Research article

Prevalence of tick-borne haemoparasites and their perceived co-occurrences with viral outbreaks of FMD and LSD and their associated factors

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ABSTRACT

Species of Theileria, Babesia, and Anaplasma are Tick-borne pathogens (TBPs) that are prevalent throughout the world, particularly in the tropical and subtropical regions. Associated diseases of Theileriosis, Babesiosis, and Anaplasmosis, respectively, represents a major threat to livestock production in many countries. TBPs have a high prevalence in different geographical locations in Egypt. Foot and mouth disease (FMD) and Lumpy skin disease (LSD) are considered endemic bovine viral diseases in Egypt. Our clinical observations during the epidemics of LSD and FMD viruses showed higher prevalence rates for the TBPs. To investigate this correlation, a total of 670 samples from cattle and buffalo were collected during the summers of 2017 and 2018 distributed throughout ranches and smallholders in two geographical locations in Egypt. Two farms with a recent clinical outbreak of LSD with a total of 270 animals, while the other location included three farms with a recent FMD outbreak with a combined 400 cattle. Examined animals were classified mainly according to age, gender, species, breed (native versus crossbred), and the presence of ticks. Whole blood samples were collected for TBPs and viral (LSD and FMD) examinations, while tissue specimens were collected for detection of FMD and LSD viruses by real-time PCR.

Our results confirmed significantly higher prevalence rates for the TBPs in LSD-positive than LSD-negative animals, while no significant difference could be detected for the prevalence rate of the TBPs in the FMD positive and negative groups. The prevalence of Babesia and Theileria was significantly ($P < 0.05$) higher in crossbreeds than native cattle. Infections with Anaplasma and co-infections with Babesia-Anaplasma and Theileria-Anaplasma were significantly higher in native than cross-breeds cattle. The intensity of parasitic infection (parasitemia) has a significant difference in the positive groups for the two viruses compared to the negative groups. These results collectively confirming the enhancing role of LSD on the prevalence rate of the haemoprotozoal infections leading to more serious outcomes to the livestock infections, and therefore the control of haemoprotozoal infections should be implemented as a part of viral epidemics control.

1. Introduction

Tick-borne diseases (TBDs) constitute a major constraint to livestock production and have a considerable economic impact in affected countries [1]. In general, tick-borne pathogens (TBPs) such as Theileria and Babesia, and Rickettsia of the genus Anaplasma cause diseases that are major health threats to cattle and small ruminants in Africa, Australia, Asia, and Latin America [2, 3, 4, 5, 6]. These diseases have a serious economic impact on livestock production [7]. Bovine babesiosis is caused by the intraerythrocytic hemoprotozoa of B. bigemina and B. bovis, which

are the primary species that affect bovine animals in tropical and subtropical regions [8]. Bovine tropical theileriosis is a tick-borne disease caused by T. annulata; with primary clinical features of fever, anorexia, and swelling of the superficial lymph nodes [9]. On the other hand, anaplasmosis is a vector-borne disease of cattle, sheep, and goats [10]. The common etiological agent of anaplasmosis in bovines is A. marginale, while cattle are also affected with A. caudatum, which may result in severe disease, and Anaplasma centrale that generally resulting in a milder form of the disease [7].

In Egypt, babesiosis is an endemic disease among cattle and buffalo and has a serious economic impact on the livestock industry [11]. Mortality rates are higher in imported cattle breeds when compared to the native breeds [12]. Theileriosis is also an endemic disease among cattle and water buffalo in Egypt [13]. Bovine cases of anaplasmosis are also endemic among cattle and buffalo herds and are usually caused by two *Anaplasma* species: *A. marginale* and *A. centrale* [14].

Foot and mouth disease (FMD) and Lumpy skin disease (LSD) are common bovine viral diseases that affect cattle, with severe impact on the animal's production, health, and immune status. FMD is classified as an endemic disease in Egypt despite the mandatory vaccination routine, and focal outbreaks still occur in many parts of the country [15]. Egypt suffered from several FMD outbreaks starting from 1958 with serotypes A and O and recently in 2012–2013 with SAT strain, with annual outbreaks at different localities in Egypt [16]. Lumpy skin disease is also a devastating emerging viral disease of cattle, which is currently endemic in most African countries and the Middle East region including Egypt [17]. An enhancing role for these bovine viral diseases to the concurrent bacterial and parasitic infections has long been proposed, which mainly attributed to their direct effect on the host immune response. Immunological studies in cattle naturally infected with LSD revealed the immunosuppressive effect of the virus that was marked within two weeks post-infection and showed a significant decrease in lymphocyte transformation rate, and percentage of phagocytic and killing capabilities [18]. Similarly, recent reports indicated the immunosuppressive effect induced by FMDV that marked with a reduction of T cell function which attributed to increment in the production of IL- 10 [19].

