Results

The present study was carried out on:

1- Fifty five native breed cattle suspected to be infected with babesiosis (presence of ticks on the cattle or in the surrounding environment, history of bloody urine, fever and icterus); their ages were from 2 to 4 years. Twenty were males and thirty five were females. Five controls –ve cattle were selected to be free from external and blood parasites on their blood films as well as free from circulating antibodies against babesiosis by indirect fluorescent antibody test (IFAT).

2- Eighty human cases which were in contact with studied cattle, their ages were from 17 to 65 years. Sixty eight were males and twelve were females. Ten control –ve human cases were selected with no history of contact with cattle, ticks bite or blood transfusion and free from blood parasites on their blood films as well as free from circulating antibabesial antibodies by IFAT.

3- Eighty engorged female ticks which were collected either from the studied cattle or the surrounding environment.

Results of the present study on animal group (cattle):

(a) Clinical manifestations in infected cattle:

The clinical signs detected in suspected cattle with ***B.bigemina*** infection, as recorded by the help of veterinarians, included fever (39.1-39.8 ^{o}C), anorexia, depression, weakness, rough hair coat, pale mucous membranes, haemoglobinuria, accelerated respiratory and heart rates and decreased body weight gain.

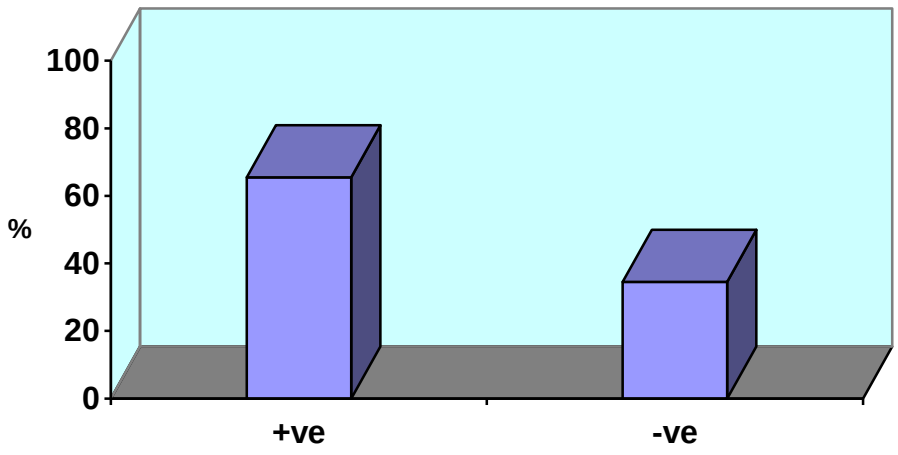
(b) Results of the blood film examination of the studied cattle:

Two thin blood film smears stained with Giemsa stain were examined for every case under oil immersion lens in cross-sectional method. Among 55 examined cattle, 36 cattle (65.5%) were positive for ***B.bigemina*** infection by blood film examination with parasitemia percentage ranging from 0.5% to 2% and 19 cattle (34.5%) were negative (table 1and figure 1).

Table (1): Infection rate of *B.bigemina* detected by blood film examination among studied cattle.

Cases / Test	+ve		-ve	
	No	**%**	**No**	**%**
Blood film	36	65.5	19	34.5

Figure (1) Infection rate of Babesia bigemina detected by blood film examination among studied cattle



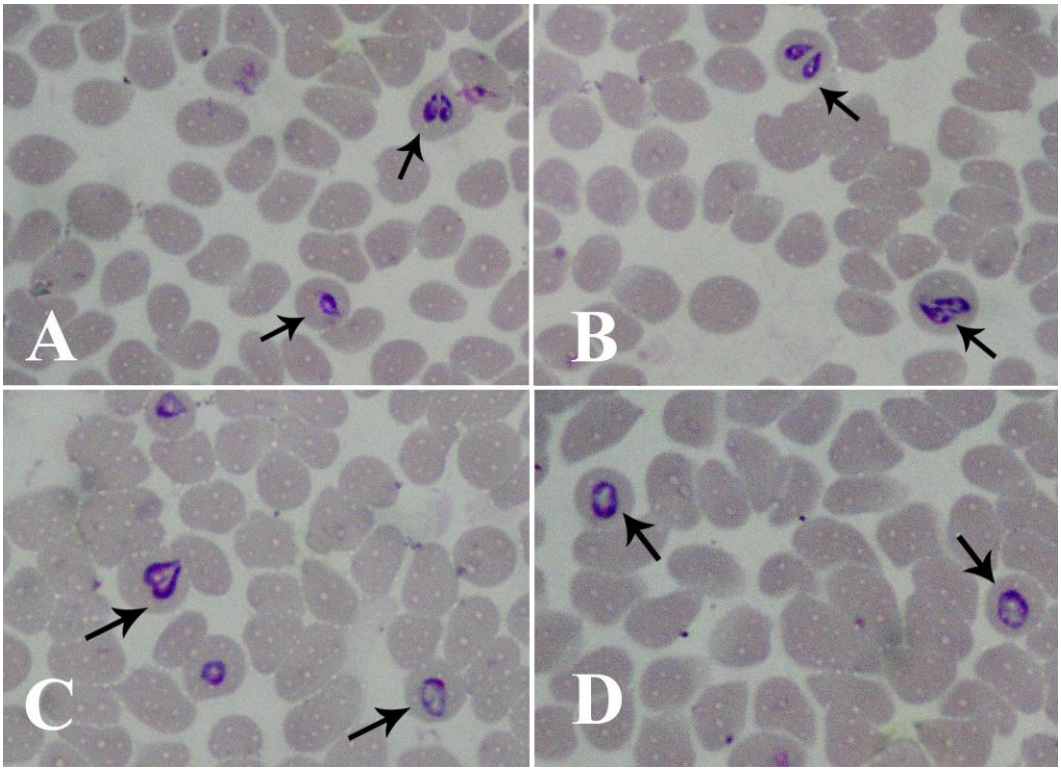


Figure (2) Giemsa-stained thin blood smears of *B.bigemina* parasites in cattle erythrocytes (×1000**) (A,B)** single and double pear shaped trophozoites**. (C,D)** Dividing forms of ***B.bigemina*** trophozoites.

