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Molecular, Epidemiological, and Clinical Investigations of *Anaplasma marginale* Infection in Cattle at Qena Governorate, Upper Egypt

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Abstract

Bovine anaplasmosis is one of the most important diseases that threaten livestock production worldwide especially in developing countries, in cattle mainly caused by obligate intra-erythrocytic *Anaplasma marginale*. *A. marginale* is transmitted biologically by ticks (*Hyalomma, Rhipicephalus*). Bovine anaplasmosis causes mild to severe clinical signs ranging from anorexia, fever, anemia, and respiratory manifestations to icterus and death. Molecular detection is the best method for *Anaplasma* diagnosis because of its ability to detect sub-clinical and carrier hosts. This study investigated the occurrence of *A. marginale* infection among cattle in the Qena governorate utilizing a molecular assay based the msp5 gene. A total of 100 whole blood samples were collected randomly from apparently healthy and diseased cattle. Such cattle were examined clinically, and their samples were included for microscopic examination. PCR screening of the tested cattle showed 23% (23/100) as a positive rate. While 6 samples from 100 (6%) showed *A. marginale* parasite in the microscopic examination. Several risk factors were analyzed in the current study, higher incidence rates were detected in animals less than 2 years than older than 2 years, Holstein-Friesian breeds than crossbreeds and in animals kept in small farms than in the mass farming system. Clinical and hematological variables were also investigated in several infected and non-infected cattle based on PCR reactivity. Fever, anorexia, respiratory manifestations, enlarged lymph nodes, pale or icteric mucous membranes and digestive disorders were reported in infected cattle (n= 23) but not in non-infected animals (n=77). Consistently, hematological variables in infected cattle (n = 10) revealed significantly lower RBCs count and hemoglobin content than those in the non-infected group (n=20) indicating hemolytic anemia. This study shows the high prevalence of *A. marginale* in cattle in Qena governorate associated with health hazards and multi-risk factors, so frequent usage of acaricides, regular examination of cattle, and successful chemoprophylaxis are recommended.

KEYWORDS
Cattle, *Anaplasma marginale*, PCR, Anaplasmosis, Diagnosis, Epidemiology

INTRODUCTION

Livestock production is an essential part of the agricultural economy and overall economy in most countries of the world, especially in developing countries (Sansoucy, 1995). This is challenged by numerous risks including malnutrition, management system, climatic changes and infectious or non-infectious diseases. Bovine anaplasmosis is one of the tick-borne pathogens (TBPS) that adversely affects the cattle industry resulting in substantial economic losses (Selim *et al*., 2021). *Anaplasma marginale* is an intraerythrocytic rickettsial pathogens transmitted biologically by more than twenty tick species including mainly (*Hyalomma*, *Rhipicephalus*, and *Ixodes*) and is responsible for several outbreaks of bovine anaplasmosis in tropical and sub-tropical regions (Kocan *et al*., 2010). *A. marginale* can be transmitted mechanically through biting flies like as horse, deer and stable flies, as well as blood contaminated objects especially contaminated needles, and surgical instruments used for castration, ear tagging, and dehorning, transplacental transmission incriminated in anaplasmosis transmission (Aubry and Geale, 2011; Henker *et al*., 2020).

As an intra-erythrocytic pathogen, hemolytic anemia and icterus are the main characteristic signs. Other symptoms include fever, appetence, weight loss, depression, weakness, excessive salivation, rapid respiration, enlargement of superficial lymph nodes decreasing milk production, abortion, temporary infertility in males, anestrus in females and death occur especially in exotic high-producing dairy cows (Birdane *et al*., 2006; Bigalke and Verwoerd, 2008). Cattle that recover from the acute stage of disease usually remain persistently infected with *A. marginale* independently of re-exposure to the rickettsia and serve as reservoirs of infection for naïve cattle (Kieser *et al*.,1990; Palmer *et al*. 1999; Kocan *et al*., 2015).

