قسم : طب الحيوان.
كلية : الطب البيطري ـ جامعة أسيوط.
رئيس القسم : أ.د. ابراهيم سيد أحمد.

صورة الخلايا الدموية البيضاء في الدورة الطرفية

بعد التحصين بمادة الـ B.C.G. والعدوى الصناعية بالبابيزيا في الحمير

ثروت نافع ، أحمد عامر

تم تقييم تأثير الحقن بمادة الـ B.C.G على تنشيط المناعة الخلوية ضد العدوى الصناعية بالبابيزيا في مجموعة من الحمير الذكور قبل وبعد العدوى. وكذلك بعد ازالة الطحال في مجموعة منهم.

وأوضحت النتائج أن حقن مادة الـ B.C.G. قد وفر الحماية ضد البابيزيا في الحمير الغير مزال طحالها بينما نفقت الحمير المزال طحالها. وأوضحت النتائج بعض التغيرات المعنوية في نسب الخلايا البيضاء الكلية والنوعية قبلوبعد العدوى وكذلك بعد ازالة الطحال.

Dept. of Vet. Med.,
Fac. of Vet. Med., Assiut University,
Head of Dept. Prof. Dr. I.S. Abdallah.

CIRCULATING LEUKOCYTES AFTER B.C.G. AND EXPERIMENTAL BABESIA EQUI INFECTION IN DONKEYS

(With Two Tables and 4 Figures & 3 Plates)

By
TH.S. NAFIE and A.A. AMER
(Received at 2/5/1987)

SUMMARY

A group of 6 donkeys were used to evaluate the effect of B.C.G. injection on the activation of non-specific cell mediated immunity against the experimental infection of Babesia equi.

The experimental donkeys were previously treated against internal parasites until proved its freedom from infestation. The animals were proved to be free from tuberculosis using tuberculin test.

The experimental animals were injected by B.C.G. intradermally. After one month they were challenged experimentally by B.equi infected blood (98% parasitaemia) intravenously.

Out of the experimental group three donkeys were splenectomized to induce relapses.

All animals were clinically observed, blood samples and bone marrow smears were routinly examined for estimation of leukocytic picture.

The study revealed that the injection of B.C.G. had protected donkeys from acute babesiasis, however it failed to protect splenectomized animals from acute relapses and severe parasitaemia was observed, and all splenectomized animals were died.

The observations of leukograms revealed a highly significant leukocytaemia to reach maximum ($19.2 \pm 3.4 \times 10^3$/ul) two weeks post B.C.G. injection, and one week post experimental babesial infection.

The relative count revealed a highly significant neutrophilia and eosinophilia post B.C.G. up to one month post infection with the increase of the relative number of non sigmented cells, however, monocytes achieved non significant increase in the peripheral blood.

Leukograms of splenectonized animals revealed a highly leuocyttosis (47×10^3/ul) in two of them, however, the 3rd one showed severe leukopenia (2.7×10^3/ ul). The relative values of segmented and non sigmented neutrophils achieved a highly significant increase (neutrophilia), while the relative number of lymphocytes was decresed, however, the relative values of eosinophils and monocytes were significantly increased.

It was observed that the phagocytic processes were highly activated post experimental infection. Neutrophils were able to engulf more than one parasitic cell in the peripheral blood, however monocytes were able to phagocyte a lot of parasitic cells and others could

engulf more than one of the infected erythrocytes (erythrophagocytosis) especially in bone marrow smears.

INTRODUCTION

Equine piroplasmosis [Babesiasis]; a world-wide disease was diagnosed in Assiut province, A.R.E. (NAFIE, 1980). Since that date, several studies were conducted on the pathological effects of Babesia equi in donkeys.

The effect of induced equine piroplasmosis on the haematological picture and some biochemical constituents in splenectomined and non- splenectomired donkeys was conducted by NAFIE et al. (1982). It was observed that the increase in total leukocytes was more prominant in splenectomized animals than in non-splenectomized ones. Russian investigators (ERCHOV, 1965) found a shift to the left in the neutrophils and a mild lymphocytosis as well as monocytosis in 50% of the diseased animals. ROBERTS et al. (1962) observed leukopenia, neutropenia and lymphopenia just prior to the appearnce of parasites in the blood. The same observations were recorded by TAYLOR et al. (1969) in the United States, Transient leukopenia and lymphopenia which progressed to lymphocytosis in about 10 days was observed by the authors. However, RUDOLPH et al. (1975) observed wide variation in the leukograms however, monocytosis and eosinopenia being the most common variations.