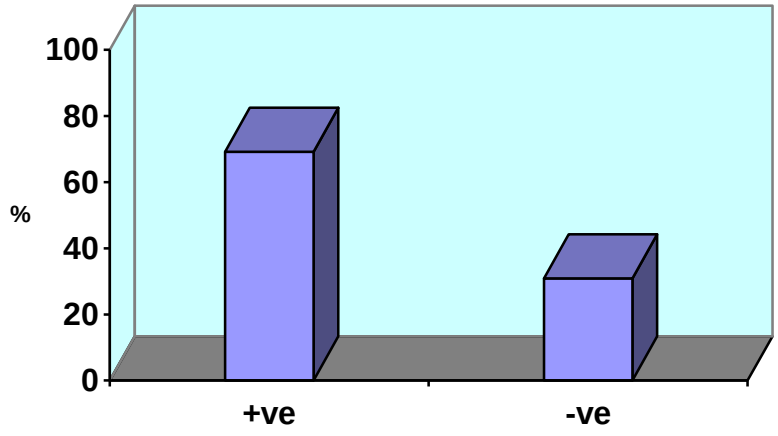
(c) Results of the indirect fluorescent antibody test (IFAT) for IgG among the studied cattle:

Tested cattle serum samples were diluted to be 1/40 for screening of positivity for ***B.bigemina*** infection. Appearance of apple green fluorescing parasites was considered positive reaction. The results clarified that among 55 examined cattle, 38 cattle (69.1%) were positive and 17 cattle (30.9%) were negative for ***B.bigemina*** IgG by IFAT (table 2 and figure 3).

Table (2): Infection rate of *B.bigemina* detected by IFAT (IgG) among studied cattle.

Cases / Test	+ve		-ve	
	No	%	No	%
IFAT (IgG)	38	69.1	17	30.9

Figure (3) Infection rate of B.bigemina detected by IFAT (IgG) among studied cattle



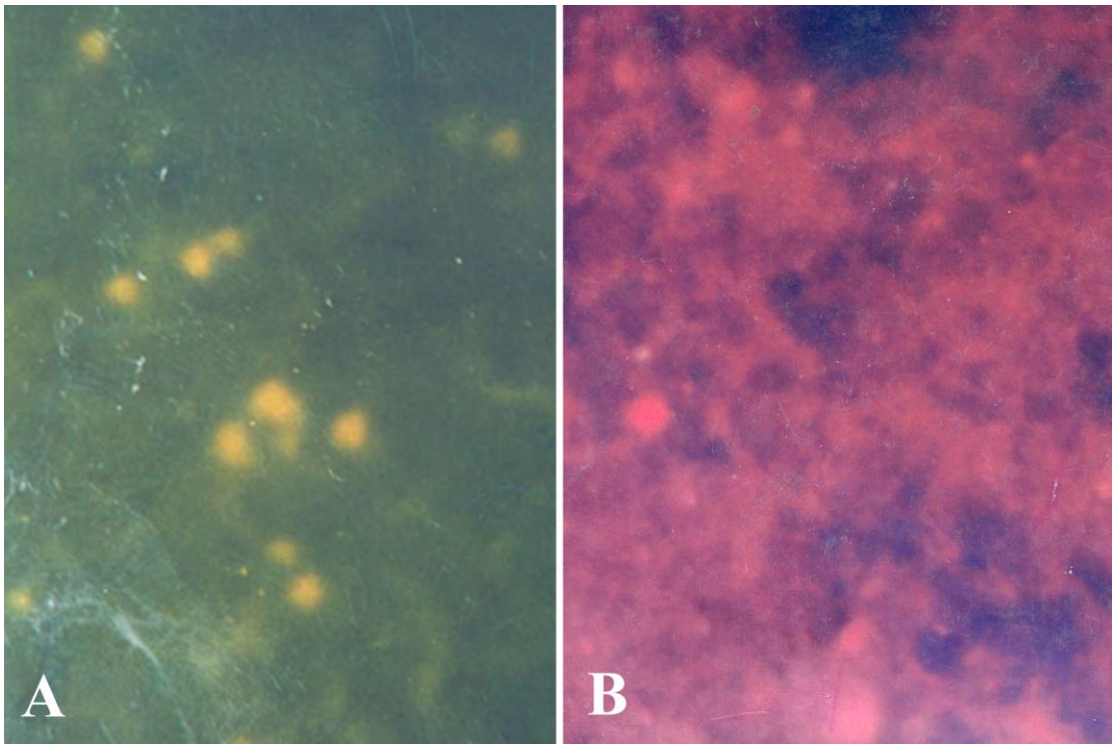


Figure (4) A- Positive indirect fluorescence reaction of B.bigemina piroplasmic antigen with cattle sera. B- Negative indirect fluorescence reaction of B.bigemina piroplasmic antigen with cattle sera.

Table (3): Distribution of Babesia bigemina positive and negative cases among the studied cattle by direct and indirect techniques.

Test / Cases	Blood film only		IFAT (IgG) only		Both blood film & IFAT (IgG)		Total n. = 55	
	No.	%	No.	%	No.	%	No.	%
+ ve	2	3.6	4	7.3	34	61.8	40	72.7
- ve	19	34.5	17	30.9	15	27.3	15	27.3

Table (3) and figure (5) show that out of 55 cattle examined, two cattle (3.6%) were positive only by Giemsa stained thin blood film examination, four cattle (7.3%) were positive only by IFAT for ***B.bigemina*** IgG and thirty four cattle (61.8 %) were positive by both blood film examination and IFAT for IgG for ***B.bigemina*** infection. The total number of ***Babesia bigemina*** infected cattle was 40 cattle and the infection rate was 72.7 % .

Figure (5) Distribution of Babesia bigemina positive and negative cases among studied cattle by direct and indirect techniques.

