

Molecular Isolation of *Theileria* and *Babesia* Species and the Risk Factors Associated with their Infection in Cattle at Makurdi and Abinsi in Benue State, Nigeria

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ABSTRACT

The study was conducted to investigate the Molecular Isolation of *Theileria* and *Babesia* Species and the Risk Factors associated with their infection in cattle at Makurdi and Abinsi in Benue State, Nigeria. Blood samples were collected randomly from 160 cows in three locations in Makurdi (Wadata, Wurukum, North bank and one location in Abinsi and examined for *Theileria* and *Babesia* infection using molecular methods. A questionnaire was used to assess the risk factors associated with the infections. The result of the PCR reaction indicated the presence of *Theileria orientalis* and *Babesia bovis* at 335 and 278 base pair respectively. Chi-Square statistical analysis was used to determine the significance of the prevalences of the infections among cattle. The results showed that *Theileria orientalis* was more prevalent than *Babesia bovis* with the prevalences of 11.25 and 7.50 respectively. The results of multivariate logistic regression analysis showed that management practices, acaricidal control on the farm, and the presence of other animals are statistically significant risk factors associated with an increased likelihood of infections in cattle. However, age and location was not statistically significant and sex showed marginal significance. In conclusion therefore, there was high prevalences of *Babesia* and *Theileria* species in cattle in Makurdi and Abinsi. Based on Molecular description it is therefore recommended that farmers should implement more effective tick control measures in the Abinsi and Makurdi area of Benue State, Nigeria to reduce the vectors responsible for transmitting *Theileria* and *Babesia* species furthermore, farmers should be educated to know the importance of regular tick control practices against the infection.

INTRODUCTION

The prevalence of haemoparasites of cattle in Nigeria is generally considered to be very high due to the preponderance of their arthropod vectors (Musa *et al.*, 2014). The prevalence of various genera of haemoparasites of cattle (Trypanosomes, Babesia, Anaplasma, and Theileria) has been reported in different parts of the country (Okorafor and Nzeako, 2014 and Qadeer *et al.*, 2015) and elsewhere in the world (Alim *et al.*, 2012; Velusamy *et al.*, 2014). *Babesia* and *Theileria* species are listed among the most economically important genera of haemoparasites in Nigeria (Velusamy *et al.*, 2014) and their impact on cattle production and productivity accounts for heavy economic losses to livestock producers in the tropics and subtropics (FAO, 2017). They are responsible for destruction of erythrocytes leading to anaemia, jaundice, anorexia, weight loss and infertility in livestock (Okorafor and Nzeako, 2014).

Babesia and *Theileria* species infect a wide range of both domestic and wild animals (Gupta *et al.*, 2017). *Babesia divergens*, *Babesia bigemina*, *Babesia bovis*, and *Babesia major* have been associated with Babesiosis in cattle (Jane and Adam, 2014; Abdela *et al.*, 2018). For theileriosis, *Theileria annulata*, *Theileria orientalis* and *Theileria parva* are responsible for bovine theileriosis

The diagnosis of these important blood protozoa are mainly based on clinical symptoms and microscopic

examination of Giemsa-stained blood smears. Several researchers identified bovine piroplasms using microscopic examination for epidemiological investigation (Al Mahmud *et al.*, 2015). In some studies serological examination has been used to confirm the tentative diagnosis of the infections (Ali *et al.*, 2016 and Rahman *et al.*, 2015). However, these studies are only reliable for the diagnosis of acute cases but have limited value in subclinical cases due to low parasitaemia (Hosen *et al.*, 2020). Compared to serological testing and microscopic identification of piroplasms, molecular detection of piroplasms infection by polymerase chain reaction (PCR) has proven to be more sensitive, accurate, and the gold standard test particularly in detecting *Babesia* and *Theileria* in carrier cattle (Belotindos *et al.*, 2014).

In recent years, molecular studies have been performed to detect piroplasmiasis in cattle in many part of the world (Roy *et al.*, 2018; Le Huy *et al.*, 2020). However, these studies were on a few tick-borne diseases with inadequate information on their genotypes or even knowledge on their molecular epidemiology, which is critical for the control and prevention of these diseases.

