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Clinical and laboratory diagnosis of some blood parasites in dairy cows in Qena governorate

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Abstract

Bovine theileriosis, babesiosis and anaplasmosis are tick-borne hemoparasitic diseases and they are responsible for huge economic losses in livestock sector in Egypt. Currently, a total number of 110 dairy cows from different regions of Qena governorate, Egypt, were clinically and laboratory investigated for diagnosis of theileriosis, babesiosis and anaplasmosis using Giemsa stained blood film and Polymerase Chain Reaction (PCR) assay during the period from January 2019 to December 2019. On the basis of the obtained results, the overall prevalence of theileriosis, babesiosis and anaplasmosis among the screened cattle was 21.81%, 9.09% and 25.45 respectively. Furthermore, mixed infections were seen in nineteen cases (17.27%) on the basis of blood film examination. PCR assay results revealed that, the infection rate with theileriosis, babesiosis and anaplasmosis was 5.12%, 10.25% and 35.89%, respectively. While, 3 cows (7.69%) were found to harbor a mixed infection. Additionally, hemato-biochemical alternations in theileriosis, babesiosis and anaplasmosis infected cows were also detected in this study. It could be concluded that PCR assay was the most sensitive test in the detection of the infection in all cases of the disease (acute, chronic and carriers) as once animals infected, they become carriers with low parasitemia after recovery and this low parasitemia cannot be detected by traditional examination of Giemsa stained thin blood smears.

Keywords:
Anaplasmosis, Babesiosis, Hemato-biochemical, PCR, Theileriosis

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Introduction

Tick-borne diseases obstruct the growth and the flourish of the livestock sector and affect the health and productivity of domesticated cattle in tropical and sub-tropical regions of the world (de Castro, 1997). Therefore, the infection with hemoprotozoan parasites or *Anaplasma (A.) marginale* should be considered as a real threat for the food industry in the whole world (Fereig et al., 2017).

Bovine tropical theileriosis in cattle is caused by the protozoan parasite, *Theileria (T.) annulata* (Gharbi et al., 2012). Theileriosis in cattle shows anorexia, emaciation, depressed rumination, ocular signs, nasal discharges, diarrhea and high fever may reach up to 40–41.5 ˚C (Kundave et al., 2013).

Bovine babesiosis in cattle is caused mainly by the protozoan parasites, *Babesia (B.) bovis and B. bigemina* (El Moghazy et al., 2014). Babesiosis causes anemia, icterus, hemoglobinuria, and death (Wagner et al., 2002). *B. bovis and B. bigemina* are responsible for high mortality rates (may reach up to 50%) in susceptible herds (Antoniassi et al., 2009).

Bovine anaplasmosis is caused by *A. marginale* which is rickettsial microorganism (Aubry and Geale, 2011). Anaplasmosis shows progressive haemolytic anaemia, fever, jaundice, decreased milk production, abortions and sudden death may occur (Constable et al., 2017).

The aims of this work are to diagnose theileriosis, babesiosis and anaplasmosis in cows in Qena governorate, Egypt, by using Giemsa-stained thin blood smears and PCR assay, also the present study describes the clinical signs and haemato-biochemical alterations that may occur in cows naturally infected with theileriosis, babesiosis and anaplasmosis.

Materials and methods

1. Study area

Qena is a governorate in the south of Upper Egypt. Economically, Qena is an agricultural and also industrial governorate. It ranks first in production of sugarcane, bananas, tomatoes, sesame, and hibiscus. The total growing region comes to nearly 291,700 hectares, of which sugarcane makes up almost 60%. Qena region is responsible for 64% of sugar production in all of Egypt.

Qena has a hot climate with very hot summers and very little precipitation year around. Winters are warm during the day, but become quite cool at night, generally the climate of the region is defined as cold winter, hot summer, cool-wet spring and autumn with a wide range of temperatures.

This study was carried out during the period from January 2019 to December 2019 in the department of Animal Medicine, Faculty of Veterinary Medicine, South Valley University; Animal health researches institute, Qena laboratory and the Biotechnology unit in Reference laboratory for veterinary quality control on poultry production (Animal health research institute, Dokki, Giza, Egypt).

2. Ethics Statement:

All dairy cows, included in this study, (n=110) were handled according to the regulations of the Animal Ethics Committee at the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt, with good animal practice following the guidelines of The Research Code of Ethics (RCOE-SVU) at the South Valley University.

3. Animals

One hundred-ten female dairy cows (62 from a dairy farm and 48 from small holders) with ages ranging from 2-5 years, from different localities at villages and cities of Qena Governorate, Egypt, were examined clinically and laboratory generally for the presence of Tick borne hemoparasitic infections. 30 out of 110

dairy cows were apparently healthy.

