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Sero epidemiological study on bovine babesiosis in cattle and buffaloes in Sharkia Governorate, Egypt

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Abstract

Background: Bovine babesiosis represents a serious challenge for animal health, herd production, and profitability. Understanding the epidemiology and risk factors associated with babesiosis is critical to reduce their negative impacts.

Aim: Investigation of the seroprevalence and risk factors associated with *Babesia bigemina* (*B. bigemina*) and *Babesia bovis* (*B. bovis*) in five districts in Sharkia governorate using ELISA.

Methods: Across-sectional research was conducted to determine the seropositivity of babesiosis by collecting a total of 352 blood samples from 250 cattle and 102 buffaloes. A multivariate logistic regression model was implemented to evaluate the strength of the risk factors associated with both Babesia species infection.

Results: The seroprevalence of *B. bigemina* and *B. bovis* was 42.6% and 17.0 %, respectively. The prevalence of babesiosis in cattle was found to be 48.8% for *B. bigemina* and 16.8% for *B. bovis*. Inclusive, in buffaloes, the prevalence was 27.5% for *B. bigemina* and 17.6% for *B. bovis*. Adult animals were more vulnerable to infection with babesia than young animals by 3–5 times, respectively. Males were more susceptible to *B. bigemina* and *B. bovis* than females by 3.7 and 3.5 times. Similarly, the odds of infection in infested animals with ticks were 2–4 times higher than in animals without ticks.

Conclusion: The obtained results revealed that age, sex of the animal, and tick infestation were major risk factors for the seropositivity of both Babesia species. Inclusive, there was no evidence to support the premise that seroprevalence of babesiosis is correlated with the season and species.

Keywords: *Babesia bigemina*, *Babesia bovis*, Egypt, Epidemiology, Risk factors.

Introduction

Ticks and tick-borne diseases are considered a global problem because of their detrimental effects on livestock productivity (Guswanto *et al*., 2017). Bovine babesiosis is considered the most prevalent infection detected in cattle and buffaloes, particularly in subtropical and in tropical areas (Fakhar *et al*., 2012; Jaimes-Dueñez *et al*., 2017). The clinical findings of bovine babesiosis vary from acute to subacute form (Góes *et al*., 2007). The sub-acute form usually occurs in endemic areas with previous exposure and an abundance of tick population; it is manifested only by a slight rise in body temperature (Terkawi *et al*., 2011). Babesia bovis and *Babesia bigemina* are the most popular species in Egypt because of the abundance of their vector ticks (*Rhipicephalus microplus* and Rhipicephalus annulatus) (Adham *et al*., 2009; Hassan *et al*., 2017). In Egypt, Babesia is the most endemic blood parasite in cattle and buffaloes and has a significant impact on milk, meat production, and herd management (Adham *et al*., 2009; Mahmoud *et al*., 2024). Previous studies recorded different prevalence rates of bovine babesiosis among cattle and buffaloes in Egypt (Ibrahim *et al*., 2013; Fereig *et al*., 2017; El-Bahy *et al*., 2018). The variation in the infection rate in Egypt is attributed to animal management, climatic changes, immune status, and study place (Menshawy *et al*., 2020).

Molecular and serological assays are more sensitive in detection and differentiation between babesia species in carrier animals. Furthermore, Nucleic acid-based techniques are capable of identifying one parasite per 1 million RBCs (Criado-Fornelio, 2007). Serological tests such as ELISA and IFA are widely used in large-scale surveillances (Gul *et al*., 2015). ELISA is an adequate serological method as it is less laborious than IFA and has greater sensitivity and specificity for

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detection and differentiation between *B. bovis* and *B. bigemina* (Sharma *et al*., 2013).

Nowadays, understanding the epidemiological features of babesiosis is a critical step toward the managemental control of the disease and improving our ability to detect and predict this health disorder (Aziz *et al*., 2014; Mahmoud *et al*., 2015). To the best of authors knowledge, there is limited information regarding bovine babesiosis in Sharkia governorate, Egypt. Therefore, our study focused on seroprevalence of Babesia species in large ruminants. Moreover, evaluation of the risk factors including seasonal variations, age, species, sex, and tick infestation associated with bovine babesiosis in five districts in the Sharkia governorate.

