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ORIGINAL PAPER

Serological Survey of *Babesia bigemina* and *Babesia bovis* in Cattle and Water Buffaloes from Menoufia Province, Egypt

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Abstract

Purpose Bovine babesiosis causes morbidity in tropical and subtropical countries worldwide. The present study aimed to determine the seroprevalence of *Babesia bigemina* and *B. bovis* in cattle and water buffaloes in Menoufia province, where the second-highest population of bovines in Lower Egypt are raised.

Materials and Methods A total of 506 blood samples were collected from cattle ($N=262$) and water buffaloes ($N=244$) in Menoufia province, Egypt. Seroprevalences of *B. bigemina* and *B. bovis* in the samples were determined using recombinant *Babesia* antigen-specific enzyme-linked immunosorbent assays (ELISA).

Results In cattle, the seroprevalences of *B. bigemina* and *B. bovis* were 41.60 and 38.17% (37.40 and 35.88% for IgM and 9.54 and 6.11% for IgG), respectively, whereas those of water buffaloes were 35.66 and 31.97% (27.87 and 21.72% for IgM and 15.16 and 15.16% for IgG), respectively. Statistically significant changes in the seroprevalences of the two infective agents were recorded on the basis of region and season of sample collection.

Conclusion In conclusion, babesiosis is frequent and presents a threat of an epidemic among bovines in Menoufia province. In turn, control of bovine babesiosis is required because of its potential to detrimentally affect milk and meat production in Menoufia province.

Keywords Seroprevalence · ELISA · Bovine · *Babesia bigemina* · *Babesia bovis* · Menoufia

Introduction

Babesia bigemina and *B. bovis* are tick-borne hemoprotozoan parasites causing bovine babesiosis, a disease negatively affecting livestock industry worldwide, especially in subtropical and tropical regions [1]. Economic losses caused by babesiosis are ascribed to poor feed conversion, reduced milk production, and increased mortality [2]. *Babesia* sporozoites invade the bovine red blood cells (RBCs) and transform into merozoites, which egress and cause destruction of RBCs, resulting in anemia and anemia-related clinical signs, and in some cases, may lead to neurological and respiratory syndromes [1, 3].

Proper diagnosis of bovine babesiosis plays a critical role in the management, monitoring, and control of the disease [4, 5]. Traditionally, microscopic techniques for blood examination using thick or thin smears stained with Giemsa can confirm the presence of *Babesia*, and remain the most convenient tool to diagnose acute babesiosis [6]. However, this technique has limited value in chronic babesiosis due to the low parasitemia. Moreover, the main challenge of using this method is how to differentiate different *Babesia* species. Serological diagnostic tests, including the enzyme-linked immunosorbent assay (ELISA) based on specific recombinant proteins, have been established to assess the exposure to different *Babesia* species, monitor the immune status of

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the host animals, and obtain critical data that can be used to control the disease [7–11]. The recombinant proteins including the C-terminal region of rhoptry-associated protein 1 (BbigRAP-1a) and spherical body protein-4 (BbSBP-4) are commonly used to diagnose and specifically differentiate between *B. bigemina* and *B. bovis* [12–16].

In Egypt, *B. bigemina* is transmitted by *Rhipicephalus annulatus* and *R. microplus* ticks, while *B. bovis* is transmitted to bovines exclusively by *R. microplus* ticks [17, 18]. Several studies previously detected these protozoan parasites in Egyptian cattle and water buffaloes bred in different geographical regions of Egypt [12, 19–22], including Menoufia province, where cattle and water buffaloes are widely distributed. Menoufia ranks second in bovine population in Lower Egypt [23]. However, information on the seroprevalence of bovine babesiosis in Menoufia has not been updated in recent years. The present study aimed to determine the seroprevalence of *B. bigemina* and *B. bovis* in cattle and water buffaloes in Menoufia province, Egypt through ELISA based on recombinant *Babesia* antigens.

Fig. 1 Map of sampling areas in Menoufia province, Egypt

Materials and Methods

Calculation of Sample Size

The minimum sample size required for this study was calculated in the online platform Epitools [24] by the formula: $N = \left[\frac{Z^2 \times P \times (1-P)}{e^2}\right] \div \left[1 + \frac{(Z^2 \times P \times (1-P)}{(e^2 \times n)}\right]$, where Z is the confidence level (95%), P is the expected true proportion (0.5) and e is the margin of error (±0.05). The population size (n) inputted was 7.8 million, the total population of cattle and water buffaloes in Egypt in 2017 [25]. The minimum sample size required is $N = 385$.