In the last decade, numerous studies have been reported the existence of *A. marginale* among cattle in Egypt via various meth-

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ods of detection including antigen-antibody reaction (Fereig *et al*., 2017; Tumwebaze *et al*., 2020; Selim *et al*., 2021), and molecular examinations (Radwan *et al*., 2013; El-Ashker *et al*., 2015; Selim *et al*., 2021; Ahmed *et al*., 2022). However, few reports have been linked the prevalence of *A. marginale* infection in cattle with an assessment of multiple risk factors and its impact on the clinical and hematological parameters which were assessed in detail in the current study.

MATERIALS AND METHODS

Ethics approval

The collection of cattle blood samples was performed by veterinarians with none or minimal invasive methods and approved by animal owners. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The collected samples were used specifically for this study, and approved by the Ethical Committee (Ethical approval number 36 dated on 11/5/2022) at Faculty of Veterinary Medicine, South Valley University, Egypt

Study area

This study was conducted at Qena Governorate, located in Upper (Southern) Egypt. This region is characterized by semi-arid, hot and dry. The River Nile is the main water source, where most of the livestock are gathered. Blood samples were collected from different villages in Qena governorates with similar climatic and geographical conditions.

Animals and Clinical examination

In this study, a total number of (100) cattle with various ages, breeds, sexes, management systems were collected from different areas in Qena governorate. Such animals were treated by deworming programs from owners in most cases. Physical investigation of general animal health conditions including, food and water intake, ticks infestation and management. Careful clinical examinations including animal temperature, respiratory system and rumen function were applied as reported by Radostits *et al*. (2007). In addition, mucous membrane, lymph nodes, eye, body condition were evaluated. Any clinical signs that may be related to blood parasites infection were particularly recorded.

Samples collection

Whole blood samples were collected from the jugular vein of all animals (n=100) either clinically suspected cases or apparent healthy by using sterile syringes after disinfection of the target site by alcohol 70%. The blood samples were collected on" sodium salt of EDTA "and divided into two parts after applying gentle mixing. The first part was used freshly for microscopic examination and hematological evaluation, while the other one was stored at -20°C until used for DNA extraction and molecular examination.

Microscopic examination of blood smears

Thin blood smear was prepared from freshly collected blood sample and dried by air then fixed with absolute methyl alcohol for (2-5) minutes. Afterwards, slides were stained by diluted Giemsa stain 10% for 15-20 minutes and washed with distilled water followed by air dryness. Each blood film was checked carefully under oil an immersion lens. For detection of suspected cases of *A. marginale* was based on their morphological characters (Coles, 1986).

DNA extraction

Genomic DNA was extracted from whole blood cattle samples (n=100) using a Promega DNA 16 Powerplex ® extraction kit according to the manufacturer's instructions at Animal medicine research lab, Faculty of Veterinary Medicine, South Valley University, Egypt. DNA extracts were stored at − 20°C pending genetic analysis. Blood samples typically were obtained as 1ml of whole blood stored in EDTA vacutainer tubes at room temperature. Samples were gently mixed by inversion for 5–6 times, and then centrifuged for 20 seconds at 14000 rpm in a micro-centrifuge to remove the supernatant as much as possible. For the remaining part, 300µl of Nuclei Lysis Solution was added and expose to vigorous mixing by vortex for 20 seconds until the solution become very viscous. After that, 100µl Protein Precipitation Solution was added, mixed by vortex, and centrifuge again as above for 3 minutes. The supernatant was carefully transferred to a sterilized micro-centrifuge tube, and mixed with 300µl isopropanol followed by centrifugation for 1 minute at 14000 rpm in a micro-centrifuge until the white thread-like strands of DNA form a visible mass was observed. The Supernatant was discarded carefully, and then 300µl of 70% ethanol was added followed by gently mixing and centrifugation for 1 minute at 1400 rpm. The ethanol was carefully aspirated using a sequencing pipette tip. The pellet was left to dry for 10–15 minutes by inversion of the tube on clean absorbent. Eventually, the pellet was resuspended by adding 100µl of DNA Rehydration Solution and incubated overnight at room temperature. The DNA can be used directly for PCR or stored at -20°C until use.