Material and Methods

Study design and sample size calculation

Our investigation was carried out in Sharkia governorate, particularly in five districts (Zaqaziq, Abu Hammad, Bilbis, Minya Al-Qamh, and Faqus) since April 2023 up to March 2024. Sharkia governorate is located in the northern part of Egypt in the Eastern Nile Delta (Fig. 1). A cross-sectional study has been conducted in five different districts to investigate the prevalence of babesiosis in the Sharkia governorate. In our investigation, each district was subject to a random selection of smallholder farms. These farms were for meat or milk production. Additionally, the cattle and buffaloes that were used for sampling were chosen at random from each farm (Table 1). The minimum sample size was calculated presuming a 95% confidence interval, 50% expected true population and a 0.05 margin of error (α) regarding the following formula (Thrusfield, 2018).

$$n = \frac{(1.96^2\ P\ exp\ (1 - P\ exp)}{d^2}$$

Where, n = Sample size, P exp = Expected true population, d = Desired margin of error.

Samples and data collection

A total of 352 blood samples were collected from apparently healthy 250 cattle and 102 buffaloes for diagnosis of babesiosis infection (*B. bigemina* and *B. bovis*) in Sharkia governorate. The blood samples were collected from jugular vein in sterile vacutainer tubes without anticoagulant for separation of serum. All samples were transported in an ice box to the laboratory of the Animal Medicine Department, Zagazig University, Egypt. The serum samples were separated and frozen at –20°C until further examination. The data regarding animals' species, age, sex, housing condition, and feeding management were recorded during sample collection. Moreover, all animals were inspected for the presence of ticks.

Sandwich ELISA

Babesia bigemina and *B. bovis* antibody test kits were obtained from Sinogeneclon Biotech company (Hangzhou, China). The tests were performed according to manufacturer instructions. Briefly, the antigen-coated microtiter plates were incubated with 50 µl of diluted serum samples (10:40) for 30 minutes at 37°C. The plates were washed five times with diluted washing solution and incubated with 50 µl of horseradish peroxidase conjugate for 30 minutes at

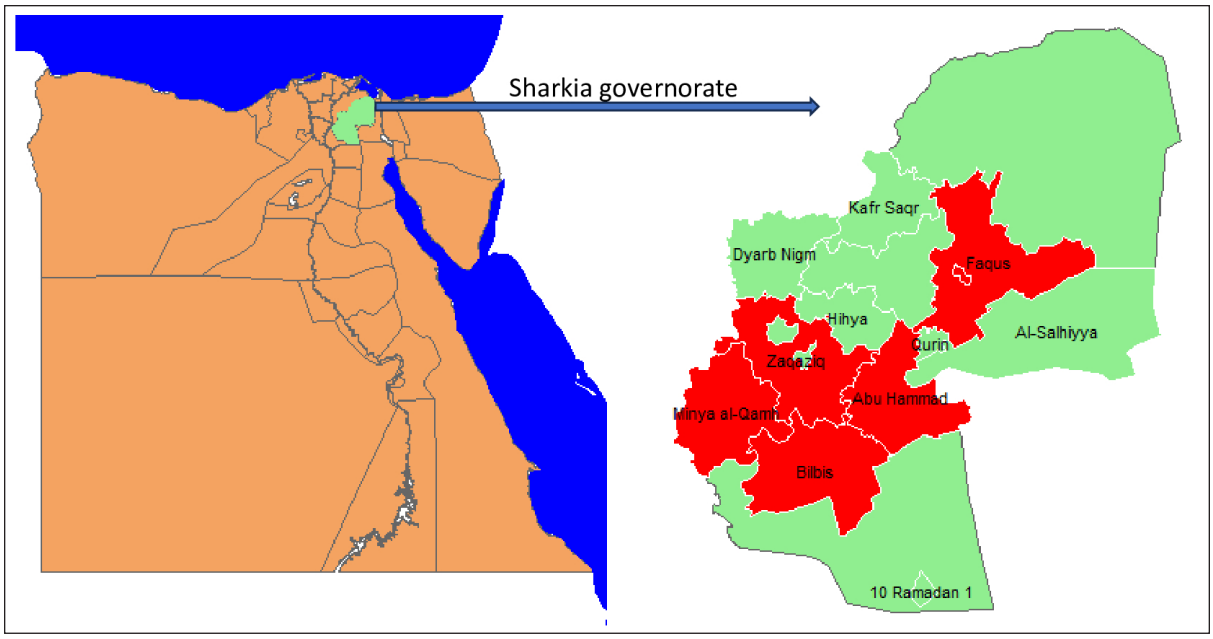

Fig. 1. Map of sampling areas in Sharkia governorate, Egypt. The five districts were Zagazig, Abu Hammad, Bilbis, Faqus and Minya Al Qamh.

