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Risk Factors Associated with Common Infectious Diseases in Beef Cattle in Menofia Governorate

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ABSTRACT

This study was conducted in Menofia governorate to determine the most common infectious diseases in beef cattle and associated risk factors. A total of 450 samples were collected from 150 diseased beef cattle of different ages, sex and seasons. Results of blood smears revealed that 14.7% were positive for blood parasites, including 9.3% and 5.4% for *Babesia* and *Theileria* infection, respectively. Meanwhile, fecal examination revealed that 67.3% were positive for at least one gastrointestinal parasite, including 18.6%, 10%, 22%, and 16.7% for *Trichostrongylus*, *Moniezia*, *Eimeria* species, and mixed infection respectively. Regarding the bacteriological examination revealed that 92% were positive for bacterial culture; *Klebsiella*, *Pseudomonas*, *Citrobacter*, *Proteus* species and mixed infection represented 56%, 15.3%, 6%, 4.7%, and 10% respectively. Risk factor analysis revealed that sex and season were significant with *Babesia* infection, as the cold season and females gender were 6 times and 3.45 times more susceptible than the warm season and males, while in *Theileria* infection older age animals were 3.27 times more susceptible than young age as well animal age was the main significant risk factor in gastrointestinal parasitic infection as older age, were 1.991 times the young age of attainment *Trichostrongylus* infection while older age was 1.946 times the young age in *Moniezia* infection. Additionally, the season was significant determinant in respiratory tract infections as cold season, was 15.27, 14.46, 8.89, 4.48, 25.08 times more susceptible than warm season for *Klebsiella*, mixed infection, *Citrobacter*, *Proteus*, and *Pseudomonas* respectively. In conclusion, this study spot highlights on the most important infectious diseases among beef cattle in Menofia Governorate.

**Keywords:** Beef cattle, *klebsiella*, blood parasite, internal parasite, risk factors.

INTRODUCTION

The population of cattle in the world is about 1.1 billion head (FAO, 2018). In Egypt, cattle population reached about 3,476,396 heads in 2019, in 2021 the cattle stock estimated about 2.82 million

heads, and about 3,54 million heads in 2023 (USDA, 2023). For cattle and buffalo herds' distribution in Egypt, Menofia occupied the third place after Behera and Beni-Suef governorates. The bovine production systems in Menofia represented as 1.4% intensive (Herd size varies from

10 up to over 1,000 heads of cattle and buffalo), 72.6% semi-intensive (Herd size can range from 10 to more than 50 heads of cattle and buffalo), and 26% extensive (Herd size varies from 1 to 10 indigenous heads of cattle and buffaloes (FAO, 2018).

Parasitic gastroenteritis, blood parasites, and bovine respiratory disease complex are among the common infectious diseases of livestock in Egypt (Radostits, 2000). The presence of helminth parasites, specifically gastrointestinal nematodes like *Trichostrongylus*, and liver flukes (specifically *Fasciola Hepatica*), poses a significant risk to the economic viability and long-term viability of ruminant livestock production and had negatively influenced growth productivity and reproductive success (Schweizer et al., 2005). Various gastrointestinal parasites, including roundworms, liver flukes, tapeworms in the small intestine, and single-celled protozoan parasites (*Coccidia*) in the lower digestive tract, have been observed in affected cattle of all age groups, with a particular impact on young cattle (William and Loyacano, 2012).

Concerning to the tick-borne pathogens, including species of *Theileria*, *Babesia*, and *Anaplasma*, are widely distributed across the globe, with a particular concentration in tropical and subtropical regions. In Egypt, tick-borne pathogens have been found to exhibit a significant prevalence in various geographical areas, especially during the epidemics of LSD and FMD viruses. This observation was reported by Abas et al. (2021), who documented high prevalence rates of tick-borne pathogens during these outbreaks.

