**
Local/Indigenous	73	13	17.8
Cross	77	15	19.5
Total	150	28	18.67
P-value (Chi-square test)			0.79
Level of significance			NS

NS means (p> 0.05)

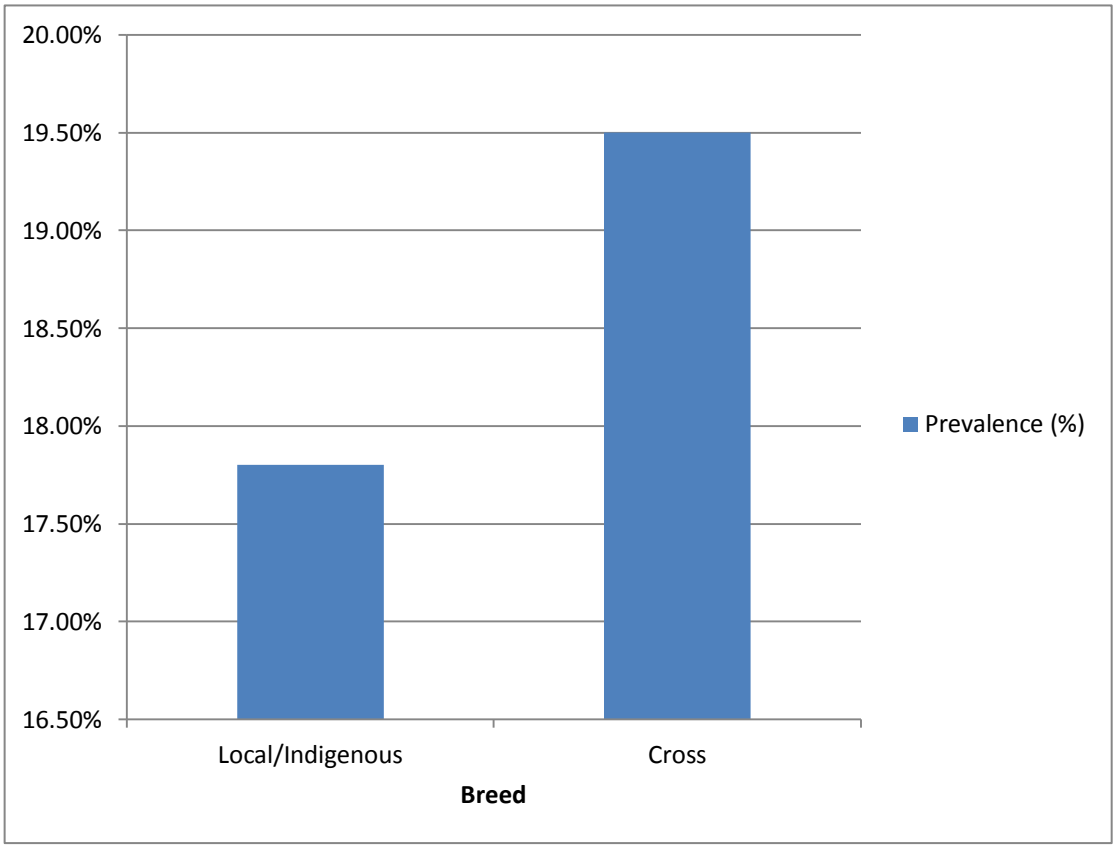

Fig. 23: Breed-wise prevalence of anaplasmosis in cattle

4.5. Therapeutic efficacy of Oxyteracycline

During the study period of 6 months the sample collection procedure was divided into 3 periods. In first period on June to July 2016 a total of 50 samples from 50 suspected animals were collected and tested. Among them, 8 animals were positive for *Anaplasma spp.* of which 5 animals were selected for therapeutic efficacy test. The selected animals were treated with Oxyteracycline (Renamycine® LA 10 mg/kg bwt). After 7 days of treatment results showed that four animals negative for *Anaplasma spp.* by 100 × objective microscopic examination.

Table-5. Efficacy of Oxyteracycline against anaplasomosis in cattle

Drug used	Animal number	Treatment Period			
		Day 0 (intensity before treatment)		Day 7 (intensity after treatment)	
Oxyteracycline (Renamycine® LA @10 mg / kg bwt)	A1	1	+	1	-
	A3	2	+++	2	+
	A12	3	++	3	-
	A20	4	++	4	-
	A46	5	+	5	-

Catagorization of infection: Thick blood films were categorized by plus system suggested in Basic Laboratory Methods in Medical Parasitology, by WHO (1991).

+ (Light)1-10 parasites per 100 thick blood film fields.

++ (Moderate) 11- 100 parasites per 100 thick blood film fields

+++ (Heavy) 1-10 parasites per thick blood film field

4.6. Therapeutic efficacy of Iimidocarb dipropionate

In second period on September to October in 2016 a total of 50 samples from 50 suspected animals were collected and tested. Among them, 10 animals were positive for *Anaplasma spp.* of which 5 animals were selected for therapeutic efficacy test.The selected animals treated with Iimidocarb dipropionate (Babenil® @ 3.5 mg/kg bwt). After 7 days of treatment results showed that two animals negative for *Anaplasma spp.* by 100x objective microscopic examination.

Table-6. Efficacy of Iimidocarb dipropionate against anaplasomosis in cattle

Drug used	Animal number	Treatment Period			
		Day 0 (intensity before treatment)		Day 7 (intensity after treatment)	
Iimidocarb dipropionate (Babenil® @ 3.5 mg/kg bwt)	A61	1	+	1	-
	A63	2	++	2	+
	A71	3	+	3	-
	A83	4	+++	4	+
	A90	5	+	5	+

Catagorization of infection: Thick blood films were categorized by plus system suggested in Basic Laboratory Methods in Medical Parasitology, by WHO (1991).

+ (Light)1-10 parasites per 100 thick blood film fields.