Animal Sampling

A total of 506 blood samples of cattle ($N = 262$) and water buffaloes ($N = 244$) were collected from open public markets in Qewaisna and Birket Al Saba, Menoufia province, Egypt (Fig. 1) during the different seasons (spring, summer, autumn and winter) of June 2017–May 2018. The markets were chosen based on convenience while sampled animals were randomly chosen. The examined animals are kept in farmers' stables in the evening and used in the fields in daytime. Cattle ages 1 month to 8 years old were classified into two groups: young (2 years or less) and adult (more than 2 years). Likewise, water buffaloes with the age range of 1 month to 10 years old were classified into two groups: young (3 years or less) and adult (more than 3 years). Blood samples were collected from the jugular or caudal vein of the animals. Then, the samples were incubated at room temperature for 1 h and centrifuged at 3000 rpm for 15 min. Sera were collected and stored at – 20 °C until use.

Recombinant *B. bigemina* RAP-1a and *B. bovis* SBP-4 Preparation

The Argentina strain of *B. bigemina* and Texas strain of *B. bovis* were continuously cultured in bovine erythrocytes (RBCs) using a micro-aerophilous stationary-phase culturing system [26]. Total RNA was isolated and cDNA was synthesized from each parasite culture. The DNA fragments encoding the *B. bigemina* C-terminal region of rhoptry-associated protein 1 (BbigRAP-1a/CT: 390–480 aa, GenBank accession number M60878) and *B. bovis* spherical body protein-4 (BbSBP-4, GenBank accession number AB594813) were amplified by PCR from *B. bigemina* and *B. bovis* cDNA, respectively, subcloned into a pGEX-4T1 plasmid vector (Amersham Pharmacia Biotech, Inc., USA) using suitable restriction enzyme sites, then, expressed as glutathione S-transferase (GST)-fusion proteins in *Escherichia coli* BL21 strain (Amersham Pharmacia Biotech, Inc.). The recombinant proteins were purified from the soluble fractions of *E. coli* lysates using Glutathione-Sepharose 4B beads (Amersham Pharmacia Biotech, Inc.) and analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis [13]. The purified recombinant proteins were used for the subsequent assays.

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA was done in accordance to the modified procedure described previously [12]. Antigen-coated wells were incubated with 50 µl of cattle or water buffalo serum samples diluted to 1:100. Either 50 µl of horseradish peroxidase (HRP)-conjugated rabbit anti-bovine IgG (Bethyl Laboratories, USA) diluted at 1:10,000 or 50 µl of HRP-conjugated rabbit anti-bovine IgM (Chongqing Biospes Co. Ltd., China) diluted at 1:10,000 was used as secondary antibody. Absorbance was detected at 405 nm using an ELISA reader (Radim Diagnostics, Italy). The results were calculated by obtaining the differences in mean optical densities between the recombinant antigens (BbigRAP-1a/CT or BbSBP-4) and those of the GST protein. The cutoff points were calculated as the OD_{405} mean value for *B. bigemina*- and *B. bovis*-negative sera plus 3 standard deviations ($n=20$): for IgG, BbigRAP-1a/CT: 0.019 and BbSBP-4: 0.018 in cattle, BbigRAP-1a/CT: 0.018 and BbSBP-4: 0.018 in water buffaloes; for IgM, BbigRAP-1a/CT: 0.019 and BbSBP-4: 0.018 in cattle, BbigRAP-1a/CT: 0.02 and BbSBP-4: 0.019 in water buffaloes. The negative sera from our standard sera stock were tested and confirmed negative by PCR [12].

Statistical Analysis

Association of the seropositivity of animals with sampling region, age, sex and season of sample collection was evaluated using the Pearson's chi-squared or Fisher's exact test in Microsoft Excel and GraphPad Prism 7. The logistic regression model was used to evaluate the differences in seroprevalence rates of animals based on age, sex and season of sample collection in IBM SPSS® version 11. The dependent variable used was the outcome of the test (seropositive or seronegative) while risk factors, i.e., age, sex and season of sample collection, were the independent variables. A p value <0.05 was considered significant.