There is undocumented evidence that the immune status of animal hosts, and the reduced immunity due to underlying viral and bacterial infections may play a role in subsequent infections by TBPs. The interdependencies of the prevalence of TBPs and viral infections in Egypt are not well investigated. In Egypt, several studies have reported on the rates of infections and prevalence for *Babesia, Theileria, and Anaplasma* in cattle populations [20, 21, 22] Nevertheless, these studies were restricted to single infection entities, with no records on co-infections, co-occurrences with other diseases particularly viral ones, and associations with different risk factors. Thus, this study was initiated with three objectives, 1) to determine the prevalence of *Babesia* spp., *Theileria* spp., and *Anaplasma* spp. infections and co-infections in relation to the status of LSD and FMD, 2) to evaluate the associations of the risk factors such as age group, gender, species, breed, and co-infection of the affected host with hard ticks, and its clinical impact for the infections and co-infections of *Babesia* spp., *Theileria* spp., and *Anaplasma* spp., and 3) to conclude the relationship (if any) between the infection intensities of blood parasites and the status of the LSD and FMD diseases.

2. Material and methods

2.1. Ethical statement

The study was approved by the Committee of Alexandria University for the Ethical Conduction on Experimental Animals. The appropriate Institutional Animal Care Guidelines were followed during all handling and procedures.

2.2. Study area

Animals examined in the current study come from ranches and smallholders mainly scattered in two geographic locations, Amreya, Alexandria Governorate (31.104538°N 29.766226°E), and Kafr El-Dawar, Behaira Governorate (31.1303°N 30.1313°E). Both municipalities were located North of Egypt, with weather conditions that were typical of Mediterranean climate (characterized by dry summers and

mild, wet winters). During the summer months (the study period), a range of annual temperature of 18 °C–45 °C was recorded.

2.3. Animals

During the summers of 2017 and 2018, a total of 670 cattle distributed throughout ranches and small holders in the two geographical locations were surveyed for the infection with TBPs. A recent clinical outbreak with LSD was recorded in two farms with total of 270 animals, while FMD cases were recorded in three farms including 400 cattle. When surveying TBPs, examined animals were classified mainly according to age into four groups including 6–12 months (M), 12–24 M, 24–36 M, and >36 M. Other variables were applied in classifying examined animals that included male versus female (gender), cattle versus buffaloes (species), native versus crossbred (breed), and whether examined animals have tick infestations upon examination and sample collection.

2.4. Clinical examination

Clinical examination of animals was basically performed according to Radostits et.al [23]. Common clinical signs of piroplasmosis and anaplasmosis were recorded from animals that included fever (40–41 °C), anorexia, cessation of rumination, pale (anemic), and icteric mucus membranes, hemoglobinuria, and enlargement of superficial lymph nodes. Other less common signs included small eruptions on the skin of the back, neck, and shoulders, frothy nasal discharge, blackish feces, corneal opacity, circling movements, respiratory distress, grinding of teeth, marked drop in the milk yield, and abortion. Sudden deaths with no apparent clinical symptoms were recorded in sporadic cases.

On the other hand, more specific and pathognomic clinical pictures of viral outbreaks with FMD and LSD were reported, including fever, depression, hypersalivation, vesicles inside the oral cavity, nose, between the toes, on the teats for FMD, while the acute form of LSD was characterized by pyrexia, lymphadenopathy, eruption of skin nodules all over the body that ends with sit-fasts formation after healing, and edema in the lower limbs and the brisket region. Some animals showed signs of severe respiratory distress due to lung edema and few cases showed bloody diarrhea before death due to lesions developed along the digestive tract.

From apparently healthy and sick animals from the same farm, samples were collected for further laboratory examinations.