In addition, respiratory tract diseases in the livestock business result in significant economic losses annually. The condition is often known as Bovine Respiratory Disease Complex (BRDC), which is frequently attributed to factors such as stress, viral agents, and bacterial

agents. In chronic cases, these agents may induce lasting lung impairment such as fibrosis, adhesions, and/or abscesses, which can have a detrimental effect on an individual's overall performance (Taylor et al., 2010). According to Cheng et al. (2018), *Klebsiella pneumoniae* is recognized as a zoonotic and foodborne threat on a global scale. In addition, Darsana et al. (2015) identified Hypervirulent *K. pneumoniae* (hvKp) as the primary pathogen responsible for bovine mastitis and pneumonia. However, Francis and Ameh (2015) found limited evidence supporting the relationship between *K. pneumoniae* and bovine respiratory disease. Therefore, the present study was planned to investigate the parasitic gastroenteritis, blood parasites, bacterial species associated with respiratory manifestation in fattening beef cattle in Menoufia governorate.

## **MATERIAL AND METHODS**

### **1-Animals and location**

A total of 450 samples consisting of (150 blood smears, 150 fecal samples, and 150 nasal swabs) were collected from 150 beef cattle, of both sex and different localities, their age ranged from six months to three years (Table 1). The examined animals were suffering from fever, respiratory manifestations, enteritis, and bloody diarrhea. This research was carried out in the Menoufia governorate throughout the period of August 2022 to March 2023. The Menoufia governorate is located in the northern region of Egypt, specifically in the Nile Delta. Geographically, the governorate is situated between two branches of the Nile River in the northern part of Egypt. The climate in Menoufia is a hot desert climate, with temperatures typically ranging from 15 to 35°C. The region under investigation is primarily characterized by agricultural activities and possesses numerous reservoirs that are conducive to the proliferation of arthropod vectors.

**Table (1):** Animal grouping according to sex, location and age:

Item	Sex		Location		age		
	Male	female	Menoufia	Desert road	Less than one year	One year	More than one year
Number	114	36	128	22	43	53	54

### **2. Clinical examination of animals**

Clinical examination of animals was performed according to (Radositis et al., 2007). The recorded clinical signs of piroplasmosis included fever (40-41C), anorexia, cessation of rumination, pale and icteric mucus membrane, hemoglobinuria and enlargement of superficial lymph nodes, dry cough, nasal discharge, and rapid respiration.

### **3. Collection of blood samples and Giemsa-stained blood smear**

Blood samples were collected from the jugular vein and ear vein of the animals and immediately preserved in a sterile tube with an anticoagulant agent (EDTA) for preparing a blood smear as described by (Soulsby, 1982, Houwen, 2000 and Harvey, 2012) for detecting the presence of blood parasites.

### **4. Collection of fecal samples and parasitological examination**

One hundred and fifty fecal samples were collected from diseased beef cattle; feces were collected directly from the rectum. As the animals were restrained properly, 20-25 grams of feces were placed in sealed plastic containers preserved in 10% formalin, labeled, and transported to the laboratory for immediate examination by direct smear examination (Demeke, et al., 2021), flotation method (Gorden and White lock 1939) and sedimentation method (Zajac and Conboy 2012). The flotation, sedimentation, and direct fecal smears techniques were performed according to (MAFF, 1986; Cringoli et al., 2004 and Zajac, et al., 2021).

### **5. Collection of nasal swabs and bacteriological examination**

One hundred and fifty nasal samples were collected from diseased beef cattle by inserting a sterile swab deeply into the nasal passage after proper and put into the labeled sterile tube containing 2ml brain heart infusion broth, then aseptically transferred to the laboratory in an ice box to be examined immediately with a minimum delay for bacteriological isolation and identification, according to standard techniques of Quinn et al. (2002).

### **6. Statistical analysis**

Data of cattle including locality, season, age, sex, and breed. The association between positive samples and these animal attributes was identified individually using a multinomial logistic regression analysis model that was carried out in IBM SPSS Statistics for Windows version 21.0(IBM SPSS Inc., Armonk, NY). The Chi-square tests were performed and differences were considered significant at  $p \leq 0.05$ .