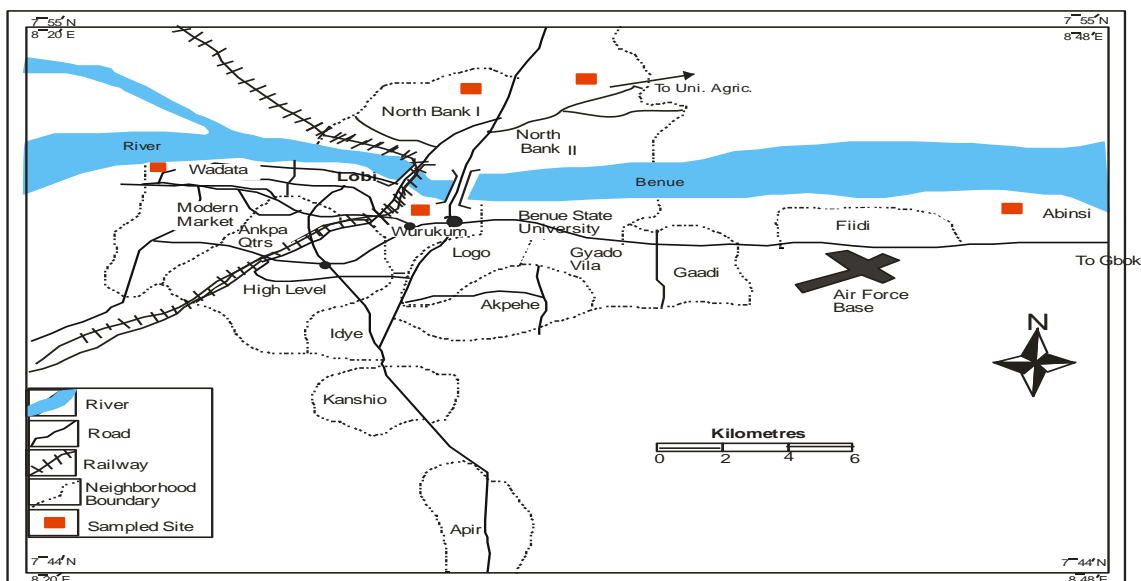
Molecular identification, with or without clinical indications of bovine piroplasmiasis, provides an alternative method for the direct detection of piroplasms in carrier animals to overcome economic losses (Moumouni *et al.*, 2015). Therefore, the present study, a molecular survey of *Babesia* and *Theileria* species based on PCR amplification was conducted in the cattle population of the Abinsi and Makurdi of Benue State. Cattle contribute significantly to food security and value chain of the Nigerian economy (Lawal-Adebawale, 2011) but their productivity is threatened by ticks and associated haemoparasitic diseases, especially Babesiosis and Theileriasis (Jatau *et al.*, 2011). The diseases condition is clinically manifested as fever, anorexia, anaemia, emaciation, threatened abortion and death in the acute form of the infections (Maharana *et al.* 2016). The effect of haemoparasitic disease of cattle causes huge loss of income to the herders, such as still birth, stunted growth and low birth rate.

The present work initialized the Prevalence of *Theileria* and *Babesia* species and the risk factor associated with Their Infection in Cattle in Makurdi and Abinsi in Benue State, Nigeria.

MATERIALS AND METHODS

Study Area

The study was conducted in Makurdi and Abinsi, Benue State. Which are located in north central Nigeria along the Benue River. They samples were collected in Northbank, Wadata, and Wurukum in Makurdi and Abinsi



Map of the Study Area Showing Sample Sites for the Collection of *Babesia* and *Theileria* Species in Cattle Sources: Ministry of Land, Survey and Solid Minerals, Makurdi

Sampling Techniques

The samples were collected from four different locations; Wadata, Wurukum, North bank, and Abinsi. One hundred and sixty (160) blood samples were collected from cattle of all ages and both sexes. The sample size was determined from previous prevalence of 15.3 % (Opara et al., 2016). The animals were restrained with rope and 5mls of blood was collected immediately from the severed jugular vein into vacutainer tubes, containing 1 mg of ethylene diaminetetra-acetic acid (EDTA) and were labelled appropriately. The age and sex of each animal were also identified based on morphometric characteristics and recorded in a case book. Cattle less than three (3) year old were categorized as young while older ones were considered as adult. The samples were transported on icepacks at 4°C to the biological laboratory, Joseph Sawuan Tarkaa University Makurdi for parasitological examinations.