37°C. The plates were washed again five times then, and 100 µl of substrate solution was added to each well for 15 minutes. Finally, 50 µl of stop solution was added to stop the reaction. The optical density was measured by a Microtiter plate reader (Biotech 808) at a wavelength of 450 nm. The cut-off value was calculated as the average of negative control well +0.15.

Statistical analysis

The statistical analysis was performed with the SPSS program (SPSS 25 for Windows, SPSS Inc., Chicago, IL, USA). The objected explanatory variables were age, sex and species of the animals, season, location, and tick infestation. The response variable was the serological status of the examined animals (either seronegative or seropositive) for both types of Babesia species. The Univariate logistic regression model was used firstly to evaluate the association between each independent variable and the serological status of *B. bigemina* and *B. bovis*. The predictors with p values ≤ 0.20 were retained in the final multivariate logistic regression model. The predictors with a p value < 0.05 were considered significant.

Ethical approval

The protocol of animal handling was approved by the Institutional Animal Care and Use Committee at Zagazig University with protocol number (ZU-IACUC/2/F/74/2024).

Results

Seroprevalence of bovine babesiosis

The prevalence of babesiosis in the Sharkia governorate was 42.6% and 17.0 % for *B. bigemina* and *B. bovis*, respectively. The number of examined animals and the prevalence of the infection in each district were documented in (Table 1). In total, 250 cattle and 102 buffaloes were tested for *B. bigemina* and *B. bovis* antibodies. The seroprevalence of *B. bigemina* and *B. bovis* in cattle was 48.8% and 16.8%, respectively. In buffaloes, the seroprevalence of *B. bigemina* and *B. bovis* was 27.5% and 17.6%, respectively (Tables 2 and 3). The seroprevalence of *B. bigemina* was (56.8%, 37.8%) while *B. bovis* was (15.9%, 17.4%) for males and females, respectively. The seroprevalence of both Babesia species was higher in the presence of ticks compared to the absence of ticks. Regarding the animal's age, the category (>2–4 years) represented the highest prevalence for *B. bigemina* and *B. bovis* with 57.7% and 24.5%, respectively. *Babesia bigemina* had the highest seroprevalence throughout the summer, with a rate of 47.3%, whereas *B. bovis* had the highest seroprevalence during autumn, with a rate of 28.2% (Tables 2 and 3). The animal-seroprevalence among the studied districts ranged from (28% to 43% for *B. bigemina*) and (10%–20% for *B. bovis*) except for Zagazig that had higher seroprevalence for *B. bigemina* (61%) (Table 1).

Univariate regression analysis

The explanatory variables were evaluated separately for their association with the seropositivity of Babesia species (*B. bigemina* and *B. bovis*). As shown in Table 2, age, sex, species, existence of ticks, and locality were considered significant risk factors for infection with *B. bigemina* ($p \leq 0.20$). Both yearling and adult animals (up to 4 years) were more susceptible to infection compared to calves. Females and buffaloes were at a lower level of risk for infection relative to males and cattle. The presence of tick infestation heightened the risk of infection compared to the absence of ticks. Animals from Minya al-Qamh had the highest risk of exposure compared with other districts. Regarding *B. bovis*, the seropositivity was significantly associated with age, sex, and tick infestation (Table 3). Animals younger than 6 months were more resistant to infection compared to older ages. Males were more vulnerable than females, and infested animals with ticks were more exhibited to infection than animals free from ticks.

Multivariate regression analysis

The final models (Table 4) revealed that the presence of ticks beside age and sex was significantly associated with babesiosis prevalence ($p < 0.05$). The probability of infection increased with age. Adult animals were more exposed to infection with *B. bigemina* and *B. bovis* than young animals by 3–5 times, respectively. The odds of infection in infested animals with ticks were 2–4 times higher than in animals without ticks. The risk of exposure to both infections was lower in females than in males. The odds of exposure to

Table 1. The number of farms and animals selected for the study and subjected to ELISA testing from Sharkia governorate, Egypt, for *B. bigemina* and *bovis* antibodies.