Results

Overall, 41.60% (109/262) and 38.17% (100/262) of cattle showed seropositivity (positive for IgM, IgG or both) for *B. bigemina* and *B. bovis*, respectively. Seroprevalence of *B. bigemina* was 37.40 and 9.54% for acute infection (IgM) and chronic infection (IgG), respectively. The number of cattle with positive reactivity against *B. bigemina* IgM was significantly higher ($p=0.0004$) in the area of Birket Al Saba (49.56%) compared to Qewaisna (28.19%) (Table 1). Seroprevalence of *B. bovis* for IgM and IgG antibodies was 35.88 and 6.11%, respectively. *B. bovis* IgM-seroreactive cattle serum samples from Qewaisna is notably higher than in Birket Al Saba while no significant differences were observed in *B. bovis* IgG-positive cattle between the two areas. Mixed infection with *B. bigemina* and *B. bovis* was detected in 24.05% (63/262) of the cattle serum samples.

In water buffaloes ($N=244$), the overall seroprevalence of *B. bigemina* was 35.66% (87/244; 27.87% for IgM and 15.16% for IgG), while the overall seroprevalence of *B. bovis* was 31.97% (78/244; 21.72% for IgM and 15.16% for IgG). *B. bigemina* seropositivity for IgM antibodies ($p=0.007$) and *B. bovis* seropositivity for IgG antibodies ($p=0.00000018$) were significantly increased in the area of Qewaisna compared to Birket Al Saba area. Mixed infection rate with *B. bigemina* and *B. bovis* was 15.16% (Table 2).

Table 1 Seroprevalence of *Babesia bigemina* and *Babesia bovis* infections in cattle from Menoufia province

Regions	*N*	*B. bigemina*		*B. bovis*		Mixed infection
		IgM	IgG	IgM	IgG	
Qewaisna	149	42 (28.19%)	14 (9.40%)	61 (40.94%)*	8 (5.69%)	35 (23.49%)
Birket Al Saba	113	56 (49.56%)***	11 (9.73%)	33 (29.20%)	8 (7.08%)	28 (24.78%)
Total	262	98 (37.40%)	25 (9.54%)	94 (35.88%)	16 (6.11%)	63 (24.05%)

Data are expressed as number of seropositive samples (%). Significant difference between regions is indicated by asterisks (*$p<0.05$; ***$p<0.001$)

Table 2 Seroprevalence of *Babesia bigemina* and *Babesia bovis* infections in water buffaloes from Menoufia province

Regions	*N*	*B. bigemina*		*B. bovis*		Mixed infection
		IgM	IgG	IgM	IgG	
Qewaisna	135	47 (34.81%)**	22 (16.30%)	29 (21.48%)	35 (25.93%)***	30 (22.22%)
Birket Al Saba	109	21 (19.27%)	15 (13.76%)	24 (22.02%)	2 (1.83%)	7 (6.42%)
Total	244	68 (27.87%)	37 (15.16%)	53 (21.72%)	37 (15.16%)	37 (15.16%)

Data are expressed as number of seropositive samples (%). Significant difference between regions is indicated by asterisks (**$p<0.01$; ***$p<0.001$)

The seroprevalences of *B. bigemina* and *B. bovis* based on age, sex and season are summarized in Table 3 for cattle and Table 4 for water buffaloes. During acute infection, the seroprevalence rates for both parasites in adult cattle (> 2 years) and water buffaloes (> 3 years) were higher than those in young animals, although not significant. Moreover, during chronic infection, the *B. bigemina* seroprevalence rates in adult animals were notably higher than that of the infection rates in the young ones. In female water buffaloes, higher seropositivity rates for all infections were observed compared to male animals ($p < 0.001$) (Table 4). Moreover, the seroprevalence was higher in female cattle during the chronic infection of *B. bigemina* and the acute infection of *B. bovis* in both animals (Tables 3 and 4). No significant differences were recorded between infection percentages of both parasites in cattle according to sex (Table 3).

In acutely *B. bigemina*-infected cattle, binary logistic regression analysis indicated that the differences in seropositivity rates in serum samples collected between winter season and spring/autumn were significant (Table 3). In water buffaloes, during acute infection, statistical analysis indicated that the seroprevalences of *B. bigemina* and *B. bovis* were significantly higher in the summer season compared to the spring and the autumn season, respectively. No significant changes were noted during chronic infection of *B. bigemina* in cattle and water buffaloes. Meanwhile, the seroprevalence of *B. bovis* chronic infection in water buffaloes was significantly ($p < 0.001$) increased to 30.0% in the summer season compared to 2.86% seropositivity rate in the winter season (Table 4).