4. Sampling and clinical analysis

The blood samples were collected from the jugular vein by using sterile sharp needle with wide pore; two samples were collected from each animal, by two types of vacutainer tubes, one coated with EDTA for blood smears, hematological studies and PCR technique. The second tube without EDTA (plain) for biochemical analysis. The collected samples were transferred in clean ice box directly to the laboratory of Department of Animal Medicine, Faculty of Veterinary Medicine, South Valley University with a minimum of delay to be immediately subjected to examination.

Furthermore, the clinical examination of all cows was carried according to procedures described by Rosenberger, 1990.

There was usually a history of tick infestations in most cases of the three forms of blood parasites (*Theileria, Babesia* and *Anaplasma*).

5. Blood films examination:

Thin blood films were prepared from whole blood samples containing EDTA according to Kelly, (1974). The parasites were identified according to the morphological characters described by Soulsby, 1982. The smears were classified as negative for piroplasms or *Anaplasma*, if no parasites were detected in about 50 oil-immersion fields under microscope (Moretti et al., 2010).

6. Polymerase chain reaction

5.1. DNA extractions:

Thirty-nine blood samples out of 110 blood samples were selected randomly for PCR assay. DNA extraction from whole blood samples was carried out according to QIAamp DNA mini kit instructions.

5.2. DNA amplification by PCR:

Three pairs of primers were supplied from Metabion (Germany). They have specific sequence and amplify specific products. For *T. annulata*, a primer (targeting and specific for *tams1gene)* was used as previously described by (Nourollahi-Fard et al., 2015). The primer sequences were as follows: forward 5'-GTAACCTTTAAAAACGT-3', reverse 5' GTTACGAACATGGGTTT 3' which permits the amplification of an approximately 721 bp. For *Babesia species*, a primer (targeting and specific for 18S rRNA gene*)* was used as previously described by (Salem and Farag, 2014). The primer sequences were as follows: forward 5'-GTCTTGTAATTGGAATGATGGTGAC-3', reverse 5'-ATGCCCCCAACCGTTCCTATTA-3' which permits the amplification of an approximately 340 bp. For *A. marginale*, a primer (targeting and specific for *msp5 gene)* was used as previously described by (Ganguly et al., 2018). The primer sequences were as follows: forward 5-ACAGGCGAAGAAGCAGACAT-3', reverse 5'-ATAAATGGGAACACGGTGGA-3' which permits the amplification of an approximately 382 bp. Then PCR reaction was performed in a total volume of 25 µl containing 12.5µl of Emerald Amp GT PCR master mix (2x premix), 6 µl of template DNA, 20 pmol of each primer and 4.5 µl of PCR grade water. Amplification was performed in a thermal cycler (T3 Thermal cycler, Biometra, Analytikjena, Germany) under the following conditions: 94°C for 5 min (initial denaturation) followed by 35 cycles of 94°C, 30 sec (denaturation), 53-55°C, 40 sec (annealing), 72°C 40 sec (extension) and a final extension of 72°C for 10 min.

The PCR products were subjected to electrophoresis in1.5 g agarose gel in 100 ml TBE. After staining with ethidium bromide, PCR products were visualized under UV light.

7. Hematological Examinations:

Haematological examinations were

carried out on whole blood with EDTA to study changes associated with blood parasites infections including red blood cells count (RBCs), white blood cells count (WBCs), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) mean corpuscular haemoglobin concentration (MCHC), platelets, polymorphs, lymphocytes, monocytes and RDW by using automated haematology analyzer (Celltac a model no. MEK-6500k).

8. Biochemical examinations:

Biochemical examinations were carried out on serum to study changes associated with blood parasites infections especially those concerning liver parameters including activities of total proteins, albumin, globulin, total bilirubin, direct bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) which were estimated by automated chemistry analyzer (Aptio, Siemens, ADVIA® 2400).

Serum globulins were determined by subtracting the obtained values of albumin from those of total proteins.

9. Statistical Analysis:

The obtained data were statistically analysed to calculate means, standard deviations and P values by means of computer based-statistical program Minitab (v17.1.0; Minitab, LLC) and GraphPad (GraphPad QuickCalcs Web site: http://www.graphpad.com/quickcalcs; GraphPad Software, Inc).

Results

1- Clinical findings:

Clinical findings of dairy cows suffering from theileriosis and babesiosis and anaplasmosis are listed in table 1.