The use of Bacillus Calmette-Guerin (B.C.G.) in the activation of non-specific cell mediated immunity against piroplasma was attempted by CLARK et al. (1975) in mice and NAFIE (1983) in donkeys and NAFIE et al. (1985). All authors reported that B.C.G. gave protection in non-splenectomized animals against acute babesial infection.

The present work was carried out to study the variations in circulating leukocytes after the immunization by B.C.G. and post challenge by Babesia equi in splenectomized and non-splenectomized donkeys.

MATERIAL and METHODS

A group of 6 donkeys aged between 3.5-4 years were used in the experimental work. All animals were treated by Ivomec* against internal parasites until it proved negative egg count by faecal examination. Scheme of the experimental work is shown in Diagrams.

Whole antieoagulated blood samples were drained from jlugular vein using disodium-Ethyline Diamine Tetra Acetate (Na-E.D.T.A.). One blood sample was taken before B.C.G. injection and the other two-after 2 and 4 weeks post injection. Animals were then infected by Babesia equi infected blood (100 ml of 98% infected blood i.v.).

Total leukocytic count and blood smears were examined daily, however, three samples every two weeks post infection were representative. In splenectomized animals blood samples were examined daily until the day of death.

Total and differential leukocytic cell count were carried out according to SCHALM (1979).

Statistical analysis was conducted using t-test according to SNEDECOR and COCHRAN (1967).

*: Developed by the research Dept. of MSD Co.

RESULTS

It was observed that the injection of B.C.G. deparred the appearance of the clinical signs of piroplasmosis in B.C.G. injected donkeys. However, in B.C.G. splenectomized animals the typical acute clinical signs of babesiasis were evident and all splenectomized animals were dead within few days. Results of the total leukocytic count and differential cell count were recorded in table (1) leukogram (1) and table (2) leukogram (2,3 & 4) for B.C.G. injected and splenectomized groups respectively.

The activity of leukocytes against babesia equi parasites were recorded in Pictures (1 & 2).

DISCUSSION

It is of important to observe that B.C.G. injection before babesial infection protected the infected animals against acute illness in non-splenectomized animals. However, in splenectomized animals, B.C.G. failed to protect them from acute relapses and the clinical signs of acute babesiasis were appeared. High temperature, severe anaemia, haemoglobinurea, yellowish mucous membranes and emaciation were the most prominant clinical signs. It was observed that all splenectomized donkeys were dead. These results could be interpretted depending on the fact that B.C.G. stimulates cell mediated immunity which depends mostly on the presence of large number of sensitized leukocytes and the presence of spleen as a lymphoid tissue which act as a precursor for leukocytes (CLARK, et al., 1975; SELBITZ et al., 1980 and NAFIE, 1983).

Regarding leukocytic count, it was observed that leukograms achieved a highly significant leukocytaemia to reach the maximum ($19.2 \pm 3.4 \times 10^3$ / ul) two weeks post B.C.G. injection and one week post experimental babesial infection. The absolute number of sigmented, non-sigmented neutrophils, eosinophils and lymphocytes were, however, significantly increased post B.C.G. injection (Table 1 & Diag. 1) and declined slowly but not reached the before injection figures. It returned to increase again post babesial infection to reach the maximum elevation at the 6th week post babesial infection with appearant shift to the left. These results coincided with those obtained by ERCHOVE (1982) and NAFIE (1980 & 1983). However, it contradict with those obtained by ROBERT et al. (1962) and TAYLOR et al. (1969), while, RUDOLPH et al. (1975) recorded a wide variation in the leukograms.

In splenectomized animals the total count reached a highly significant increase (47×10^3/ul) in two of them, however, the 3rd one showed gradual decrease to reach (2.7×10^3/ul). The absolute values of sigmented and non-sigmented neutrophils recorded a highly significant increase (Neutrophilia) while the absolute number of lymphocytes was decreased, however, the relative values of eosinophils and monocytes were significantly increased. With respect to the 3rd splenectomized donkey, it was observed that the total leukocytic count was decreased just post splenectomy. The typic count revealed a sharp decrease in the sigmented neutrophils, however the non-sigmented one showed a significant increase up to the 4th day when it began to decrease again. These picture indicated a degenerative shift to the left (Table 2 & Diags 2,3 & 4).