Discussion

In this study, we determined the seroprevalences for *B. bigemina* and *B. bovis* in cattle (41.60 and 38.17%, respectively) and water buffaloes (35.66 and 31.97%, respectively) in Menoufia province. These are higher compared to previous serological surveys conducted in other provinces of Egypt which recorded seroprevalences of 10.6–33.20% for *B. bigemina* and 9–20.43% for *B. bovis* in cattle, and 15.63–22.20% for *B. bigemina* and 11.46–22.20% for *B. bovis* in water buffaloes using ELISA [12, 22, 27]. In addition, a study in Menoufia employing PCR assays demonstrated that the *Babesia* spp. infection rate was 12.66% in cattle [20] while another study in Menoufia revealed molecular detection rates of 7.62 and 2.54% for *B. bigemina* and *B. bovis* in cattle, respectively, and 3.03% for *B. bovis* in water buffaloes [21]. Several molecular investigations in other Egypt provinces also recorded infection rates of 5.3–19.33% for *B. bigemina* and 3.97–5.88% for *B. bovis* in cattle, and 10.42 and 4.17% in water buffaloes, respectively [12, 21, 28]. Higher positivity rates reported in the current study using ELISA compared to studies which employed PCR assays indicate differences in the exposure of animals to the parasites and the presence of the parasites during the sampling [22]. Same observation has been documented from previous studies in Egypt [12, 22].

The differences in prevalences of *B. bigemina* and *B. bovis* in cattle and water buffaloes among different regions in Egypt may be attributed to the geographic distribution of tick vector [1, 29], climate condition, farm management and the time of sampling [30]. Adham *et al.* [18] demonstrated the presence of infected tick vector of bovine babesiosis in Egypt wherein *B. bigemina* was more predominant than *B. bovis* infection in *Rhipicephalus* (*Boophilus*) ticks [18]. In Mediterranean countries such as Egypt, *R.* (*Boophilus*) *annulatus* is considered as the most prevalent and main tick vector transmitting *B. bigemina* and *B. bovis* [30, 31]. Although this mirrors the higher seroprevalences of *B. bigemina* compared to that of *B. bovis* in both animals in the current study, the infection status of the tick vector of these parasites in Menoufia remains to be elucidated as this study did not evaluate tick infections.

Higher seropositivity rates were observed in adult animals with the exception during the chronic infection of *B. bovis*. This was consistent with previous studies that showed increased seroprevalence in older cattle [32–34]. Both young and adults are susceptible to babesiosis, but the stronger innate immune response in younger animals affords them with higher tolerance against *Babesia* infections [34]. In the present study, no significant changes were observed among male and female cattle. These results were consistent with previous studies carried out in cattle [35, 36].

Although seroprevalence of *B. bigemina* showed significant increase during the winter season compared to the spring and the autumn seasons during acute infection in cattle, the seroprevalences of *B. bigemina* and *B. bovis* were significantly increased during the summer season compared to those of the spring and the autumn season, respectively, during acute infection in water buffaloes. Moreover, during chronic infection of *B. bovis* in water buffaloes, the summer season prevalence was significantly higher than that of the winter season. Vieira *et al.* [37] reported that *B. bigemina* infection rate reduced significantly during the winter compared to those of spring, summer, or autumn. A previous study from Egypt revealed a significant increase in the tick infestation during summer months among cattle [38]. The increase in the tick infestation during summer months might be attributed to the increase in temperature and humidity [39]. Seasonal differences of tick infestation may lead to the varying degrees of season-related infection.

