EPIDEMIOLOGICAL STUDIES ON BOVINE BABESIOSIS AND THEILERIOSIS IN QALUBIA GOVERNORATE

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A B S T R A C T

The present work was carried out to determine the current epidemiology of bovine Babesiosis and Theileriosis in Qalubia Governorate using blood film examination and PCR technique. 167 animals in different locations in Qalubia governorate including 89 cattle and 78 buffaloes were examined clinically and microscopically. Selected 40 samples from them were examined by PCR for detection of blood parasites. Blood films examination revealed that, the infection rate with Babesiosis were 35.93% (60/167) in cattle and buffaloes, 22.47% (20/89) of cattle and 51.28% (40/78) of buffaloes, while the infection rate of Theileriosis was 11.38% (19/167) in cattle and buffaloes, 14.61% (13/89) of cattle and 7.69% (6/78) of buffaloes. Therefore, the results detected higher prevalence of Babesiosis in buffaloes than in cattle and higher prevalence of Theileriosis in cattle than in buffaloes. Mixed parasitic infection was recorded in 4.79% (8/167) of cattle and buffaloes. 40 Blood samples (20 cattle and 20 buffaloes) were examined by PCR for Babesia infection. The confirmed Babesiosis infections in cattle and buffaloes were 15/20 (75%) and 11/20 (55%) respectively by PCR assay. These results revealed that Babesiosis infection rates among selected cattle were 40% and 75% by microscopic examination and by PCR respectively. While the Babesiosis infection rates among selected buffaloes were 40% and 55% by microscopic examination of blood films and by PCR respectively. Finally, the results in this study revealed that, out of (20) apparently healthy cattle and buffaloes, PCR detect 10/20 (50%) while ME identified only 5/20 (25%) as Babesia infected animals. Out of (20) clinically infected animals, PCR detect 16 (80%) while ME identified only 11 (55%) as Babesia infected animals. In conclusions, the results revealed strong evidence that PCR is much more sensitive than ME either in clinically infected or apparently healthy animals (carriers).

Key Words: Babesia, Theileria, ME (Microscopic Examination), PCR

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1. INTRODUCTION

Nowadays Piroplasmosis is a disease with a world-wide distribution affecting many species of mammals with a major impact on cattle and human. It has increasingly been recognized throughout the world as public health problems (Schorn et al., 2011; Zanet et al., 2014). Babesia spp. and Theileria spp. are protozoan parasites transmitted mainly by ticks and able to infect erythrocytes and/or leukocytes of a wide variety of domestic and wild animals (Duh et al., 2008; Silva et al., 2010). Babesia is the second most common parasite found in the blood of mammals after trypanosomes (Yabsley and Shock, 2013). Babesiosis and Theileriosis cause direct economic losses, such as mortality, reduction in meat and milk yield,

and indirectly through control measures of ticks (Gharbi et al., 2011 ; Shahnawaz et al., 2011).These infections are characterized by anemia, icterus, hemoglobinuria, and death (Wagner et al., 2002 ; Vial and Gorenflot, 2006). Bovine babesiosis is caused by multiple species: *Babesia bigemina, Babesia divergens, Babesia bovis,* and *Babesia major*. Two species, *B. bigemina* and *B. bovis*, have a considerable impact on cattle health and productivity in tropical and subtropical countries (Iseki et al., 2010). Bovine Theileria species cause severe and mild infections in their hosts (Safieldin et al., 2011).Two of them, *T. annulata* and *T. parva*, cause lymphoprolipherative disease with high mortality and morbidity in cattle, commonly known as Tropical Theileriosis and East Coast fever, respectively. In Egypt, large numbers of cattle are infected with subclinical piroplasmosis (Adham et al., 2009). Subclinical Babesiosis and Theileriosis lead to conversion of the affected livestock to chronic carriers and in turn sources of infection for tick vectors, and cause natural transmission of the disease. Therefore, latent infections are the target in the epidemiology of the diseases (Nayel et al., 2012). Early detection of blood parasites is highly beneficial in control. Microscopy using Giemsa stained blood smears has been considered the "gold standard" for detecting Babesia and Theileria organisms in the blood of infected animals, particularly in acute cases, but not in carriers, where the parasitemia is low, with small numbers of the protozoa in the peripheral blood (Bose et al., 1995; Nayel et al., 2012). Polymerase chain reaction (PCR) is more sensitive and specific technique and offers an alternative approach for the detection of Babesiosis. (AbouLaila et al., 2010; Zulfiqar et al., 2012; Shams et al., 2013). So the present study aimed to detect piroplasmosis in animals by microscopy and PCR to detect latent infection.