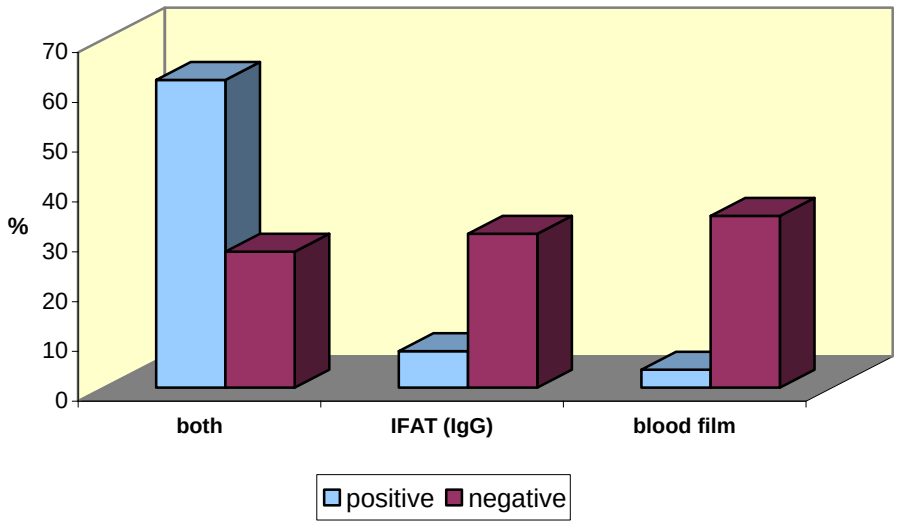
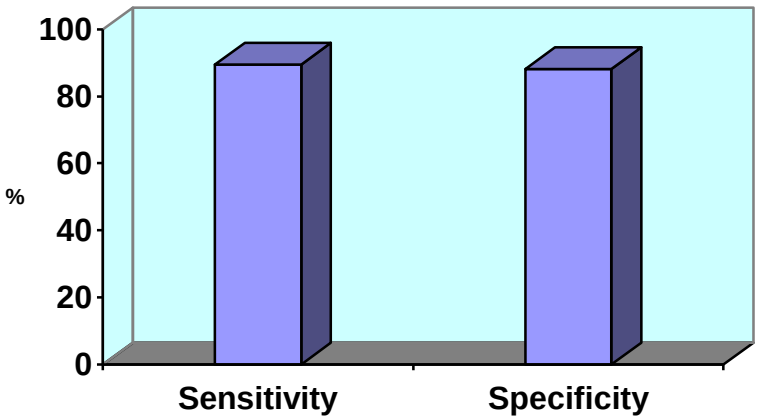


Table (4): Sensitivity and specificity of the IFAT (IgG) for the studied cattle.

Serology test / Blood film	+ve	-ve	Total
	No.	No.	No.
+ ve	34	2	36
- ve	4	15	19
Total	38	17	55
sensitivity	89.5%		
specificity	88.2%.		

Table (4) and figure (6) show that, the sensitivity of the IFAT (IgG) for the studied cattle is 89.5% and the specificity of this test is 88.2%.

Figure (6) Sensitivity and specificity of the IFAT (IgG) for the studied animals.



Results of the present study on human group:

(a) Clinical manifestations recorded in infected human cases:

Out of 80 human subjects under the present study, nine ***Babesia*** infected human cases were recorded either by blood film examination or IFAT for ***B.bigemina*** IgG. Three cases of them were symptomatic. Symptoms included fatigue, anorexia, malaise and headache. Case no. 3 was a male patient, 62 years old, working at a cattle ranch suffered from fatigue, anorexia and headache. Case no. 4 was a male patient, 47 years old, farmer suffered from fatigue and malaise. Case no. 6 was a male patient, 37 years old, employee but share in the work at his family's cattle ranch, suffered from fatigue, malaise and headache. The nine cases gave no past history of tick bite, splenectomy or blood transfusion.

Table (5): Clinical and laboratory findings in *Babesia bigemina* infected human cases.

Case number	AGE (years)	Sex	Symptoms	Tick bite	Spleen-ectomy	Blood trans-fusion	Blood film	IFAT (IgG) titre
1	44	Male	asymptomatic	_	_	_	+	_
2	35	Male	asymptomatic	_	_	_	+	_
3	62	Male	Fatigue, anorexia, headache	_	_	_	+	1/32
4	47	Male	Fatigue, malaise	_	_	_	+	_
5	21	Male	asymptomatic	_	_	_	_	1/64
6	37	Male	Fatigue, malaise, headache	_	_	_	+	1/64
7	50	Female	asymptomatic	_	_	_	+	_
8	55	Female	asymptomatic	_	_	_	+	1/32
9	59	Male	asymptomatic	_	_	_	+	_

(b) Results of Complete Blood Count for studied human cases:

C.B.C was done for all human cases under study on Cell counter and Sysmex apparatus (Technowave) and thin blood film smears stained by leishman's stain were examined for differential leucocytic count. The results are shown in tables (6), (7) and figures (7-12).

Table (6): Results of Erythrogram and platelets variables in Babesia positive versus negative human cases.

Studied human group / variables	**-ve (n. = 71) Means ± SD**	**+ ve (n. = 9) Means ± SD**	**t**	**p**
HB (gm %)	13.8 ± 1.68	14 ± 1.55	0.36	> 0.05
RBCs (× 10^6 /µL)	5.12 ± 0.98	5.24 ± 0.67	0.48	> 0.05
HCT (%)	42.39 ± 6.1	41.58 ± 5.4	0.39	> 0.05
MCV (fl)	78.11 ± 10.2	78.33 ± 4.4	0.11	> 0.05
MCH (pg)	27.22 ± 3.8	26.23 ± 2.96	0.91	> 0.05
MCHC (%)	34.09 ± 2.37	33.68 ± 2.68	0.44	> 0.05
Platelets (×10^3/ µL)	250.9 ± 44.11	245.6 ± 29.22	0.48	> 0.05

Table (6) and figure (7-10) show that no significant difference was found between infected (**+ ve**) and noninfected (-ve) human cases as regards erythrogram and platelet variables.