District	N of examined farms	No of examined animals	Positive *B. bigemnia*	Positive *B. bovis*
Bilbis	19	60	26 (43.3%)	6 (10.0%)
Faqus	11	42	14 (33.3%)	6 (14.3%)
Minya Al Qamh	26	110	38 (34.5%)	22 (20.0%)
Abu Hammad	10	42	12 (28.6%)	8 (19.0%)
Zagazig	23	98	60 (61.2%)	18 (18.4%)
Total	89	352	150 (42.6%)	60 (17.0%)

Table 2. The univariate logistic regression model for risk factors associated with *B. bigemina* infection.

Variable	No of examined animals	No of positive animals (%)	OR[1]	95% CI[2]		*P*-value[3]
Age						0.17
<6 months[4]	74	28 (37.8%)				
>6 months–2years	88	36 (40.9%)	1.137	0.464	2.786	
>2 years–4years	106	48 (57.7%)	1.985	.843	4.676	
>4 years	84	28 (33.3%)	0.676	0.326	2.069	
Sex						0.029
Female[4]	264	50 (37.8%)				
Male	88	25 (56.8%)	2.158	1.080	4.313	
Species						0.011
Cattle[4]	250	61 (48.8%)				
Buffalo	102	14 (27.5%)	0.397	0.196	0.806	
Tick						0.012
Absent[4]	156	25 (32.1%)				
Present	196	50 (51.0%)	2.208	1.189	4.100	
Season						0.33
Winter[4]	28	9 (32%)				
Autumn	39	17 (43.5%)	1.810	0.657	4.981	
Summer	84	40 (47.6%)	1.919	0.779	4.727	
Spring	25	8 (32%)	0.991	0.313	3.155	
location						0.018
Faqus[4]	42	14 (33.3%)				
Zagazig	98	60 (61.2%)	3.263	1.117	9.533	
Minya Al Qamh	110	38 (34.5%)	1.056	0.364	3.059	
Bilbis	60	26 (43.3%)	1.529	0.480	4.877	
Abu Hammad	42	12 (28.6%)	0.667	0.171	2.596	

[1]OR: Odds ratio; [2]CI, confidence interval (95%); [3]*p*-value ≤ 0.20 was used to detect which variables will be retained in the final multivariate model; [4]Reference category means the value to which the variable-level is being compared while controlling for the effect of the other predictors in the models.

B. bigemina and *B. bovis* in males were 3.7 and 3.5 times higher than in females.

Discussion

The objective of this study was to evaluate the prevalence and potential causes of bovine babesiosis in various regions within the Sharkia governorate, Egypt, using ELISA. Sharkia is the second governorate at the level of the Republic in terms of agricultural area after the Al-Behera Governorate in Egypt. This investigation was objected to this area because it is one of the most famous governorates for breeding livestock, and there is a lack of available data regarding the infection with bovine babesiosis.

In current investigation, the overall seroprevalence for babesiosis in the Sharkia governorate was 42.6% and 17.0 % for *B. bigemina* and *B. bovis*, respectively. The higher occurrence of *B. bigemina* may be due to the abundance of their main vector tick *(Rhipicephalus annulatus)* in the Sharkia governorate (Yousef, 2020) in addition to the diversity of tick species that transmit *B. bigemina* counter to *B. bovis* that is transmitted mainly by *R. microplus* (Bock *et al*., 2004). Our results agreed with (Ibrahim *et al*., 2013), who reported a higher prevalence of *B. bigemina* compared with *B. bovis* in the Behiera and Faiyum governorates, with percentages of 12.5% and 10.12%, respectively. Similarly, Mahmoud *et al*. (2015) and Fereig *et al*. (2017) reported a higher frequency of *B. bigemina* compared with *B. bovis* in different districts in lower and upper Egypt. On the other hand, a Vietnamese study recorded a higher seroprevalence of *B. bovis* (37.4%) than *B. bigemina*

Table 3. The univariate logistic regression model for risk factors associated with *Babesia bovis* infection.