We found higher levels of seropositivity for both *B. bigemina* and *B. bovis* IgM antibodies, which indicate high frequency of recent acute infections among cattle and water buffaloes in Menoufia. This finding is particularly important

Table 3 Seroprevalence of *Babesia bigemina* and *Babesia bovis* in cattle according to age, sex and season

Parameters	Total (*N*)	*B. bigemina* IgM				*B. bigemina* IgG				*B. bovis* IgM				*B. bovis* IgG			
		Positive (prevalence)	OR	95% CI	*p* value	Positive (prevalence)	OR	95% CI	*p* value	Positive (prevalence)	OR	95% CI	*p* value		Positive (prevalence)	OR	95% CI	*p* value
Age																		
Young (≤2 years)	114	43 (37.39%)	1.02	0.62–1.68	0.93	7 (6.09%)	0.47	0.18–1.12	0.14	34 (29.57%)	0.62	0.38–1.04	0.091	9 (7.83%)		1.73	0.66–4.50	0.31
Adult (>2 years)	148	55 (37.41%)				18 (12.24%)				60 (40.82%)				7 (4.76%)				
Sex																		
Male	30	16 (53.33%)	2.09	0.96–4.29	0.071	2 (6.67%)	0.65	0.15–0.20	0.75	8 (26.67%)	0.62	0.28–1.42	0.32	3 (10.0%)		1.87	0.54–6.38	0.41
Female	232	82 (35.45%)				23 (9.14%)				86 (37.07%)				13 (5.60%)				
Season																		
Summer	42	15 (35.71%)[a]	1.00		<0.001***	4 (9.52%)	1.00		0.32	19 (45.24%)	1.00		0.094	1 (2.38%)		1.00		0.42
Autumn	69	19 (27.54%)[b]	1.46	0.64–3.33		10 (14.49%)	0.62	0.18–2.12		30 (43.48%)	1.07	0.50–2.32		6 (8.70%)		0.26	0.03–2.21	
Winter	76	50 (65.79%)[a,*]	0.29	0.13–0.64		7 (9.21%)	1.04	0.29–3.77		25 (32.89%)	1.69	0.78–3.65		6 (7.89%)		0.28	0.03–2.45	
Spring	75	14 (18.67%)[b]	2.42	1.03–5.71		4 (5.33%)	1.87	0.44–7.89		20 (26.67%)	2.27	1.03–5.03		3 (4.00%)		0.59	0.06–5.81	

Different superscript letters showed significant differences between these values. OR for season was analyzed in comparison to the values of summer. Significant differences between variables are indicated by asterisks (***$p < 0.001$)

OR Odds ratio, *CI* Confidence interval

Table 4 Seroprevalence of *Babesia bigemina* and *Babesia bovis* in water buffaloes according to age, sex and season

Parameters	Total (*N*)	*B. bigemina* IgM				*B. bigemina* IgG				*B. bovis* IgM				*B. bovis* IgG			
		Positive (prevalence)	OR	95% CI	*p* value	Positive (prevalence)	OR	95% CI	*p* value	Positive (prevalence)	OR	95% CI	*p* value	Positive (prevalence)	OR	95% CI	*p* value
Age																	
Young (≤ 3 years)	88	21 (23.86%)	0.73	0.40–1.32	0.29	9 (10.23%)	0.52	0.23–1.16	0.11	19 (21.59%)	0.99	0.52–1.86	0.97	17 (19.32%)	1.63	0.80–3.30	0.17
Adult (> 3 years)	156	47 (30.13%)				26 (16.67%)				34 (21.79%)				20 (12.82%)			
Sex																	
Male	10	1 (10.0%)	0.02	0.003–0.18	< 0.001***	0	n.a.	n.a.	0.17	1 (10.0%)	0.39	0.048–3.14	0.36	1 (10.0%)	0.61	0.075–4.97	0.64
Female	234	67 (28.63%)				37 (15.81%)				52 (22.22%)				36 (15.38%)			
Season																	
Summer	40	17 (42.5%)[a,*]	1.00		< 0.001***	7 (17.5%)	1.00		0.061	14 (35.0%)[a,*]	1.00		0.019*	12 (30.0%)[a,*]	1.00		< 0.001***
Autumn	101	33 (32.67%)[a]	1.52	0.72–3.23		16 (15.84%)	1.13	0.43–2.99		15 (14.85%)[b]	3.09	1.32–7.22		23 (22.77%)[a]	1.45	0.64–3.30	
Winter	70	18 (25.71%)[a]	2.14	0.94–4.87		14 (20.0%)	0.85	0.31–2.32		13 (18.57%)[a]	2.36	0.97–5.73		2 (2.86%)[b]	14.57	3.06–69.37	
Spring	33	0[b]	n.a.	n.a.		0	n.a.	n.a.		11 (33.33%)[a]	1.08	0.41–2.85		0[b]	n.a.	n.a.	