2.5. Collection of samples

For whole blood sampling, samples collected from each animal from the jugular vein aseptically using vacutainer tubes with Ethylene Diamine Tetra Acetate (EDTA). 2 ml blood aliquots from samples were then transferred into 2-ml Eppendorf tubes with EDTA. Samples were either processed immediately for blood smears preparation and TBPs examination, or were stored at −20 °C till examination for LSD and FMD. Cases with enlarged superficial lymph nodes and apparent bovine theileriosis were diagnosed by collection of aspirations from peripheral lymph nodes, with aspirates were processed for Giemsa staining similar to the blood smears (see below).

For FMD sampling, tissue specimens from freshly ruptured oral vesicles and saturated swab of saliva were collected from the oral mucosa and tongue without contamination with ingested food. Samples were collected into tubes with buffered phosphate saline (PBS), and were stored at −20 °C or immediately processed for laboratory assay. On the other hand, LSD samples included skin biopsies from lumpy's nodules, in addition to whole non-coagulated blood samples were collected from animals with clinical signs of LSD.

2.6. Laboratory examinations

2.6.1. Examination of blood samples for Babesia spp., Theileria spp. and Anaplasma spp.

Thin blood smears were prepared from EDTA-non-coagulated blood and stained with Giemsa's stain according to the standard laboratory protocols [24]. After fixation, air-drying and staining, smears were examined by using oil immersion and 1000X magnification to detect intraerythrocytic stages of piroplasms and *Anaplasama*. Haemoprotozoan parasites were identified and categorized into three genera (*Babesia*, *Theileria*, or *Anaplasma*) based on morphological features [25, 26]. Negative records were reported when no forms of protozoans were detected after examining two triplicate sets of smears independently by two researchers/technicians.

2.6.2. Lymph node biopsy examination

Bovine theileriosis was diagnosed by collection of aspirations from peripheral lymph nodes. Smears from aspirate samples were processed and Giemsa-stained according to the standard procedures [26].

2.7. Laboratory diagnosis of FMD and LSD

FMD and LSD were clinically diagnosed based on typical clinical symptoms and according to the clinical hallmarks of these diseases [27]. FMD and LSD diagnosis was confirmed in the laboratories of the Ministry of Agriculture (El Abasia and El Dokki, Egypt), by the reverse transcriptase- quantitative polymerase chain reaction (RT-qPCR).

2.8. Detection of FMD viral RNA by RT-PCR

2.8.1. FMD viral RNA extraction

FMD viral RNA was extracted from collected samples, tissue of oral vesicles, oral swabs and blood by QIAamp viral RNA mini kit (Qiagen, Valencia, California, USA) according to the manufacturer's instructions.

2.8.2. RT-PCR for FMDV

FMD viral RNA was detected by RT-qPCR, reaction mix was performed according to the manufacturer's instructions of Quantitect probe RT-PCR kit (Qiagen, Valencia, California, USA). Primers targeting FMDV RNA polymerase gene (3D) (GenBank AF189157) were applied, with sequences were, 5′ ACTGGGTTTTACAAACCTGTGA-3′ as a forward primer, 5′-GCGAGTCCTGCCACGGA-3′ as a reverse primer, with TaqMan probe 5′-FAM-TCCTTTGCACGCCGTGGGAC-TAMRA-3' [28]. RT-qPCR was done in a thermocycler (Bio-Rad, USA), starting the one-step real-time RT-PCR amplification with reverse transcription at 60 °C for 1 h, followed by PCR: 55 cycles of denaturation for 2 s at 95 °C, 60 s at 60 °C (annealing and extension). In each reaction, one positive control (2.5 µl of RNA samples) and negative control (deionized sterile water) were included. Levels of fluorescence were measured at the end of each cycle using the RT System (Bio-Rad, USA).

2.9. Detection of LSD viral DNA by RT-qPCR

2.9.1. Viral DNA extraction

Skin biopsy homogenate and blood samples were used for the LSDV DNA extraction using QIAamp DNA Mini Extraction Kit (Qiagen, Germany) according to the manufacturer's instructions. Positive control of LSDV reference strain was used, while deionized sterile water was used as control negative. Extracted DNA aliquots of 50 µl were stored at −20 °C until further analysis.

2.9.2. RT-qPCR for LSDV

RT-qPCR100 reactions kits (GPS, Alicante, Spain) were used for LSDV DNA amplification, the dried mixture of the specific primers with the labeled probe were used according to the kit's manual. The test was done according to the method of Dejan et al. [29].