2. MATERIALS AND METHODS

2.1. Animals:

The present work was carried out on 89 cattle and 78 buffaloes of different ages, sexes and breeds from different localities and different breeding farms in Qalubia Governorate. These animals were examined clinically and laboratory to determine current epidemiological situation of Babesiosis and Theileriosis. Generally, most of the examined animals were suffered from one or more of these complains mainly fever (40-41°C), emaciation, anemia, various degrees of jaundice (icterus) from paleness in mild cases to severe yellow discoloration of conjunctival and vaginal mucous membranes in more progressive cases., haemoglobinuria, accelerated heart and respiratory rates, ocular problems, enlargement of superficial lymph nodes and drop in milk production. History of examined animals revealed that, it were exposed to bad management. Various degrees of tick infestations with different life stages were present around groins, horns, Inter-mandibular space, and ears, while others were apparently healthy without tick infestation (31 cattle and 44 buffaloes).

2.2. Blood samples:

Ninety two blood samples were collected on anticoagulant (sodium salt of EDTA) by using sterile syringe from jugular vein of clinically suspected cases with blood parasites and other 75 blood samples from contact animals (apparently healthy).

2.3. Blood films examination :

Thin blood films were prepared from blood samples, air dried, fixed with absolute methyl alcohol for 15 minutes, and then stained by Giemsa stain 10% for 30 minutes then examined microscopically using oil immersion lens (x1000) of a light microscope according to (Zafar et al., 2006). The parasites were identified according to the characters described by (Soulsby, 1982). The smears were recorded as negative for piroplasms if no parasites

were detected in 50 oil-immersion fields. (According to Moretti et al., 2010).

2.4. Primers : (Bioneer Company, Germany)

Primer	Sequence
Babesia forward	5′-GTC TTG TAA TTG GAA TGA TGG-3′
Babesia reverse	5′-CCA AAG ACT TTG ATT TCT CTC-3′

PCR Product size of Babesia primers was 350 bp.

2.5. Detection of Babesiosis using Polymerase Chain Reaction (PCR):

The DNA samples were tested in 50 µl reaction volume in a PCR tubes. The PCR protocol were carried out according to (Adaszek and Winiarczyk 2008).

3. RESULTS:

3.1. Result of epidemiological aspects of bovine piroplasmosis:

3.1.1. Incidence:

Out of 167 examined animals for piroplasmosis, 60 animals (20 cattle and 40 buffaloes) were found to be infected with Babesiosis representing 35.93 % and 19 animals (13 cattle and six buffaloes) were found to be infected with Theileriosis representing 11.38 %. Ticks were found on 30.38 % (24/79) of the infected animals.

3.1.2. Clinical findings:

Babesiosis

Water buffaloes and cattle which infected with Babesiosis were suffering from marked rise in body temperature, reaching (40-41°c) , loss of appetite, cessation of rumination, labored breathing, various degrees of jaundice (icterus) from paleness in mild cases to severe yellow discoloration There was significant relationship between incidence of infection of blood parasite and breed , also between incidence of infection and age. There was no significant relationship found between blood parasites of conjunctival and vaginal mucous membranes in more progressive cases, as shown in Photo.1., haemoglobinuria, progressive haemolytic anaemia. Accelerated heart and respiratory rates, weakness and a reluctance to move are the symptoms developed especially in more protracted cases**.** The fever during infections in some cases cause abortion to pregnant cattle.

Theileriosis

Clinical signs on the infected animals were pyrexia (40- 41.7 °c), enlar-gement of superficial lymph nodes, as shown in Photo.2., nasal and ocular discharges, salivation, anemia, respiratory distress and eye lesions as shown in Photo.3.

3.1.3. Seasonal incidence:

Table (1) showed that, the peak of infection of Babesiosis in cattle was recorded in summer (33.33%), while autumn season recorded the highest infection rate of Babesiosis in buffaloes (75%). While the peak of infection of tropical Theileriosis in cattle and buffaloes was recorded in spring 40% and 20% respectively. There was no significant relationship between incidence of infection of blood parasite and season.

3.1.4. Age, sex and breed susceptibility:

infection and sex. Analysis of data revealed that, adult animals (36/60) (60%) were more infected by Babesiosis as compared with calves (24/60) (40%). Further analysis

Table.1. Seasonal incidence of Babesiosis and Theileriosis among suspected cattle and buffaloes using blood smears.