++ (Moderate) 11- 100 parasites per 100 thick blood film fields

+++ (Heavy) 1-10 parasites per thick blood film field

4.7. Therapeutic efficacy of Diminazine aceturate

In third period on November to December in 2016 a total of 50 samples from 50 suspected animals were collected and tested. Among them, 10 animals were positive for *Anaplasma spp.* of which 5 animals were selected for therapeutic efficacy test.The selected animals treated with Diminazine aceturate (Berenil® 3.5mg/kg bwt)..After 7 days of treatment results showed that only one animal negative for *Anaplasma spp.* by 100 × objective microscopic examination.

Table-7. Efficacy of Diminazine aceturate against anaplasomosis in cattle

	Animal number	Treatment Period			
		Day 0 (intensity before treatment)		Day 7 (intensity after treatment)	
Diminazine aceturate (Berenil® @ 3.5mg/kg bwt)	A103	1	+	1	+
	A124	2	++	2	+
	A129	3	+	3	+
	A137	4	+	4	-
	A144	5	+++	5	++

Catagorization of infection: Thick blood films were categorized by plus system suggested in Basic Laboratory Methods in Medical Parasitology, by WHO (1991).

+ (Light)1-10 parasites per 100 thick blood film fields.

++ (Moderate) 11- 100 parasites per 100 thick blood film fields

+++ (Heavy) 1-10 parasites per thick blood film field

CHAPTER 5

DISCUSSION

CHAPTER 5

DISCUSSION

The prevalence obtained in this study was found relating to the stock density in different upazila (Report of DLS and statistical pocketbook, 2011). High number of cattle population with tick, high number of lowland/ flood plain based area/ ponds and veterinary practitioners in Ullaparas upazila favored the transmission of organism among animals probably causing high prevalence. The overall prevalence of anaplasmosis in this study 18.67 % supports the earlier report of Anaplasma infection in Bangladesh (Talukdar *et al.,* 2001) who was observed higher prevalence of anaplasmosis in cattle that was 33 % in Baghabari (Shahjadpur) Milk Shed Area. The occurrence of subclinical Anaplasma infection in 5.93 % cattle has been reported from Bangladesh (Samad *et al.,* 1989). The present study also support with the report of Arunkumar *et al.* (2013) who observed that the overall prevalence rate of *A. marginale* infection was 19.3 % in Chennai district of Tamil Nadu in India.

Anaplasma spp. can cause infections in bovine of all age categories where severity and mortality rate increases with augmentation of animal age (Richey 1984). In divergence with the current study, Khan *et al.* (2004) and Atif *et al.* (2012) found adult population more prone to bovine anaplasmosis. Age-wise occurrence of anaplasmosis recorded in this study support the report of Chakraborti (2002) who recorded animals over 3 years of age are highly affected by anaplasmosis. Observation of this study also supported by the findings of Kamani *et al.* (2010) who observed higher prevalence in adult than young cattle. Endemic instability of the study areas might responsible for frequent infections in adult cattle where newborn calves were protected by colostral immunity (Cynthia *et al.,* 2011). It is hypothesized that the strong innate immunity and age resistance of young cattle are responsible for their less vulnerability to tick infestation (Sarkar, 2007) and in such way, leads to less ectoparasitic burden.

In the present study, higher prevalence of Anaplasma infections in crossbreed cattle as compared to local cattle was found in agreement with the reports of Radostits *et al.* (2000). Variation in geoclimatic condition, breed, exposure of vectors and age of the animals might contribute to variable prevalence of hemoprotozoan diseases in the study areas Muhanguzi *et al.,* 2010). Constant exposure of infections and development of immunity against such infections might responsible for lower prevalence in indigenous

cattle (Siddiki *et al.,* 2010). On the contrary, more attention in the management of HF crossbred cattle gives less chance of pre exposure of vectors and develop no or less immunity, resulting frequent occurrence of such diseases (Chowdhury *et al.,* 2006; Ananda *et al.,* 2009; Siddiki *et al.,* 2010).

The report of Sarkar (2007) who reported the prevalence of ectoparasites were significantly higher in female than male. The prevalence of hemoprotozoan diseases in female cattle of this investigation showed uniformity with the report of Kamani *et al.* (2010). Higher prevalence in female cattle possibly due the fact that they were kept longer for breeding and milk production purpose, supplied insufficient feed against their high demand (Kamani *et al.,* 2010).

During therapeutic efficacy test, 1st group was treated with a single dose of oxytetracycline LA (10 mg / kg body weight). In 1st group four animal recovered completely, one moderately. This was related to (Urquhart *et al,* 1988) who used oxetetracycline for the treatment of anaplasmosis. In case of 2nd group complete recovery was recorded in two animal, moderate recovery in one animal. 2nd Group was treated with Iimidocarb dipropionate (Babenil® @ 3.5 mg/kg bwt) administered as a single dose. This study is in line with Chakrabarti, Production. India.(1996), McDougald and Edward, (1988) and Robertson, (1976), who also used Imidocarb at the rate of 5 mg/kg body weight. In case of 3rd group one animal recovered completely. One animal recovered moderately and three animals not response. 3rd group was treated with a single dose of Diminazene acceturate (3.5 mg / kg body weight). The findings are in accordance with Nasir (2000), who reported 55 percent efficacy. In contrast Muhammad *et al,* (1999) reported 93 percent efficacyof Buparvaquone and Oxytetracycline in Faisalabad, the difference may be due to the use of Oxytetracycline with Buparvaquine, While in India, Anonymous, (1993) reported 98.8 percent cure rate and Patil *et al*, (1995) recorded 80 percent cure rate.

CHAPTER 6

CONCLUSIONS

CHAPTER 6

CONCLUSIONS

On the basis of present findings the following conclusions may be inferred:

1. Blood samples collected from cattle reveals the highest prevalence of anaplasmosis were found at Ullahpara Upazila than Rayganj Upazila.
2. The prevalence of anaplasmosis varies in all age groups and the highest prevalence was observed above 2 to 3 years age group followed by moderate in above 3 years and less in 6 months to 2 years age group.
3. According to sex the prevalence of anaplasmosis was higher in female than male.
4. Cross breed animals were more prone to anaplasmosis infection than the local cattle.
5. Treatment with Oxytetracycline was most effective against anaplasmosis in cattle followed by moderate with imidocarb dipropionate and less effective by diminazene aceturate treatment.

