Journal of Current Veterinary Research

ISSN: 2636-4026

Journal home page: http://www.jcvr.journals.ekb.eg

*Internal medicine & Infectious disease*

# Bovine Tick Born Blood Parasites in Egypt: Vectors and Associated Risk Factors

Mohamed A. Hashad[1*], Ahmed Zaghawa[1], Mohamed A. Nayel[1], Mohamed AboElkhair[2], Akram A. Salama[1], Ahmed Osman[3], and Walid Saad Mousa[1]

*(1) Department of Animal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt.*

*(2) Department of Virology, Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt.*

*(3) Department of Parasitology, Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt.*

**Corresponding author:* Mohamed hashad 414@yahoo.com

*Received: 8/11/2023*

*Accepted: 26/11/2023.*

## ABSTRACT

The current study intended to evaluate the involvement of ticks and mosquitos in Babesia and Theileria transmission. A total of 520 ticks and 280 mosquitoes were collected and pooled (80 pools) from 6 localities in Menofia between May 2019 and May 2021. Ticks and mosquitoes pools were molecularly examined using universal GF2/GR2 Babesia/Theileria primers,PCR revealed thatout of 80 pools19 (23.7%) were positive for piroplasms.One positive pool was subjected to sequencing,the results of the sequence analysis revealed *Babesia bovis*. Risk factor analysis revealed that animal-keeping togethered has a significant effect onpiroplasms transmission, on the other hand breed, sex, age of animals, season and location have no significant effect on piroplasms transmission. The prevalence of piroplasms infection in animal-keeping togethered was (15time) higher than individual animals, higher in summer season (25.7%) than winter season (10%), higher in animals >3 year (26.3%) compared to the other age groups, in female was (1.6 time) higher than in male, and higher (1.4 times) in imported breeds than native breeds.

**Keywords**: Bovine piroplasmosis, ticks and mosquito, Transmission and PCR.

## INTRODUCTION

Tick-borne protozoa called piroplasmids, belonging to the genera Babesia and *Theileria*, can cause fatal blood cell disorders in cattle and other mammals. Bovine piroplasmids are widespread throughout the Mediterranean region and are endemic in Egypt (Ibrahim et al., 2009). *Babesia* and *Theileria* spp are Apicomplexa protozoan intraerythrocytic parasites and more than 100 species have been reported thus far, mostly based on physical traits, and Babesia spp are strictly host specific (Ahmad et al., 2014). Because these infections can

result in anemia, icterus, hemoglobinuria, and death, they are significant global health risks that affect many regions of the world (Nayel et al., 2012). Vector-borne sickness disseminated by a variety of biting, blood-feeding arthropods that causes emaciation, hide damage, infertility, mastitis, reduction of milk supply, and mortality of up to 20% (Anonymous, 1988). The Piroplasmosis include two primary genera (*Babesia* and *Theileria*) that cause economically significant diseases in both domestic and wild animals. Piroplasmid species are continuously being discovered, and their full biology is not fully understood. Many parasites in this group were formerly classified based on morphology, host cells where schizogony occurs, the presence of piroplasms in red blood cells linked with disease manifestation, and host-vector specificity. Finding blood parasites, or babesia, is crucial for an early diagnosis (Nayel et al., 2012). The earlier the pathogen is detected, the better the prognosis.

Numerous tests have been developed for diagnosis. The clinical evidence is confirmed by examining Giemsa-stained blood smears (Trees, 1974). An alternate method for the identification of babesiosis is molecular diagnosis, such as polymerase chain reaction (PCR), which can detect the parasite in the early stages of the illness and is more sensitive and specific (Abou Laila et al., 2010).

Each element of the vector-borne system, the pathogen, vector, and reservoir must work together effectively for vector transmission to occur. However, it also depends on how these elements interact with one another in their environment, which may have a direct or indirect impact. Since not simply any pathogen can be transmitted by any vector and be harbored by every animal, their genotypes can also affect the success of transmission (Kuleš et al., 2016).

Vectors are living organisms that can transmit infectious diseases between animals. Many of these vectors are bloodsucking insects, such as mosquitoes, sandflies, ticks, bugs, flies, fleas, lice, and some freshwater aquatic snails, that feed on disease-causing microorganisms from an infected host (human or animal) and then inject them into their next victim during their next blood meal (Eassa & Abd El-Wahab, 2022).

Ticks are hematophagous arthropods that parasitize the majority of vertebrate species worldwide, including people and animals. Given that ticks host and spread a variety of illnesses that are dangerous to both humans and animals, ticks are the second-largest vector for human vector-borne diseases, after mosquitoes. Tick bites can also irritate people or induce paralysis or serious allergies (De La Fuente et al., 2007).

Correlation and frequency of piroplasmosis in animal population of the districts affected by variety of hosts and their environment-related variables. To ascertain a correlation with the prevalence of piroplasmosis, factors such as host-related determinants (animal species, breed, sex, and age) and husbandry practices, such as animal-keeping (togethered or opened), housing (closed, semi-closed, or open), hygiene (ranked 1-10 for poor, very poor, and good), floor pattern (cemented, partially cemented, or non-cemented, and seasons of the year

should be investigated and considered in any survey (Rao et al., 2020). Furthermore, the age of the animal plays a significant role since the innate resistance is reinforced by mother antibodies that are transferred to calves through colostrum, the infection rate is low in young animals. This resistance gradually decreases, leaving the animal more susceptible to illness (Fadly, 2012). As well as breed of cattle from *Bos taurus* genus are more common than those from the *Bos indicus* genus (Radostits et al., 2007). Additionally, native breeds are less susceptible to infection than non-native breeds due to long-term exposure to tick populations in nature, which led to the development of either innate immunity to the tick or intrinsic resistance (Wodaje et al., 2019).

The peak of the tick population and climatic variations both affect the frequency of infection and tick activity (Menshawy et al., 2018) Babesia spp. infection in cattle reaches peak in the summer season (El Bahy et al., 2018).
According to Magona et al. (2011), the prevalence of theileriosis varies by geographic area and a number of other variables, including tick density, climatic circumstances, age, gender, management practices, and immunity, either passive or active. Due to the warmth and humidity that encourage tick proliferation and subsequent parasite transmission, the incidence rate is higher during the monsoon season (Vahora et al., 2012). In addition to tick resistance and inherent vulnerability to infection, cow breed has an impact on prevalence (Muhammad et al., 2008).