Table 1: Main clinical findings in clinically healthy cows and diseased ones.

Clinical Findings	Healthy cows	Theileriosis	Babesiosis	Anaplasmosis
1-Body condition	Good, active and move well	Emaciation and weakness especially in late stages		
2-Mucous membranes	Rosy red, moisted, filled episcleral blood vessels	Paleness or congestion of the visible mucous membranes and Petechiae on the mucous membranes	Paleness with empty episcleral blood vessels in mild cases to severe yellow discoloration (icteric)	
3-Oculonasal discharge and salivation	Absent	Present (main clinical sign) with corneal opacity, conjunctivitis and obvious lacrimation	Present	
4-Rectal temperature (°C)	38.7 °C	> 40 °C		
5-Heart rate	57 beats / minute	Tachycardia		
6-Respiratory manifestations	Absent	Present		
7-Lymph nodes	No swelling, movable, hot-less and painless.	Greatly swollen, painful and hot during palpation.	No swelling, movable and hot-less.	
8-Urine	Light yellow	Straw yellow	Hemoglobinuria	Often brown
9-Milk production	Normal	Decreased		
10-Cough	Absent	-----	Present	Present
11-Ruminal motility	Normal	-----	Cessation of rumination	Decreased
12-Bloody feces	Absent	-----	-----	Present

Sporadic cases of sudden death were recorded (3 cases showed fever and hemoglobinuria before death).

2-Survey findings:

On the basis of blood film analysis, out of 110 examined animals for piroplasmosis, 10 animals were found to be infected with babesiosis representing 9.09 % and 24 animals were found to be infected with theileriosis representing 21.81 %. On the other hand, 28 cows were found to be infected with anaplasmosis giving an overall prevalence of 25.45%. Additionally, the mixed infection was recorded in nineteen cows (17.27%) including 4 *A. marginalis* & *Babesia* spp. + 4 *Theileria spp.* & *Babesia spp.* + 11 *A. marginalis* & *Theileria spp*.

Currently, the results of PCR technique revealed, 2 cows (5.12%) were found to be infected with theileriosis (Fig. 1 and 2), 4 cows (10.25%) were found to be infected with babesiosis (Fig. 3 and 4), and 14 cows (35.89%) were found to be infected with anaplasmosis (Fig. 5, 6 and 7). Whereas, 3 cows (7.69 %) were found to harbor a mixed infection (2 *A. marginalis* & *Babesia spp.* + 1 *Theileria spp.* & *Babesia spp.*).

In this study, the highest occurrence of blood parasitic infections was observed in summer months (June - September).

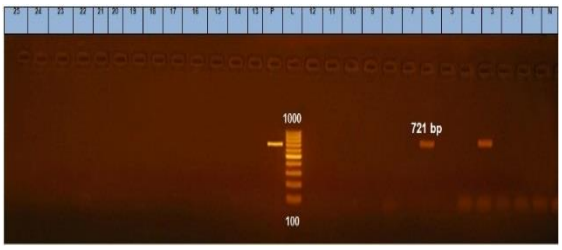

Fig. 1. *Theileria* spp. Lane L(100-1000 bp DNA ladder), Lane (3 and 6) positive PCR product amplified from study blood samples, Lane P (control positive), Lane N (control negative), Lane (25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 5, 4, 2 and 1) negative study blood sample.

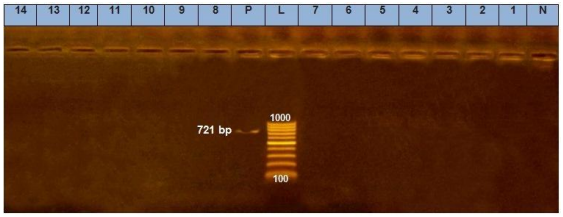

Fig. 2. *Theileria* spp. Lane L(100-1000 bp DNA ladder), Lane P (control positive), Lane N (control negative), Lane (14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 and 1) negative study blood samples.

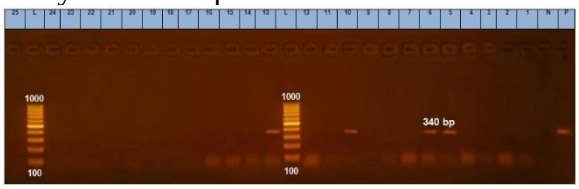

Fig. 3. *Babesia* spp. 2 Lane L (100-1000 bp DNA ladder) , Lane (13, 10, 6 and 5) positive PCR product amplified from study blood samples, Lane P (control positive), Lane N (control negative), Lane (25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 12, 11, 9, 8, 7, 4, 3, 2 and 1) negative study blood sample.