Season	Cattle					Buffaloes				
	No.	Babesiosis		Theileriosis		No.	Babesiosis		Theileriosis	
		+ve	%	+ve	%		+ve	%	+ve	%
Winter	22	4	18.18	3	13.64	13	6	46.15	1	7.69
Spring	15	3	20	6	40	20	12	60	4	20
Summer	11	5	33.33	2	18.18	29	10	34.48	0.0	0.0
Autumn	41	8	19.51	2	4.88	16	12	75	1	6.25
Total	89	20	22.47	13	14.61	78	40	51.28	6	7.69

No. = number of examined animals +ve. = Positive. % = Percent

Table.2. Prevalence of Babesiosis and Theileriosis using blood smears in calves and adults of cattle and buffaloes in Qalubia Governorate.

Age		Babesiosis		Theileriosis		Total positive smears
		Cattle	Buffaloes	Cattle	Buffaloes	
Calves	No.	17	7	10	1	35
	%	21.5%	8.9%	12.7%	1.3%	44.30%
Adult	No.	3	33	3	5	44
	%	3.8%	41.8%	3.8%	6.3%	55.69%
Total number		20	40	13	6	79

No. = number of examined animals. % = Percent

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Table.3. Prevalence of Babesia in cattle and buffaloes by microscopy and PCR.

Species	Cattle (N=20)		Buffaloes (N=20)	
	Blood smear (%)	PCR (%)	Blood smear (%)	PCR (%)
Babesia.sp	40%	75%	40%	55%

Table.4. Results of PCR assay and microscopical examination (ME) for detection of Babesia from clinically infected and apparently healthy cattle and water buffaloes from Qalubia Province of Egypt.

Species	Total examined number	PCR/ME +/+	PCR/ME +/-	PCR/ME -/+	PCR/ME -/-	Total
cattle (N = 20)	Clinically infected (10)	6	3	0	1	10
	Apparently healthy (10)	2	4	1	3	10
Water buffaloes (N = 20)	Clinically infected (10)	5	2	0	3	10
	Apparently healthy (10)	3	1	2	4	10
Total		16	10	3	11	40

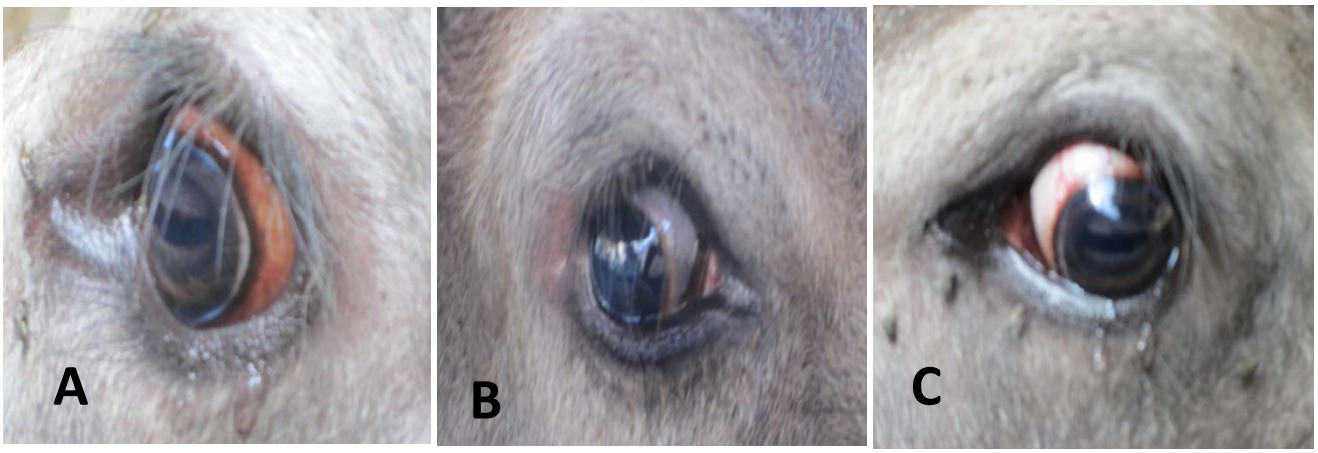

Photo.1. Showing different clinical signs in a buffaloes suspect to be affected with blood parasites. (A) Congestion of the conjunctival mucous membrane. (B) Severe lacrimation. (C) Paleness of the conjunctival mucous membrane.