The purpose of this study was to identify the roles that various arthropod species play in the transmission of piroplasmids as well as associated risk factors.

## MATERIALS AND METHODS

### ***Animals***

Samples were collected from clinically diseased and apparently healthy cattle and buffalo of different ages,breeds and sex in 6 different localities in Menofia Governorate (Menouf, Shebein El-Kom, Tala, Berkt El sabaa, Al shohada and Ashmoon). Every animal was inspected for tick infestations.

**Table (1):** Data related to examined animals.

| Type of data | | No of collected samples |
|---|---|---|
| **Animals status** | Diseased animals | 34 |
| | Apparently healthy | 46 |
| **Samples type** | Ticks | 52 |
| | Mosquitoes | 28 |
| **Season** | Summer | 70 |
| | Winter | 10 |
| **Animal breed** | Native breeds | 64 |
| | Foreign breeds | 16 |

| | | |
|---|---|---|
| **Age of animals** | < 1year | 17 |
| | 2-3 year | 44 |
| | > 3 year | 19 |
| **Sex of animals** | Females | 58 |
| | Males | 22 |
| **Location** | Menouf | 20 |
| | Shebein El-Kom | 5 |
| | Tala | 5 |
| | Al-shohada | 10 |
| | Berkt-El Sabaa | 25 |
| | Ashmoun | 15 |

***Samples***

***Ticks And Mosquitoes***

From the period of May 2019 to May 2021, A total of 520ticks and280 mosquitoes were collected from 6 different localities in Menofia Governorate.

Ticks were divided into pools (52) each pool containing about 10 ticks. Mosquitoes were divided into 28 pools.

The collected ticks and mosquitoes were properly transferred on ice tothe laboratory for immediatemorphological identificationusing stereomicroscope according to (Walker 2000). After identification, ticks and mosquitoes were pooled in 1.5 ml Eppendorf tubes each tube contains 10 ticks or mosquito, stored frozen at -80°C for the molecular examination.

***Clinical examination of animals.***

Cattle and buffalo were clinically examined according to (Radostits et al. 2000). Data of cattle including locality, season, age, sex, breed and animal-keeping togethered were collected.

***Processing of ticks and mosquitoes for pcr***

After being collected, ticks, and mosquitoes were rinsed three times in phosphate buffer saline (PBS). After being cleaned, the ticks and mosquitoes were combined, crushed in a mortar, and centrifuged for 15 minutes at 2000 rpm to extract DNA.

***Extraction of* DNA**

DNA of all the processed ticks and mosquitoes were extracted usingQIAamp RNA/DNA mini kit (INTRON biotechnology®) according to manufacture instructions.

**PCR *and sequencing***

PCR amplification performed using PCR master mix (Promega)®in a total volume of 25µl/reaction. The PCR primer set targets ~550 bp for Babesia and ~570 for TheileriaThe primers used were with the following sequences; BAB GF2: (5′-GTC TTG TAA TTG GAA TGA TGG-3′) and BAB GR2: (5′-CCA AAG ACT TTG ATT TCT CTC-3′) (Adaszek and Winiarczyk 2008). The PCR amplicon was electrophoresed at 100 volts for 45 minutes in 2% agarose gel containing ethidium bromide and then seen in a trans-illuminator according to the manufacturer's recommendations. Using a gel extraction kit (Qiagen),

***Sequence analysis***

The obtained sequence using an AB1 PRISM 3100 genetic analyzer (Applied Biosystems, USA, gene link DNA Sequencing service, New York, USA)

was edited and aligned with other sequences retrieved from the GenBank using CLUSTALW algorithms available in the Molecular Evolutionary Genetic Analysis (MEGA version X) software and blasted using NCBI Nucleotide BLAST. The phylogenetic trees were built in MEGA version X using the Neighbor-Joining method. The evolutionary distances were computed using the Jukes-Cantor method. One thousand bootstrap replicates were conducted to assess statistical support for the tree topology (Tamura et al., 2004).

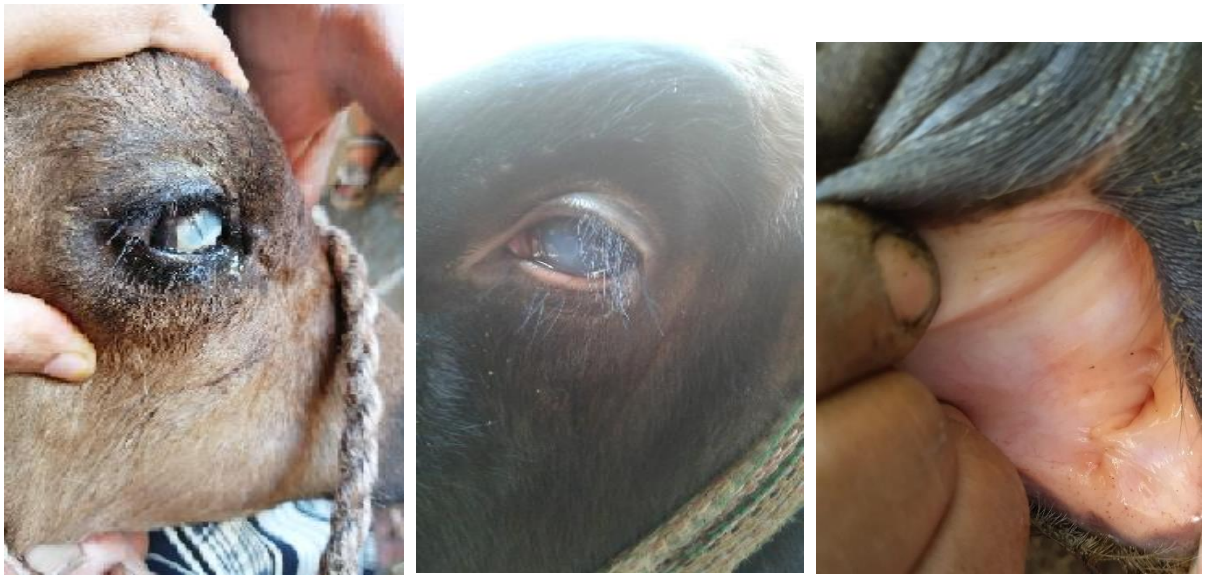
**Statical analysis**

The association between positive pools and animal attributes was identified individually using multinomial and univariate logistic regression analysis model that was carried out in IBM SPSS statistics for Windows version 21.0 (Wilson 1927).