## **RESULT**

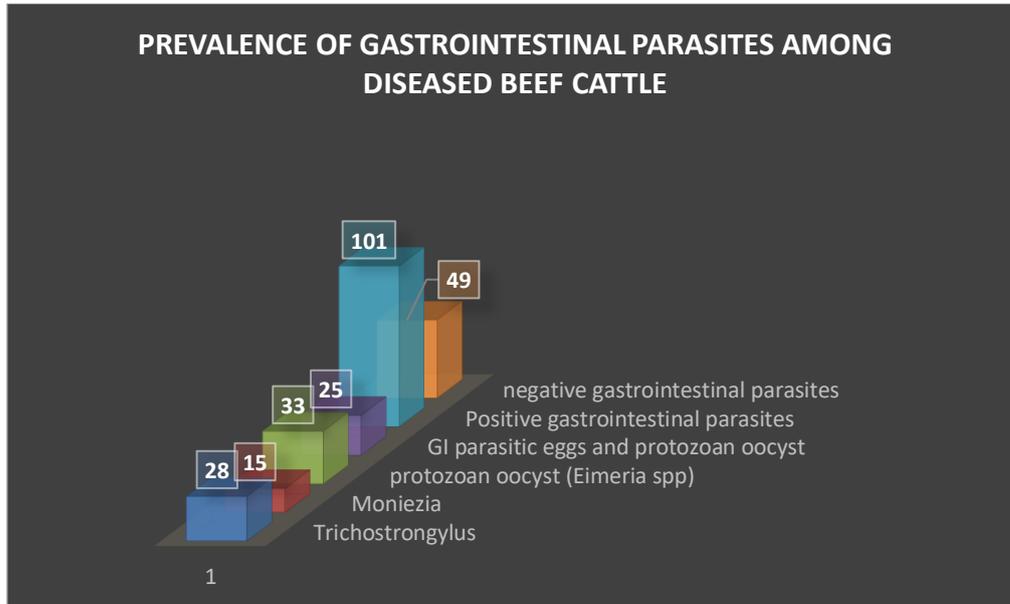
### **1. Prevalence of tick-borne blood parasites among diseased beef cattle**

Out of 150 blood smears, 22/150 (14.7%) were positive for one species of blood parasite, and 128/150 (85.3%) samples were negative. Form the positive samples, 14/150 (9.3%) were identified as *Babesia* species and 8/150 (5.4%) samples were identified as *Theileria* species.

**2. Prevalence of gastrointestinal parasites among diseased beef cattle**

Out of the 150 fecal samples, examined by different parasitological methods, 101 (67.3%) were positive for at least one enteric parasites, while 49 (32.7 %) were negative. The most commonly observed gastrointestinal

parasite eggs were *Trichostrongylus* 28 (18.6%), followed by *Moniezia* 15 (10%), Mixed infection of gastrointestinal parasites while *Eimeria* species oocyst 33 (22%). and protozoan oocyst was 25 (16.7%), as illustrated in Figure 1.