The reaction for qPCR was done in 10 µl volume consisting of 2 µl master mix, 0.5 µl of primers and probe mix with the reference dye FAM and ROX, 2.5 µl of DNA template, up to 10 µl with distilled DNase and RNase-free water. For the thermocycling conditions: Initial denaturation at 95 °C for 15 min, followed by 35 cycles of 95 °C for 15 s (denaturation) and 60 °C for 60 s (combined annealing/extension). Levels of fluorescence were measured at the end of each cycle, with RT System (Bio-Rad, USA) was used for the analysis.

2.10. Statistical analysis

Correlations between the prevalence of blood parasites and infection status by LSD and FDM were analyzed using the univariate analysis of chi-square test. Strengths of correlation between the incidence of infections and co-infections and dependent and independent variables (risk factors) were estimated by multivariate binary logistic regression analysis, and after adjustment of odds ratio (OR). As measures of significance, *P values* were set at 5% (p ≤ 0.05). On the other hand, strengths of correlation between *Babesia* infections and status of viral infections (LSD and FMD) were calculated by Pearson's correlation analysis, with significance cut-offs of p ≤ 0.05, confidential interval (CI 95%) >1, and OR ≥1. Data processing and analysis for significance were performed in the statistical package SPSS for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Prevalence of hemoprotozoan infections and its correlation with the incidence of LSD and FMD

For the LSDV samples, and from the qPCR data, 148 cases were positive for the LSDV out of 270 cases examined (Table 1). On the other hand, and from the examined samples for FMDV, 213 cases were confirmed positive to FMDV out of 400 cases examined (Table 2).

When these same cases were examined for TBPs, variable rates of infections were recorded in cattle distributed throughout ranches and smallholders in the two surveyed locations. In the LSD-affected farms (270 animals), 31.85%, 4.45%, and 13.33% of animals were tested positive for *Babesia*, *Theileria*, and *Anaplasma* infections, respectively. Lower rates of 0.75%, 5.20% and 1.5% were recorded for mixed infections with *Babesia/Theileria*, *Babesia/Anaplasma*, *Theileria/Anaplasma*, respectively (Table 1). Out of 270 LSD-positive and negative cases surveyed, and apart from single *Theileria* and mixed *Babesia/Theileria* infections, significantly higher prevalence rates were recorded for the TBPs in LSD-positive than LSD-negative animals (P < 0.05). The prevalence rates included 80 (54.05%) for *Babesia*, 24 (16.22%) for *Anaplasma*, 8 (5.41%) for *Babesia/Anaplasma*, and 4 (2.70%) for *Theileria/Anaplasma* concurrent with LSD, when compared with 6 (4.92%), 12 (9.84%), 6 (4.92%), and 0 (0%) in the absence of LSD, respectively (Table 1).

In the FMD-affected farms, 15.75%, 0.75%, and 6.25% of animals were tested positive for *Babesia*, *Theileria*, and *Anaplasma* infections, and 2% for mixed *Babesia/Anaplasma* infection, respectively. No mixed infections with *Babesia/Theileria* and *Theileria/Anaplasma* were reported (Table 2). Higher rates for single and mixed TBPs infections were reported in FMD-positive animals than negative ones. Of the 213 FMD-positive animals, *Babesia* was detected in 60 cases (28.18%), *Theileria* in 2 cases (4.05%), *Anaplasma* in 25 cases (11.75%); and *Babesia/Anaplasma* in 6 cases (2.82%) compared to 1.60%, 0.53%, 0% and 1.07%, respectively in FMD negative cattle (Table 2). No single case of *Theileria/Anaplasma* or *Babesia/Theileria* mixed infections was reported whether the animal tested positive or negative for FMD (Table 2). Nevertheless, and in contrast to the LSD data, no significant difference could be detected for the prevalence rate of the TBPs between the FMD positive and negative groups (P > 0.05).

Table 1. Prevalence of *Babesia* spp., *Theileria* spp., and *Anaplasma* spp. infections and co-infections in relation to LSD status.