Further investigation using modern serological and molecular techniques with large number of samples for the identification of carriers, tick vectors and particularly hematophagic flies are needed. Further study can be done for molicular charactarization (PCR) and other different drugs effect.

REFERENCES

REFERENCES

Abbott, J.R., Palmer, G.H., Kegerreis, K.A., Hetrick, P.F., Howard, C.J., Hope, J.C., Brown, W.C., 2005. Rapid and long-term disappearance of CD4+ T lymphocyte responses specific for Anaplasma marginale major surface protein-2 (MSP2) in MSP2 vaccinates following challenge with live A. marginale. Journal of immunology (Baltimore, Md. : 1950) 174: 6702-6715.

Adam, K. M. G., Paul, J., and Zaman, V., 1971. Medical and Veterinary Parasitology, Edinburgh & London: Churchill Livingstone.1st ed., 167-168.

Ahmed AKNU, 1976. Blood parasites of domestic animals in Bangladesh. Bangladesh Veterinary Journal, 10: 69-71.

Alim MA, S Das, K Roy, M Masuduzzaman, S Sikder, MM Hassan, AZ Siddiki and MA Hossain, 2012. Prevalence of hemoprotozoan diseases in cattle population of Chittagong division, Bangladesh. Pak Vet J, 32(2): 221-224.

Ananda KJ, PE Souza and GC Puttalakshmamma, 2009. Prevalence of Haemoprotozoan diseases in crossbred cattle in Banglore north. Veterinary World, 2: 15-16.

Anonymous, 1993. Tropical Animal Health and Production. India.

Anthony, D.W., Roby, T.O. 1962. Anaplasmosis studies with Dermacentor variabilis (Say) and Dermacentor andersoni Stiles (= D. venustus Marx) as experimental vectors. Proceedings of the 4th national anaplasmosis conference. In: Proceedings of the 4th national anaplasmosis conference, p. 78-81.

Arunkumar S and K Nagarajan, 2013. A study on prevalence of *Anaplasma marginale* infection among cattle population of Kancheepuram and in around Chennai district of Tamil Nadu. International Journal of Food, Agriculture and Veterinary Sciences, 3: 155-157.

Atif FA, MS Khan, F Muhammad and B Ahmad, 2013. Sero-epidemiological study of *Anaplasma marginale* among cattle. The Journal of Animal and Plant Sciences, 23: 740-744.

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

Baumgartner, W., Schlerka, G., Fumicz, M., Stoger, J., Awad-Masalmeh, M., Schuller, W., Weber, P., 1992. Seroprevalence survey for Anaplasma marginale-infection of Austrian cattle. Zentralblatt fur Veterinarmedizin. Reihe B. Journal of veterinary medicine. Series B 39: 97-104.

Blouin, E.F., Kocan, K.M., 1998. Morphology and development of Anaplasma marginale (Rickettsiales: Anaplasmataceae) in cultured Ixodes scapularis (Acari: Ixodidae) cells. Journal of medical entomology 35: 788-797.

Boone, D.R., Castenholz, R.W., Garrity, G.M., Brenner, D.J., Krieg, N.R., Staley, J.T., 2005. The Proteobacteria. Part C The Alpha,- Beta,- Delta-, and Epsilonproteobacteria. . Bergey's Manual of Systematic Bacteriology 2.

Boulanger, P., Ruckerbauer, G.M., Bannister, G.L., MacKay, R.R., Peter, N.H., 1971. Anaplasmosis: control of the first outbreak in Canada by serological identification and slaughter. Canadian journal of comparative medicine. Revue canadienne de medecine comparee 35: 249-257.

Brown, W.C., Norimine, J., Goff, W.L., Suarez, C.E., McElwain, T.F., 2006. Prospects for recombinant vaccines against Babesia bovis and related parasites. Parasite Immunology 28: 315-327.

Carreno, A.D., Alleman, A.R., Barbet, A.F., Palmer, G.H., Noh, S.M., Johnson, C.M., 2007. In vivo endothelial cell infection by Anaplasma marginale. Veterinary pathology 44: 116-118.

Carson, C.A., Weisiger, R.M., Ristic, M., Thurmon, J.C., Nelson, D.R., 1974. Appendage-related antigen production by Paranaplasma caudatum in deer erythrocytes. American journal of veterinary research 35: 1529-1531.

Chakrabarti, A (1996). A Text Book of Preventive Veterinary Medicine. 1st Ed.

Chakraborti A., 2002. A Textbook of Preventive Veterinary Medicine, 3rdedition, Kalyani Publishers, New Delhi. pp. 683.

Chowdhury S., Hossain M.A., Barua S.R., Islam S. 2006. Occurrence of common blood parasites of cattle in Sirajgoni Sadar of Bangladesh. Bangl. J. Vet. Med 4(2): 143–145.

Cossart, P., Lecuit, M., 1998. Interactions of Listeria monocytogenes with mammalian cells during entry and actin-based movement: bacterial factors, cellular ligands and signaling. The EMBO journal 17: 3797-3806.

Cynthia M, MA Kahn, S Line, E Susan and BS Aiello, 2011. Marck Veterinary Manual, Online Ed. Merck Sharp and Dohme Corp, a subsidiary of Merck and Co., Inc. Whitehouse Station, NJ, USA.

De la Fuente, J., Almazan, C., Blouin, E.F., Naranjo, V., Kocan, K.M., 2005a. RNA interference screening in ticks for identification of protective antigens. Parasitology research 96: 137-141.

De la Fuente, J., Garcia-Garcia, J.C., Blouin, E.F., Kocan, K.M., 2001a. Differential adhesion of major surface proteins 1a and 1b of the ehrlichial cattle pathogen Anaplasma marginale to bovine erythrocytes and tick cells. International journal for parasitology 31: 145-153.