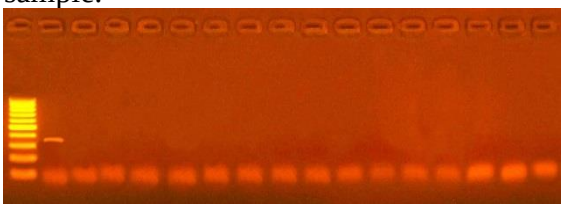

Fig. 4. *Babesia spp.* On the left, Lane L(100-1000 bp DNA ladder) followed by Lane P (control positive), Lane N (control negative), Lane (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14) negative study blood sample.

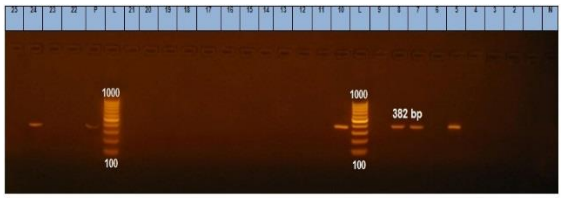

Fig. 5. *Anaplasma marginale* Lane L(100-1000 bp DNA ladder) , Lane (24, 10, 8, 7 and 5) positive PCR product amplified from study blood samples, Lane P (control positive), Lane N (control negative), Lane (25, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 9, 6, 4, 3, 2 and 1) negative study blood sample.

Table 2: Microscopical and PCR examination results.

	Blood film examination (n= 110)	PCR assay (n= 39)
Theileriosis	24 infected cows (21.81 %)	2 infected cows (5.12%)
Babesiosis	10 infected cows (9.09 %)	4 infected cows (10.25%)
Anaplasmosis	28 infected cows (25.45%)	14 infected cows (35.89%)
Mixed infection	19 infected cows (17.27%)	3 infected cows (7.69 %)

3-Morphological results:

The shape of the different blood parasites inside the RBCs are presented in Table 3 and Fig. 6.

Table 3: Morphological features of the recovered blood parasites.

Blood parasites	Morphological results
***Theileria* organisms**	rod or round shaped organisms
***Babesia* organisms**	pyriform in shape and in pairs at an obtuse angle
***Anaplasma* organisms**	dense, deep purple, vacuole-bound, near-circular inclusion bodies and located near the margin of the cell

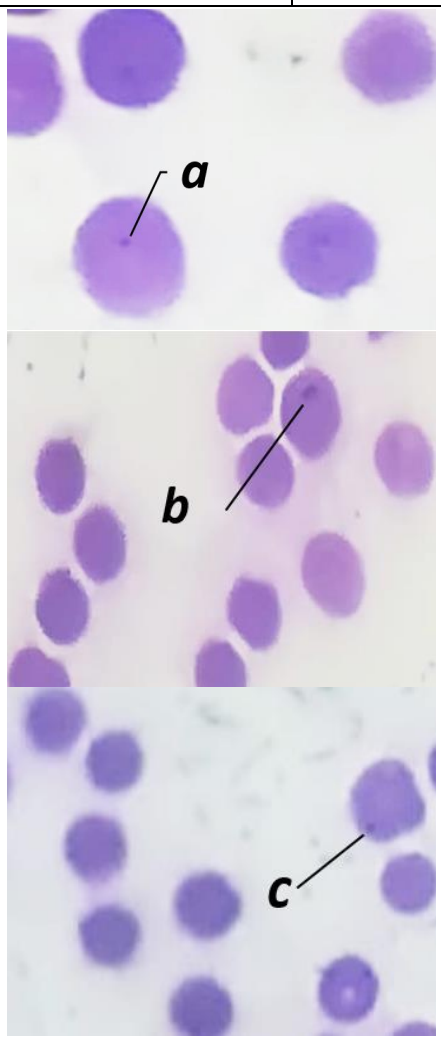

Fig. 6. showed the recovered blood parasites, a) *Theileria* spp., b) *Babesia* spp. and c) *Anaplasma marginalis*

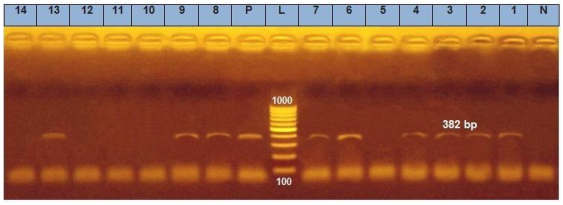

Fig. 7. *Anaplasma marginale*. Lane L(100-1000 bp DNA ladder), Lane (13, 9, 8, 7, 6, 4, 3, 2 and 1) positive PCR product amplified from study blood samples, Lane P (control positive), Lane N (control negative), Lane (14, 12, 11, 10 and 5) negative study blood sample.