PCR Amplification

One pair of primers set F: 5′-GCT CTA GCA GGT TAT GCG TC-3′ R: 5′-CTG CTT GGG AGA ATG CAC CT-3′ (Wuyts *et al*., 1994) was designated using the contribution of gene-bank based on the msp5 gene sequence of *Anaplasma* spp. 12.5 µl 2X master mix (Qiagen), 1 µl 1F (10 pmol/µl), 1 µl R(10 pmol/ µl), 2 µl DNA template, and nuclease free water up to 25 µl were gently mixed and centrifuged. All mixtures were placed in a thermal cycler which was optimized and started with an initial denaturation for 60 sec at 96°C followed by 35 cycles of denaturation for 15 min at 96°C, annealing for 1 min at 60°C, and extension for 30 sec at 72°C. The final extension occurred at 72ºC was allowed to proceed for 10 min. PCR amplicons were electrophoresed for 90 minutes on 1.5% agarose gel with ethidium bromide and photographed using UV trans-illuminator.

The animal number was subdivided into 2 groups based on PCR results of *A. marginale* detection; PCR negative as non-infected group (n=77) and PCR positive as the infected group (n=23).

Hematological investigation

Various hematological parameters were assessed for a number of animal cases that randomly selected from animals negative to PCR, microscopic and clinical examinations referred as control negative group (n=20) and a number of positive cases for PCR, microscopic and clinical examinations referred as control positive group (n=10). Analyses were conducted on numerous hematological variables including hemoglobin concentration

(Hb), total erythrocyte count (TEC), packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV), total leucocytes count (TLC), differential leucocytes count (DLC), and percentages of neutrophils, lymphocytes, monocytes, eosinophils and basophils.

Statistical analysis

A P value of < 0.05 was considered as a statistically significant value. The 95% confidence intervals of a proportion including continuity correction and odds ratios were calculated using www.vassarstats.net. Differences in the incidence of *A. marginale* infection in cattle were detected using a chi-square (Pearson) test for clinical examination and using Fisher Exact Probability Test for molecular detection. Statistical analyses of hematological variables between infected and non-infected groups were measured by Student t test with Microsoft Excel 2010.

RESULTS

Infection rate and risk factors of A. marginale among cattle at Qena

Among 100 randomly selected cattle, microscopic examination of prepared blood films revealed that 6 (6%, Confidence intervals at 95% = 2.46-13.12) showed *A. marginale* in thin blood smears (Table 1). The organisms were observed at the periphery of erythrocytes (Figure 1). In addition, PCR analysis revealed that 23/100 (23%, 95% CI = 15.42-32.69) samples appeared to be positive by molecular investigations (Table 1). PCR analysis results revealed the obtaining of specific band at the expected molecular size 256 base pair of the target msp5 gene sequence (Figure 2). Among all tested animals (n=100), 12 (12%, 95% CI = 6.63-20.4) have been shown one or more clinical signs relevant to *A. marginale* infection. Such cases 12 (12%) were also positive to PCR screening and 6 from them (n=12) were also positive for microscopic investigations. Noteworthy, 6 cases (6%, 95% CI = 2.46-13.12) were simultaneously positive to PCR screening, microscopic examination and showed a number of BA relevant signs (Table 1). These results demonstrated the high incidence rate of *A. marginale* infection among cattle at Qena governorate, southern Egypt.

Considering the molecular examination, univariable statistical modeling revealed that the risk of infection was significantly associated with age, breed, and management system as predisposing factors for *A. marginale* infection. Higher infection rate was observed in animals less than 2 years (5/10, 50%) showed than older ones (18/90, 20%; odds ratio [OR] = 4; P = 0.047). In case of breeds, infection rates were higher in Holstein-Friesian breeds (8/19, 42.1%; OR = 0.28; P = 0.029) when compared to cross breeds (13/79, 17.1%). In addition, cattle bred in mass or extensive farms showed lower infection rate (16/84, 19%) than those located in individual or small holder farms (7/16, 43.8%; OR = 3.31; P = 0.048). On the contrary, different sex, tick infestation or using acaricide did not have significant effect of infection rate of *A. marginale* infection among tested cattle population in our study. Thus, age, breed, and management system can affect the

Table 1. Prevalence of *Anaplasma marginale* among cattle in Qena governorate, Egypt.