De la Fuente, J., Garcia-Garcia, J.C., Blouin, E.F., Kocan, K.M., 2003. Characterization of the functional domain of major surface protein 1a involved in adhesion of the rickettsia Anaplasma marginale to host cells. Veterinary microbiology 91: 265-283.

De la Fuente, J., Garcia-Garcia, J.C., Blouin, E.F., Rodriguez, S.D., Garcia, M.A., Kocan, K.M., 2001b. Evolution and function of tandem repeats in the major surface protein 1a of the ehrlichial pathogen Anaplasma marginale. Animal health research reviews / Conference of Research Workers in Animal Diseases 2: 163-173.

De la Fuente, J., Garcia-Garcia, J.C., Blouin, E.F., Saliki, J.T., Kocan, K.M., 2002a. Infection of tick cells and bovine erythrocytes with one genotype of the intracellular ehrlichia Anaplasma marginale excludes infection with other genotypes. Clinical and diagnostic laboratory immunology 9: 658-668.

De la Fuente, J., Kocan, K.M., Garcia-Garcia, J.C., Blouin, E.F., Claypool, P.L., Saliki, J.T., 2002b. Vaccination of cattle with Anaplasma marginale derived from tick cell culture and bovine erythrocytes followed by challenge-exposure with infected ticks. Veterinary microbiology 89: 239-251.

De la Fuente, J., Lew, A., Lutz, H., Meli, M.L., Hofmann-Lehmann, R., Shkap, V., Molad, T., Mangold, A.J., Almazan, C., Naranjo, V., Gortazar, C., Torina, A., Caracappa, S., Garcia-Perez, A.L., Barral, M., Oporto, B., Ceci, L., Carelli, G., Blouin, E.F., Kocan, K.M., 2005b. Genetic diversity of anaplasma species major surface proteins and implications for anaplasmosis serodiagnosis and vaccine development. Animal health research reviews / Conference of Research Workers in Animal Diseases 6: 75-89.

De la Fuente, J., Massung, R.F., Wong, S.J., Chu, F.K., Lutz, H., Meli, M., von Loewenich, F.D., Grzeszczuk, A., Torina, A., Caracappa, S., Mangold, A.J., Naranjo, V., Stuen, S., Kocan, K.M., 2005c. Sequence analysis of the msp4 gene of Anaplasma phagocytophilum strains. Journal of clinical microbiology 43, 1309-1317.

De la Fuente, J., Naranjo, V., Ruiz-Fons, F., Hofle, U., Fernandez De Mera, I.G., Villanua, D., Almazan, C., Torina, A., Caracappa, S., Kocan, K.M., Gortazar, C., 2005d. Potential vertebrate reservoir hosts and invertebrate vectors of Anaplasma marginale and A. phagocytophilum in central Spain. Vector borne and zoonotic diseases (Larchmont, N.Y.) 5: 390-401.

De la Fuente, J., Ruybal, P., Mtshali, M.S., Naranjo, V., Shuqing, L., Mangold, A.J., Rodriguez, S.D., Jimenez, R., Vicente, J., Moretta, R., Torina, A., Almazan, C., Mbati, P.M., de Echaide, S.T., Farber, M., Rosario-Cruz, R., Gortazar, C., Kocan, K.M., 2007. Analysis of world strains of Anaplasma marginale using major surface protein 1a repeat sequences. Veterinary microbiology 119: 382-390.

De la Fuente, J., Torina, A., Caracappa, S., Tumino, G., Furla, R., Almazan, C., Kocan, K.M., 2005e. Serologic and molecular characterization of Anaplasma species infection in farm animals and ticks from Sicily. Veterinary parasitology 133: 357-362.

De la Fuente, J., Torina, A., Naranjo, V., Caracappa, S., Vicente, J., Mangold, A.J., Vicari, D., Alongi, A., Scimeca, S., Kocan, K.M., 2005f. Genetic diversity of Anaplasma marginale strains from cattle farms in the province of Palermo, Sicily. Journal of veterinary medicine. B, Infectious diseases and veterinary public health 52: 226-229.

De la Fuente, J., Van Den Bussche, R.A., Garcia-Garcia, J.C., Rodriguez, S.D., Garcia, M.A., Guglielmone, A.A., Mangold, A.J., Friche Passos, L.M., Barbosa Ribeiro, M.F., Blouin, E.F., Kocan, K.M., 2002c. Phylogeography of New World isolates of Anaplasma marginale based on major surface protein sequences. Veterinary microbiology 88: 275-285.

De la Fuente, J., Van Den Bussche, R.A., Kocan, K.M., 2001c. Molecular phylogeny and biogeography of North American isolates of Anaplasma marginale (Rickettsiaceae: Ehrlichieae). Veterinary parasitology 97: 65-76.

Dohoo I, W Martin and H Stryhn, 2003. Veterinary Epidemiologic Research. AVC Inc, Prince Edward Island, Canada

Drummond, R. O., R.A. Bram and N. Konnerup. (1978). Animal pests and world food production.In: Pimental,(Editor),World food, Pest losses and the environment West view Press, Boulder, Co, pp. 204.

Dumler, J.S., Barbet, A.F., Bekker, C.P., Dasch, G.A., Palmer, G.H., Ray, S.C., Rikihisa, Y., Rurangirwa, F.R., 2001. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combinations and designation of Ehrlichia equi and 'HGE agent' as subjective synonyms of Ehrlichia phagocytophila. International journal of systematic and evolutionary microbiology 51: 2145-2165.

Dunning Hotopp, J.C., Lin, M., Madupu, R., Crabtree, J., Angiuoli, S.V., Eisen, J.A., Seshadri, R., Ren, Q., Wu, M., Utterback, T.R., Smith, S., Lewis, M., Khouri, H., Zhang, C., Niu, H., Lin, Q., Ohashi, N., Zhi, N., Nelson, W., Brinkac, L.M., Dodson, R.J., Rosovitz, M.J., Sundaram, J., Daugherty, S.C., Davidsen, T., Durkin, A.S., Gwinn, M., Haft, D.H., Selengut, J.D., Sullivan, S.A., Zafar, N., Zhou, L., Benahmed, F., Forberger, H., Halpin, R., Mulligan, S., Robinson, J., White, O., Rikihisa, Y., Tettelin, H., 2006. Comparative genomics of emerging human ehrlichiosis agents. PLoS genetics 2, e21.