A close-ended questionnaire was developed and filled in at flock level by interviewing the herders during sampling in order to assess potential risk factors for Theileriosis and Babesiosis. Those included flock size, type of management (extensive: free ranging without supplementary feed; semi-intensive: supplementary feed provided), source of grazing/browsing place.

LABORATORY ANALYSIS

Haematological Examination

Thin blood smears were prepared using the method described by Ademola and Onyiche (2013). The smears were examined at ×100 magnification (oil immersion) on an Olympus microscope for the presence and identification of blood parasites according to keys and descriptions as given by Soulsby (1982) and Taylor *et al.* (2016).

Sample Preparation for Polymerase Chain Reaction (PCR)

Blood samples collected were transported to the Molecular Biology Laboratory for PCR diagnosis. Genomic DNA was isolated using ZYMO DNA MINI KIT USA according to the manufacturer’s instructions for solid tissue samples.

Collected samples were treated with lysis buffer and Proteinase K to remove potential inhibitors of PCR present in the blood. For each sample, 500 µl of blood was aliquoted into a microfuge tube filled with 1ml lysis buffer (0.22 % NaCl, 0.015 % saponin, 1 mM EDTA, pH 7.5).

Tube contents were mixed and centrifuged at 9,300Xg for 5 minutes and the resulting supernatant discarded; pellets were resuspended in 750 µl of lysis buffer, centrifuged and supernatants discarded; this process was repeated until the pellet was clear of haemoglobin. The final pellet was resuspended in 100 µl of 50 mM KCl, 10 mM tris-HCl pH 8.0, 0.5 % Tween 20 and 100 µg proteinase K per ml. The tubes were then incubated in a water bath at 56°C for 2 hours after which they were immediately stored at -20°C. 1µl of the lysate was used as a template for PCR reactions.

PCR Assay for Diagnosis of Tick-Borne Pathogens and Validation by Sequencing

A semi-nested PCR was used for the detection of TBP based on methodology and primer sets previously developed and validated with species specific DNA samples. A primary reaction was carried out using universal primer sets designed against the 18S rRNA gene for *T. annulata*, *T. orientalis*, *B. bigemina* and *B. bovis*. (Simuunza 2011 and Ghaemi 2012). The set of primer for primary reaction of *Theileria* and *Babesia* showing the forward and reverse reaction are shown below:

PRIMER SET 1			
<i>Theileria/Babesia</i> universal	F: 5'-GACACAGGGAGGTAGTGACAAG-3' R: 5'CTAAGAATTTACCTCTGACAGT3'	385 to 429	Simuunza <i>et al.</i> (2011)

The primer use for species identification are:

<i>T. orientalis</i>	F: 5 ['] -ACATTCTCTTGTTGAGT-3 [']	335	Ghaemi <i>et al.</i> (2012)
<i>B. bovis</i>	R: 5 ['] -GCTCAATTATACAGGCGAAACCTGC-3 [']	278	Simuunza <i>et al.</i> (2011)

Each PCR reaction was carried out in a 25 µl reaction volume containing 10 pmol/µl of each primer, 1 µl of template (DNA in sample lysate) and 12.5 µl of 2X GoTaq1 Green Master Mix (Promega Corporation). Negative controls consisted of water or *E. coli* DNA. Thermal cycler conditions for the primary universal reactions were: 35 cycles of 30 sec at 94°C, 30 sec at 57°C. Species specific semi-nested PCR, primary reaction samples generating positive PCR products were diluted 1:100 and 2 µl of diluted primary reaction mix was used as a template for the semi-nested secondary reaction.