Variable	No of examined animals	No of positive animals (%)	OR[1]	95% CI[2]		*P*-value[3]
Age						0.123
<6 months[4]	74	8 (10.8%)				
>6 months–2 years	88	14 (15.9%)	1.338	0.347	5.157	
>2 years–4 years	106	26 (24.5%)	3.094	0.936	10.224	
>4 years	84	12 (14.3%)	1.146	0.238	4.633	
Sex						0.107
Female[4]	264	46 (17.4%)				
Male	88	14 (15.9%)	1.964	0.439	5.417	
Species						0.89
Cattle[4]	250	42 (16.8%)				
Buffalo	102	18 (17.6%)	1.061	0.865	4.460	
Tick						0.007
Absent[4]	156	44 (22.4%)				
Present	196	16 (10.3%)	3.684	1.422	9.542	
Season						0.226
Winter[4]	28	4 (14.2%)				
Autumn	39	11 (28.2%)	2.259	0.634	8.047	
Summer	84	12 (14.3%)	0.958	0.281	3.263	
Spring	25	3 (12%)	0.750	0.151	3.732	
location						0.602
Faqus[4]	42	6 (14.3%)				
Zagazig	98	18 (18.4%)	2.195	0.433	11.123	
Minya Al Qamh	110	22 (20.0%)	3.00	0.612	14.715	
Bilbis	60	6 (10.0%)	1.481	0.247	8.901	
Abu Hammad	42	8 (19.0%)	1.667	0.249	11.133	

[1]OR: Odds ratio; [2]CI, confidence interval (95%); [3]*p*-value ≤ 0.20 was used to detect which variables will be retained in the final multivariate model; [4]Reference category means the value to which the variable-level is being compared while controlling for the effect of the other predictors in the models.

(9.3%) in the studied buffaloes (Li *et al*., 2014). This fluctuation may be attributed to the environmental and managemental differences between Egyptian and other foreign districts and the geographic distribution of tick vectors.

The investigated cattle had a higher serological prevalence for *B. bigemina* than water buffaloes, with percentages of 42.6% and 27.5%, respectively. Meanwhile, both animal species were nearly similar in their seropositivity to *B. bovis*. The variation may be attributed to the thinner skin of cattle compared with buffaloes. Thus, cattle are more preferable to tick vectors and more susceptible to infection with blood parasites than buffaloes Siddique *et al*., (2020). These findings coincide with what was mentioned by Jacob *et al*. (2020) in their meta-analysis; they reported that cattle are more susceptible to babesiosis than buffaloes. In the univariable analysis, cattle were more vulnerable to infection with *B. bigemina* compared to buffaloes. These results came in accordance with (Ibrahim *et al*., 2021), who found that water buffaloes revealed a lower percentage of infection with *B. bigemina* (35.6%) compared with cattle (41.6%). On the other hand, Abas *et al*. (2021) recorded a higher prevalence of babesiosis in buffaloes compared to cattle. The seroprevalence of *B. bigemina* and *B. bovis* was higher in males than females. The univariable analysis revealed that the odds of infection in males were 2.15 and 1.9 times higher than in females for *B. bigemina* and *B. bovis*, respectively. The obtained results agreed with Fereig *et al*. (2017), who found that the investigated males had a higher prevalence of *B. bovis* compared to females,

Table 4. The final multivariate logistic regression model for risk factors associated with *B. bigemina* and *B. bovis* infection.

Variable	Level	*B. Bigemina*		*B. bovis*	
		OR[1] (CI 95%)[2]	*p*-value[3]	OR[1] (CI 95%)[2]	*p*-value[3]
Age	<6 months[4]		0.021		0.02
	>6 months–2 years	0.71 (0.24–2.10)		0.89 (0.21–3.72)	
	>2 years–4 years	3.46 (1.24–9.66)		5.25 (1.37–20.07)	
	>4 years	1.18 (0.41–3.43)		1.74 (0.38–7.87)	
Sex	Female[4]		0.011		0.021
	Male	3.7 (1.35–10.21)		3.5 (1.21–10.16)	
Tick	Absent[4]		0.025		0.006
	Present	2.35 (1.11–4.96)		3.9 (1.47–10.66)	
Constant		0.208	0.03		

[1]OR: Odds ratio; [2]CI, confidence interval (95%); [3]*p*-value < 0.05 were considered significant. [4]Reference category means the value to which the variable-level is being compared while controlling for the effect of the other predictors in the models.

with an odds ratio of 4.4. On the contrary, Hamsho *et al*. (2015) and Siddique *et al*. (2020) reported that females had a higher risk for infection with bovine babesiosis than males. However, another Egyptian study mentioned that sex did not represent any risk for babesia infection (Rizk *et al*., 2017).