	Number	Babesia	Theileria	Anaplasma	Babesia/Theileria	Babesia/Anaplasma	Theileria/Anaplasma	X^2	p-value
LSD-Positive	148	80* (54.05%)**	6 (4.05%)	24 (16.22%)	0 (0%)	8 (5.41%)	4 (2.70%)	27.20	0.018
LSD-Negative	122	6 (4.92%)	6 (4.92%)	12 (9.84%)	2 (1.64%)	6 (4.92%)	0 (0%)		
Total	270	86	12	36	2	14	4		

The chi-square statistic is 27.1954. The p-value is .01811. The result is significant at p < .05.

* Number of infected animals.

** Percentage of the infected animals out of the total examined animals.

Table 2. Prevalence of *Babesia* spp., *Theileria* spp., and *Anaplasma* spp. infections and co-infections in relation to FMD status.

	Number	Babesia	Theileria	Anaplasma	Babesia/Theileria	Babesia/Anaplasma	Theileria/Anaplasma	X^2	p-value
FMD-Positive	213	60* (28.18%)**	2 (4.05%)	25 (0.94%)	0 (0%)	6 (2.82%)	0 (0%)	12.27	0.15
FMD-Negative	187	3 (1.60%)	1 (0.53%)	0 (0%)	0 (0%)	2 (1.07%)	0 (0%)		
Total	400	63	3	25	0	8	0		

The chi-square statistic is 12.2745. The p-value is .15422. The result is significant at p < .05.

* Number of infected animals.

** Percentage of the infected animals out of the total examined animals.

Table 3. Prevalence of *Babesia* spp., *Theileria* spp., and *Anaplasma* spp. infections and co-infections according to selected risk factors.

	No	Babesia (149)*	Theileria (15)	Anaplasma (61)	Babesia/Theileria (2)	Babesia/Anaplasma (22)	Theileria/Anaplasma (4)	AOR** (95% CI)	p-value
Age group									
6–12 M	208	42 (20.2%)	0 (0.0%)	22 (10.6%)	0 (0.0%)	5 (2.4%)	0 (0.0%)	1.30 (0.75–7.52)	0.290
12–24 M	145	34 (23.4%)	6 (4.1%)	12 (8.3%)	0 (0.0%)	11 (7.6%)	3 (2.1%)	2.10 (0.75–3.25)	
24–36 M	207	39 (18.8%)	7 (3.4%)	21 (10.2)	2 (1.0%)	5 (2.4%)	0 (0.0%)	2.80 (1.75–13.75)	
>36 M	110	34 (30.9%)	2 (1.8%)	6 (5.4%)	0 (0.0%)	1 (0.9%)	1 (0.9%)	Reference	
Gender									
Male	295	72 (24.4%)	4 (1.4%)	23 (7.8%)	2 (0.7%)	8 (2.7%)	1 (0.3%)	2.25 (1.05–9.75)	0.691
Female	375	77 (20.5%)	11 (2.9%)	38 (10.1%)	0 (0.0%)	14 (3.7%)	3 (0.8%)		
Species									
Cattle	490	107 (21.8%)	4 (0.8%)	49 (10.0%)	2 (0.4%)	20 (4.0%)	4 (0.8%)	0.95 (2.25–7.75)	0.110
Buffaloes	180	42 (23.3%)	11 (6.1%)	12 (6.7%)	0 (0.0%)	2 (1.1%)	0 (0.0%)	Reference	
Cattle breed									
Native	250	27 (10.8%)	1 (0.4%)	35 (14%)	1 (0.4%)	16 (6.4%)	3 (1.2%)	0.80 (0.75–2.90)	0.040
Crossbred	240	80 (33.3%)	3 (1.3%)	14 (5.8%)	1 (0.4%)	4 (1.7%)	1 (0.4%)	Reference	
Hard Ticks									
Infected	328	127 (38.7%)	9 (2.7%)	53 (16.2%)	2 (0.6%)	20 (6.1%)	4 (1.2%)	0.55 (0.25–2.10)	0.292
Non-infected	342	22 (6.4%)	6 (1.8%)	18 (5.3%)	0 (0.0%)	2 (0.6%)	0 (0.0%)	Reference	

*Number of the positive blood parasite cases.