Different superscript letters showed significant differences between these values. OR for season was analyzed in comparison to the values of summer. Significant differences between variables are indicated by asterisks (*$p < 0.05$; ***$p < 0.001$)

OR Odds ratio, *CI* Confidence interval, *n.a.* data cannot be analyzed

and calls for intensive implementation of control measures for bovine babesiosis in the area.

In conclusion, the current study indicated that cattle and water buffalo babesiosis is frequent and presents a threat of an epidemic in Menoufia province. The current study provides additional information on the bovine *B. bigemina* and *B. bovis* infections in Egypt and will assist in developing strategies for controlling the disease. Further investigations are needed to understand the reasons behinds the high rates of these parasitic infections in Menoufia province, Egypt.

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Author Contributions Conceptualization and design of the study: HMI and XX. Sample collection: HMI and NMB. Laboratory assays: HMI, HMA, DSM, KM-G, SKS and AG-K. Data analysis: HMI and EMG. Drafting of the manuscript: HMI, EMG and MAT. Editing and revising: HMI, EMG, MAT, BB, LM and XX. All authors approved the final version of the manuscript.

Compliance with Ethical Standards

Conflict of interest None of the authors of this work has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Ethical approval Sampling in the public markets in Menoufia was permitted by the local administrators of the market. The purpose and procedures involved in the present study were explained and informed consent from all farmers was obtained prior to blood sample collection of their animals. All field sampling and laboratory procedures were carried out according to the ethical guidelines for use of animal samples of Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan (Permit for animal experiment: 19-15) and the Institutional Animal Ethical Committee, Menoufia University, Egypt (approval ID: MUFS/F/IM/2/17).