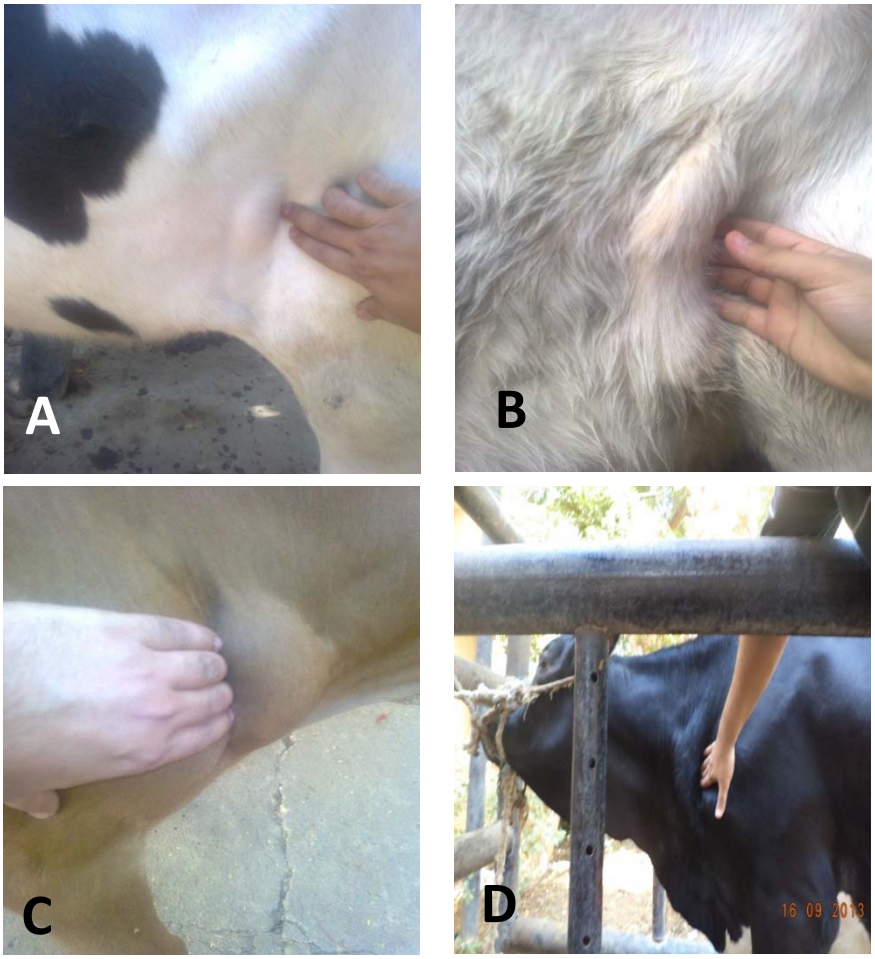

Photo.2. Showing (A) enlarged prefemoral lymph node in 3 years mixed breed female cattle. (B) Enlarged prefemoral lymph node of 1.5 years mixed breed male cattle. (C) Enlarged prescapular lymph node of 4 month native breed male calf. (D) Enlarged prescapular lymph node of 4.5 years female cattle

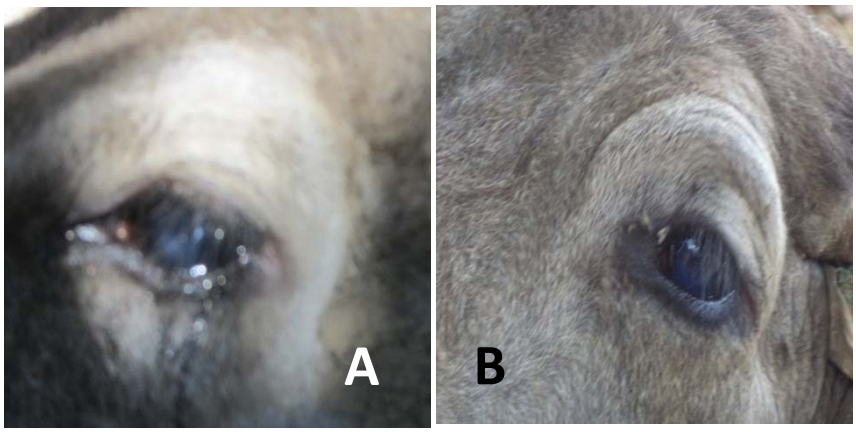

Photo. 3. Showing eye lesions in a buffaloes suspect to be affected with blood parasites. (A) Corneal opacity with severe lacrimation. (B) Corneal opacity