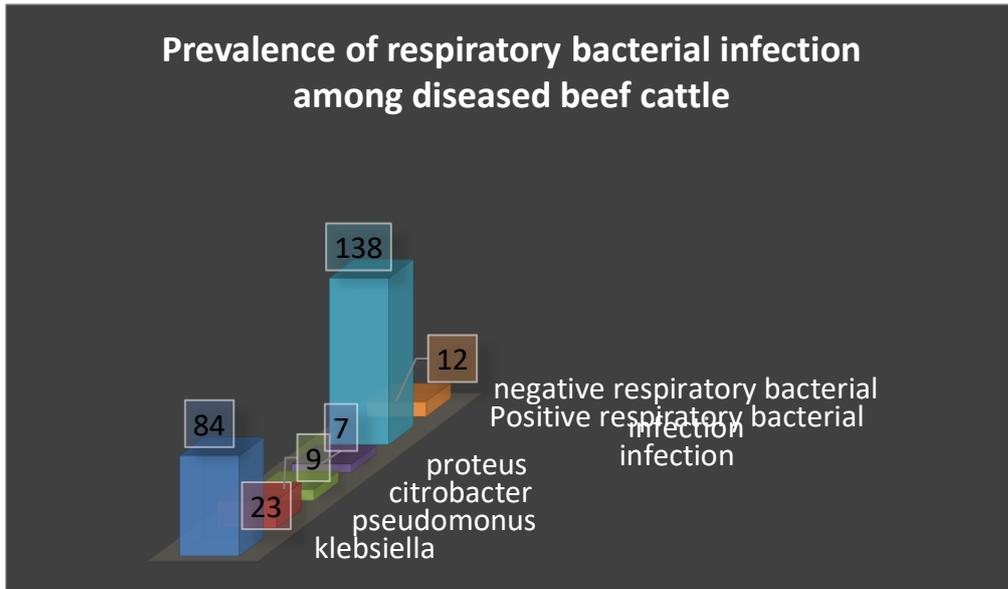


**Figure (1):** Prevalence of gastrointestinal parasites among diseased beef cattle.

**3. Prevalence of respiratory bacterial species among diseased beef cattle**

One hundred and fifty nasal swabs collected from diseased beef cattle were subjected to bacteriological isolation and identification, the results revealed that 138/150 (92%) were positive for

culture. The most identified species were *Klebsiella* 84/150 (56%), *Pseudomonas* 23/150(15.3%), *Citrobacter* 9/150 (6%), *Proteus* 7/150 (4.7%) and mixed infections 15/150 (10%), while negative culture were 12/150(8%) as showed in figure 2.



**Figure (2):** Prevalence of respiratory bacterial infection among diseased beef cattle.

**4. Risk factors associated with tick-borne blood parasites among diseased beef cattle**

Breed, age, and location variables were not significant risk factors for *Babesia* infection despite that Babesiosis in older age animals was 1.1 times as in young ones. The prevalence of Babesiosis in the cold season was almost 6 times more than in the warm season and 3.45 times higher in females gender than males which was

almost significant at  $p < 0.06$  and  $p < 0.07$  respectively. While, the prevalence of Theileriosis in older ages was 3.27 times higher than in younger which was significant at  $p < 0.05$ . Other risk factors (Breed, sex, age, and location) were not significantly associated with Theileriosis, despite that the prevalence was higher in native breed than Charolais and Mobiliar and in cold than warm seasons as illustrated in Table 2.

**Table (2):** Risk factors associated with tick-borne blood parasites among diseased beef cattle.

Blood parasite	Wald	Sig.	OR	95% Confidence Interval for OR	
				Lower Bound	Upper Bound
<i>Babesia</i>	Intercept	.175	.676		
	Age	.194	.660	1.114	.689 1.800
	Sex	3.453	.063	3.450	.934 12.736
	Season	3.186	.074	6.146	.018 1.207
	Breed	.000	.998	4.439E-9	.000 .
	Location	.	.	.	. .
<i>Theileria</i>	Intercept	11.172	.001		
	Age	6.340	.012	3.271	1.300 8.227
	Sex	.120	.729	.723	.115 4.549
	Season	.032	.857	1.162	.227 5.932

Breed	.	.	1.888E-8	1.888E-8	1.888E-8
Location	.	.	.	.	.

**5. Risk factors associated with associated with gastrointestinal parasites among diseased beef cattle**

Sex, season, breed, and location variables were not significant risk factors for the prevalence of internal gastrointestinal parasites among examined animals. The older age was 1.991 times more *Trichostrongylus* infected than the young age which was almost significant at  $p < 0.008$ . While, in *Moniezia* infection in

the older age animals was 1.946 times more infected than young age which was almost significant at  $p < 0.038$ . Concerning to *Eimeria* and mixed infections, all the variables including age, sex, season, Breed, and location were not significant risk factors as illustrated in Table 3.