**RESULT**

***Clinical examination of the infected animals:***

The clinically diagnosed animals with babesiosis sufferedfromfever 41°C, loss of appetite, cessation of rumination, emaciation, sometimes hemolytic anemia, various degrees of jaundice (Icterus) from paleness in mild cases to severe yellow discoloration of conjunctival and vaginal mucous membranes in more progressive cases haemoglobinuria, Theileriosis clinically diagnosed animals suffered from ever 41°C, loss of appetite, cessation of rumination, emaciation, and, corneal opacity (fig.1).



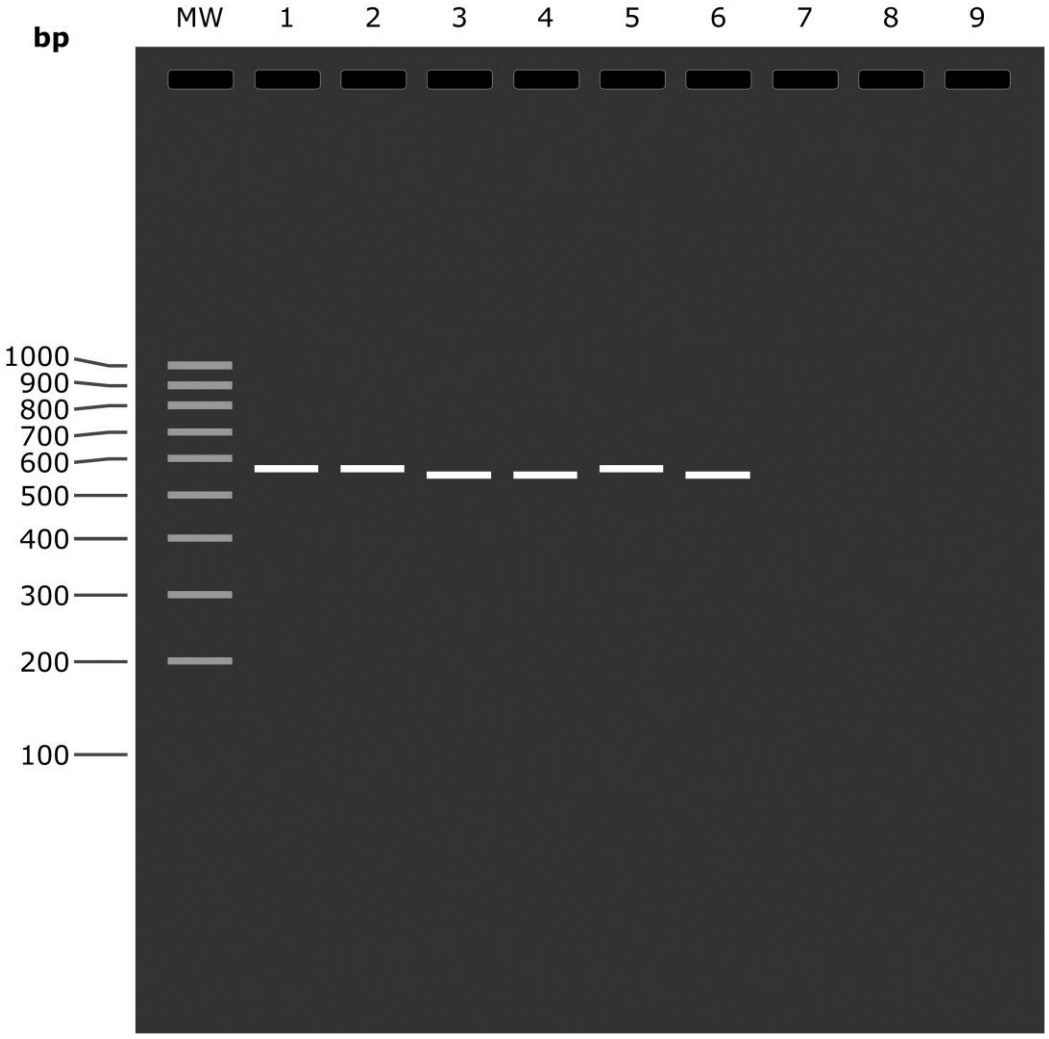
**(a)** **(b)** **(c)**

**Figure (1):** Corneal opacity **(a&b)**, vaginal mucous membrane paleness in a cow **(c)** in clinically suspected animals with blood parasites.

***Molecular identification of babesia and Theileria by PCR: -***

Out of 80 tick and mosquito samples from (34) diseased cattle and buffalo exhibiting clinical signs and (46) non diseased animals 19 (23.7 %) were positive by PCR 14

samples were positive from (34) clinically diseased animals and 5 positive samples from (46) apparently healthy animals using the babesia and The PCR primer set targets ~550 bp for Babesia and ~570 for Theileria (Fig. 4) all the positive samples were from ticks pools and we not found any positive samples from mosquitoes pools.




**(Fig.2):** - PCR results of the samples using primers for Babesia and Theileria (~550 and ~570 bp). (MW) 100 bp DNA marker, Lanes 1, 2, 5 are positive for Theileria.while Lanes 3,4, 6 positive for Babesia.