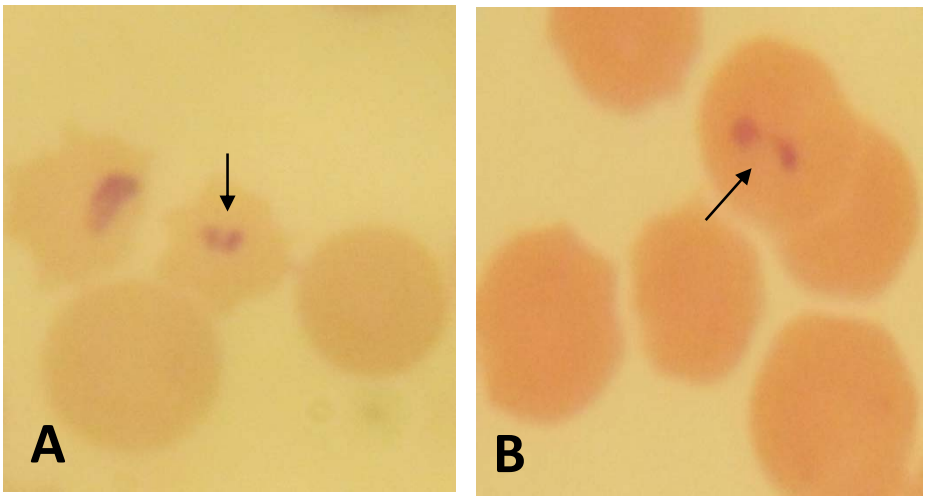

Photo.4. Leishman stained microplate showing characteristics of *Babesia* infection. Thin arrows shows *Babesia* sp. inside the bovine erythrocytes: *B.bigemina* having paired structure at an acute angel to each other. *B.bovis* having paired form at an obtuse angel to each other.

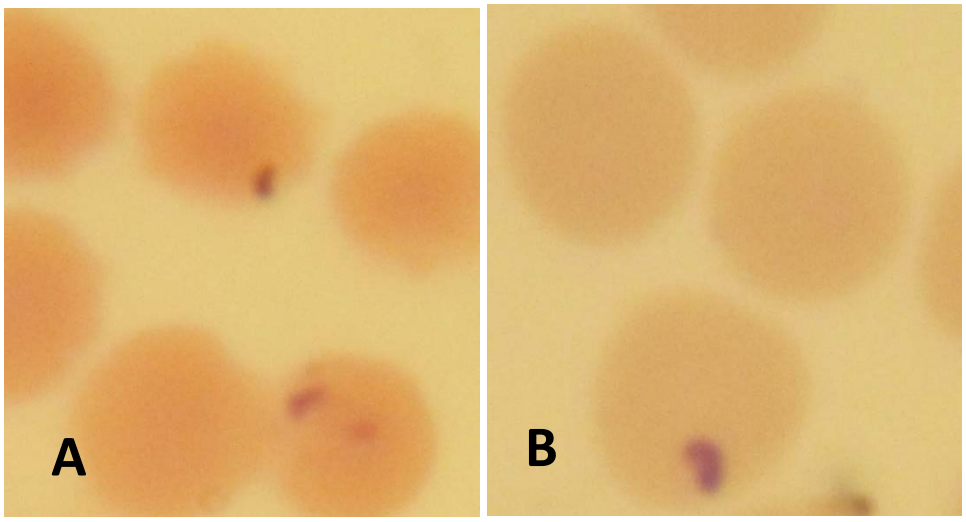

Photo.5. Showing: Theileria protozoan parasite of different shapes inside red blood cells in Leishman-stained blood smear.