**Table (3):** Risk factors associated with associated with gastrointestinal parasites among diseased beef cattle.

Internal parasite	Wald	Sig.	OR	95% Confidence Interval for OR	
				Lower Bound	Upper Bound
<i>Trichostrongylus</i>	Intercept	6.513	.011		
	Age	6.995	.008	1.991	1.195 3.317
	Sex	.000	.985	1.012	.283 3.624
	Season	.014	.906	.928	.269 3.200
	Breed	.242	.623	.650	.116 3.628
	Location	.	.	.	.
<i>Eimeria oocyst</i>	Intercept	2.061	.151		
	Age	12.326	.000	.299	.152 .586
	Sex	.255	.613	1.449	.344 6.099
	Season	1.827	.177	2.304	.687 7.734
	Breed	1.034	.309	1.854	.564 6.091
	Location	.	.	.	.
mixed infection	Intercept	.096	.756		
	Age	1.594	.207	.740	.463 1.181
	Sex	.045	.832	1.151	.313 4.232

	Season	1.286	.257	1.925	.621	5.967
	Breed	.	.	9.869E-10	9.869E-10	9.869E-10
	Location	.	.	.	.	.
<i>Moniezia</i>	Intercept	1.016	.314			
	Age	4.320	.038	1.946	1.039	3.647
	Sex	1.240	.265	.374	.066	2.113
	Season	1.721	.190	.227	.025	2.082
	Breed	.894	.344	.340	.036	3.184
	Location	.	.	.	.	.

**6. Risk factors associated with respiratory bacterial infections among diseased beef cattle**

Breed, age, location, and sex variables were not significant risk factors for respiratory bacterial infections among the diseased beef cattle, while season was the main important risk factor. The

prevalence of *klebsiella*, *Citrobacter*, *Proteus*, *Pseudomonas*, and mixed bacterial was higher in the cold season 15.271, 4.482, 8.896, 25.078, and 14,461 times than warm season which was significant at  $p < 0.001$ ,  $p < 0.133$ ,  $p < 0.067$ ,  $p < 0.003$  and  $p < 0.011$  respectively, as showed in Table 4.

**Table (4):** Risk factors associated with respiratory bacterial infections among diseased beef cattle.

Nasal swab		Wald	Sig.	OR	95% Confidence Interval for OR	
					Lower Bound	Upper Bound
<i>Klebsiella</i>	Intercept	2.897	.089			
	Location	1.982	.159	.222	.027	1.805
	Breed	.	.	.	.	.
	Sex	1.229	.268	2.895	.442	18.958
	Age	.008	.930	1.027	.573	1.839
	Season	10.175	.001	15.271	2.861	81.525
<i>Citrobacter</i>	Intercept	3.219	.073			
	Location	.	.	1.584E-9	1.584E-9	1.584E-9
	Breed	.	.	.	.	.
	Sex	.013	.909	.865	.072	10.435
	Age	.860	.354	1.432	.670	3.061
	Season	2.255	.133	4.842	.618	37.945
	Intercept	3.369	.066			

<b><i>Proteus</i></b>	Location	.575	.448	.313	.016	6.309
	Breed	.	.	.	.	.
	Sex	.473	.492	2.496	.184	33.879
	Age	.026	.873	1.072	.458	2.508
	Season	3.364	.067	8.896	.861	91.947
<b><i>Pseudomonas</i></b>	Intercept	6.197	.013			
	Location	.363	.547	.509	.057	4.576
	Breed	.	.	.	.	.
	Sex	.665	.415	.312	.019	5.125
	Age	.344	.557	1.238	.607	2.522
	Season	8.562	.003	25.078	2.897	217.052
mixed infection	Intercept	5.292	.021			
	Location	1.114	.291	.257	.021	3.208
	Breed	.	.	.	.	.
	Sex	.430	.512	2.117	.225	19.918
	Age	.185	.667	1.169	.573	2.387
	Season	6.537	.011	14.461	1.866	112.098

## DISCUSSION

Babesiosis and Theileriosis are parasitic protozoa belonging to the apicomplexan group, specifically the hemoprotozoan subclass responsible for significant losses to livestock production, particularly cattle. These illnesses possess global relevance and are characterized clinically by anemia, icterus, hemoglobinuria, and fatality particularly in tropical and temperate countries (Silva, et al., 2010). In the current study, the prevalence rate of blood parasites was 14.7% out of 150 samples and Babesia species was prevalent in 9.33%. Similar findings were reported by Aldham et al., (2009), Adel (2007), EL-fayomie et al., (2013), and Abdel Aziz et al. (2014) they recorded Babesiosis infection rate 13% in Giza, 11.1% in Gharbia and 13% in Port Said and 12% in Sharkeya respectively. On the other hand, lower prevalence rate than Yasser (2014) who recorded (68.9%) in Egypt, (22.47%) by Mohamed and Ebied (2014) in Qalubia governorate, (31.7%) by EL-Seify (1989) in Beni-Suef governorate. While theileriosis was