**Table (2):** The prevalence of blood parasites carried by ticks in samples taken:

| **Type of samples** | **Examined samples** | | **Positive by PCR** | |
|---|---|---|---|---|
| | No | % | No | % |
| **Ticks** | 52 | 65 | 19 | 36.5 |
| **Mosquitoes** | 28 | 35 | 0 | 0 |
| **Total** | 80 | 100 | 19 | 23.75 |

***4.3.3. Sequencing and phylogenic analysis of babesia and Theileria:***

Typing by nucleotide sequencing of purified PCR product of the piroplasm detected in this study showed significant nucleotide sequence homology with 97 to 100% nucleotide identity. it was shown that the piroplasm sequences obtained were nearly equivalent to those of the isolates of Babesia bovis B bo16 (EF458216) and Babesia bovis (BBOV2) (L19077). In contrast Suhag isolate (LC653103) appeared to be in a separate clade Fig (3)

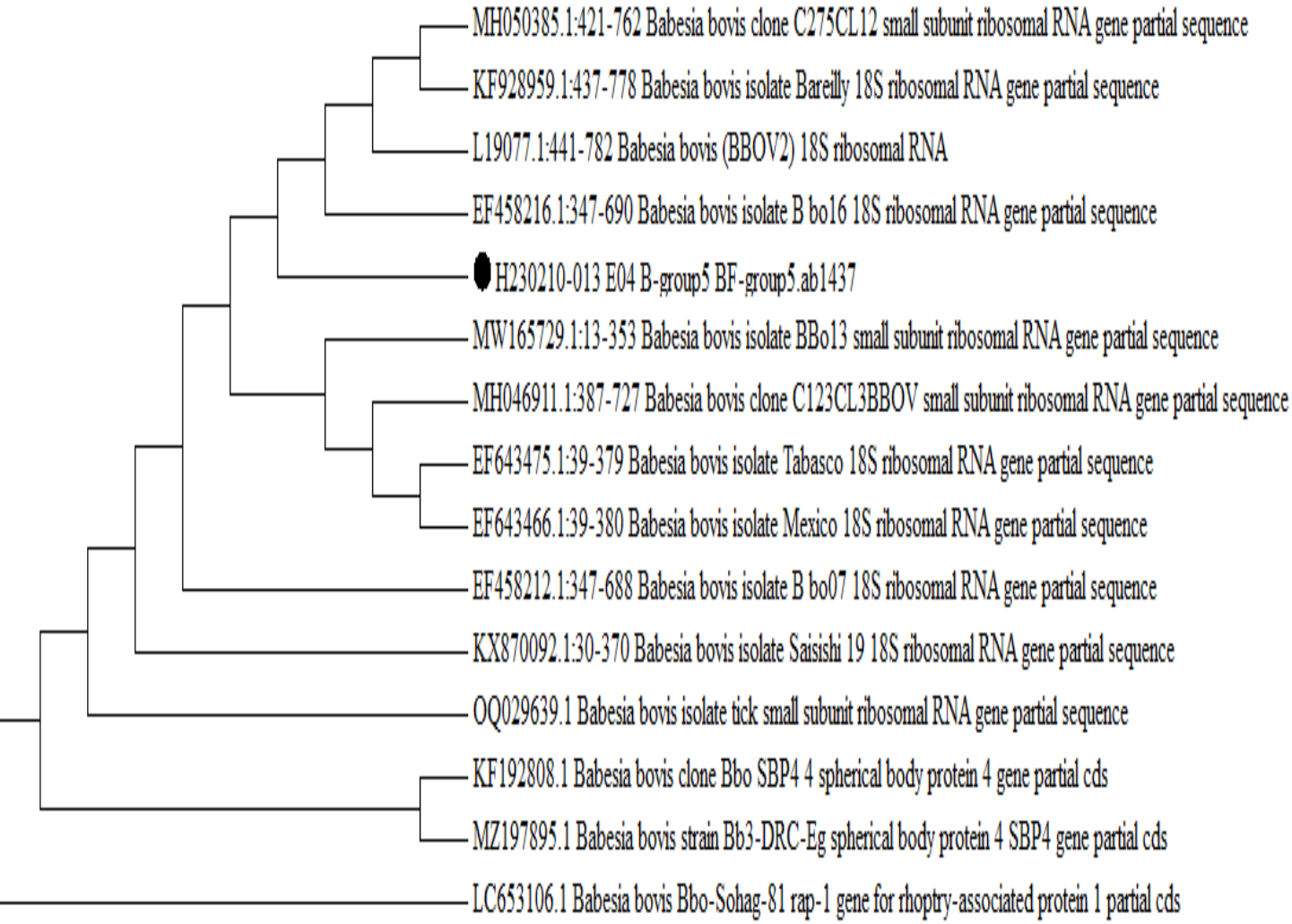



**Fig (3).** 18S rRNA based phylogenetic analysis of genotypes identified in this study. Phylogenetic tree highlighting the position of Babesia sp. in the present study in relation to other Babesia sp. available in GenBank.

***4.4.3. Risk factor associated with tick borne blood parasite***

Breed, sex, age of animals, season and contact with other animal's variables were investigated in the current study. The results revealed animal-keeping togethered has a significant effect for piroplasms infection, on the other hand breed, sex, age of animals and season variables have no significant effect for piroplasms prevalence (Tab.3).