Detection method	No. of tested	No. of negative (%)	No. of positive (%)	95% CI
Molecular examination	100	77 (77)	23 (23)	15.42-32.69
Microscopical examination	100	94 (94)	6 (6)	2.46-13.12
Clinical examination	100	88 (88)	12 (12)	6.63-20.4
Molecular and microscopic	100	94 (94)	6 (6)	2.46-13.12
Clinical and microscopic	100	94 (94)	6 (6)	2.46-13.12
Clinical and molecular examination	100	88 (88)	12 (12)	6.63-20.4
Clinical, microscopical and molecular examination	100	94 (94)	6 (6)	2.46-13.12

95% CI, confidence interval at 95% measured by http://vassarstats.net/ access time was June 04, 2022

Table 2. Univariable analysis of risk factors associated with *A. marginale* infection in cattle in Qena governorate, Egypt.

		Molecular examination (n = 100)				
Cattle group		No. of tested	No. negative	No. positive (%)	OR (95% CI)	*P - value*
Age	< 2 years old	10	5	5 (50%)	4 (1.04-15.32)	0.047
	> 2 years old	90	72	18 (20%)		
Gender	Male	14	10	4 (28.6%)	1.41 (0.39-5.01)	0.732
	Female	86	67	19 (22.1%)		
Breeds	Native	5	3	2 (40%)	0.31 (0.047- 2.04)	0.229
	Holstein-Friesian	19	11	8 (42.1%)	0.28 (0.09- 0.84)	0.029
	Cross breeds	76	63	13 (17.1%)	-	
Tick infestation	Yes	88	67	21 (21.4%)	1.57 (0.32-7.72)	0.728
	No	12	10	2 (16.7%)	-	
Using of acaricides	Yes	89	69	20 (22.5%)	0.77 (0.19-3.19)	0.999
	No	11	8	3 (27.3%)	-	
Management system	Individual owner /small holders	16	9	7 (43.8%)	3.31 (1.07- 10.21)	0.048
	Mass farming	84	68	16 (19%)	-	

OD, Odd ratio, molecular examination P- value was assessed by two-tailed Fisher Exact Probability Test. Access time of http://vassarstats.net/ was June 04, 2022.

infection rate of *A. marginale* infection in cattle.

Effect of A. marginale infection on clinical and blood pictures in cattle

As previously described, 100 tested cattle that have shown PCR positive results 23 (23%) and 12 (12%) of cattle exhibited clinical signs relevant to *A. marginale* infection (Table 1). In PCR positive animals (n=23), systematic clinical examination to the signs related to *A. marginale* demonstrated that cattle showing some clinical symptoms including fever 12 (52.2%), changes in mucus membranes as pale 11 (47.8%), icteric 4 (17.4%), petechial 3 (13%), enlargement of superficial lymph nodes 5 (21.7%), respiratory manifestations 11 (47.8%), eye congestion and lacrimation 5 (21.7%), anorexia 12 (52.2%), emaciation 3 (2.9%), red urine 1 (4.3%), recumbency 2 (8.7%), diarrhea 2 (8.7%), constipation 8 (34.8%), and 12 (52.2%)cases suffered from tick infestations (Tables 3 and 4). Noteworthy, all cases that were considered positive clinically for *A. marginale* infection showed also rise in body temperature (40.2-41.6o C), tick infestation and positive PCR results. In addition, some PCR negative cattle cases showed variable clinical signs that also observed in *A. marginale* infected cattle (Table 3). However, the frequency of each exhibited clinical sign in PCR negative was not comparable to those of PCR positive cases suggesting the correlation of such signs to *Anaplasma* infection in cattle (Table 4).