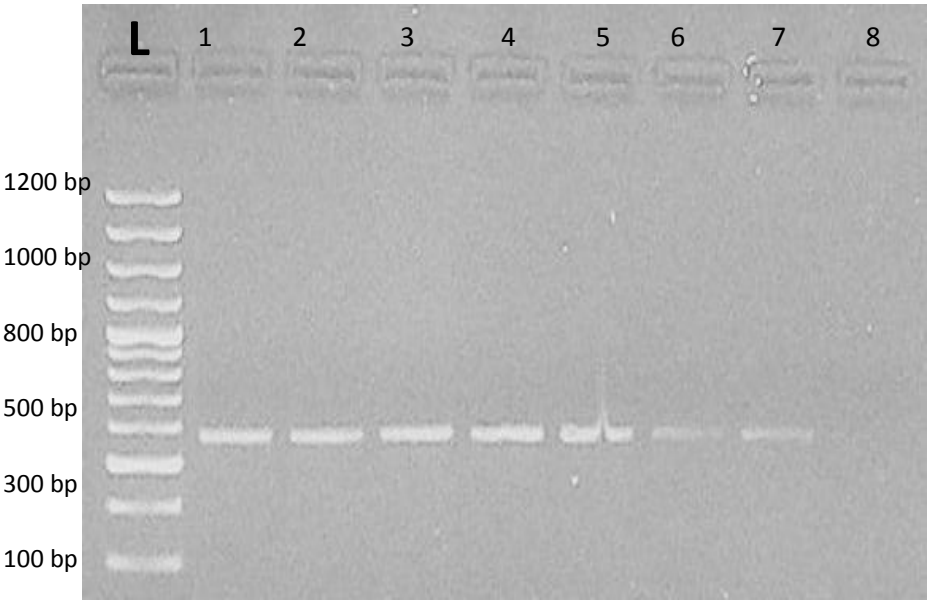

Photo.6. PCR result showing positive pool samples on gel at 350pb for *Babesia*. Lane L: 100-1200 bp DNA Ladder, Lane (1, 2, 3, 4, 5, 6, 7): Positive PCR product amplified from field blood samples, Lane (8): Negative PCR product samples.

showed that, the calves of cattle (17/60) (28.33%) were more prone to Babesiosis than their adults (3/60) (5%). While calves (11/19) (57.89%) were more infected by Theileriosis as compared with adult animals (8/19) (42.11%). Further analysis showed that, the adult buffaloes (5/19) (26.32%) were more prone to Theileriosis than their calves (1/19) (5.26%)**.** As shown in Table.2.

3.2. Results of blood film examination:

Parasitological examination of (167) randomly selected cattle and buffaloe, by direct microscopy using Giemsa and/or Leishman-stained thin blood films, revealed that (79) had intra-erythrocytic stages of piroplasms of both Babesia and Theileria spp. with an overall prevalence of (47.30 %). Among those, 60 (35.93 %) were infected by Babesia sp., 19 (11.38 %) had Theileria sp., and eight cases (4.79%) showed mixed infection. *B. bigemina* is larger in size and having paired structure at an acute angel to each other while *B. bovis* is smaller in size and having paired forming an obtuse angel to each other Photo.4. Giemsa and/or Leishman-stained blood smears from piroplasm infected animals showed intra-erythrocytic piroplasms of Theileria that were in the form of comma, oval and round shape, as shown in Photo.5.

3.3. Results of molecular diagnosis (PCR):

Regardless the presence or absence of clinical signs of Babesiosis, PCR revealed that, the prevalence of Babesiosis in cattle 15/20 (75%) was higher than the prevalence in buffaloes 11/20 (55%). Moreover, the prevalence in cattle with clinical signs 9/10 (90%) was higher than the prevalence in buffaloes with clinical signs 7/10 (70%). Out of (20) apparently healthy cattle and buffaloes, PCR detect 10/20 (50%) while ME identified only 5/20 (25%) as Babesia infected animals. Out of (20) clinically infected animals, PCR detect 16 (80%) while ME identified only 11 (55%) as Babesia infected animals, as shown in Table.3., Table.4. & photo.6.

4. DISCUSSION:

Regarding the incidence of Babesiosis, out of 167animals examined for piroplasmosis in Qalubia Governorate from March 2013 to April 2014, 60 animals (20 cattle and 40 buffaloes) were found to be infected with Babesiosis representing 35.93 %. This

result was nearly similar to the results obtained by EL-Seify, (1989) who recorded in Beni-Suef governorate that, 31.7% of cattle were infected with *B. bigemina.* For the incidence of Theileriosis, out of 167animals examined for piroplasmosis, 19 animals (13 cattle and six buffaloes) were found to be infected by Theileriosis representing 11.38 %. Similar results were obtained by Adel, (2007) who reported that, 11.31% of farm animals were infected with *T. annulata* in Gharbia governorate, Also Abu El-Magd, (1980) reported that, 11.1% of animals were infected with *T. annulata* in Quena governorate and Salem et al., (1993) reported that, 10% of imported cattle and 8.75% of native cattle in Quena, were infected with *T.annulata* respectively. On other hand these results were disagreed with results of Gamal EI-Dien, (1993) who recorded in EI-Behera Province the incidence of *T. annulata* was 65.4%.It was notable that water buffaloes identified as Babesia-infected showed a milder form (less severe) of clinical signs in comparison to the clinical signs appeared on Babesia-infected cattle. This result was in agreement with that reported previously by (Mahmmod, 2014).