4- Hemato-biochemical results:

The only one pure case of babesiosis showed a marked decrease in WBCs (4.90 10^3cells/mm^3), and increase in lymphocytes (80.90 10^3cells/mm3) and monocytes (8.90 10^3cells/mm3) and a marked decrease in polymorphs (10.20 10^3cells/mm^3). RBCs (3.24 10^6cells/mm^3), Hb (4.70 g/dl) and PCV (15.50 %) values showed marked decreases but there was an increase in MCV (47.80 fl) and almost no noticeable changes in MCH (14.50 pg) and MCHC (30.30 %).

The only one pure case of theileriosis showed a marked decrease in RBCs (4.17 10^6cells/mm3), in Hb (6.4 g/dl) and in PCV (21.3 %). There was increase in MCV (51.1 fl) and a slight increase in MCH (15.3 pg) and MCHC (30.0 %). There were decreases in WBCs (6.0 10^3cells/mm^3) and polymorphs (20.3 10^3cells/mm^3) and

increases in lymphocytes (63.5 10^3cells/mm^3) and monocytes (16.2 10^3cells/mm^3).

As showed in Table 4, in anaplasmosis cases there were highly significant decreases in RBCs (4.20 10^6cells/mm^3), Hb (6.58 g/dl), and PCV (22.00 %) and significant increase in MCV (55.35 fl) and MCH (16.66 pg). Monocytes (11.92 10^3cells/mm3) showed highly significant increase, while there were non-significant changes in WBCs (6.82 10^3cells/mm^3), polymorphs (24.58 10^3cells/mm^3) and lymphocytes (65.82 10^3cells/mm^3).

Mixed infection also showed highly significant decreases in RBCs (2.61 10^6cells/mm3), Hb (4.63 g/dl), and PCV (12.83 %). There was significant increase in MCV (53.63 fl) but there were non-significant changes in MCH (25.77 pg) and MCHC (44.95 %). There were significant decreases in WBCs (6.13 10^3cells/mm^3) and polymorphs (12.93 10^3cells/mm^3) and highly significant increases in lymphocytes (77.67 10^3cells/mm^3) and monocytes (9.23 10^3cells/mm^3).

Table 4: Hematological parameters in clinically healthy and infected cattle.

Parameters	Control	*Anaplasma sp.*	Mixed Infections
	Healthy (n= 10)	Infected Group (n=12)	Infected Group (n=3)
RBCs (10^6cells/mm^3)	7.11 ± 0.58	4.20 ± 1.84**	2.61 ± 1.71**
Hb (g/dl)	9.98 ± 0.64	6.58 ± 2.24**	4.63 ± 1.20**
PCV (%)	31.96 ± 2.07	22.00 ± 6.97**	12.83 ± 7.28**
MCV (fl)	45.08 ± 3.73	55.35 ± 10.95*	53.63 ±10.89*
MCH (pg)	14.06 ± 1.11	16.66 ± 2.62*	25.77 ± 19.17 NS
MCHC (%)	29.45 ± 5.69	29.73 ± 1.71 NS	44.95 ± 23.73 NS
WBCs (10^3cells/mm^3)	7.62 ± 0.81	6.82 ± 2.34 NS	6.13 ± 1.15*
Polymorphs (10^3cells/mm^3)	34.25 ± 11.97	24.58 ± 14.48 NS	12.93 ± 8.70*
Lymphocytes (10^3cells/mm^3)	59.66 ± 2.00	65.82 ±15.51 NS	77.67 ± 8.01**
Monocytes (10^3cells/mm^3)	6.09 ± 0.71	11.92 ± 2.96**	9.23 ± 1.77**

** Highly significant ($P < 0.001$), * Significant ($P < 0.05$), NS Non significant
Mixed infection = 2 *A. marginalis* & *Babesia* spp. + 1 *Theileria* spp. & *Babesia* spp.

The only free case of babesiosis showed high increase in AST (95 IU/L) and ALP (42 IU/L) and decreases in ALT (16 IU/L) and GGT (11 IU/L).There were also decreases in albumin (2.7 g/dl), globulin (2.4 g/dl), total protein (5.1 g/dl) and no changes in A/G ratio (1.13) and total bilirubin (0.67 mg/dl).