Eriks, I.S., Stiller, D., Palmer, G.H., 1993. Impact of persistent Anaplasma marginale rickettsemia on tick infection and transmission. Journal of clinical microbiology 31: 2091-2096.

Estrada-Pena, A., Acevedo, P., Ruiz-Fons, F., Gortazar, C., de la Fuente, J., 2008. Evidence of the importance of host habitat use in predicting the dilution effect of wild boar for deer exposure to Anaplasma spp. PloS one 3, e2999.

Farhan Ahmad Atif, Muhammad Sarwar Khan, Hafiz Javed Iqbal, Ghulam Murtaza Arshad, Ejaz Ashraf and Sami Ullah, 2012. Prevalence of Anaplasma marginale, Babesia bigemina and Theileria annulata infections among cattle in Sargodha District, PakistanAfrican Journal of Agricultural Research Vol. 7(22), pp. 3302-3307.

French, D.M., McElwain, T.F., McGuire, T.C., Palmer, G.H., 1998. Expression of Anaplasma marginale major surface protein 2 variants during persistent cyclic rickettsemia. Infection and immunity 66: 1200-1207.

Fyumagwa, R.D., Simmler, P., Meli, M.L., Hoare, R., Hofmann-Lehmann, R., Lutz, H., 2009. Prevalence of Anaplasma marginale in different tick species from Ngorongoro Crater, Tanzania. Veterinary parasitology 161: 154-157.

Ghaidaa Abbas Jassem and Olaa A. Agaar,2015, Molecular and biochemical study of Anaplasma marginale in cattle in Wassit Province of Iraq. Journal of Bacteriology Research Vol. 7(4), pp. 36-41.

Grau, H.E., Filho, C.N., Pappen, F.G., Farias, N.A., 2013. Transplacental transmission of Anaplasma marginale in beef cattle chronically infected in southern Brazil. Revista brasileira de parasitologia veterinaria = Brazilian journal of veterinary parasitology : Orgao Oficial do Colegio Brasileiro de Parasitologia Veterinaria 22: 189-193.

Hawkins, J.A., Love, J.N., Hidalgo, R.J., 1982. Mechanical transmission of anaplasmosis by tabanids (Diptera: Tabanidae). American journal of veterinary research 43: 732-734.

Hornok, S., Micsutka, A., Fernandez de Mera, I.G., Meli, M.L., Gonczi, E., Tanczos, B., Mangold, A.J., Farkas, R., Lutz, H., Hofmann-Lehmann, R., de la Fuente, J., 2012. Fatal bovine anaplasmosis in a herd with new genotypes of Anaplasma marginale, Anaplasma ovis and concurrent haemoplasmosis. Research in veterinary science 92: 30-35.

Hosmer D and S Lemeshow, 1989. Applied Logistic Regression. Wiley, New York

Howell, J.M., Ueti, M.W., Palmer, G.H., Scoles, G.A., Knowles, D.P., 2007. Persistently infected calves as reservoirs for acquisition and transovarial transmission of Babesia bovis by Rhipicephalus (Boophilus) microplus. Journal of clinical microbiology 45: 3155-3159.

Jonsson, N.N., Reid, S.W.J., 2000. Global Climate Change and Vector Borne Diseases. The Veterinary Journal 160: 87-89.

Kakarsulemankhel JK, 2011. Re-description of existing and description of new record of tick [*Hyalomma* (*Euhyalomma*) *schulzei*] from Pakistan. International Journal of Agriculture and Biology, 13: 689–694.

Kamani J, A Sannusi, OK Eqwu, GI Dogo, TJ Tanko, S Kemza, AE Takarki and DS Gbise, 2010. Prevalence and significance of haemoparasitic infections of cattle in North-Central, Nigeria. Vet World, 3: 445-448.

Kamani., A.Sannusi., O.K.Egwu., G.I.Dogo., T.J.Tanko., S.Kemza., A.E.Tafarki and D.S. Gbise 2010. Prevalence and Significance of Haemoparasitic Infections of Cattle in North- Central, Nigeria Veterinary World, Vol. 3(10):445-448

Khan MQ, A Zahoor, M Jahangir and MA Mirza, 2004. Prevalence of blood parasites in cattle and buffaloes. Pakistan Veterinary Journal, 24: 193-195.

Kieser, S.T., Eriks, I.S., Palmer, G.H., 1990. Cyclic rickettsemia during persistent Anaplasma marginale infection of cattle. Infection and immunity 58: 1117-1119.

Kocan KM, de la Fuente, AA Guglielmone and RD Melendez, 2003. Antigens and alternatives for control of *Anaplasma marginale* infection in cattle. Clinical Microbiological Review, 16: 698-712.

Kocan, K., de la Fuente, J., Step, D.L., Blouin, E., Coetzee, J.F., Simpson, K.M., Genova, S.G., J., B.M., 2010a. Current Challenges of the Management and Epidemiology of Bovine Anaplasmosis. The Bovine practitioner. 44, 93-102.

Kocan, K., Venable, J.H., Konrad, C., Brock, W.E., 1978a. Ultrastructural localization of antigens (Pawhuska Isolate) with Ferritin-conjugated antibody. American journal of veterinary research 7: 1131-1135.

Kocan, K.M., Blouin, E.F., Barbet, A.F., 2000. Anaplasmosis control. Past, present, and future. Annals of the New York Academy of Sciences 916: 501-509.

Kocan, K.M., Blouin, E.F., Palmer, G.H., Eriks, I.S., Edwards, W.L., 1996. Strategies to interrupt the development of Anaplasma marginale in its tick vector. The effect of bovine-derived antibodies. Annals of the New York Academy of Sciences 791: 157-165.

Kocan, K.M., de la Fuente, J., 2003. Co-feeding studies of ticks infected with Anaplasma marginale. Veterinary parasitology 112: 295-305.