Thermocycler conditions for the nested PCR reactions were similar to primary reaction except the annealing temperature for the *Theileria annulata* and *Theileria orientalis* primer sets were 48°C for 90 sec and 55°C for 90 sec, respectively. PCR products were separated by gel electrophoresis on a 1.5 % agarose gel and visualized by ethidium bromide stain under UV using a Gel Doc (Syn-gene) apparatus. The primers used in the studies were tested for specificity previously using combinations of field isolates and standards but as an additional confirmation, positive PCR reactions were validated for amplification of the species-specific target gene by direct sequencing of two representative amplicons for each species-specific primer set. Amplified DNA samples were sent for direct sequencing (Eurofins Scientific India Pvt. Ltd.) and sequences were validated by NCBI BLAST searches (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to validate identity to the target species as the top hit.

Data Analysis

The prevalence rates among the different categories were expressed as percentage of the total animals sampled. The Chi-square test multivariate logistic regression analysis was used to test for significant differences in risk factors associated with Theileriosis and Babesiosis among cattle in the study area at 0.05 level of significance. All analysis was done using SPSS, version.

RESULTS

PCR Screening Results for Different Parasites in Four Different Locations

The species *T. orientalis* had a high prevalence among the examined cases. There were 18 cases of *T. orientalis* infection identified, which accounts for 11.25 of the total number of cases. (Table 1) In contrast, *B. bovis* had a low prevalence. There were 12 cases of *B. bovis* infection, which represents 7.5 of the total cases. This means that approximately 7.5 of the sampled cattle were found to be infected with *B. bovis*

According to the result in Plate 1: The PCR detection of surveyed *Theileria* and *Babesia* species of cattle in Northbank, Wadata, Wurukum and Abinsi showed the presence of both genera found in the first primary reaction. The expected size of the PCR fragment was between 385-439bp. This result indicates that there was either a deletion or alteration of the gene during the PCR amplification.

Plate 2 shows the secondary PCR reaction surveyed to detect the specific species presence in either *Theileria* or *Babesia*. The results indicated the presence of *Theileria orientalis* with 335pb fragment.

Plate 3 shows that another secondary PCR reaction surveyed to detect the specific species present in *Babesia*. The result indicated the presence of *Babesia bovis* with 278bp fragment.

Table 1: PCR screening results for different parasites in four different Locations

Location	Number Examined	Positive	<i>B. bovis</i>	<i>T. orientalis</i>
Wadata	40	5(3.12)	2(1.25)	3(1.88)

Northbank	40	9(5.63)	4(2.50)	5(3.13)
Wurukum	40	7(4.38)	4(2.50)	3(1.88)
Abinsi	40	9(5.63)	2(1.25)	7(4.38)
Total	160	30(18.75)	12(7.50)	18 (11.25)

Prevalences of *Theileria* and *Babesia* Species in Relation to Sex of Cattle in Makurdi and Abinsi

The result in Table 2 indicate that out of 160 samples, an overall prevalence of 30 (18.75) was recorded. The highest prevalence was observed among male cattle. Out of the male cattle sampled, 16(23.2) were found to be positive with *Theileria and Babesia species*. The prevalences among the female cattle in the sample shows 14(15.4) these means there was no significant difference between the prevalences in male and female cattle ($p>0.05$). This indicates that, the observed differences in prevalences between male and female cattle are not statistically significant. In other words, the variation in prevalence between the two groups could have occurred by chance and may not be a meaningful difference.

Prevalences of *Theileria orientalis* and *Babesia bovis* species in Makurdi and Abinsi

The results in Table 3 reveal that *Theileria* species had the highest infection rate among the cattle samples. Specifically, 14 cases of *Theileria* infection were identified, which amounts to 8.8 of the total number of cattle samples examined. The second highest infection rate was recorded for *Babesia* species. In this case, 12 cases of *Babesia* infection were identified, accounting for 7.8 of the total cattle samples. In addition to single infections with either *Babesia* or *Theileria* species, the study also noted cases of mixed infections. These occurred in 4 cases, which represent 2.5 of the total cattle samples. A mixed infection means that the same cattle were found to be infected with both *Babesia* and *Theileria* species simultaneously.

Table 2: Prevalences of *Theileria* and *Babesia* Species in Relation to Sex of Cattle in Makurdi and Abinsi.