detected with 8 (5.33%) in our study result. This was lower than results obtained by Adel, (2007) who reported 11.31% in Gharbia governorate, and Salem et al., (1993) who reported 10% of imported cattle and 8.75% of native cattle in Quena, while, Gamal EI-Dien, (1993) recorded higher prevalence 65.4%.in EI-Behera governorate. The variance between the studies may be explained as difference in the environmental, geographic lactation, hygienic measures, cattle breeds and veterinary care.

Regarding to the results of gastrointestinal parasites infestation in the current study, 101 (67.3%) were positive for at least one genus of enteric parasites. Conversely. This observation is consistent with previous studies conducted by Biffa et al. (2007) who recorded that the prevalence of helminths was 82.2% in Ethiopia as well as Fabiyi and Adeleke (1982) reported a prevalence rate of 65.4% among cattle. The prevalence of trichostrongylus was (18.6%) in the current study which was higher than that recorded by Yuwajita *et al.*, (2014) who

recorded (10.76%) in Udon Thani, Thailand, and less than (26.29%) recorded by Kaewnoi, *et. al.*, (2020) in beef cattle. Additionally, Muktar, *et al.* (2015) recorded (15.19%) prevalence of *Trichostrongylus* at Dire Dawa, Ethiopia. The variation in results may be due to differences in management and husbandry practices, climate, and pastures (Smith, 2009). According to the current study, the prevalence of *Eimeria spp* oocyst was 22%, and mixed infection of gastrointestinal parasitic eggs was 16.7%. This was nearly similar with (Melo *et al.*, (2022) who recorded 17.12% prevalence rate of *Eimeria* species among cattle. Higher prevalence was recorded by Hillesheim and Freitas (2016) who reported 48.2% prevalence of *Coccidia* among the cattle farms in Brazil. The lower prevalence rate was recorded by Lopez-Osorio *et al.* (2020) in Colombia and by Hastutiek *et al.* (2019) in Indonesia, with 75.5% and 53.42% respectively.

The occurrence of respiratory diseases are prevalent among various household animal species that are influenced by multiple factors including the interaction between infectious pathogens as well as the host's defensive mechanisms and environmental conditions (Lacasta *et al.*, 2008; Holman *et al.*, 2017). In this current study, 138 (92%) were positive for bacterial species and *Klebsiella* species (56%), *Pseudomonas* species (15.3%), *Citrobacter* species (6%), *Proteus* species (4.7%) were the most frequently isolated bacteria and mixed infection was evident in (10%). In a related study conducted by Francis, and Ameh, (2015) revealed that *S. aureus* (18.67%), *K. pneumonia* (16.67%), *S. pneumonia* (12.00), *Proteus Bulgaria* (11.33%), *P. multocida* (11.33%), *E. coli* (10%), *Corynebacterium spp* 12 (8%), *Salmonella spp.* 12(8%) and *Enterobacter spp.* 6 (4%) were the identified prevalence bacterial species from 150

pneumonic lung. In addition, Venkatesakumar *et al.*, (2020) recorded that *P. multocida* (55.95%), *Pseudomonas aeruginosa* (16.67%), *K. pneumoniae* (9.52%), and *E. coli* (8.33%) were isolated from bronchoalveolar lavage fluid. In other studies, Mahmoud *et al.* (2005) and Saleh and Allam (2014) found that *K. pneumoniae* was the most prevalent bacteria with 48%, followed by *S. aureus* (44%) and *Proteus spp* (20%). On the other hand, Lim *et al.*, (1995), and Wills *et al.*, (1997) isolated *K. pneumonia*, *P. Multocida*, *Pseudomonas aeruginosa*, and *Strep pneumonia* from the upper respiratory tract of dairy cattle. The fluctuation in the proportion of isolation can be attributed to variations in sanitary measures, stress factors, modifications in management practices, and the immune condition cattle herds (Sedeek and Thabet, 2001). However, many bacterial pathogens include *Mycobacterium*, *Acinetobacter baumannii*, *K. pneumoniae*, and *Streptococcus. Pneumoniae*, *H. influenza*, *E. coli*, *S. aureus*, and *Pseudomonas aeruginous* are among the primary bacterial strains that have been extensively documented as causing respiratory infections associated with morbidity and mortality in developing nations (Woldemeskel *et al.*, 2002).