**Table (3)** Multivariate analysis of risk factors associated with piroplasmosis by SPSS program.

| Variables in the Equation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | B | S.E. | Wald | df | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
| | | | | | | | | Lower | Upper |
| **Step 1[a]** | contact (1) | 2.728 | 1.069 | 6.511 | 1 | .011 | 15.296 | 1.882 | 124.305 |
| | sex | .572 | .687 | .692 | 1 | .406 | 1.771 | .460 | 6.816 |
| | age | .012 | .479 | .001 | 1 | .980 | 1.012 | .396 | 2.588 |
| | location | -.164- | .186 | .775 | 1 | .379 | .849 | .589 | 1.223 |
| | season | -.540- | 1.199 | .203 | 1 | .652 | .583 | .056 | 6.104 |
| | breed | -.170- | .906 | .035 | 1 | .852 | .844 | .143 | 4.980 |
| | Constant | -2.996- | 1.473 | 4.139 | 1 | .042 | .050 | | |
| **Step 2[a]** | contact (1) | 2.728 | 1.069 | 6.518 | 1 | .011 | 15.307 | 1.885 | 124.314 |
| | sex | .576 | .671 | .735 | 1 | .391 | 1.778 | .477 | 6.627 |
| | location | -.165- | .183 | .815 | 1 | .367 | .848 | .592 | 1.213 |
| | season | -.541- | 1.199 | .204 | 1 | .652 | .582 | .056 | 6.098 |
| | breed | -.161- | .836 | .037 | 1 | .848 | .852 | .166 | 4.382 |
| | Constant | -2.972- | 1.138 | 6.819 | 1 | .009 | .051 | | |
| **Step 3[a]** | contact (1) | 2.722 | 1.068 | 6.490 | 1 | .011 | 15.205 | 1.873 | 123.412 |
| | sex | .622 | .629 | .976 | 1 | .323 | 1.862 | .543 | 6.390 |
| | location | -.176- | .174 | 1.020 | 1 | .312 | .839 | .596 | 1.180 |
| | season | -.501- | 1.181 | .180 | 1 | .671 | .606 | .060 | 6.130 |
| | Constant | -2.984- | 1.138 | 6.868 | 1 | .009 | .051 | | |
| **Step 4[a]** | contact (1) | 2.762 | 1.065 | 6.731 | 1 | .009 | 15.837 | 1.965 | 127.649 |
| | sex | .665 | .622 | 1.144 | 1 | .285 | 1.944 | .575 | 6.577 |
| | location | -.178- | .173 | 1.058 | 1 | .304 | .837 | .597 | 1.175 |
| | Constant | -3.067- | 1.132 | 7.341 | 1 | .007 | .047 | | |
| **Step 5[a]** | contact (1) | 2.762 | 1.064 | 6.738 | 1 | .009 | 15.834 | 1.967 | 127.447 |
| | sex | .685 | .614 | 1.244 | 1 | .265 | 1.983 | .595 | 6.607 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Constant | -3.568- | 1.051 | 11.517 | 1 | .001 | .028 | | |
| **Step 6[a]** | contact (1) | 2.726 | 1.059 | 6.626 | 1 | .010 | 15.273 | 1.916 | 121.724 |
| | Constant | -3.332- | 1.018 | 10.721 | 1 | .001 | .036 | | |
| a. Variable(s) entered on step 1: type, sex, age, location, season, breed. | | | | | | | | | |

***4.4.3.1. The univariate analysis of the risk factors***

Association between prevalence rate of piroplasms infection and risk factors was examined by univariate analysis.

***4.4.3.1.1. Effect of animal-keeping togetheredon prevalence of piroplasms infection***

A statistically significant correlation was observed between the frequency of piroplasms infection and animal-keeping togethered, the prevalence of piroplasms infection in animals animal-keeping togethered was (15time) higher than individual animals (table 4).

**Table 4:** the univariate analysis of the animal-keeping togetheredwith prevalence of piroplasms infection

| **Variables in the Equation** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **B** | **S.E.** | **Wald** | **df** | **Sig.** | **Exp(B)** | **95% C.I.for EXP(B)** | |
| | | | | | | | | **Lower** | **Upper** |
| **Step 1[a]** | Contact (1) | 2.726 | 1.059 | 6.626 | 1 | .010 | 15.273 | 1.916 | 121.724 |
| | Constant | -3.332- | 1.018 | 10.721 | 1 | .001 | .036 | | |
| a. Variable(s) entered on step 1: contact. | | | | | | | | | |

***4.4.3.1.2. Effect of the sex***

Though the predominance of piroplasmsillness in female was higher (1.6 time) than in male (table 5).

**Table 5:** analysis of the sex with predominance of piroplasms infection

| **Variables in the Equation** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | B | S.E. | Wald | Df | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
| | | | | | | | | Lower | Upper |
| **Step 1[a]** | sex(F) | .475 | .541 | .771 | 1 | .380 | 1.608 | .557 | 4.639 |
| | Constant | -1.340- | .339 | 15.647 | 1 | .000 | .262 | | |
| a. Variable(s) entered on step 1: sex. | | | | | | | | | |

***4.4.3.1.3. Effect of season***

There was no statistically significant association between the prevalence of piroplasms infection and season. Although, the prevalence of piroplasms infection is higher in summer season (25.7%) than winter season (10%) but non statistical no difference (table 6).

**Table 6:** the univariate analysis the association of the season with prevalence of piroplasms infection

| Variables in the Equation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | B | S.E. | Wald | df | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
| | | | | | | | | Lower | Upper |
| Step 1[a] | season (1) | 1.136 | 1.089 | 1.089 | 1 | .297 | 3.115 | .369 | 26.331 |
| | Constant | -2.197- | 1.054 | 4.345 | 1 | .037 | .111 | | |
| Step 2[a] | Constant | -1.166- | .263 | 19.711 | 1 | .000 | .311 | | |
| a. Variable(s) entered on step 1: season. | | | | | | | | | |

***4.4.3.1.4. Effect of breed***

There was no statistically significant association between the prevalence of piroplasms infection and animal breeds, the prevalence of piroplasms infection is higher (1.4 times) in imported breeds than native breeds (table 7).

**Table 7:** Univariate analysis association of animal breed with prevalence of piroplasms infection

| Variables in the Equation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | B | S.E. | Wald | df | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
| | | | | | | | | Lower | Upper |
| **Step 1[a]** | breed (1) | .368 | .703 | .274 | 1 | .601 | 1.444 | .364 | 5.724 |
| | Constant | -1.466- | .641 | 5.241 | 1 | .022 | .231 | | |
| **Step 2[a]** | Constant | -1.166- | .263 | 19.711 | 1 | .000 | .311 | | |
| a. Variable(s) entered on step 1: breed. | | | | | | | | | |

***4.4.3.1.5. Effect of age***

There was no statistically significant association between the prevalence of piroplasms infection and animal age, all age groups of animals were susceptible to piroplasms, but it has been usually showed more frequently in group (>3 year) 26.3% compared to the other age groups table.8.