Kocan, K.M., de la Fuente, J., Blouin, E.F., 2008. Advances toward understanding the molecular biology of the Anaplasma-tick interface. Frontiers in bioscience : a journal and virtual library 13: 7032-7045.

Kocan, K.M., de la Fuente, J., Blouin, E.F., Coetzee, J.F., Ewing, S.A., 2010b. The natural history of Anaplasma marginale. Veterinary parasitology 167: 95-107.

Kocan, K.M., de la Fuente, J., Blouin, E.F., Garcia-Garcia, J.C., 2004. Anaplasma marginale (Rickettsiales: Anaplasmataceae): recent advances in defining host-pathogen adaptations of a tick-borne rickettsia. Parasitology 129 Suppl, S285-300.

Kocan, K.M., de la Fuente, J., Guglielmone, A.A., Melendez, R.D., 2003. Antigens and alternatives for control of Anaplasma marginale infection in cattle. Clinical microbiology reviews 16: 698-712.

Kocan, K.M., Ewing, S.A., Hair, J.A., Barron, S.J., 1984. Demonstration of the inclusion appendage of Anaplasma marginale in nymphal Dermacentor andersoni. American journal of veterinary research 45: 1800-1807.

Kocan, K.M., Goff, W.L., Stiller, D., Claypool, P.L., Edwards, W., Ewing, S.A., Hair, J.A., Barron, S.J., 1992a. Persistence of Anaplasma marginale (Rickettsiales: Anaplasmataceae) in male Dermacentor andersoni (Acari: Ixodidae) transferred successively from infected to susceptible calves. Journal of medical entomology 29: 657-668.

Kocan, K.M., Hair, J.A., Ewing, S.A., Stratton, L.G., 1981. Transmission of Anaplasma marginale Theiler by Dermacentor andersoni Stiles and Dermacentor variabilis (Say). American journal of veterinary research 42: 15-18.

Kocan, K.M., Halbur, T., Blouin, E.F., Onet, V., de la Fuente, J., Garcia-Garcia, J.C., Saliki, J.T., 2001. Immunization of cattle with Anaplasma marginale derived from tick cell culture. Veterinary parasitology 102: 151-161.

Kocan, K.M., Stiller, D., Goff, W.L., Claypool, P.L., Edwards, W., Ewing, S.A., McGuire, T.C., Hair, J.A., Barron, S.J., 1992b. Development of Anaplasma marginale in male Dermacentor andersoni transferred from parasitemic to susceptible cattle. American journal of veterinary research 53: 499-507.

Kocan, K.M., Venable, J.H., Brock, W.E., 1978b. Ultrastructure of anaplasmal inclusions (Pawhuska isolate) and their appendages in intact and hemolyzed erythrocytes and in complement-fixation antigen. American journal of veterinary research 39: 1123-1130.

Kreier, J.P., Ristic, M. 1974. The rickettsias: Family III. Anaplasmataceae. in Buchanan, R.E., Gibbons, N.E. (ed): Bergey's Manual of Determinative Bacteriology, ed. 8 Baltimore, In: Williams & Wilkins Co., 907-908.

Kreier, J.P., Ristic, M., 1963. Anaplasmosis. XI. Immunoserologic characteristics of the parasites present in the blood of calves infected with the Oregon strain of Anaplasma marginale. American journal of veterinary research 24: 688-696.

Kumar PP and AK Sangwan, 2010. Comparative prevalence of subclinical bovine anaplasmosis under different cattle management systems in Haryana. Haryana Veterinarian, 49: 1-5.

Kuttler KL 1980. Pharmacotherapeutics of drugs used in treatment of anaplasmosis and babesiosis. J Am Vet Med Assoc.. 176(10 Spec No):1103-8.

Kuttler, K.L., 1984. Anaplasma infections in wild and domestic ruminants: a review. Journal of wildlife diseases 20: 12-20.

M. Alonso, C. Arellano-Sota, V.H. Cereser, C.O. Cordoves, A.A. Guglielmone, R. Kessler A.J. Mangold, A. Nari, J.H. Patarroyo, M.A. Solari, C.A. Vega, O. Izcaíno and E. Camus, 1992. Epidemiology of bovine anaplasmosis and babesiosis in Latin America and the Caribbean, Rev. sci. tech. Off. int Epiz. 11 (3): 713 733

Makala LH, P Mangani, K Fujisaki and H Nagasawa, 2003. The current status of major tick borne diseases in Zambia. Veterinary Research, 34: 27-45.

Maldonado, J., Coronado, A., Kowalski, A., Medina, J., 2012. Evidencia molecular de transmisión transplacentaria de Anaplasma marginale en becerros neonatos cebú de Venezuela. Zootecnia Tropical 30: 109-114.

McDougald, L.R AND L.R. Edward (1988). Antiprotozoan drug, Veterinary Pharmacology and Therapeutics 6th. Ed. IOWA State University Press.

Md. Abdul Alim, Shubhagata Das, Krisna Roys, Md. Masuduzzaman, Suchandan Sikder1, Mohammad Mahmudul Hassan2, A. Z. Siddiki and Mohammad Alamgir Hossain (2011). Prevalence of Hemoprotozoan Diseases in Cattle Population of Chittagong Division, Bangladesh, research group, Centre for Communicable Diseases, ICDDRB, Dhaka, Bangladesh.

Miller, J.G., 1956. The prevention and treatment of anaplasmosis. Annals of the New York Academy of Sciences 64: 49-55.

Minjauw B, McLeod A (2003). Tick borne disease and poverty. The impact of ticks and tick-borne disease on the livelihoods of small scale and marginal livestock owners in India and eastern and Southern Africa. Research report, DFID, Animal Health Programme, Centre for Tropical Veterinary Medicine, University of Edinburgh

Mohammed, G., 1999. Clinico-epidemiologicaland therapeutic aspects of Bovine Theileriosis Pakistan. Vet. J 19(2): 64-71.