In both infections, the animals aged from 2 to 4 years had the highest seroprevalence for *B. bigemina* and *B. bovis* with percentages of 57.7% and 24.5%, respectively, followed by the age group (>6 months–2 years). In the univariate analysis, the animal's age was a significant risk factor associated with exposure to the infection ($p < 0.20$). These findings were in the same line with Wesonga *et al*. (2017), who found that the seroprevalence of *B. bigemina* was higher in adult animals. Other studies in Pakistan and Sudan by Atif (2012) and Shuaib *et al*. (2015) didn't detect any association between age and babesiosis. On the other hand, Fereig *et al*. (2017) mentioned that younger ages had a higher risk of *B. bovis* infection. Regarding tick infestation, the risk of infection to Babesia parasites extended with the presence of ticks. The odds of exposure to both Babesia species were 2.2 and 1.4 times in infested animals related to free animals for both Babesia species. In agreement with our results, previous reports in different governorates in Egypt reported that ticks exaggerated the presence of babesiosis (Fadly, 2012).

In the current study, the highest seroprevalence of *B. bigemina* and *B. bovis* was in summer and autumn, respectively. However, there weren't any significant seasonal variations regarding the seroprevalence of both Babesia species. These results coincided with El-Moghazy *et al*. (2014), who reported a higher prevalence of bovine babesiosis in summer and autumn without statistical differences between different seasons. In contrast, several studies reported that the risk of exposure to babesia parasites increased significantly during the summer season (Siddique *et al*., 2020; Ibrahim *et al*., 2021). The close seroprevalence of babesiosis among different seasons may be due to the humid, hot climate in the studied area and the availability of tick vectors most of the year. Moreover, Bock *et al*. (2004) mentioned that the antibodies of both Babesia species may be detected for up to 7 months post- natural infection. The highest seroprevalence of *B. bigemina* and *B. bovis* was recorded in Zagazig and Minya al-Qamh, respectively. There was no significant difference among the studied districts for the distribution of babesiosis except for Zagazig, which had higher seroprevalence for *B. bigemina* (61%). The high prevalence in our investigation may be due to the nature of animal housing in the studied cities, which contributes to tick infestation and impedes effective tick control.

The final multivariate logistic analysis was used to visualize the combined effect of the significant variables in each babesia species. Interestingly, age, sex, and tick infestation are significant risk factors for the seropositivity of both Babesia species. It is clear from the obtained results that the prevalence increased with age. Adult animals are more exposed to infection with *Babesia bigemina* and *B. bovis* than young animals by 3–5 times, respectively. This observation suggests the accumulated parasite exposure by getting older. Furthermore, the sex and existence of tick vectors are an influential factor in the seropositivity of both babesia species. Males were 3.7 and 3.5 times more likely to be exposed to *B. bovis* and *B. bigemina*, respectively, than females. Similarly, the odds of infection in infested animals with ticks were from 2 to 4 times higher than in animals without ticks. In current investigation, most males are fattened and reared in closed houses made from unshelled bricks. Inclusive, these types of houses besides high humidity, are considered a suitable environment for the tick population. Thus, males have a higher risk of exposure to tick infestation and,

accordingly, babesia parasites than females which are reared in an open housing system.

Conclusion

In this study, age, and sex, besides tick infestation, are considerable independent factors that could be used for the prediction of the occurrence of bovine babesiosis in the investigated population. Understanding babesiosis epidemiology can inform future management and control strategies for large ruminants, reducing negative impacts on animal production and profitability. More future studies are recommended for a better understanding of the epidemiological status of bovine babesiosis in Sharkia governorate, Egypt.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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Data availability

All data findings during our investigation are available within the manuscript.

Author contributions

SGY and MHE were involved in data curation, samples collection, formal analysis, discussion of results and prepared the initial draft. NMS and HG were involved in samples collection, discussion of results and review. All authors approved the submitted form.

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