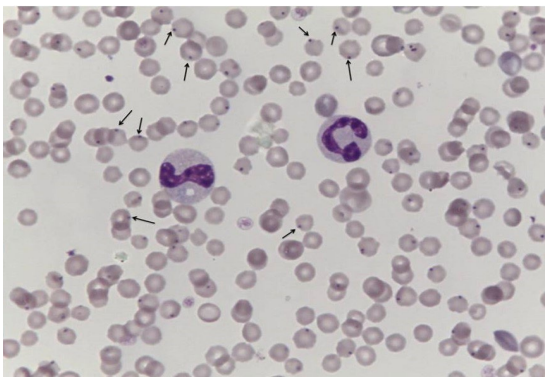

Fig. 1. Microscopical picture of *Anaplasma marginale* infected cattle blood. Giemsa stained blood smear shows *A. marginale* at the periphery of erythrocytes of cattle. Arrows indicate the infected RBCs with *A. marginale*.

Table 5 shows the effect of *A. marginale* infection on hematological profiles in tested cattle population (n=100). Two groups were considered in this assessment, negative group (n=20) in which cattle were selected randomly among those of PCR and microscopic negative results, and no recorded clinical abnormal

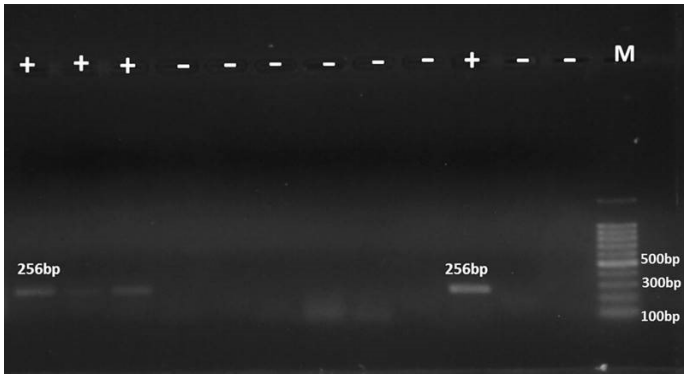

Fig. 2. PCR amplification of *Anaplasma marginale* msp5 gene. Lanes: M: 100 bp DNA marker; +: *A. marginale* positive control; -: *A. marginale* negative control. The band appeared at 265 bp is similar as expected size.

ities. Whilst another group was specified to the positive group (n=10) which included the randomly selected cattle of PCR positive and showing a variety of relevant clinical signs of anaplasmosis in cattle. Significant decrease in RBCs count, Hb content, PCV and eosinophils count was observed in infected animals when compared with control negative group. However, no significant differences were observed in other parameters including MCH, MCHC, MPV, TLC, or neutrophils, monocytes, and basophils counts among the control positive or negative groups (Table 5). While significant decrease was observed in lymphocytes count, in the control positive (5.601±0.214×100cells/mm3) than negative groups (8.205±0,876×100cells/mm3). These results indicate the remarkable suffering of infected *A. marginale*-infected cattle from hemolytic anemia associated with mild immunopathology.

DISCUSSION

Bovine anaplasmosis is a tick-borne disease that causes significant economic losses in many countries especially in tropical and subtropical areas regions (Kocan *et al*., 2010), and in Egypt (El-Dakhly *et al*., 2020). To date, there is a lack of reports and scientific research about anaplasmosis existence, epidemiology and impacts in most regions of Egypt particularly in Upper/ Southern governorates. In a previous report by Fereig *et al*. (2017), sera from 90 cattle at Qena governorate were tested for antibodies against *A. marginale* using competitive ELISA, and 25 (28%) of them were positive . In a more recent study, the microscopic examination of blood smears from cattle and buffaloes in Qena governorate revealed that 7.5% had an *Anaplasma* infection (Mahmoud *et al*., 2022). Additionally, Ahmed *et al*. (2022) revealed that the infection rate of *Anaplasma* in dairy cows using PCR was 35.89 %. Thus, in the current study, we have attempted to confirm the endemic situation of anaplasmosis in cattle using the molecular detection and to provide a detailed description of

Table 3. Effect of *Anaplasma* marginal infection on clinical picture among tested cattle.