Moura, A.B., Vidotto, O., Yamamura, M.H., Vidotto, M.C., Luz Pereira, A.B., 2003. Studies on the Anaplasma marginale Theiler, 1910 infection in Boophilus microplus (Canestrini, 1887) using 'nested' PCR. Brazil. J. Vet. Parasitol. 12: 27-32.

Muhammad Sohail Sajid, Rao Muhammmad Siddique, Sohail Ahmad Khan, Zafar Iqbal and Muhammad Nisar Khan, 2014. Prevalence and Risk Factors of Anaplasmosis in Cattle and Buffalo Populations of District Khanewal, Punjab, Pakistan ,Global Veterinaria 12 (1): 146-153

Muhanguzi D, K Ikwap, K Picozzi and C Waiswa, 2010. Molecular characterization of *anaplasma* and *ehrlichia* species in different cattle breeds and age groups in Mbarara district (Western Uganda). International Journal of Animal and Veterinary Advances, 2: 76-88.

Munderloh, U.G., Lynch, M.J., Herron, M.J., Palmer, A.T., Kurtti, T.J., Nelson, R.D., Goodman, J.L., 2004. Infection of endothelial cells with Anaplasma marginale and A. phagocytophilum. Veterinary microbiology 101: 53-64.

Nasir, A.A. (2000). Effect of Theileriosis on blood parameters of exotic cattle and efficacy of Buparvaquone and Oxytetracycline. Pak. J. Bio. Sci., 3(6): 1027-1028.

Nelson, K.J., Knutson, S.T., Soito, L., Klomsiri, C., Poole, L.B., Fetrow, J.S., 2011. Analysis of the peroxiredoxin family: using active-site structure and sequence information for global classification and residue analysis. Proteins 79: 947-964.

Niyonzema A and HH Kiltz, 1986. Control of ticks and tick-borne diseases in Burundi. Australian Center for International Agricultural Research, 17: 16-17.

Patil, N.A., 1995. Efficacy of Butalexre (buparvaquone) in the treatment of clinical cases ofbovine tropical Theileriasis, Indian Journal of animal search 29(1): 59-61.

Potgieter, F.T., Sutherland, B., Biggs, H.C., 1981. Attempts to transmit Anaplasma marginale with Hippobosca rufipes and Stomoxys calcitrans. The Onderstepoort journal of veterinary research 48: 119-122.

PSAC (President's Science Advisory Committee). 1967. Report of the panel on World Food Supply., Vol: II. The White House, USA

Radostits OM, DC Blood and CC Gay, 2000. Veterinary Medicine: A text book of disease of cattle, sheep, pigs, goats and horse. 9th Ed, BaillereTindall Publication, London, pp: 1172-1173; 1289-1290.

Rahman, Sumon, Khan and Islam, 2015. Current status of subclinical form of babesiosis and anaplasmosis in cattle at Rangpur district in Bangladesh Progressive Agriculture 26: 51-59.

Razzak A and H Shaikh, 1969. A survey on the prevalence of ticks on cattle in East Pakistan. Pakistan Journal of Veterinary Science, 3: 54-60.

Rees, C.W., Avery, J.L., 1939. Experiments on the hereditary transmission of anaplasmosis by ticks. North American Veterinarian, 35-36.

Rey Valeiron, C., Aso, P.M., Coronado, A., 2003. Prevalence of Anaplasma marginale and specific antibodies in new born calves. Acta cientifica venezolana 54: 121-126.

Ribeiro, M.F., Lima, J.D., 1996. Morphology and development of Anaplasma marginale in midgut of engorged female ticks of Boophilus microplus. Veterinary parasitology 61: 31-39.

Richey EJ, 1984. Management of anaplasmosis in beef cattle. Animal Nutrition for Health, 12: 10-14.

Robertson,A. (1976). Handbook of animal diseases in Tropics, 3rd Ed. British Veterinary Association London

Samad MA and OP Goutam, 1984. Prevalence of *Theileria annulata* infection in cattle of Bangladesh. Indian Journal of Parasitology, 7: 61-63.

Samad MA, SA Bashar, M Shahidullah and MU Ahmed, 1989. Prevalence of haemoprotozoan parasites in cattle of Bangladesh. Indian Journal of Veterinary Medicine, 13: 50-51.

Sarkar M, 2007. Epidemiology and pathology of ectoparasitic infestation in Black Bengal Goats in Bangladesh. M.Sc. thesis. Department of Parasitology, Bangladesh Agricultural University, Mymensingh.

Scoles, G.A., Miller, J.A., Foil, L.D., 2008. Comparison of the efficiency of biological transmission of Anaplasma marginale (Rickettsiales: Anaplasmataceae) by Dermacentor andersoni Stiles (Acari: Ixodidae) with mechanical transmission by the horse fly, Tabanus fuscicostatus Hine (Diptera: Muscidae). Journal of medical entomology 45: 109-114.

Shkap, V., Kocan, K., Molad, T., Mazuz, M., Leibovich, B., Krigel, Y., Michoytchenko, A., Blouin, E., de la Fuente, J., Samish, M., Mtshali, M., Zweygarth, E., Fleiderovich, E.L., Fish, L., 2009. Experimental transmission of field Anaplasma marginale and the A. centrale vaccine strain by Hyalomma excavatum, Rhipicephalus sanguineus and Rhipicephalus (Boophilus) annulatus ticks. Veterinary microbiology 134: 254-260.

Siddiki AZ, MB Uddin, MB Hasan, MF Hossain, MM Rahman, BC Das, MS Sarker and MA Hossain, 2010. Coproscopic and haematological approaches to determine the prevalence of helminthiasis and protozoan diseases of Red Chittagong Cattle (RCC) breed in Bangladesh. Pakistan Veterinary Journal, 30: 1-6.

Siddiki AZ, MB Uddin, MB Hasan, MF Hossain, MM Rahman, BC Das, MS Sarker and MA Hossain, 2010. Coproscopic and haematological approaches to determine the prevalence of helminthiasis and protozoan diseases of Red Chittagong Cattle (RCC) breed in Bangladesh. Pakistan Vet J, 30(1): 1-6.