Figure(7) Means of HB of Babesia positive and negative cases in human group

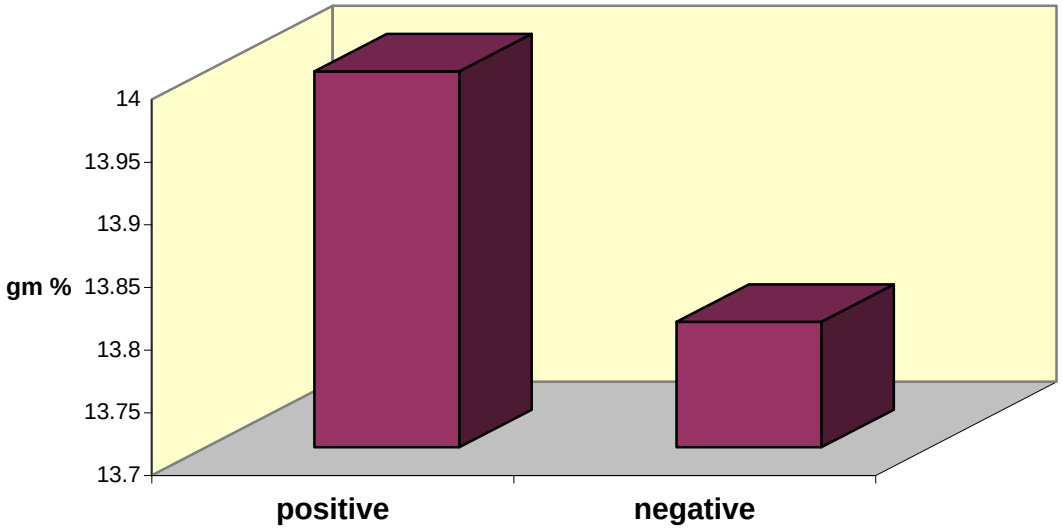


Figure (8) Means of RBCs of *Babesia* positive and negative cases in human group

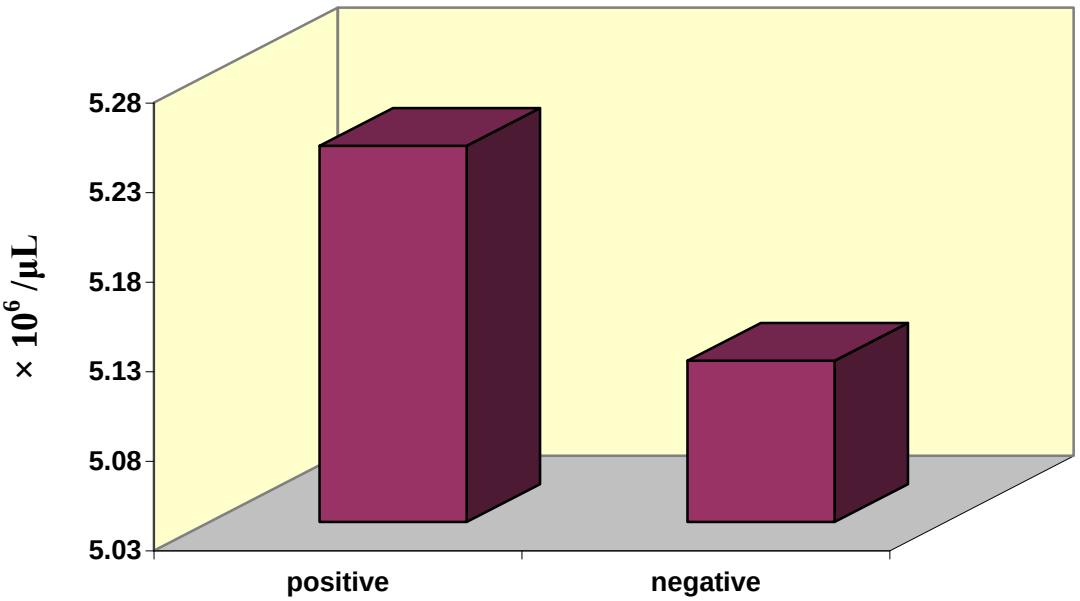


Figure (9) Means of HCT and MCHC of *Babesia* positive and negative cases in human group

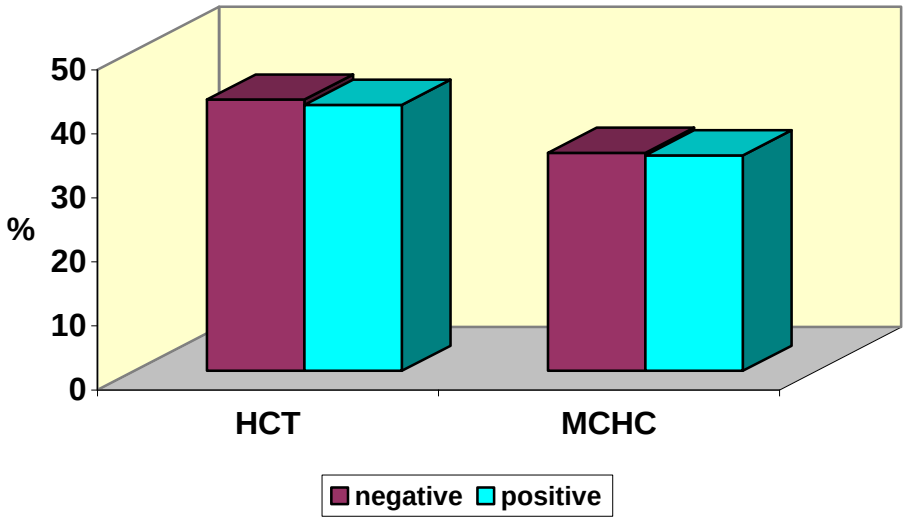


Figure (10) Means of platelets of *Babesia* positive and negative cases in human group

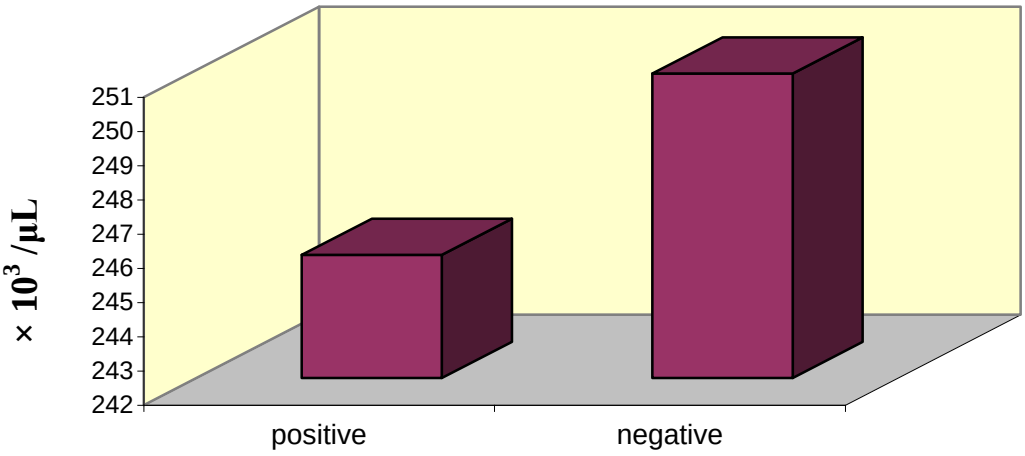


Table (7): Results of leucogram variables in *Babesia* positive versus negative human cases.