Gender	Number Examined	Positive	Prevalence (%)
Male	69	16	23.2
Female	91	14	15.4
Total	160	30	18.8

$$\chi^2 = 1.57, df= 1, p= 0.21$$

Table 3: Prevalences of *Theileria orientalis* and *Babesia bovis* Species in Makurdi and Abinsi

Organism	Number Examined	Positive	Prevalence (%)
<i>Theileria orientalis</i>	160	14	8.8
<i>Babesia bovis</i>		12	7.8
Mixed infection		4	2.5

$$\chi^2 = 5.97, df= 2, p= 0.05$$

Classification of Cattle as Babesiosis Positive or Negative in Relation to Different Risk Factors

The result in Table 8 shows the risk factors associated with the occurrence of babesiosis in cattle. The odds ratio for age was 1.06, indicating that for every one-unit increase in age, the odds of cattle contracting babesiosis increase by a factor of 1.06. However, the p-value for age was 0.784, which is not statistically significant. The 95 % confidence interval for age ranged from 0.68 to 1.65. Cattle of a male sex had an odds ratio of 1.81, suggesting that they had a higher odds of contracting babesiosis compared to the female sex. The

p-value for sex was 0.06, which is marginally significant. The 95 % confidence interval for sex ranged from 0.99 to 3.30. Cattle subjected to free range management practices had an odds ratio of 5.18, signifying that cattle in free range practices were associated with significantly higher odds of babesiosis. The p-value for management practices was 0.001, indicating strong statistical significance. The 95 % confidence interval for management practices ranged from 2.74 to 9.79. Cattle in farms without acaricidal control measures had an odds ratio of 4.34, indicating a substantial increase in the odds of babesiosis. The p-value for not applying acaricidal control on the farm was 0.001, signifying statistical significance. The 95 % confidence interval for acaricidal control ranged from 2.34 to 8.04.

The presence of other animals was associated with an odds ratio of 1.74, suggesting that it increased the odds of babesiosis. The p-value for the presence of other animals was 0.001, indicating strong statistical significance. The 95 % confidence interval for the presence of other animals ranged from 1.25 to 2.44. The odds ratio for location was 1.12, implying that the odds of *babesiosis* varied by location. However, the p-value for location was 0.09, which is not statistically significant. The 95 % confidence interval for location ranged from 0.04 to 1.28.

Table 4: Classification of cattle as Babesiosis Positive or Negative in Relation to Different Risk Factors

Risk factor	Category	Babesiosis		OR	CI	P
		+ve (n=18)	-ve (n=142)			
Age	1-2	4	30	1.06	0.68–1.65	0.784
	3-4	6	42			
	5>	8	70			
Sex	Male	10	59	1.81	0.99–3.30	0.06
	Female	8	83			
Management practices	Free range	14	106	5.18	2.74–9.79	0.001
	Intensive	4	36			
Acaricidal control in the farm				4.34	2.34–8.04	0.001
	Yes	4	36			
	No	14	106			
Presence of other animal				1.75	1.25–2.44	0.001
	Yes	14	106			
	No	4	36			
Location				1.12	0.048-1.28	0.096
	Wadata	3	37			
	Northbank	5	35			
	Wurukum	3	37			
	Abinsi	7	33			

Abbreviations: CI, confidence interval at 95 %; OR odds ratio; P, P value

Classification of Cattle as *Theileriosis* Positive or Negative in Relation to Different Risk Factors

The result in Table 9 shows the risk factors associated with the occurrence of *Theileriosis* in cattle. The odds ratio for age was 1.06, indicating that for every one-unit increase in age, the odds of cattle contracting

babesiosis increase by a factor of 0.64. However, the p-value for age was 0.22, which is not statistically significant. The 95 % confidence interval for age ranged from 0.31 to 1.30. Cattle of a male sex had an odds ratio of 0.67, suggesting that they had a higher odds of contracting Theileriosis. Compared to the female sex. The p-value for sex was 0.72, which is marginally significant. The 95 % confidence interval for sex ranged from 0.45 to 1.00. Cattle subjected to free range management practices had an odds ratio of 6.19, signifying that cattle in free range practices were associated with significantly higher odds of Theileriosis. The p-value for management practices was 0.001, indicating strong statistical significance. The 95 % confidence interval for management practices ranged from 3.13 to 12.24. Cattle in farms without acaricidal control measures had an odds ratio of 7.97, indicating a substantial increase in the odds of Theileriosis. The p-value for not applying acaricidal control on the farm was 0.001, signifying statistical significance. The 95 % confidence interval for acaricidal control ranged from 3.96 to 16.03. The presence of other animals was associated with an odds ratio of 3.83, suggesting that it increased the odds of Theileriosis. The p-value for the presence of other animals was 0.001, indicating strong statistical significance. The 95 % confidence interval for the presence of other animals ranged from 2.62 to 5.60. The odds ratio for location was 3.83, implying that the odds of babesiosis varied by location. However, the p-value for location was 0.112, which is not statistically significant. The 95 % confidence interval for location ranged from 2.62 to 5.60.