The only free case of theileriosis showed high increase in ALT (21 IU/L), AST (259 IU/L), GGT (184 IU/L) and ALP (50 IU/L). There were also decreases in total protein (6.1 g/dl), albumin (2.8 g/dl) and slight decreases in globulin (3.3 g/dl) and A/G ratio (0.85). There were also increases in total bilirubin (1.5 mg/dl) and direct bilirubin (0.57 mg/dl).

As showed in Table 5, In anaplasmosis, there were significant increases in AST (85.83 IU/L) and GGT (41.58 IU/L) and almost no change in ALP (29.08 IU/L) and non-significant increase in ALT (23.17 IU/L). There was also a significant increase in total protein (7.34 g/dl) and highly significant increase in globulin (4.29 g/dl) while albumin (3.05 g/dl) showed highly significant decrease. There were almost no changes in total bilirubin (0.75 mg/dl) and direct bilirubin (0.37 mg/dl).

In mixed infection, there were significant increases in ALT (25.67 IU/L) and AST (155.33 IU/L) but there were non-significant increases in GGT (20.67 IU/L) and ALP (34.67 IU/L). There was also a significant decrease in total protein (5.93 g/dl) and high significant decrease in albumin (2.63 g/dl). There were almost no changes in total bilirubin (0.67 mg/dl) and direct bilirubin (0.30 mg/dl).

Table 5: Serum biochemical constituents in infected and healthy cattle.

Parameters	Control	*Anaplasma sp.*	Mixed Infections
	Healthy (n= 10)	Infected Group (n=12)	Infected Group (n=3)
ALT (IU/L)	19.60 ± 3.95	23.17 ± 7.06 NS	25.67 ± 3.21*
AST (IU/L)	50.30 ± 12.76	85.83 ± 39.99*	155.33 ± 92.09*
GGT (IU/L)	16.30 ± 6.31	41.58 ± 31.87*	20.67 ± 12.06 NS
ALP (IU/L)	29 ± 1.94	29.08 ± 15.14 NS	34.67 ± 8.62 NS
Total protein (g/dl)	6.94 ± 0.19	7.34 ± 0.55*	5.93 ± 0.85*
Albumin (g/dl)	3.65 ± 0.13	3.05 ±0.17**	2.63 ± 0.15**
Globulin (g/dl)	3.29 ± 0.20	4.29 ± 0.49**	3.30 ± 0.72 NS
A/G ratio	1.11 ± 0.09	0.72 ± 0.07**	0.82 ± 0.14*
Total bilirubin (mg/dl)	0.77 ± 0.20	0.75 ± 0.09 NS	0.67 ± 0.010 NS
DB (mg/dl)	0.34 ±0.17	0.37 ± 0.15 NS	0.30 ± 0.07 NS

** Highly significant (P < 0.001), * Significant (P < 0.05), NS Non significant
Mixed infection = 2 *A. marginalis* & *Babesia* spp. + 1 *Theileria* spp. & *Babesia* spp.

Discussion

In Egypt, Tick-borne infections are considered as destructive infections to the programs of livestock improvement and cause serious health problems leading to lower animal productivity and great economic losses.

The hot and humid climate favours the multiplication and survivability of ticks (Kholi et al., 2014).

The results of this study revealed that the percentage of infection of *Theileria* spp. in 110 dairy cows by using Giemsa stained blood film examination method was 21.81%. Kohli (2014) from India recorded 27.2%, Acici (1995) from Turkey recorded 17 % and Nayel et al., (2012) from Egypt recorded 16.05 %. While Gamal EI-Dien (1993) from Egypt, found that the prevalence of theileriosis was 65.4 % by using stained blood films method.

The results of this study revealed that the percentage of infection of *Babesia* spp.

in 110 dairy cows was (9%) in agreement with Nayel et al., (2012) from Egypt who recorded 8.1% and Mazyad and Khalaf (2002) from Egypt also who recorded 9.9 % and 8.1 % respectively while the percentage was lower than that recorded by Saad (2015) from Pakistan who recorded 24%, Osaki et al., (2002) from Brazil who recorded 64 % and Battsetseg et al., (2002) from Brazil also who recorded 64 % by using blood stained films method.

The percentage of infection of *Babesia* spp. (9%) in 110 dairy cows by using blood stained films method was higher results than that recorded by Fethu et al., (2016) from Ethiopia who recorded 4.4%.

The results of this study revealed that the percentage of infection of *A. marginalis* in 110 dairy cows was (25.4%) and this is in agreement with the study of Ybañez et al., (2013) wherein stained blood smears technique revealed 25% but this study disagreed with Aquino et al., (2018).