Studied human group / Leucogram variables		-ve (n. = 71) Means ± SD	+ ve (n. = 9) Means ± SD	t	p
WBCs (x 10^3 μL)		7.37 ± 2.05	7.38 ± 1.31	0.02	> 0.05
Relative differential leucocytic count (%)	Neutrophiles	57.15 ± 10.78	49.89 ± 10.96	1.88	> 0.05
	Lymphocytes	38.24 ± 10.99	45.89 ± 10.88	1.98	> 0.05
	Monocytes	1.54 ± 0.88	1 ± 1	1.55	> 0.05
	Esinophiles	2.24 ± 1.74	3.11 ± 2.03	1.23	> 0.05
	Basophiles	0.23 ± 0.42	0.11 ± 0.33	0.99	> 0.05

Table (7) and figure (11-12) show that no significant difference was found between infected (**+ ve**) and noninfected (-ve) human cases as regards leucogram variables.

Figure (11) Means of WBCs of Babesia positive and negative cases in human group

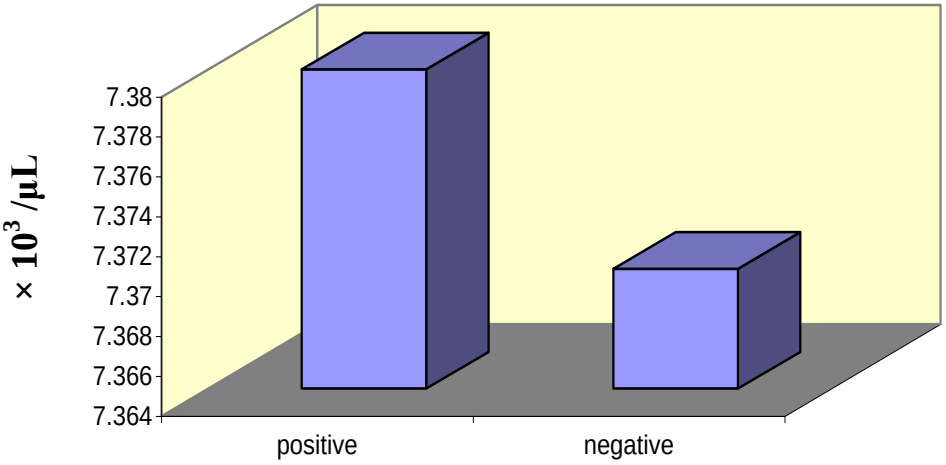
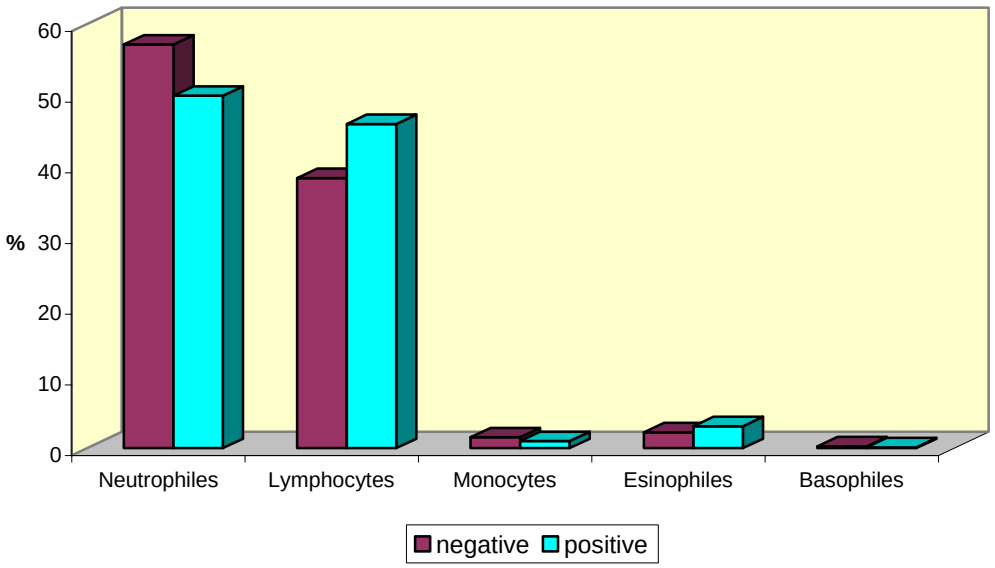


Figure (12) Leucogram of *Babesia* positive and negative cases in human group



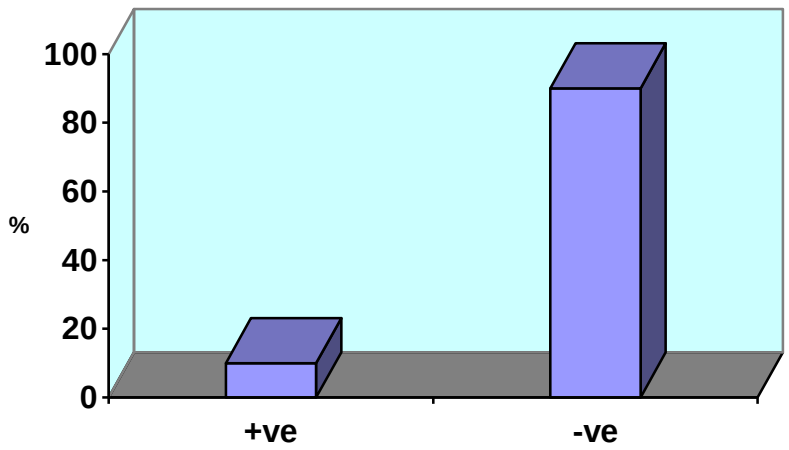
(c) Results of the blood film examination of the studied human cases:

Four thin blood film smears stained with Giemsa stain were examined for every case under oil immersion lens in cross=sectional method. Among 80 examined human cases, 8 cases (10%) were positive for ***Babesia*** infection with parasitemia percentage not more than 0.1% and 72 cases (90%) were negative.

Table (8): Infection rate of *Babesia* detected by blood film examination among studied human cases.