Table 5. Classification of Cattle as Theileriosis Positive or Negative in Relation to Different Risk Factors

Risk factor	Category	Theileriosis		OR	CI	P
		+ve (n=12)	-ve (n=148)			
Age	1-2	3	31	0.64	0.31–1.30	0.22
	3-4	5	43			
	5>	4	74			
Sex	Male	6	63	0.67	0.45–1.00	0.72
	Female	6	85			
Management practices	Free range	11	109	6.19	3.13-12.24	0.001
	Intensive	1	39			
Acaricidal control in the farm	Yes	1	39	7.97	3.96-16.03	0.001
	No	11	109			
Presence of other animal	Yes	11	109	3.83	2.62–5.60	0.001
	No	1	39			
Location	Wadata	2	38	3.83	2.62–5.60	0.112
	Northbank	4	36			
	Wurukum	4	36			
	Abinsi	2	38			

Abbreviations: CI, confidence interval at 95 %; OR odds ratio; P, P value

DISCUSSION

The overall prevalence of *Theileria* and *Babesia* was 30 (18.8 %) out of 160 samples examined. This implies

that *Theileria* and *Babesia* species are prevalent in the area. The prevalence of 18.8 % suggests that nearly one-fifth of the cattle in the study area were carrying these parasites. These parasites can have various effects on cattle, including causing diseases and affecting their overall health. The result indicates that there is a noteworthy presence of *Theileria* and *Babesia* in this area, which may require attention in terms of prevention, management or potential treatments for affected cattle. However, the overall prevalence of (18.8 %) gotten in this study is lower than that recorded by Kamani *et al.*, (2010) who reported the overall higher prevalence of 25.7 % for *Theileria* and *Babesia* in North-central, Nigeria and also Zawua *et al.*, (2015) who recorded an overall prevalence of 28.9 % in slaughtered cattle from Gboko, Benue State. The difference in the prevalence rate observed in this area may be attributed to differences in geographical location and the husbandry systems. However, Okorafor and Nzeako (2014) and Ademola and Onyiche (2013) reported lower prevalences of *Theileria* and *Babesia* species, 6.7 % and 5 in Oyo State. This high disparity in prevalence values could be attributed to local differences in prevalence of *Theileria* and *Babesia* due to variations in geographical location (Velusamy *et al.*, 2014).

The study also recorded a higher prevalence of *Theileria* and *Babesia* species in male cattle 16(23.2 %), than female 14(15.4 %). However, there was no significant difference between the prevalence in male and female cattle ($p>0.05$). In other words, the variation in prevalence between the two groups could have occurred by chance and may not be a meaningful difference. The high prevalence in male may be attributed to their type of grazing. Most of the male cattle were allowed to graze freely in the field. While the female cattle were restricted in a semi-intensive rearing especially during lactation period. Also the results highlight a significant gender-based difference in parasite prevalence, with a higher prevalence observed in male cattle compared to female cattle. These differences might be due to various factors, such as variations in immune response, behavior or exposure to the parasites. Understanding these gender-specific differences in parasite prevalences is important for managing the health and well-being of cattle populations, as it can inform targeted interventions and strategies for disease control and prevention in different groups of cattle. This result differs from the study of Yu Chen *et al* (2021), who reported that the prevalence of *Theileria* and *Babesia* species was higher in female (48.9 %) than that in male (45.8 %). This difference may be attributed to the geographic location and the type of husbandry.