Respecting to the seasonal incidence of piroplasmosis, the seasonal prevalence of Babesiosis and Theileriosis using blood smears examination showed that, the peak of infection of Babesiosis in cattle was recorded in summer (33.33%), while autumn season recorded the highest infection rate of Babesiosis in buffaloes (75%). These results were similar with results of El-Sawalhy, (1987) who recorded that, the highest infection rate of Babesiosis was recorded in both summer and autumn and was less in spring and low in winter in both cows and buffaloes. The seasonal prevalence in this study also revealed that, the peak of infection of Tropical Theileriosis in cattle and buffaloes was recorded in spring season 40% and 20% respectively. However its maximum prevalence in cattle were in spring 6/15 (40%) then in summer 2/11 (18.18%). This in accordance with observations that obtained by Qayyum et al., (2010) who recorded highest incidence of Theileriosis during spring to late summer. Where the bi-monthly monitoring revealed that the incidence of the disease was highest during June-July (59%) followed by May-April (31%) and August-September 25%). While these results were in contrast to that of El Mentenawy, (2000) who reported that Theileriosis prevalence reached a maximum (84.3%) in both autumn and summer seasons.

Analysis of data revealed that, adult animals (36/60) (60%) were more infected by Babesiosis as compared with calves (24/60) (40%). Further analysis showed that, the calves of cattle (17/60) (28.33%) were more prone to Babesiosis than their adults (3/60) (5%). This in accordance with Ruprah, (1985) and Roy et al., (2004) who reported highest prevalence in animals aged more than 3 years followed by the lowest prevalence in less than one year age group. Also These results agreed with that obtained by Gattas, (1990) who found that incidence of *B. bigemina* among buffaloes were to be lower in young animals than in older ones. On the other hands, these results were differ with Zulfiqar et al., (2012) who reported that, calves (28%) were more infected by *B. bovis* as compared with adult animals (15%) and further analysis showed that the calves of buffaloes (50%) were more prone to Babesiosis than their adults (0%).

In this study, calves (11/19) (57.89%) were more infected by Theileriosis as compared with adult animals (8/19) (42.11%). Further analysis showed that the adult of buffaloes (5/19) (26.32%) were more prone to Theileriosis than their calves (1/19) (5.26%). These results were disagreed with Abdel- Kader, (1991) who reported that the susceptibility of clinical theileriosis was low among calves of age less than one year old and increased in age of 1-3 years old.

In the present study, the results obtained from microscopical examin-ation of blood smears revealed that, parasitological

examination of (167) selected cattle and buffaloes, by direct microscopy using Giemsa and/or Leishman-stained thin blood films, revealed that (79) had intra-erythrocytic stages of piroplasms of both Babesia and Theileria spp. with an overall prevalence of (47.30 %). Among those, 60 (35.93 %) were infected by Babesia sp., 19 (11.38 %) had Theileria sp. This result nearly similar to the results obtained by EL-Seify, (1989) in Beni-Suef governorate, as 31.7% of cattle were infected with *B. bigemina*. In addition, these results coincided with results of Adel, (2007) and Abu El-Magd, (1980) who reported that, 11.31% and 11.1% of farm animals were infected with *T. annulata* in Gharbia and Quena governorates respectively. The present investigation showed that mixed infection, using Giemsa-stained blood smears of Babesia and Theileria spp. appeared in eight (4.8%) cases. These results coincided with those obtained by Salama, (2009) and Nayel et al., (2012) who reported that, 11 (2.72 %) of 405 examined cattle showed mixed infection. *B. bigemina* is larger in size and having paired structure at an acute angel to each other. *B. bovis* is smaller in size and having paired form at an obtuse angel to each other. However, it is hard to differentiate the two species by microscopic examination. These results were coincided with Chaudhry et al., (2010) and Shams et al., (2013). Intra-erythrocytic stages of *Theileria* appear as comma, oval and round shape. This results agreed with (Radostitis et al., 2007; Salama, 2009).