Clinical parameters	Negative PCR (n=77)	Positive PCR (n=23)
Body temperature	38.6 °C (38.2 ° C -38.9 °C)	41.2 °C (40.2 ° C -41.6 °C)
Respiratory disorders	Normal, polypnea	Exaggerated, polypnea or dyspnea
Mucus membrane	Bright-rosy red, pale, no lesions	Pale, congested, icteric or petechial hemorrhage
Abnormalities of lymph nodes	None	Moderate enlarged, slightly movable
Eye lesions	Lacrimation	Congested, or peri-ocular edema
Appetite	Anorexia	Anorexia
Urine	Normal	Hemoglobinuria or dark yellow
Body condition	Good	Emaciated, Weak
Abnormal posture	None or sternal recumbency	Sternal, lateral recumbency, staggering gait
Tick infestation	None, few to moderate	None, moderate to heavy
Digestive disorders	Constipation, watery diarrhea	Constipation, bloody diarrhea

Table 4. Frequency of exhibited clinical disorders among *Anaplasma marginale* infected and non-infected groups.

Clinical signs	Frequency among negative PCR cases (n=77), (%)	Frequency among positive PCR cases (n=23), (%)
Fever	7 (9.1%)	12 (52.2%)
Pale mucus membrane	3 (3.9%)	11 (47.8%)
Petechial hemorrhage	0	3 (13%)
Icteric mucus membrane	0	4 (17.4%)
Enlarged lymph nodes	0	5 (21.7%)
Respiratory manifestations	3 (3.9%)	11 (47.8%)
Eye congestion-lacrimation	2 (2.6%)	5 (21.7%)
Anorexia	5 (6.5%)	12 (52.2%)
emaciation	8 (10.4%)	3 (2.9%)
Red urine	0	1 (4.3%)
recumbency	1 (1.3%)	2 (8.7%)
Diarrhea	6 (7.8%)	2 (8.7%)
Constipation	1 (1.3%)	8 (34.8%)
Tick infestation	69 (89.6%)	12 (52.2%)

Table 5. Hematological findings in a group of negative and positive *A. marginale* infected cattle.

Hematological parameters	Negative PCR (n=20)	Positive PCR (n=10)	*P*-value
HB (g/dl)	9.925±1.016	4.92±1.943	0.009
RBCS (10^6cells/mm^3)	7.25±1.022	3.38±2.274	0.036
PCV (%)	32.85±3.73	13.4±9.029	0.022
MCV (fl)	45.89±6.43	47.1±5.82	0.105
MCH (pg)	13.89±1.88	28.22±31.8	0.190
MCHC (%)	30.26±0.72	50.94±54.84	0.236
Platelets(10^3cells/mm^3)	311.04±159.98	366.4±456.78	0.299
MPV (fl)	8.021±0.55	7.9±0.77	0.444
TLC (10^3cells/mm^3)	13.035±2.674	10.775±2.529	0.084
Neutrophils (10^3cells/mm^3)	3.81±1.726	4.021±1.832	0.073
Lymphocyte (10^3cells/mm^3)	8.205±0,876	5.601±0.214	0.013
Monocyte (10^3cells/mm^3)	0.965±0.496	1.013±0.784	0.149
Eosinophils (10^3cells/mm^3)	0.055±0.060	0.14±0.046	0.060
Basophils (10^3cells/mm^3)	0	0±1.22	_

HB: Hemoglobin; RBCs: Red blood cells count; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MPV: Mean platelet volume; TLC: Total leukocytes count.
P ≤0.05 significant, P >0.05 not significant

risk factors and health impact on cattle. In this study, the results of molecular investigation of *A. marginale* revealed that clinical examination is not sufficient to confirm the *Anaplasma* infection because of most of clinical signs are very similar with some infectious and non-infectious diseases. In addition, microscopic examination showed lower infection rate that PCR testing which might be correlated to lower sensitivity of microscopy compared to PCR detection method (El-Dakhly *et al*., 2020).