**Table 8:** The univariate analysis of the association of animal age with prevalence of piroplasms infection

| Variables in the Equation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | B | S.E. | Wald | df | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
| | | | | | | | | Lower | Upper |
| **Step 1[a]** | age | | | .095 | 2 | .954 | | | |
| | age (1) | -.045- | .676 | .004 | 1 | .947 | .956 | .254 | 3.593 |
| | age (2) | .149 | .774 | .037 | 1 | .847 | 1.161 | .255 | 5.286 |
| | Constant | -1.179- | .572 | 4.249 | 1 | .039 | .308 | | |
| **Step 2[a]** | Constant | -1.166- | .263 | 19.711 | 1 | .000 | .311 | | |
| a. Variable(s) entered on step 1: age. | | | | | | | | | |

## DISCUSSION

Piroplasmosis is a disease with a world-wide distribution affecting many species of mammals with a major impact on cattle and human. It has increasingly been recognized throughout the world as public health problems (Hazem et al., 2014). Identification of VBDs in vectors can provide valuable information for developing control methods (Ozan et al., 2019). This study investigated the kinds of ticks and mosquitos gathered from different animal farmsand individual cases in six localities in Menofia governorates, and used molecular techniques to determine the prevalence of babesia and Theileria in the specimens collected. Veterinarians and physicians are showing an increased level of interest in vector-borne diseases (VBDs) that afflict animals, as their distribution has expanded recently. Therefore, it is essential to diagnose and identify vector-borne diseases (VBDs) in order to create an epidemiological map of these disorders. This can be done by advancing molecular biology (Abdullah et al., 2021). Clinical examination of animals revealed that the affected animals suffered from pyrexia 41°C, sometimes hemolytic anemia, jaundice (Icterus) in more advanced cases hemoglobinuria and in accordance with those previously shown by (Fadly, 2012). Egypt's climatic circumstances, as well as inadequate preventive and control efforts, provide an ideal setting for numerous tick species, yet there is little information on the occurrence and distribution of tick species and their associated infections in the country (AL-Hosary et al., 2021).

In our study, out of 80ticks and mosquitoes samples from (34) diseased cattle and buffalo exhibiting clinical signs and (46) non diseased animals, 19 (23.7 %) from total samples were positive by PCR using the babesia and Theileria primer. This result was nearly similar as Babesia sp. infection rates were reported to be 25.33 percent in Egypt by Rania (2009), 20 percent in France by De Vos and Potgieter (1994), and 8 out of 30 cattle (26.7%) in Brazil by Costa-Junior et al. (2006) using PCR. On the other hand this result differ with (Nayel et al. 2012) who found that 20 of 158 animals (12.66 %) were positive for Babesia.

Typing by nucleotide sequencing andPhylogenetic analysis it was shown

that the piroplasm sequences obtained were nearly equivalent to those of the isolates of Babesia bovis B bo16 (EF458216) and Babesia bovis (BBOV2) (L19077). In contrast Suhag isolate (LC653103) appeared to be in a separate clade.

Regarding to the effect of some risk factor associated with piroplasms infection revealed that the warm seasons almost 3 time more than cold season of getting piroplasms with no statistically significant. This was in contact with (Qayyum et al., 2010; Naz et al., 2012; Patel et al., 2017; Zaman et al.,2022) indicated that Theileriosis and babesiosis were most prevalent in the summer, with lower incidence in the autumn, spring, and winter. This could be because of the ideal environmental conditions for the vectors' growth and development, but in some cases, slightly different trends were noted, which could be caused by fomites.The sex of affected animals has been found non-significant effect on piroplasms infection and recorded 1.6 time higher in female than in the male population, which was agree with some reports (Alim et al., 2012; Atif et al., 2012; Zaman et al., 2022).This could be because it is customary to engage women for field ploughing and other transportation tasks. The higher infection rates in females than in males may be caused by factors such as breeding stress, milk production, pregnancy, parturition, inadequate food, ageing, hormonal changes, increased medication stress, and use for draught purposes in later life(Maharana et al., 2016; Bary et al., 2018).

Although, there was no statistically significant between the prevalence of piroplasms infection and animal breeds, the prevalence of piroplasms infection was higher (1.4 times) in imported breeds than native breeds and this is similar to (Zaman, et al., 2022) recorded that prevalence of Babesiosis and theileriosis in cattle in Holstein Friesian breed was found significant ($P < 0.05$).

According to the influence of age and its relation with the piroplasms infection, it was no statistical significance association between the prevalence of piroplasms infection and animal age, all age groups of animals were susceptible to piroplasms, but it has been usually showed more frequently in group (>3 year) and group (1-3 y) compared to the other age group (<1 y) This is consistent with the findings of Ibrahim et al. (2000) and El Bader et al. (2009), but it is not consistent with the findings of Cleon (1988), who claimed that animals between the ages of 6 and 12 months had a higher prevalence than animals in other age groups.

The infection rate was low among young animals may be due to young calves possess innate resistance enhanced by maternal antibodies, these resistance declined gradually leaving the animal with high susceptibility to the disease (Fadly, 2012). Finally, A statistically significant correlation was observed between the frequency of piroplasms infection and animal-keeping togethered, The prevalence of piroplasms infection in contact animals (15.2 time) higher than non-contact animals.

## REFERENCES

Abdullah, H. H. A. M., Amanzougaghene, N., Dahmana, H., Louni, M., Raoult, D., & Mediannikov, O. (2021). Multiple vector-borne pathogens of domestic animals in egypt. *PLoS*

*Neglected Tropical Diseases*, *15*(9). https://doi.org/10.1371/JOURNAL.PNTD.0009767

AbouLaila, M., Yokoyama, N., Igarashi, I. 2010. Development and evaluation of two nested PCR assays for the detection of Babesia bovis from cattle blood. Vet Parasitol, 172:65–70.

Adaszek L, Winiarczyk S (2008) Molecular characterization of Babe- sia canis canis isolates from naturally infected dogs in Poland. Vet Parasitol 152:235–241

Ahmad, I., Khawja, A., Shams, S., Ayaz, S., Khan, S., & Akbar, N. (2014). Detection of babesiosis and identification of associated ticks in cattle. *International Jornal of Bioassays*, *3*(7), 3195–3199.https://doi.org/http://dx.doi.org/10.21746/ijbio.2014.07.006

AL-Hosary, A., Răileanu, C., Tauchmann, O., Fischer, S., Nijhof, A. M., & Silaghi, C. (2021). Tick species identification and molecular detection of tick-borne pathogens in blood and ticks collected from cattle in Egypt. *Ticks and Tick-Borne Diseases*, *12*(3). https://doi.org/10.1016/j.ttbdis.2021.101676

Alim, M. , Das, S. , Roy, K. , Masuduzzaman, M. , Sikder, S. , Hassan, M. M. , & Hossain, M. A. (2012). Prevalence of hemoprotozoan diseases in cattle population of Chittagong Division, Bangladesh. Pakistan Veterinary Journal, 32(2)

Anonymous D. (1988). Lumpy skin disease.Vol.1.No.l, Paris: O.I.E. Disease Information.