With respect to the results of molecular diagnosis, for a more accurate detection of animal infection with Babesia, (40) samples (20 cattle and 20 buffaloes) were chosen for polymerase chain reaction (PCR) assay. Regardless the presence or absence of clinical signs of Babesiosis, PCR was more sensitive in the detection of Babesia with 75% and 55% in cattle and buffaloes respectively, while Microscopic Examination (ME) identified only 40 % of Babesia infection in cattle and 40% in buffaloes. This result nearly similar to the results obtained by (Salem, 1998) who recorded that, the incidence of *B. bigemina* in Giza governorate was 40.9%, while the incidence of *B. bovis* were 30% as measured by PCR technique. While this result was disagreed with Salama, (2009) and Nayel et al., (2012) who revealed that 54 (19%) were positive using direct smear and 62 (22.5%) were positive using PCR. These results were also in contradicting with the findings obtained by Rania, (2009) who mentioned that, rate of infection for *Babesia* was 25.33% and Liu et al., (2014) who revealed that 7.3% (19/260) and 5.8% (15/260) of cattle were positive for *B. bigemina* and *B. bovis*. Out of (20) apparently healthy cattle and buffaloes, PCR detect 10/20 (50%) while ME identified only 5/20 (25%) as Babesia infected animals. Out of (20) clinically infected animals, PCR detect 16 (80%) while ME identified only 11 (55%) as Babesia infected animals. This finding is strong evidence that PCR is much more sensitive than ME either in clinically infected or apparently healthy animals (carriers) corroborating previous observations (Oliveira-Sequeira et al., 2005; Brito et al., 2010 ; Brito et al., 2013).

In conclusions, microscopic examination is not suitable for detecting the carrier or chronic phases of piroplasmosis. However, it remains the most rapid confirmatory method for detecting this infection in acute phase of the disease. While PCR turned out to be a sensitive and accurate method for diagnosis of Babesiosis in animals in the early phase of infection and in carrier animals by DNA amplification.

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دراسات وبائية لمرض البابيزيوزيس والثيليريوزيس للأبقار والجاموس فى محافظة القليوبية

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الملخص العربي

تم إجراء هذه الدراسة بمحافظة القليوبية لمعرفة نسبة الإصابة بمرضى البابيزيوزيس والثيليريوزيس في الأبقار والجاموس. أجريت هذه الدراسة على عدد167حيوان (89 من الأبقار و78 من الجاموس) من مناطق مختلفة بمحافظة القليوبية وذلك بفحص مسحات الدم المختلفة بالميكروسكوب الضوئي واختيار عينات محددة للفحص بواسطة اختبار تفاعل البلمرة المتسلسل . وقد أظهرت هذه الدراسة بفحص شرائح الدم أن نسبة الإصابة بمرض البابيزيوزيس 35.93٪ (167/60) في الأبقار والجاموس، في حين أن نسبة الإصابة في الأبقار بمفردها كانت 22.47٪ (20/ 89) والجاموس كانت 51.28٪(78/40) بينما كانت نسبة الإصابة بمرض الثيليريوزيس في الأبقار والجاموس كانت 11.38٪ (167/19) أما في الأبقار كانت 14.61٪ (13/ 89) وفي الجاموس كانت 7.69 ٪ (6 /78). لذلك كانت معدلات الإصابة بالبابيزيوزيس أعلى في الجاموس عنها في الأبقار وكانت معدلات الإصابة بالثيليريوزيس أعلى في الأبقار عنها في الجاموس وكانت نسبة الإصابة المختلطة في الأبقار والجاموس معا 4.79 ٪ (8/ 167). كما تم استخدام اختبار تفاعل البلمرة المتسلسل على عدد 40 حاله (20 من الأبقار و20 من الجاموس) وتم التعرف على وجود البابيزيا في دم الأبقار والجاموس بنسبة 75 ٪، 55٪ على التوالي. وتبين أن نسبة الإصابة بمرض البابيزيوزيس في الأبقار40٪ بفحص شرائح الدم وكانت75 ٪باستخدام اختبار تفاعل البلمرة المتسلسل. بينما كانت نسبة الإصابة بمرض البابيزيوزيس في الجاموس 40٪ بفحص شرائح الدم وكانت 55٪ باستخدام اختبار تفاعل البلمرة المتسلسل. وقد أظهرت النتائج أن نسبة الإصابة بمرض البابيزيوزيس في الأبقار والجاموس السليمة ظاهريا والحاملة للمرض بفحص شرائح الدم 25٪ وكانت50٪باستخدام اختبار تفاعل البلمرة المتسلسل. بينما كانت نسبة الإصابة بمرض البابيزيوزيس في الأبقار والجاموس ذات الاعراض المرضية المميزة للإصابة بالمرض بفحص شرائح الدم 55 ٪ وكانت 80٪ باستخدام اختبار تفاعل البلمرة المتسلسل. أوضحت هذه الدراسة أن استخدام اختبار تفاعل البلمرة المتسلسل أكثر حساسية من فحص شرائح الدم سواء في الحالات ذات الاعراض المرضية أو في الحالات السليمة ظاهريا (العدوى الكامنة أو العدوى المزمنة).

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