Taking in account the PCR screening, the obtained infection rate 23% was higher than those El-Ashker *et al.* (2015) conducted on cattle of Dakahlia, El-Dakhly *et al.* (2020) on cattle of various regions of middle Egypt, Tumwebaze *et al.* (2020) on cattle of Menofia, and Selim *et al.* (2021) on cattle from some Delta region governorates that recorded positive rates as 20.1%, 10.6%, 15.2%, and 19.8%, respectively. While, our results was lower than those reported by Radwan *et al.* (2013) on cattle from Kaliobia, ElHariri *et al.* (2017) on buffaloes of Giza, Qalyoubia, El-Wadi El-Gadeed and Menofia governorates, Al-Hosary *et al.* (2020) on cattle of EL-Minia, Assiut, EL-Fayoum, and New Valley, Al-Hosary *et al.* (2021) on cattle of Faiyum, Assiut and Kharja, Ahmed *et al.* (2022) on dairy cows of Qena governorate where they reported positive infection rate as 26%, 69.3%, 68.3%, 92.7%, and 35.89 %, respectively. These variations might be related to the differences in sampled animals, location, seasons, and timing of sample collection.

Herein, we demonstrated that age, breeds and management or breeding system are considered as risk factors for *A. marginale* infection in cattle. Infection rate in cattle less than 2 years old was higher than older one. This result agreed with Younis *et al.* (2009) and conflicted with Al-Hosary *et al.* (2021) and Selim *et al.* (2021) who found that older cattle are more susceptible to *Anaplasma* infection than in younger cattle less than 2 years old. This might be caused by the premunition phenomenon that is characteristic in some blood parasites and anaplasmosis (Palmer *et al.*, 1999), where the previously infected animals showed variable degrees of resistance to the re-infection that explaining the higher infection rate in young, aged animals than older ones. Consistently, our study revealed the Holstein breed as a more susceptible cattle breed than native ones as reported also by Selim *et al.* (2021). Similarly, Al-Hosary *et al.* (2021) reported that infection rate in Holstein-Friesian breed was higher than native one. Regarding management or breeding system, our results revealed that infection rate was higher in farms with small herd size than those recorded in mass or extensive farms. This result was consistent with that obtained by Selim *et al.* (2021).

In case of assessing the effect of *A. marginale* infection on clinical profile in cattle, this study revealed that a variety of clinical signs that were higher in severity and frequency in PCR pos-

itive group than that of PCR negative group. This clinical picture includes fever, respiratory, nervous, and digestive disorders, and abnormalities in urine, mucous membranes, eye and lymph nodes. Most of these clinical signs induced by *A. marginale* infection in cattle had been already reviewed by Kocan *et al.* (2010). Similarly, in this study, hemogram revealed that bovine anaplasmosis caused dramatic changes in hematological parameters. Hematological picture in PCR positive group demonstrated the suffering of cattle from hemolytic anemia indicated in lower number of RBCs, hemoglobin content and PCV. This result agreed with many previous reports (Arunkumar and Nagarajan, 2013; Abdel Hamid *et al*., 2014; El-Ashker *et al*., 2015; Ahmed *et al*. 2022). Regarding the effect of *A. marginale* infection on leucogram, no significant effect was reported among the control negative or positive group except than recording marked lymphopnea in *A. marginale* infected than non-infected group. Such result might suggest the suffering of infected animals from long-term stress which usually associated with decrease in number of lymphocytes triggered by the action of glucocorticoids (Coles, 1986).

CONCLUSION

High infection rate of *A. marginale* among tested cattle population in Qena governorate, Southern Egypt. Based on the obtained data, molecular detection based on msp5 gene showed higher positive results than microscopic and clinical signs investigations indicating higher sensitivity. Also, molecular assay provides more accurate results that allowed verified diagnosis for identification of carrier animals and subclinical cases which is very important aspect for epidemiological investigation of bovine anaplasmosis. Age, breed, and management system are reported as risk factors for *A. marginale* infection in cattle in our investigated region. We also detected that *A. marginale* infection can affect adversely cattle health and perhaps the production via affecting clinical and hematological statuses. Accordingly, *A. marginale* infection should be taken in account in case of suspicion of tick-borne pathogens and other similar cases for anaplasmosis in cattle in Southern Egypt.

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CONFLICT OF INTEREST

The authors declare that no competing interests exist.

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