Cases / Test	+ve		-ve	
	No	**%**	**No**	**%**
Blood film	8	10	72	90

Figure (13) Infection rate of Babesia detected by blood film examination among studied human cases



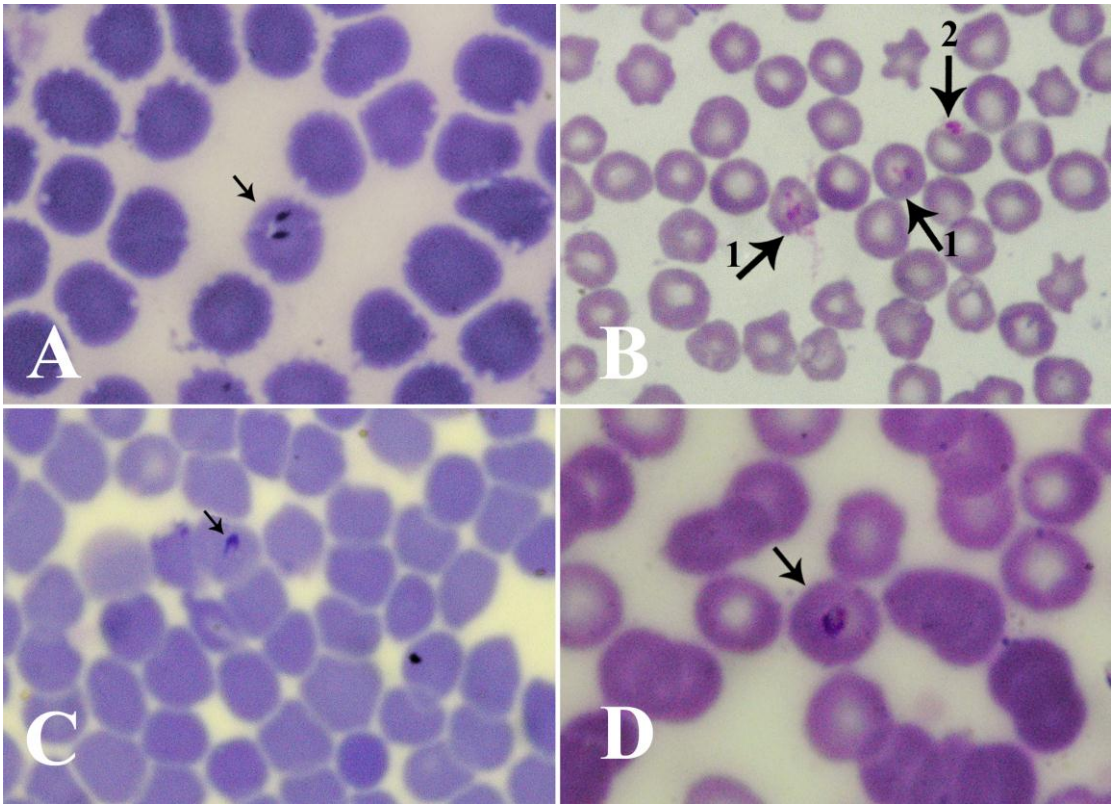


Figure (14): Giemsa-stained thin blood smears of pyriform parasites of *Babesia* species without pigment in human erythrocytes (×1000**) (A)** Double pear shaped trophozoites. **(B) 1-** single pear shaped trophozoites and **2-** exo-erythrocytic merozoite **(C,D)** single pear shaped trophozoites of ***Babesia*** parasites.

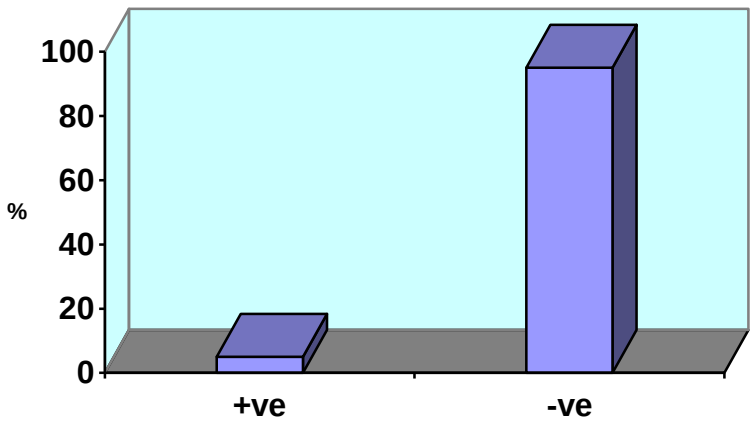
(c) Results of the indirect fluorescent antibody test (IFAT) for IgG among the studied human cases:

Tested human sera were double folds diluted beginning from 1: 16 to 1: 160 for screening of positivity for ***B.bigemina*** infection, cut off value was ≥ 1: 32. Appearance of apple green fluorescing parasites was considered positive reaction. The results clarified that among 80 examined human cases, 4 cases (5%) were positive and 76 cases (95%) were negative for ***B.bigemina*** (IgG) by IFAT.

Table (9): Infection rate of *B.bigemina* detected by IFAT (IgG) among studied human cases.

Cases / Test	+ve		-ve	
	No	%	No	%
IFAT (IgG)	4	5	76	95

Figure (15) Infection rate of B.bigemina detected by IFAT (IgG) among studied human cases



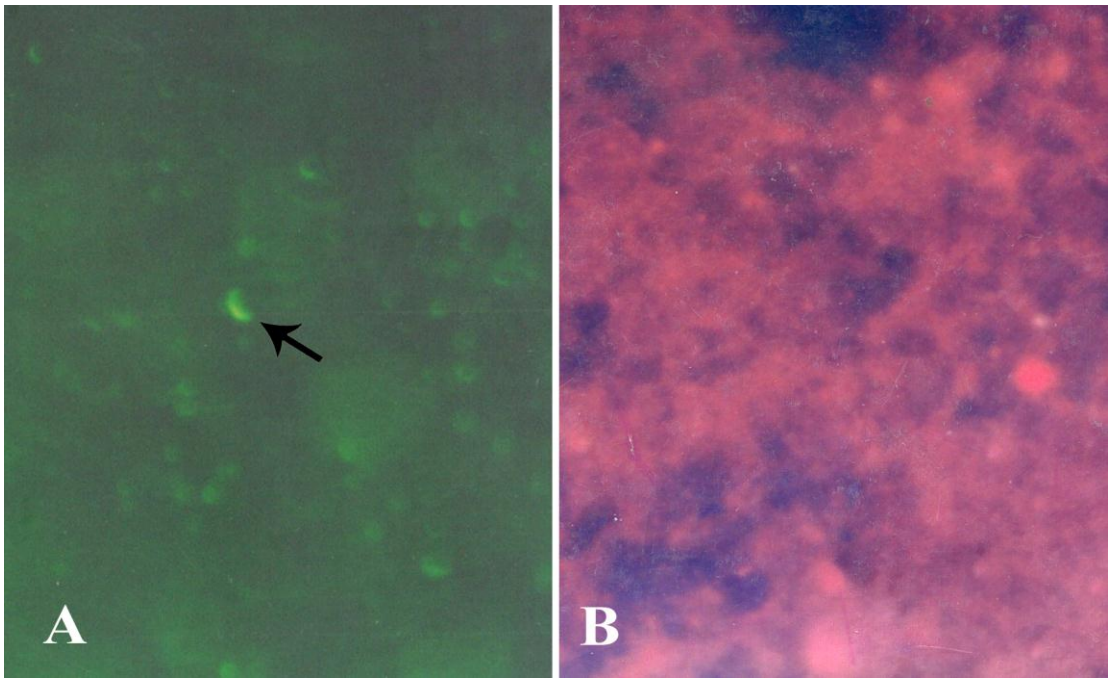


Figure (16): A- Positive indirect fluorescence reaction of B.bigemina piroplasmic antigen with human sera. B- Negative indirect fluorescence reaction of B.bigemina piroplasmic antigen with human sera.