The results of this study revealed that the percentage of infection of *Theileria* spp. in 39 dairy cows by using PCR technique was (5.12%) that was lower than results revealed by Farooqi et al., (2017) who revealed that the infection percentage of *Theileria* spp. was 18.88% and Kohli et al., (2014) who recorded 32.5% and also Abdel-Rady (2010) who recorded a higher percentage (65.6%).

The results of this study revealed that the percentage of infection of *Babesia* species in 39 dairy cows was (10.25%) in agreement with El-Ashker et al., (2015) who recorded 8.5 %, Ibrahim et al., (2013) who recorded 9.27% and El-Bahy et al., (2018) who recorded 9.42%. The results of this study disagreed with some researchers who recorded higher results like El-Fayomy et al., (2013) from Egypt who recorded 23% and Rania (2009) who recorded 25.33 %.

The results of this study revealed that the percentage of infection of *A. marginalis* in 39 dairy cows was (35.89%) in agreement with El-Ashker et al., (2015) who showed a percentage of 21.3% but the results of this study were not in agreement with Aquino et al., (2018) who recorded higher results (67.3%) and Younis et al., (2009) who recorded lower results (3.68%).

Nayel et al., (2012) mentioned that variation in prevalence rates between studies could possibly be attributed to an abundance of the vectors as a result of high temperature and humidity.

In PCR examination, there were 2 blood samples, negative to blood parasites, these two samples were positive to *Anaplasma* spp. in blood film examination. 4 blood samples were also negative to blood parasites in PCR examination, these 4 samples were positive for *Theileria sp.* in blood film examination. Blood parasitic structures recognized in RBCs are often difficult to be differentiated from some other RBCs inclusions like Heinz bodies, Howell-Jolly bodies or staining artifacts (Ge et al., 1995).

Tick borne infections may cause important changes in hematological and biochemical parameters. In this study, the only one pure case of theileriosis showed a marked decrease in RBCs (4.17 10^6cells/mm3), in Hb (6.4 g/dl) and in PCV (21.3 %). There was increase in MCV (51.1 fl) and a slight increase in MCH (15.3 pg) and MCHC (30.0 %). There were decreases in WBCs (6.0 10^3cells/mm3) and polymorphs (20.3 10^3cells/mm3) and increases in lymphocytes (63.5 10^3cells/mm3) and monocytes (16.2 10^3cells/mm3).

Such changes in Leucogram may be attributed to the persistent harmful effects of *Theileria* toxic metabolites on the haemopiotic organs (bone marrow) and their obstruction to the process of leucogenesis. The increase in numbers of lymphocytes and monocytes may act as a

compensatory mechanism as target cells in response to the invasion of *Theileria* protozoan.

This decrease in RBCs, Hb and PCV may be attributed to destruction of RBCs by macrophages in the spleen and other mononuclear phagocyte system organs (Singh et al., 2001).

A leukopenia in theileriosis was reported by Aulakh and Singla (2006) and Modi et al., (2015) in cattle while Mehta and colleagues (1988) reported a leukocytosis in theileriosis.

Acharya, et al., (2015) showed a significant decrease in MCHC and MCV and showed non-significant decrease in MCH. Ibrahim et Al., (2009) showed non-significant increase in MCV and non-significant decrease in MCHC while Nazifi et al., (2010) revealed a significant elevation in MCV and MCH.

This study recorded only one pure case of free *Babesia* without another mixed infection. This only one case of babesiosis showed a marked decrease in WBCs (4.90 10^3cells/mm3) and increase in lymphocytes (80.9 10^3cells/mm3) and monocytes (8.9 10^3cells/mm3) and a marked decrease in polymorphs (10.20 10^3cells/mm3). This could be explained as the destruction of RBCs by *Babesia* sp. stimulates the phagocytic cells such as lymphocytes and monocytes to clear the body from the toxic remnants of destroyed RBCs. This is in agreement with Guglielmone et al. (1996), who reported that babesiosis leads to stimulation of defense mechanism of animal body to produce antibodies against *Babesia* antigens. RBCs (3.24 10^6cells/mm3), Hb (4.70 g/dl) and PCV (15.50 %) values showed marked decreases but there was an increase in MCV (47.80 fl) and almost no noticeable changes in MCH (14.50 pg) and MCHC (30.30 %).

In Sharma et al., (2016) results, haematological parameters showed significant ($P< 0.05$) decrease in the RBCS count, PCV, Hb and MCV and also showed significant increase ($P< 0.05$) in MCH and MCHC.