The current study investigated some risk factors associated with blood parasites infestation and the results indicated significant effect of season on the prevalence of Babesiosis and Theileriosis which is 6 times more prevalent in cold seasons than in warm seasons. This was disagreed with other studies (Qayyum *et al.*, (2010); Naz *et al.*, (2012); Patel *et al.*, (2017); and Zaman *et al.*, (2022), they demonstrated the high prevalence of Babesiosis and Theileriosis in the summer season, followed by autumn, spring, and winter. Additionally, certain variations observed in our study, which could possibly be attributed to the role of fomites as well as climate change

that suggested to change the tick habitats and supposed the higher prevalence of Babesiosis and Theileriosis during colder seasons compared to warmer seasons particular in regions where ticks were not previously observed (Martínez-García et al., 2021).

Furthermore, the animal sex had a significant association with the prevalence of Babesiosis as it found to be 3.45 times higher in females compared to males. This finding is consistent with reports by Zaman et al. (2022), and Atif et al. (2012). However, it contradicts the results of previous studies conducted by Hussein et al. (2017) and Idris et al. (2018). The observed disparity in our study could potentially be attributed to some factors such as breeding stress, milk production, pregnancy, parturition, poor feeding, older age, hormonal changes, and the usage of females for draught purposes (Maharana et al., 2016; Bary et al., 2018). While this finding was not in contact with Gray and Murphy (1985) who noticed that there is no observed variation in susceptibility between breeds or sexes.

The effect of age was also investigated and the older age females were 1.1 times more susceptible than young age in *Babesia* infestation with statistically significant effect. This result was in consistent with the previous reports of Kaur et al. (2016). Meanwhile, inconsistent with Zaman et al., 2022; Muhanguzi et al. (2010) and Swami et al. (2019) who recorded greater infection rate in calves compared to adults which may be attributed to several factors, including the presence of softer skin in young animals, reduced immunity, inadequate nutrition due to underfeeding, suboptimal housing conditions, and the prevalence of diseases in specific geographic areas (Lawrence et al., 2019 and Zeb et al., 2020). Additionally, Chauvin et al. (2009) revealed correlation between the age of cattle and the clinical

stages of Babesiosis, and young animals 3 - 9 months had greater resistance compared to adult animals

The current study investigated some risk factors (age, sex, season, Breed, and location) that associated with gastrointestinal parasites infestation and the results indicated non-significant effect of these parameters. Even though, (Edosomwan et al, 2012) demonstrated that numerous risk factors are known to exert an influence on the occurrence and severity of gastrointestinal helminth infections such as age, gender, meteorological conditions, and husbandry or management practices and considered the key contributing variables for the development of parasite infections.

This study revealed that season is the main important risk factor in the prevalence of bacterial infection as the cold season is almost 15.271 times more than the warm season for *Klebsiella* infection with highly significance. The same pattern for *Citrobacter*, *Proteus* and *Pseudomonas* species recording 4.482 and 25.078 and 14,461 time respectively in cold season than warm season (Callan and Garry, 2002). It has been revealed that environmental risk factors such as humidity, severe fluctuations in environmental temperatures and elevated concentrations of harmful gases like ammonia are considered risk variables that can potentially elevate the density of pathogens or raise the pathogen exposure. The current study reported a correlation between the occurrence of pneumonia and colder seasons, which previously reported by Catania et al. (2020). In contrast, Fanelli et al. (2021) proposed that the epidemic usually occur due to major fluctuations in temperature, rather than cold temperatures.

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