Table (10): ***Distribution of Babesia bigemina positive and negative cases among*** **studied human cases** ***by direct and indirect techniques*****.**

Test / Cases	Blood film only		IFAT (IgG) only		Both blood film & IFAT (IgG)		Total	
	No	%	No	%	No	%	No	%
+ ve	5	6.3	1	1.3	3	3.8	9	11.3
- ve	72	90.0	76	95	71	88.8	71	88.8

Table (10) and figure (17) show that out of 80 examined human cases, five cases (6.3 %.) were positive only by Giemsa stained thin blood film examination for ***Babesia*** infection, one case (1.3 %) was positive only by IFAT for ***B. bigemina*** IgG and three cases (3.8 %.) were positive by both blood film and IFAT for ***B. bigemina*** IgG. The total number of ***Babesia bigemina*** infected human cases was 9 cases and the infection rate was 11.25%.

Figure (17) Distribution of Babesia bigemina positive and negative cases among studied human cases by direct and indirect techniques

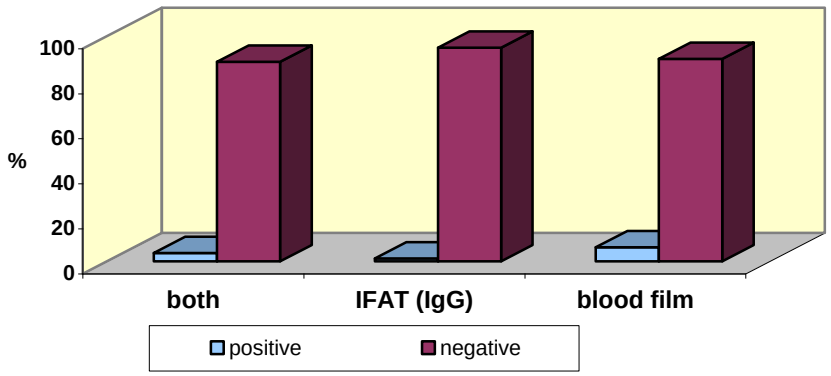


Table (11): Sensitivity and specificity of the IFAT (IgG) for the studied human cases.

IFAT (IgG) / Blood film	+ve	-ve	Total
	No.	No.	No.
+ ve	3	5	8
- ve	1	71	72
Total	4	76	80
sensitivity	75%		
specificity	93.4%.		

Table (11) and figure (18) show that the sensitivity of the IFAT (IgG) in human cases was 75% and the specificity of this test in human cases was 93.4%.

Figure (18) Sensetivity and specificity of IFA test for the studied human cases

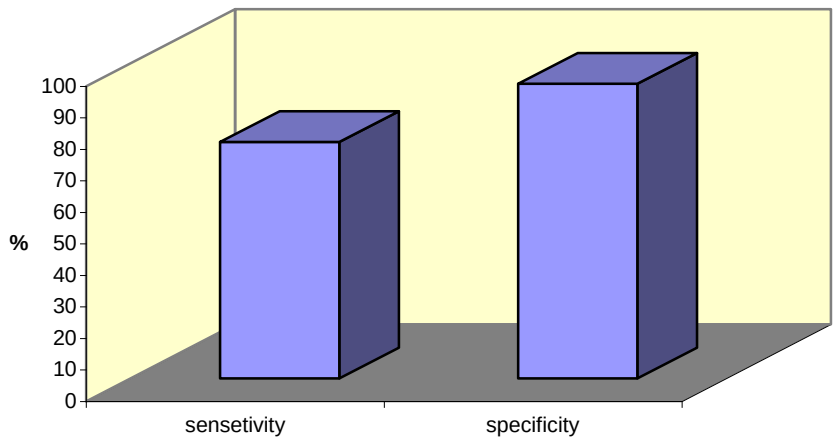


Table (12): Relation between Babesia infection and age in the studied human cases.

Studied human cases / Age groups (years)	Total (n.=80)	+ ve (n.=9)		-ve (n.=71)	
	No	No	%	No	%
< 40 (17-39)	39	3	7.7	36	92.3
≥ 40 (40 – 65)	41	6	14.6	35	85.4
Z		0.93		0.33	
P		> 0.05		> 0.05	

Table (12) and figure (19) show that out of 80 examined human cases, 39 were in the age group 17-39 (< 40y) and 41 cases were in the age group 40-65 (≥ 40y). Out of 39 cases in the age group (< 40y) 3 cases (7.7 %) were infected and out of 41cases in the age group (≥ 40y) 6 cases (14.6 %) were infected. The second group showed higher rate of infection (14.6 %) but no significant correlation between age and infection was found