References

1. Bock R, Jackson L, De Vos A, Jorgensen W (2004) Babesiosis of cattle. Parasitology 129(1):S247–S269. https://doi.org/10.1017/S0031182004005190
2. Dantas-Torres F, Alves LC, Uilenberg G (2016) Babesiosis. In: Marcondes CB (ed) Arthropod borne diseases, 1st edn. Springer, Switzerland, pp 347–354. https://doi.org/10.1007/978-3-319-13884-8
3. Everitt JI, Shadduck JA, Steinkamp C, Clabaugh G (1986) Experimental *Babesia bovis*infection in holstein calves. Vet Pathol 23:556–562. https://doi.org/10.1177/030098588602300503
4. Morzaria S, Katende J, Kairo A, Nene V, Musoke A (1992) New methods for the diagnosis of *Babesia bigemina* infection. Mem Inst Oswaldo Cruz 87(3):201–205. https://doi.org/10.1590/S0074-02761992000700033
5. de Vos AJ, Potgieter FT (1994) Bovine Babesiosis. In: Coetzer JAW, Thomson GR, Tustin RC (eds) Infectious diseases of livestock: with special reference to southern Africa, vol 2. Oxford University Press, Cape Town, South Africa, pp 278–294
6. Moody AH, Chiodini PL (2000) Methods for the detection of blood parasites. Clin Lab Haem 22:189–202
7. Mahoney DF, Ross DR (1972) Epizootiological factors in the control of bovine babesiosis. Aust Vet J 48:292–298. https://doi.org/10.1111/j.1751-0813.1972.tb05160.x
8. Mahoney DF (1975) The diagnosis of babesiosis in Australia. In: Wells EA (ed) Workshop on hemoparasites (anaplasmosis and babesiosis). Centro Internacional de Agricultura Tropical, Cali, Colombia, pp 49–62
9. Weiland G, Reiter I (1988) Methods for the measurement of serological response to *Babesia*. In: Ristic M (ed) Babesiosis of domestic animal and man. CRC Press Inc, Boca Raton, Florida, pp 143–158
10. Bose R, Jacobson RH, Gale KR, Waltisbuhl DJ, Wright IG (1990) An improved ELISA for the detection of antibodies against *Babesia bovis* using either a native or a recombinant *B. bovis* antigen. Parasitol Res 76:648–652. https://doi.org/10.1007/BF00931081
11. Araujo FR, Madruga CR, Leal CR, Schenk MA, Kessler RH, Marques AP, Lemaire DC (1998) Comparison between enzyme-linked immunosorbent assay, indirect fluorescent antibody and rapid conglutination test in detecting antibodies against *Babesia bovis*. Vet Parasitol 74:101–108. https://doi.org/10.1016/S0304-4017(97)00171-4
12. Ibrahim HM, Adjou Moumouni PF, Mohammed-Geba K, Sheir SK, Hashem IS, Cao S, Terkawi MA, Kamyingkird K, Nishikawa Y, Suzuki H, Xuan X (2013) Molecular and serological prevalence of *Babesia bigemina* and *Babesia bovis* in cattle and water buffalos under small-scale dairy farming in Beheira and Faiyum Provinces, Egypt. Vet Parasitol 198:187–192. https://doi.org/10.1016/j.vetpar.2013.08.028
13. Terkawi MA, Huyen NX, Shinuo C, Inpankaew T, Maklon K, Aboulaila M, Ueno A, Goo YK, Yokoyama N, Jittapalapong S, Xuan X, Igarashi I (2011) Molecular and serological prevalence of *Babesia bovis* and *Babesia bigemina* in water buffalo in the northeast region of Thailand. Vet Parasitol 178:201–207. https://doi.org/10.1016/j.vetpar.2011.01.041
14. Terkawi MA, Huyen NX, Wibowo PE, Seuseu FJ, Aboulaila M, Ueno A, Goo YK, Yokoyama N, Xuan X, Igarashi I (2011) Spherical body protein 4 is a new serological antigen for the global detection of *Babesia bovis* infection in cattle. Clin Vaccine Immunol 18:337–342. https://doi.org/10.1128/CVI.00388-10
15. Terkawi MA, Seuseu FJ, Eko-Wibowo P, Huyen NX, Minoda Y, Aboulaila M, Kawai S, Yokoyama N, Xuan X, Igarashi I (2011) Secretion of a new spherical body protein of *Babesia bovis* into the cytoplasm of infected erythrocytes. Mol Biochem Parasitol 178:40–45. https://doi.org/10.1016/j.molbiopara.2011.02.006
16. Guswanto A, Allamanda P, Mariamah ES, Sodirun S, Wibowo PE, Indrayani L, Nugroho RH, Wirata IK, Jannah N, Dias LP, Wirawan HP, Yanto R, Tuvshintulga B, Sivakumar T, Yokoyama N, Igarashi I (2017) Molecular and serological detection of bovine babesiosis in Indonesia. Parasit Vectors 10:550. https://doi.org/10.1186/s13071-017-2502-0
17. Abdel-Shafy S, Mahmoud MS (2002) Morphological and ultrastructural studies on *Babesia bigemina* local strain in haemolymph and salivary glands of infected *Boophilus annulatus* females. J Egypt Vet Med Assoc 62:247–256
18. Adham FK, Abd-el-Samie EM, Gabre RM, El-Hussein H (2009) Detection of tick blood parasites in Egypt using PCR assay I-*Babesia bovis* and *Babesia bigemina*. Parasitol Res 105:721–730. https://doi.org/10.1007/s00436-009-1443-8
19. Mahmmod YS (2012) Molecular detection of natural *Babesia bovis* infection from clinically infected and apparently healthy water buffaloes (*Bubalis bubalis*) and crossbred cattle. J Buffalo Sci 1:55–60. https://doi.org/10.6000/1927-520X.2012.01.01.10