** AOR = adjusted odds ratio; CI = Confidence interval.

3.2. Risk factors and their correlation with TBPs prevalence

No significant correlations (p > 0.05) were detected between variables such as animal age, gender, species, and status of ticks infestation, with the prevalence of hemoprotozoan infections (Table 3). The prevalence of *Babesia* and *Theileria* was significantly (P < 0.05) higher in cross-breeds than native cattle. *Anaplasma* infections, and co-infections with *Babesia-Anaplasma* and *Theileria-Anaplasma* were significantly higher in native than cross-bred cattle, while equal infection rates were recorded for *Babesia-Theileria* co-infections (Table 3).

3.3. LSD and FMD incidences and their correlation with intensity (Parasitemia) of Babesia infection

Babesia was categorized into low (1–20%), Medium (20–50%), and High (>50%), according to percentages of infected red blood cells (RBCs). Data, as presented in Table 4, revealed significant differences (p < 0.05) between the LSD positive and negative groups. 46.25%, 32.5%, and 21.25% of high, medium, and low parasitemia were recorded in LSD-positive cases when compared with 0%, 66.67%, and 33.33% in LSD-negative animals (Table 4). Statistically significant differences (p < 0.05) were also recorded for FMD. These were 48.33%, 13.33%, and

Table 4. Infection intensity (Parasitemia) of *Babesia* in relation to LSD and FMD status.

	Intensity of Parasitemia*			AOR**	CI 95%***	p-value
	Low (1–20%)	Medium (20–50%)	High (>50%)			
	No/%	No/%	No/%			
LSD-positive (80 *Babesia* cases)	17/21.25%	26/32.50%	37/46.25%	4.86	1.80–10.50	0.013
LSD-negative (6 *Babesia* cases)	2/33.33%	4/66.67%	0/0.00%	Reference		
FMD-positive (60 *Babesia* cases)	23/38.33%	8/13.33%	29/48.33%	2.70	0.77–8.75	0.040
FMD-negative (3 *Babesia* cases)	2/66.67%	0/0.00%	1/33.33%	Reference		

* Parasitemia intensities were measured by dividing the number of infected RBCs (RBCs with piroplasm bodies) per the total number of counted RBCs.
** AOR = adjusted odds ratio.
*** CI = Confidence interval.

38.33%, for FMD-positive animals; while 33.33%, 0%, and 66.67% for FMD-negative cases, respectively.

4. Discussion

Previous reports in Egypt discussed the detailed epidemiological situation of FMD, LSD, and blood parasites infections [16, 21, 30]. During these studies, clinical observations during and after the epidemics of such viral diseases in Egypt greatly hinted at a correlation between those viral infections and the blood parasite infections. Thus, this study aimed to evaluate the effect of the two endemic viral diseases in Egypt, the FMD and LSD on the prevalence of haemoprotozoan infections and the risk factors implicated in such correlation. Firstly, we investigated the effect of the LSD and FMDV on the prevalence of haemoprotozoal diseases. Independent from viral diseases correlation, results for the blood parasites infections revealed prevalence rates that were coherent to the recent reports studied the prevalence of blood parasites infection in the lower Egypt and Delta area, for *Babesia* [21, 31, 32], *Theileria* [33, 34], and *Anaplasma* [35, 36]. Such agreement can be explained by exposure to the same risk factors under the same environmental conditions in these studies.

For the first surveyed group, with recent LSD outbreak, we confirmed significant higher prevalence rates for the TBPs in LSD-positive than LSD-negative animals. Prevalence data confirmed a direct correlation between the LSD and haemoprotozoal prevalence. It is indisputable that most of the viral diseases have a direct or indirect immune suppressive effect to the host, with many reports indicated that LSDV infected cattle showed marked leucopenia in early stages and a pronounced immunosuppression effect during the late stages of the disease [18]. This immune suppressive effect which potentially enhances the vulnerability of the host to other concurrent infections especially when environmental risk factors are ideal for that, i.e., the season and existence of vectors in case of our study or due to reactivation of already existing latent infection. The induced viral leucopenia of LDSV results in macrophages depletion which plays a crucial role in resistance and infection control of *Babesia* species, which mainly attributed to diminishing Th1 cell cytokines production including gamma interferon (IFN- α) and the tumor necrosis factor-alpha (TNF-α). Therefore, macrophages depletion could potentially exaggerated the pathogenesis of haemoprotozoal infection [37]. Thus, data strongly recommend that the increased prevalence rate of haemoprotozoal infection in the LDSV-positive group was mainly due to the immune-suppressive effect of the virus which makes the hosts more vulnerable to external infection or reactivation of latent one. This is particularly plausible, especially when LSD and haemoprotoazal infections have the same seasonal incidence due to the vector availability.