Figure (19) Age and *Babesia* infection in studied human cases

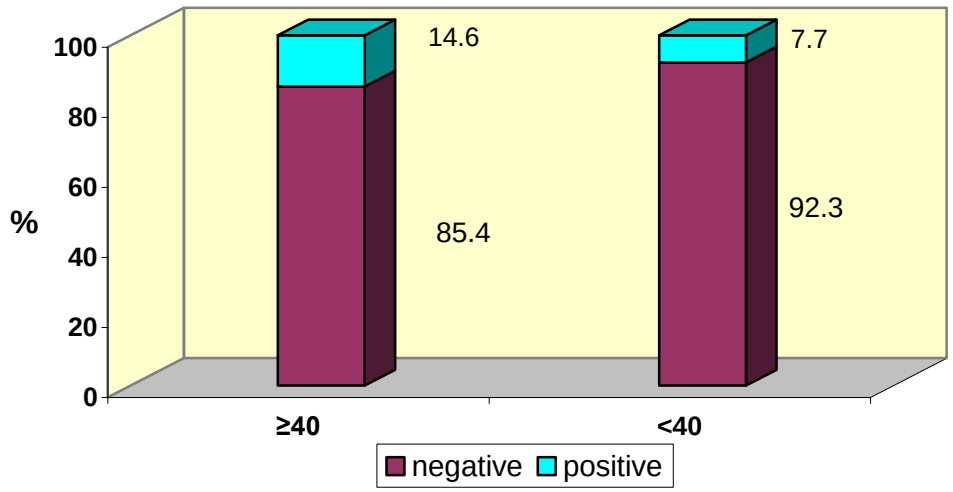
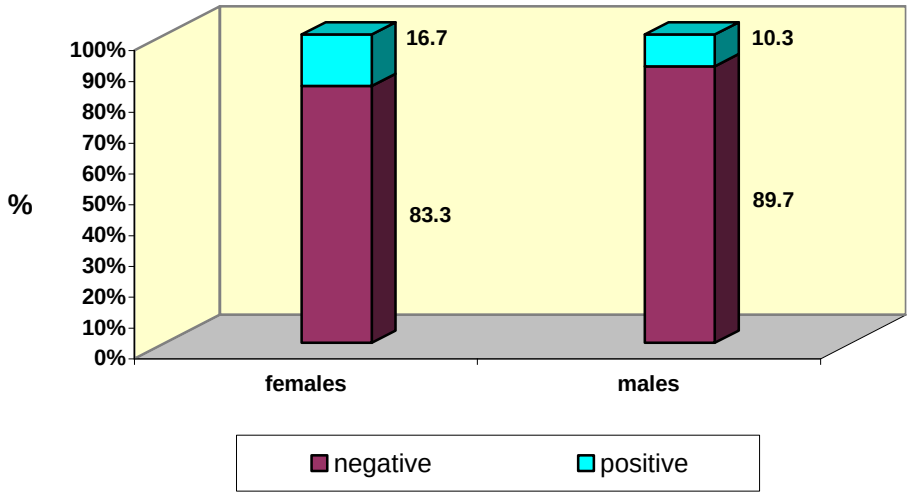


Table (13): *Relation between Babesia infection and sex* in the studied human cases.

Studied human cases / Sex	Total (n.=80)	+ ve (n.=9)		-ve (n.=71)	
	No.	No.	%	No.	%
Males	68	7	10.3	61	89.7
Females	12	2	16.7	10	83.3
Z		0.61		0.22	
P		> 0.05		> 0.05	

Table (13) and figure (20) show that out of 80 examined human cases, 68 cases were males and 12 were females. Out of 68 male cases, 7 cases (10.3%) were infected and out of 12 female cases, 2cases (16.7%) were infected but no significant correlation between sex and infection rate was found

Figure (20) Sex and *Babesia* infection in human group



Results of the present study on the collected ticks:

a) Identification:

The isolated ticks were ***Boophilus annulatus*** (shown in figure 21)

Points of identification were

- ***Boophilus annulatus*** is a relatively large brownish tick.
- Inflexible dorsal scutum covers the anterior part of idiosoma of the female.
- Capitulum is anterior and mouth parts are terminal and visible from above.
- Palpi are short and ridged.
- Eyes are located on the margins of the scutum and spiracular plate is nearly round.
- Anal groove is absent or very indistinct.
- No festoons
- Females when unfed are flat but swell to enormous size when fully engorged resembling beans.

Classification of ***Boophilus annulatus***

Phylum	***Arthropoda***
Class	***Arachnida***
Order	***Acarina***
Suborder	***Mesostigmata***
Family	***Ixodidae***
Genus	***Boophilus***
Species	***annulatus.***

Figure (21): showing engorged female ***Boophilus annulatus*** tick

b) Results of the haemolymph smears examination:

Haemolymph smears prepared and stained by Giemsa stain were examined under oil immersion lens and 80% of the examined ticks showed kinetes of ***Babesia bigemina*** shown in figure (22)

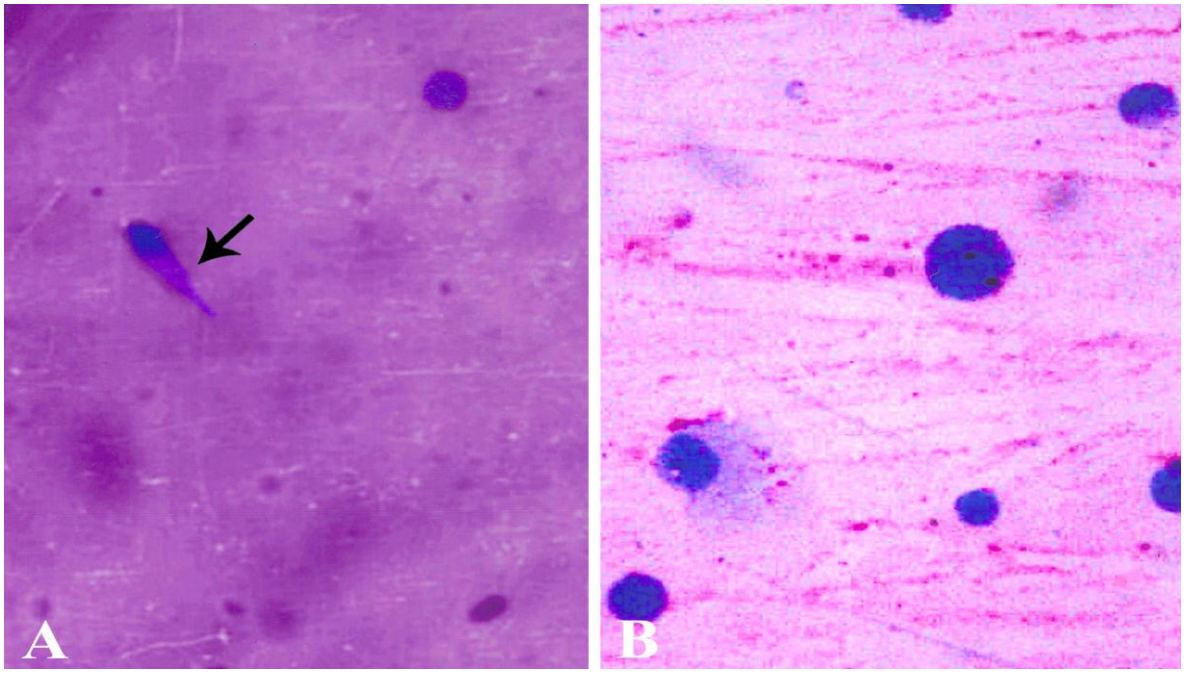


Figure (22): Giemsa stained haemolymph smear of ***Boophilus*** ticks A- Positive smear showing kinetes of ***Babesia bigemina***
B- Negative smear