Atif, Farhan Ahmad, et al. (2012): Prevalence of Anaplasma marginale, Babesia bigemina and Theileria annulata infections among cattle in Sargodha District, Pakistan." *Afr. J. Agric. Res* 7.22 (2012): 3302-3307.

Bary, M. A., Ali, M. Z., Chowdhury, S., Mannan, A., Nur e Azam, M., Moula, M. M., ... & Hossain, M. A. (2018). Prevalence and molecular identification of haemoprotozoan diseases of cattle in Bangladesh. *Adv. Anim. Vet. Sci*, *6*(4), 176-182.

Chen, Z., Liu, Q., Liu, J., Xu, B., Lv, S., Xia, S., & Zhou, X. (2014). Tick-borne pathogens and associated co-infections in ticks collected from domestic animals in central China. 1–8.

Costa- Junior. L. M., M. L. Élida, O. A. Rabelo and F. B. Múcio. 2006. Comparison of different direct diagnostic methods to identify Babesia bovis and Babesia bigemina in animals vaccinated with live attenuated parasites. Vet parasitol, 139 : 231-36

Cleon, V. (1988): In "diseases of sheep" Third edition. Lea, Fembigen. Philagelphia.

Chen, Z., Liu, Q., Liu, J., Xu, B., Lv, S., Xia, S., & Zhou, X. (2014). Tick-borne pathogens and associated co-infections in ticks collected from domestic animals in central China. 1–8.

Costa- Junior. L. M., M. L. Élida, O. A. Rabelo and F. B. Múcio. 2006. Comparison of different direct diagnostic methods to identify Babesia bovis and Babesia bigemina in animals vaccinated with live attenuated parasites. Vet parasitol,

139 : 231-36

Eassa, S., & Abd El-Wahab, E. (2022). Vector-Borne diseases in Egypt: Present status and accelerating toward elimination. *Journal of Vector Borne Diseases*, *59*(2), 127–138. https://doi.org/10.4103/0972-9062.321759

El Badr, A.; Ibrahim, A.; Tolba, M.; Metwally, A.; Zaghlol, D. and Osman, A. (2009): Piroplasmosis in cattle and buffaloes in south valley Governorates, Egypt. EVMSPJ, 5: 83– 89.

El-Bahy, N. M., Menshawy, S. M., Goda, W. M., Nasr, S. M., AbouLaila, M. R. Bazh, E. K. and Abou-Rwash, A. A. (2018): Molecular detection of Babesia bigemina and Babesia bovis in cattle in Behaira Governorate.ejpmr. 5(12): 441-446

EL-Seify, M.A. 1989. Incidence and geographical distribution of some blood parasites among cattle and buffaloes in Beni-Suef Governorate. Vet. Med. J. Giza. 37(3):611-622.

Elsify, A., Sivakumar, T., Nayel, M., Salama, A., Elkhtam, A., Rizk, M., Mosaab, O., Sultan, K., Elsayed, S., Igarashi, I., & Yokoyama, N. (2015). An epidemiological survey of bovine Babesia and Theileria parasites in cattle, buffaloes, and sheep in Egypt. In *Parasitology International* (Vol. 64, Issue 1). https://doi.org/10.1016/j.parint.2014.10.002

Fadly, R. S. (2012). Prevalence of Blood Parasites of Some Farm Animals At Behera Province. *Assiut Veterinary Medical Journal*, *58*(134), 1–7. https://doi.org/10.21608/avmj.2012.173780

Hazem M. El Moghazy , Ebied, M.H, Mohamed, A. G., M El Haz, oghazy M., & -l, S. E. (2014). EPIDEMIOLOGICAL STUDIES ON BOVINE BABESIOSIS AND THEILERIOSIS IN QALUBIA GOVERNORATE ziz A Abdel Amr and , 2. *Benha Veterinary Medical Journal*, *27*(1), 36–48. http://www.bvmj.bu.edu.eg/issues/27-1/4.pdf

Hussein, Hala E., et al. (2017): "The Babesia bovis hap2 gene is not required for blood stage replication, but expressed upon in vitro sexual stage induction." *PLoS Neglected Tropical Diseases*11.10 (2017): e0005965.

Ibrahim, M.S.; El Seify, M.A.; Hafez, A.M.; Deghidy, N.S. and Harfoush, M.A. (2000): Studies on blood parasites in sheep. The 1st scientific congress for provincial laboratonries May 15-7.

Ibrahim, A. k., EL Behairy, A. M., Mahran, K. M. A., & Awad, W. . (2009). Clinical and laboratory diagnosis of piroplasmids in naturally infected cattle in egypt. *J. Egypt. Vet. Med. Assoc*, *203*(2), 191–203.

Kuleš, J., Horvatić, A., Guillemin, N., Galan, A., Mrljak, V., & Bhide, M. (2016). New approaches and omics tools for mining of vaccine candidates against vector-borne diseases. *Molecular BioSystems*, *12*(9), 2680–2694. https://doi.org/10.1039/c6mb00268d

Liu, G. P. , Ren, Q. M. , He, S. X. , Wang, F. , & Yang, J. (2008). Distribution and medical importance of ticks in three provinces of northeast China. Chinese Journal of Hygienic Insecticides & Equipments, 14(1), 39–42.

Mahmmod, Y. (2014). Natural babesia bovis infection in water buffaloes (bubalus bubalis) and crossbred cattle under field conditions in Egypt: A preliminary study. *Journal of Arthropod-Borne Diseases*, *8*(1), 1–9.

Maharana, B. R., Kumar, B., Prasad, A., Patbandha, T. K., Sudhakar, N. R., Joseph, J. P., & Patel, B. R. (2016). Prevalence and assessment of risk factors for haemoprotozoan infections in cattle and buffaloes of South-West Gujarat, India. Indian Journal of Animal Research, 50(5), 733-739.