SM Shariful Hoque Belal, Md. Abdullah-Al-MAHMUD and Mst. Jannatul Ferdous, 2015. Prevalence of anaplasmosis in cattle in sirajganj district of bangladesh, Res. Agric., Livest. Fish. Vol. 1, No. 1, December 2014: 97-103

Smith, K., Kilborne, F.L., 1893. Investigations into the nature, causation and prevention of southern cattle fever. In: 8th and 9th Am. Rep. Bur. An. Ind. 177–1893.

Smith, R.D., Levy, M.G., Kuhlenschmidt, M.S., Adams, J.H., Rzechula, D.L., Hardt, T.A., Kocan, K.M., 1986. Isolate of Anaplasma marginale not transmitted by ticks. American journal of veterinary research 47: 127-129.

Soulsby, E.J.L. 1982. Helminths, Arthropods and Protozoa of Domestic Animals.7th Ed. Lea & Febiger Philadalphia,Great Britain.

Stich, R.W., Kocan, K.M., Damian, R.T., Fechheimer, M., 1997. Inclusion appendages associated with the intraerythrocytic rickettsial parasiteAnaplasma marginale are composed of bundled actin filaments. Protoplasma 199: 93-98.

Stich, R.W., Kocan, K.M., Palmer, G.H., Ewing, S.A., Hair, J.A., Barron, S.J., 1989. Transstadial and attempted transovarial transmission of Anaplasma marginale by Dermacentor variabilis. American journal of veterinary research 50: 1377-1380.

Stich, R.W., Olah, G.A., Brayton, K.A., Brown, W.C., Fechheimer, M., Green-Church, K., Jittapalapong, S., Kocan, K.M., McGuire, T.C., Rurangirwa, F.R., Palmer, G.H., 2004. Identification of a novel Anaplasma marginale appendage-associated protein that localizes with actin filaments during intraerythrocytic infection. Infection and immunity 72: 7257-7264.

Stiller, D., Kocan, K.M., Edwards, W., Ewing, S.A., Barron, J.A., 1989. Detection of colonies of Anaplasma marginale in salivary glands of three Dermacentor spp infected as nymphs or adults. American journal of veterinary research 50, 1381-1385.

Talukdar MH and MJ Karim, 2001. Subclinical Anaplasma infection in crossbred cattle in Bangladesh. Bangladesh Veterinary Journal, 35: 159-160.

Tarek R. Abou-Elnaga., Mona A. Mahmoud., Wafaa A. Osman., Azza S. A Goda, 2009. Serological Survey of Anaplasma Marginale (Rickettsia) Antibodies In Animals By Major Surface Protein 5 Competitive Inhibition Enzyme-Linked Immunosorbent Assay Scvmj, IVX (1).

Tarek R. Abou-Elnaga., Mona A. Mahmoud., Wafaa A. Osman., Azza S. A Goda, 2009. Serological Survey of Anaplasma Marginale (Rickettsia) Antibodies In Animals By Major Surface Protein 5 Competitive Inhibition Enzyme-Linked Immunosorbent Assay Scvmj, IVX (1).

Tarun Kumar, Neelesh Sindhu, Gaurav Charaya, Ankit Kumar, Parmod Kumar, Gauri Chandratere, Divya Agnihotri, and Rajesh Khurana 2015. Emerging status of anaplasmosis in cattle in Hisar ; Vet World. 8(6): 768–77

Theiler, A., 1910. Anaplasma marginale (gen. and spec. nov.): the marginal points in the blood of cattle suffering from a specific disease. Report of the Government Veterinary Bacteriologist for the year 1908-1909. Transvaal Department of Agriculture. Pretoria, South Africa. 7-64.

Theiler, A., 1911. Further investigation into anaplasmosis of South African cattle. First report of the Director of Veterinary Research. Department of Agriculture of the Union of South Africa. 7-32.

Theiler, A., 1912. Übertragung der Anaplasmosis mittels Zecken. Zeitschrift für Infektionskrankheiten, 105–116.

Thrusfield M, 1995. *Veterinary Epidemiology*. Blackwell Science Ltd, a Blackwell Publishing Company, pp. 39-41.

Tilak Chandra Nath, Md. Jamal Uddin Bhuiyan, Md. Shafiul Alam(2013). A Study on the Prevalence of Common Blood Parasites of Cattle in Sylhet District of Bangladesh Journal home International Journal of Animal & Fisheries Science, 1 (1) 8- 13

Torina, A., Alongi, A., Naranjo, V., Estrada-Pena, A., Vicente, J., Scimeca, S., Marino, A.M., Salina, F., Caracappa, S., de la Fuente, J., 2008. Prevalence and genotypes of Anaplasma species and habitat suitability for ticks in a Mediterranean ecosystem. Applied and environmental microbiology 74: 7578-7584.

Uilenberg G, 1995. International collaborative research: significance of tick-borne hemoparasitic diseases to world animal health. Veterinary Parasitology, 57: 19-41.

Urquhart, G. M., J. Armour, J. L. Duncan, A.M. Dunn and F.W. Janings. (1988). Vet. Parasitology. Ist Ed: ELBS, Longman, London, UK.

Wamsley, H.L., Alleman, A.R., Johnson, C.M., Barbet, A.F., Abbott, J.R., 2011. Investigation of endothelial cells as an in vivo nidus of Anaplasma marginale infection in cattle. Veterinary microbiology 153: 264-273.

Zahang, L., Wang, Y., Cai, D., He, G., Cheng, Z., Liu, J., Meng, K., Yang, D., Wang, S., 2013. Detection of Anaplasma marginale in Hyalomma asiaticum ticks by PCR assay. Parasitology research 112: 2697-2702.

Zaugg, J.L., 1985. Bovine anaplasmosis: transplacental transmission as it relates to stage of gestation. American journal of veterinary research 46: 570-572.

Zivkovic, Z., Nijhof, A.M., de la Fuente, J., Kocan, K.M., Jongejan, F., 2007. Experimental transmission of Anaplasma marginale by male Dermacentor reticulatus. BMC veterinary research 3: 32.