For the second FMD-plagued farms, no significant difference for prevalence rates of the TBPs (single and mixed infections) was reported from FMD-positive animals than negative ones. FMDV, similar to LSDV, induces an immunosuppressive effect, with FMDV-infected cattle showed alteration in the frequency and function of conventional dendritic cells (cDC) and plasmacytoid dendritic cells (pDC) that peaked on the 3rd and

4th days post-infection. This in turn induces immunosuppression effect during FMDV infection, which is mainly reflected as increment in IL-10 production, which in turn impairs T cell function [38]. Nevertheless, the lympho-tropism of LSDV can explain the significant effect of such virus on the prevalence rates of the TBPs versus to the FMD, which can be attributed to greater immunosuppression effects than FMDV infected groups.

The severity of the symptoms of blood parasites infection depends on several host factors such as immune status and concurrent possible infections by other pathogens [39]. Duration of haemoprotozoal latency is a crucial factor in the establishment of new infections, as symptoms can occur after a long period of latency in case of *Anaplasma* infection [36]. Infection with *Babesia* species, on the other hand, can persist for 2–3 years and can be easily reactivated throughout this period [40]. Thus, depending on the aforementioned data, we strongly support the hypothesis of latent infection reactivation for the haemoprotozoal infection during the viral epidemics. This is usually accompanied with immune suppression, especially in the geographical area under investigation in this study which has a higher prevalence of blood parasite infection. This could help to explain the higher prevalence of the haemoprotozoal infection during those viral epidemics. The study outcomes can be further supported by the reports studied the prevalence rates of the latent blood parasites infections which represented up to 15% of all sampled animals in case of babesiosis tested by PCR [41]. For animals showing no clinical signs, which represented a considerable percentage, additional reports studied the duration of latency found that this period could be ranged from 2-3 years for most of the blood parasites infections [40].

Our assessment of the animal risk factors affecting TBPs prevalence such as the animal breed, age, gender, species, and status of tick infestation revealed that only the breed of the animal either native or cross-bred has a statistically significant difference in the prevalence of haemoprotozoal infection. This observation strongly supported by this recent report in Egypt for the level of animal risk factors associated with haemoprotozoal infection, which also considered the animal breed as a major determinant for the prevalence of the haemoprotozoal infection [42]. This can be attributed to genetic differences and their innate resistance to infections between the local and imported breeds. Also, the non-significance of other determinants strongly supports our notion for the strong correlation between these two viral diseases and the haemoprotozoal prevalence rates.

A further strong clue for the correlation between the outbreaks of the LSD, FMD, and hemoprotozoal infection was supported in this study, where a significant difference (p < 0.05) between the positive and negative groups for the two viruses was detected when levels of the parasite infection intensity (parasitemia) were assessed. This can be considered as a logical outcome for the immune-suppressive effect of the viral infection. In most of the haemoprotozoal infections, the level of parasitemia is directly related to the phagocytic power of leukocytes, which is dramatically impaired during those two viral infections as mentioned before. Under the normal condition, and after the parasite infection, there is a mounted increase in the innate immune response

against infected erythrocytes with chemokines will be released to attract more immune cells [43]. This mechanism is initiated during the acute infection with a high level of parasitemia to diminish the infection [44]. This normal pathogenesis is greatly impaired during those two viral infections which result in significant differences in the level of parasitemia between viral positive and negative groups.

5. Conclusions

Our results confirmed a significant higher prevalence rates for the TBPs in LSD-positive than LSD-negative animals, while no significant effect for the FMDV on TBPs prevalence was detected. Up to our knowledge, this is the first report discussing such correlations and highlight the impact of these two endemic viral diseases on TBPs prevalence. Evidence for such correlation has significant impacts due to the aggravated outcomes in case of concurrent viral and parasitic infections than the sole infection.

Babesia is the most commonly detected hemoprotozoan in the geographical area under the study. The infection rates of *Babesia* and *A. marginale* were higher among young animals over 6 months of age and declined in animals over 2 years of age, while the infection rates of *T. annulata* were lower among young animals and increased in animals above 2 years of age.

Declarations

Author contribution statement

O. Abas: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

A. Abd-Elrahman: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

A. Saleh: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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