20. Nayel M, El-Dakhly KM, Aboulaila M, Elsify A, Hassan H, Ibrahim E, Salama A, Yanai T (2012) The use of different diagnostic tools for *Babesia* and *Theileria* parasites in cattle in Menofia, Egypt. Parasitol Res 111:1019–1024. https://doi.org/10.1007/s00436-012-2926-6
21. 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. Parasitol Int 64:79–85. https://doi.org/10.1016/j.parint.2014.10.002
22. Mahmoud MS, Kandil OM, Nasr SM, Hendawy SH, Habeeb SM, Mabrouk DM, Silva MG, Suarez CE (2015) Serological and molecular diagnostic surveys combined with examining hematological profiles suggests increased levels of infection and hematological response of cattle to babesiosis infections compared to native buffaloes in Egypt. Parasit Vectors 8:319. https://doi.org/10.1186/s13071-015-0928-9
23. Africa Sustainable Livestock 2050 (2018) Livestock production systems spotlight- Egypt cattle and buffaloes, and poultry sectors. Food and Agriculture Organization, Rome, Italy
24. Sergeant ESG (2018). Epitools epidemiological calculators. Ausvet. http://epitools.ausvet.com.au
25. Food and Agriculture Organization of the United Nations (2019). FAOSTAT statistical database. http://faostat.fao.org/
26. Levy MG, Rustic M (1980) *Babesia bovis*: continuous cultivation in a microaerophilus stationary phase culture. Science 204:1218–1220. https://doi.org/10.1126/science.7355284
27. Fereig RM, Mohamed SGA, Mahmoud HYAH, AbouLaila MR, Guswanto A, Nguyen TT, Mohamed AE, Inoue N, Igarashi I, Nishikawa Y (2017) Seroprevalence of *Babesia bovis*, *B. bigemina*, *Trypanosoma evansi*, and *Anaplasma marginale* antibodies in cattle in southern Egypt. Ticks Tick Borne Dis 8:125–131. https://doi.org/10.1016/j.ttbdis.2016.10.008
28. El-Dakhly KM, Arafa WM, Soliman S, Abdel-Fatah OR, Wahba AA, Esteve-Gasent MD, Holman PJ (2020) Molecular detection, phylogenetic analysis, and genetic diversity of *Theileria annulata*, *Babesia bigemina*, and *Anaplasma marginale* in cattle in three districts of Egypt. Acta Parasitol. https://doi.org/10.2478/s11686-020-00189-z
29. McCosker PJ (1981) The global importance of babesiosis. In: Ristic M, Krier JP (eds) Babesiosis. Academic Press, New York, pp 1–24
30. World Organization for Animal Health (2008) OIE manual of diagnostic tests and vaccines. Office International des Epizooties. https://www.oie.int/doc/ged/D7710.PDF. Accessed 1 Jun 2020
31. Ica A, Vatansever Z, Yildirim A, Duzlu O, Inci A (2007) Detection of *Theileria* and *Babesia* species in ticks collected from cattle. Vet Parasitol 148:156–160. https://doi.org/10.1016/j.vetpar.2007.06.003
32. Applewhaite LM, Craig TM, Wagner GG (1981) Serological prevalence of bovine babesiosis in Guyana. Trop Anim Health Prod 13:13–18. https://doi.org/10.1007/BF02237880
33. Sukanto IP, Payne RC, Partoutomo S (1993) Bovine babesiosis in Indonesia. Prev Vet Med 16:151–156. https://doi.org/10.1016/0167-5877(93)90085-8
34. Terkawi MA, Alhasan H, Huyen NX, Sabagh A, Awier K, Cao S, Goo YK, Aboge G, Yokoyama N, Nishikawa Y, Kalb-Allouz AK, Tabbaa D, Igarashi I, Xuan X (2012) Molecular and serological prevalence of *Babesia bovis* and *Babesia bigemina* in cattle from central region of Syria. Vet Parasitol 187:307–311. https://doi.org/10.1016/j.vetpar.2011.12.038
35. Abdo Rizk M, Salama A, El-Sayed SA, Elsify A, El-Ashkar M, Ibrahim H, Youssef M, El-Khodery S (2017) Animal level risk factors associated with *Babesia* and *Theileria* infections in cattle in Egypt. Acta Parasitol 62:796–804. https://doi.org/10.1515/ap-2017-0096
36. Tembue AAM, Silva FJM, Silva JB, Santos TM, Santos HA, Soares CO, Fonseca AH (2011) Risk factors associated with the frequency of antibodies against *Babesia bovis* and *Babesia bigemina* in cattle in southern Mozambique. Pesq Vet Bras 31:663–666. https://doi.org/10.1590/S0100-736X2011000800005
37. Vieira LL, Canever MF, Cardozo LL, Cardoso CP, Herkenhoff ME, Neto AT, Vogel CIG, Miletti LC (2019) Prevalence of *Anaplasma marginale*, *Babesia bovis*, and *Babesia bigemina* in cattle in the Campos de Lages region, Santa Catarina state, Brazil, estimated by multiplex-PCR. Parasite Epidemiol Control 6:e00114. https://doi.org/10.1016/j.parepi.2019.e00114
38. Asmaa NM, ElBably MA, Shokier KA (2014) Studies on prevalence, risk indicators and control options for tick infestation in ruminants. Beni-Suef Univ J Basic Appl Sci 3:68–73. https://doi.org/10.1016/j.bjbas.2014.02.009
39. Khan MN, Hayat CS, Iqbal Z, Hayat B, Naseem A (1993) Prevalence of ticks on livestock in Faisalabad. Pak Vet J 13:182e4