Menshawy, S. M., Bazh, E. K. and Abou-Rawash, A. A. (2018): Dynamics of Boophilus Ticks and its Role in Transmission of Piroplasm at Behaira District. AJVS. 56 (1): 137-144.

Mohamed, G., and M. Ebied. 2014. Epidemiological studies on bovine babesiosis and thrileriosis in Qalubia Governorate. Benha Veterinary Medical Journal, 27(1):36-48.

Magona, J.W., Walubengo, J., Olaho-Mukani, W., Jonsson, N.N., Welburn, S.W., Eisler, M.C.( 2011): Spatial variation of tick abundance and sero conversion rates of indigenous cattle to *Anaplasma marginale*, *Babesia bigemina* and Theileria parvainfections in Uganda. Exp. Appl. Acarol., 55: 203–213

Muhammad, G., Naureen, A., Firyal, S., Saqib, M. (2008): Tick control strategies in dairy production medicine. *Pak. Veter. J.*, 28(1): 43–50.

Nayel, M., & El-dakhly, K. M. (2012). *The use of different diagnostic tools for Babesia and Theileria parasites in cattle in Menofia , Egypt*. https://doi.org/10.1007/s00436-012-2926-6

Naz S, Maqbool A, Ahmed S, Ashraf K, Ahmed N, Saeed K, Latif M, Iqbal J, Ali Z, Shafiand K, Nagra IA (2012). Prevalence of theileriosis in small ruminants in Lahore. Pak J Vet. Animal Science 2:16–20

Ozan, E., Albayrak, H., Gumusova, S., Bolukbas, C. S., Kurt, M., Pekmezci, G. Z., Beyhan, Y. E., Kadi, H., Kaya, S., Aydin, I., & Yazici, Z. (2019). A study on the identification of five arboviruses from hematophagous mosquitoes and midges captured in some parts of Northern Turkey. *Journal of Arthropod-Borne Diseases*, *13*(2), 224–233. https://doi.org/10.18502/jad.v13i2.1249

Patel MD, Kumar N, Rathod PH, Tyagi KK, & Sorathiya LM (2017). Incidence and Haematological Changes in Haemoprotozoan Infections in Bovines of South Gujarat. Ind J Vet Sci Biotechnol 13(2).

Qayyum A, Farooq U, Samad HA and Chauhdry HR (2010). Prevalence, clinicotherapeutic and prophylactic studies on Theileriosis in district Sahiwal, Pakistan. J Anim Plant Sci 20:266–270

Radostits, O., Gay, G., Hinchiff, K. and Con, P. (2007): Veterinary Medicine: A text book of the disease of cattle, sheep, goat, pigs and horses. 10th edition London: Saunders Elsevier, PP: 1110-1489

Radostits, O.; Gay, C.; Blood, D. and Hinchcliff, K. (2000): Veterinary Medicine, 9th Ed. Blaillier, London, Philadelphia, New York.

Rania YE (2009) Some studies on diagnosis

on babesiosis. M.V.Sc. thesis, Faculty ofVeterinary Medicine, Benha University, Benha, Egypt

Rao Muhammad Siddique, Muhammad Sohail Sajid, , Zafar Iqbal, Muhammad Saqib ,(2020) association of different risk factors with the prevalence Of babesiosis in cattle and buffalos *pak.* J. Agri. Sci., Vol. 57(2), 517-524; 2020

Rouby, S. R., Hussein, K. H., Aboelhadid, S. M., & El-Sherif, A. M. (2017). `QWET6UOP]\78+Role of rhipicephalus annulatus tick in transmission of lumpy skin disease virus in naturally infected cattle in Egypt. In *Advances in Animal and Veterinary Sciences* (Vol. 5, Issue 4). https://doi.org/10.17582/journal.aavs/2017/5.4.185.191

Salem, A.A., Hassan, M.S., Hosein, H.I., Abo-El-Magd, M.M., Mahmoud, S.Z. 1993. Some studies on blood parasites in cattle. Beni-Suef, Vet. Med. Res. III, 1: 86-94

Silva MG, Marques PX, Oliva A (2010) Detection of Babesia and Theileria species infection in cattle from Portugal using a reverse line blotting method. Vet Parasitol 174(3-4): 199-205.

Soulsby, E. J. L. (1982). Helminths. Arthropods and Protozoa of domesticated animals, 291.

Tamura K, Nei M, and Kumar S, 2004. Prospects for inferring very large phylogenies by using neighbor joining method. Proceedings of the National Academy of Sciences (USA), 101: 11030-11035.

Trees, A. J. 1974. The application of acridine orange staining to quantitate low levels of *Babesia divergens* parasitaemias. Trans. R. Soc. Trop. Med. Hyg. 68**:**277.

Tuppurainen, E. S. M., Lubinga, J. C., Stoltsz, W. H., Troskie, M., Carpenter, S. T., Coetzer, J. A. W., Venter, E. H., & Oura, C. A. L. (2013). Mechanical transmission of lumpy skin disease virus by Rhipicephalus appendiculatus male ticks. *Epidemiology and Infection*, *141*(2), 425–430. https://doi.org/10.1017/S0950268812000805

Vahora, S.P., Patel, J.V., Parel, B.B., Patel, S.B., Umale, R.H. (2012): Seasonal incidence of hemoprotozoan disease in crossbred cattle and buffalo in Kaira and Anand district of Gujarat, India. Veter World,5(4): 223–225.

Walker JB, Keirans JE, Horak IG, The Genus Rhipicephalus (Acari, Ixodidae), Guide to the BrownTicks of the World. Cambridge University Press, Cambridge, 2000.

Wilson EB. Probable inference, the law of succession, and statistical inference. J Am Statist Assoc 1927; 22: 209–12.

Wodaje, A.; Biyansa Adudna and Hamid, M. (2019):A Review on Bovine Babesiosis. Int. J. Adv. Res. Biol. Sci. 6 (1): 63-70.

Zaman, M. A., Atif, F. A., Abbas, R. Z., Shahid, Z., Mehnaz, S., Qamar, Q., Qamar, M. F., & Hussain, K. (2022). Climatic Regions Based Molecular Prevalence of Babesiosis and Theileriosis in Cattle and Water-Buffalo in Pakistan. Research Square, 1, 1–18.