The current study revealed an insignificant decrease in WBCs count (6.81 10^3cells/mm3) and in polymorphs (24.58 10^3cells/mm3), an insignificant increase in lymphocytes (65.81 10^3cells/mm3) and a highly significant increase in monocytes (11.91 10^3cells/mm3) in anaplasmosis.

In this study also, there was a highly significant decrease in RBCs (4.20 10^6cells/mm3), Hb (6.58 g/dl) and PCV (22.00 %) in anaplasmosis. MCV (55.35 fl) and MCH (16.65 pg) values showed significant increases.

El-Ashker et al., (2015), De et al. (2012) and Abdel Hamid et al., (2014) reported that the levels of PCV, RBCs count and Hb were significantly decreased in infected cattle.

In this study, the only one pure case of theileriosis showed high increase in ALT (21 IU/L), AST (259 IU/L), GGT (184 IU/L) and ALP (50 IU/L). There were also decreases in total protein (6.1 g/dl), albumin (2.8 g/dl) and slight decreases in globulin (3.3 g/dl) and A/G ratio (0.85). There were also increases in total bilirubin (1.5 mg/dl) and D.B (0.57 mg/dl). The elevation in serum AST activity is due to muscle trauma caused by prolonged recumbency caused by theileriosis as explained by omer et al., (2002).

Hussein et al., (2007) showed significant increases in AST, GGT, hypoproteinemia, hypoalbuminemia, and decreased A/G ratio in theileriosis infected cattle. This may be attributed to the harmful effect of *Theileria* toxic metabolites on liver cells.

In this study, in the only free case of Babesiosis, there were high increase in AST (95 IU/L) and ALP (42 IU/L) and decreases

in ALT (16 IU/L) and GGT (11 IU/L).There were also decreases in albumin (2.7 g/dl), globulin (2.4 g/dl), total protein (5.1 g/dl) and no changes in A/G ratio (1.13). The changes in the protein picture caused by *Babesia* sp. could be caused by decreased protein production as a result of deprivation of protein diet resulting from fever accompanied infection also, defected hepatic functions and destructed RBCs and its excretion in urine may play a role (Al-Aboud et al., 2005).

Hussein et al., (2007) and Yeruham et al., (2003) recorded significant increases in AST, GGT, hypoalbuminemia, hypoproteinemia, and decreased A/G ratio in Babesiosis infected cattle.

In anaplasmosis, this study revealed significant ($p<0.05$) increase in AST (85.83 IU/L) and a significant increase in GGT (41.58 IU/L) and non-significant increases in ALT (23.17 IU/L) and ALP (29.08 IU/L) and there were highly significant decrease in Albumin (3.05 g/dl), highly significant increase in globulin (4.28 g/dl), significant increase in total protein (7.34 g/dl) and highly significant decrease in A/G ratio (0.71). Henley and Judith (1985) explained that hypoalbuminemia may occur as a result of decreased protein synthesis capacity of the defected liver and its excretion in urine (albuminuria) in addition to the malnutrition occurs during the disease.

Subramanian, B. et al., (2019) recorded that the mean of total protein, albumin, globulin, albumin: globulin ratio, total bilirubin, direct bilirubin, indirect bilirubin and ALT did not show any significant changes from that of their control values.

The differences between results of researches may be occurred due to geographical differences and distribution of tick vector and differences in the resistance of breeds against infection in different studying areas.

Under the conditions of this study and according to data obtained, it was concluded that bovine theileriosis, babesiosis and anaplasmosis are of the most economically important diseases of bovines. Besides, limiting the movement of cattle between countries.

PCR is the most accurate and beneficial technique for diagnosis of infection till date than stained blood films examination by microscopy. PCR can help in avoiding transmission of infection to original inhabitant animals in the farm.

Present work showed the haematological and biochemical alterations caused by theileriosis, babesiosis and anaplasmosis in affected cows.

Therefore, based on the above conclusions the following recommendations can be proposed:

1- It is recommended that Egyptian management programs should concentrate on implementing surveillance systems to stop and prevent such diseases from becoming established in the country through the application of recent accurate molecular techniques such as PCR especially in asymptomatic carriers that are an important reservoir of infection in most areas of Egypt.

2- Researches should focus on designing new drugs or vaccines having various modes of action, easily applying, effective and with low cost.

3- In the endemic areas, the owners should be learned about the importance of ticks not only as external parasites but also as vectors of diseases and how can they get rid of through application proper drugs or sanitation methods.

4- Continuous and programmed acaricide dipping and spraying should be implemented as a prevention and control strategy in endemic areas by competent authorities.

Conflict of interest statement

The authors declare that they do not have any conflict of interest.

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