In respect to age the result show that most of the infected cattle were 5 years and above 12(15.4 %) followed by those within 3-4 years 11 (22.9 %) and 1-2 years 7(20.6 %), although there was no significant difference ($p>0.05$). The high prevalence in older cattle may be as a result of their ability to graze in free range to a long distance while the younger ones are confined in a semi-intensive system. This result agrees with several studies as reported by Kamani *et al.*, (2010); Alim *et al.*, (2012); Ademola and Onyiche, (2013); Okorafor and Nzeako, (2014). In their findings, older cattle had a higher prevalence of *Theileria* and *Babesia* species compared to their younger counterparts which is in contrast with Ademola *et al.*, (2013) who observed that prevalence of haemoparasites in ruminants decreased with increasing age. This could be as a result of immunity acquired from previous infection by the adult cattle. Kamani *et al.*, (2010) however reported higher prevalence in older Cattle and stated that this could be due to the fact that adults are readily susceptible to *Theileria* and *Babesia* compared to younger ones because of their longer period of exposure to the arthropod vectors.

Result of PCR amplification in this study shows that *Theileria orientalis* species is (11.25 %) and is nearly similar with results of a study conducted by traditional diagnostic PCR method in middle Delta of Egypt with prevalences of (9.18 %; Nayel *et al* 2012). However a lower prevalence of (5.30 % and 3.97 %) was documented in west Delta and Upper Egypt (Ibrahim *et al* 2012). On the other hand in other tropical and subtropical areas, PCR showed a prevalence of 26.7 in Brazil (Costa *et al* 2006). 18.8 % in Philippines (Yu *et al* 2013) 29 % in Pakistan (Chaudhry *et al* 2010) and 78.5 % in Portugal (Silva *et al* 2009) such fluctuation in the prevalence might be attributed to the differences in animal gender, breed, ecology of the area, management practice and irregular use of antiprotozoal and acaricidal drugs. The higher prevalence of *T. orientalis* infection than *B.bovis* detected in this study is in agreement with previous surveys performed in Egypt and other locations in Africa. (Ibrahim *et al* 2009, and Mohammed 2012). The prevalence of *theileria* species in this investigation is parallel with those previously recorded in different provinces. In part of Egypt Quena (11.1 %), Gharbia (11.3 %) and menofia (16.05 %) (Nayel *et al* 2012) and that obtained in Turkey 17 % (Acici 1995).

On the contrary, high infection rates (65 %) of theileriosis was detected in Egypt by using Giemsa stained blood smear (EL- fayomy *et al* 2013). Furthermore, prevalences of *theileria* species higher than 20 % were identified in Tanzania (Ogden *et al* 2003). Turkey (Altay *et al* 2008) and Tunisia (M'gherbi *et al* 2008). Inconstancy in theileriosis and Babesiosis prevalence might be attributed to the disparities in animal age, ecological area, management practice, irregular use of antiprotozoal and presence of other animal species

Among the risk factors that predisposed cattle to infection with *Babesiosis* and *Theileriosis*, management practices significantly influence the risk of *Babesiosis* and *Theileriosis* infections. In particular, cattle that are allowed to graze freely in a range are at a higher risk of contracting these diseases. This means that animals with unrestricted movement and access to different areas are more likely to become infected. The use of acaricides, which are chemicals or treatments aimed at controlling ticks and mites, is another significant factor. The statement suggests that farms where acaricidal control measures are not practiced have a higher risk of *Babesiosis* and *Theileriosis* infection. In essence, the absence of tick and mite control measures on farms increases the susceptibility of cattle to these parasitic infections. The presence of other animal species in the same farm is also highlighted as a significant factor. Farms that have mixed animals (different species of animals) tend to record higher infection rates of *Babesiosis* and *Theileriosis*. This implies that the coexistence of various animals in the same environment can contribute to the spread of these diseases. This result is consistent with the finding of Mohamed *et al* (2017) whose findings revealed that management practices, acaricidal control and presence of other animals were significant risk factors in the infection of *Babesiosis* and *Theileriosis*.

CONCLUSION

The findings revealed the presence of *Theileria* and *Babesia* species infection in the study area underscoring the importance of regular surveillance and control measures to manage these infections in the cattle population. Furthermore, the identification of risk factors, such as sex (male or female cattle) species and management practices highlights the need for targeted interventions to mitigate the spread of these parasites.

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