These pictures were similar to those obtained by NAFIE (1980); NAFIE et al. (1982) and SALEM et al. (1986).

The carefull examination of stained blood and bone marrow smears (Fig. 1 & 2) revealed that the phagocytic characters of neutrophils and monocytes were highly activated post B.C.G. injection. Neutrophils thus could engulf and lyse a great number of babesial agents, while monocytes were able to phagocyte more than one parasitic cell. Furthermore, monocytes

were able to engulf infected erythrocytes (Target cells). More than one monocytic cell coalleased with each other to increase the phagocytic affinity. Similar observations were recorded by ALLEN et al. (1975) and NAFIE (1980 and 1983).

The overall observations revealed that the use of B.C.G. activated leukocytes through the mechanism of cell mediated immunity and increased the total number of circulating leukocytes specially segmented, non-segmented and lymphocytes (The cells which are encountered in the cell mediated immunity). The functions of these cells were supported by increasing the number of monocytes and to a little extent by eosinophilic cells.

REFERENCES

Allen, P.C.; Frerichs, W.M. and Holbrook, A.A. (1975): Experimental acute Babesia caballi infections. 1- Red blood cell dynamics. Experimental Parasitol. 37; 67-77.

Clark, I.A.; Allison, A.C. and Cox, F.E. (1975): Protection of mice against babesia and plasmodium with B.C.G. Nature, Vol. 259 (1) 29; 309-311.

Erchov, V.S. (1965): Parasitology and parasitic diseases of livestock. Moscow. State Publishing House for Agricultural Literature.

Nafie, Th.S. (1980): Effect of blood parasitic infestation-Equine piroplasmosis- on the picture of some trace elements in Equine. Master degree Thesis, Vet. Med. Dept.; Fac. of Vet. Med. Assiut Univ. Egypt.

Nafie, Th.S.; El-Allawy, T.; Mottelib, A.A. and El-Amrousi, S. (1982): Effect of induced equine piroplasmosis on some blood constituents. Vet. Med. J. Vol. 9, No. 17 & 18; 123.

Nafie, Th.S. (1983): Evaluation and liver and kidney function tests in donkeys piroplosmosis. Ph. D., Thesis, Dept. of Vet. Med. Fac. of Vet. Med. Assiut Univ. Egypt.

Nafie, Th.S.; Amer, A.A. and Hafiz, A.M. (1986): Red blood cells indices in relation to experimental Babesia equi infction in donkeys. Second scientific Congress, Fac. of Vet. Med., Assiut Univ. 22-23 th March, 1986.

Roberts, E.D.; Morehouse, L.G.; Gainer, G.H. and McDaniel, H.A. (1962): Equine piroplasmosis. J.A.V.M.A. Vol. 141, No. 11; 1323-1324.

Rudolph, W.; Correa, J.; Zurita, L. and Manley, W. (1975): Equine piroplasmosis: Leukouytic response to Babesia equi infection in Chile. Br. Vet. J., 131; 601-

Salem, A.E.; El-Battawy, G.; El-Seify, M.A.; Selim, M.K. and Hosny, Z. (1986): Preliminary investigations on haematological and biochemical pictures of blood of premunized donkeys against Babesia equi. Second Scientific Congress, Fac. of Vet.Med., Assiut Univ. 22-23th March, 1986. pp. 148-157.

Schalm, O.W. (1979): "Veterinary Haematology". 4th. Ed. Balliere, Tindall and Cassell, ltd. London.

Selbitz, H.J.; Voigt, A. and Schonberr, W. (1980): "Uber moglichkeiten der nutzing der interferone in der veterinärmedizin". Possible uses of interferone in veterinary medicine. Mh. Vet. Med. 35; 769-771.

Snedecor, G.W. and Cochran, W.G. (1967): "Statistical Methods". 6th Ed. Iowa State College Press, Iowa, U.S.A.

Taylor, W.M.; Bryant, J.E.; Anderson, J.B. & Willers, H.K.H. (1969): J. Am. Vet. Med. Ass. 155, 915. (Cited in Biochemistry book of biomerieux Co. 1980, Bains France).

Table (1): Total and absolute differential leukocytic count of BCG injection experiment

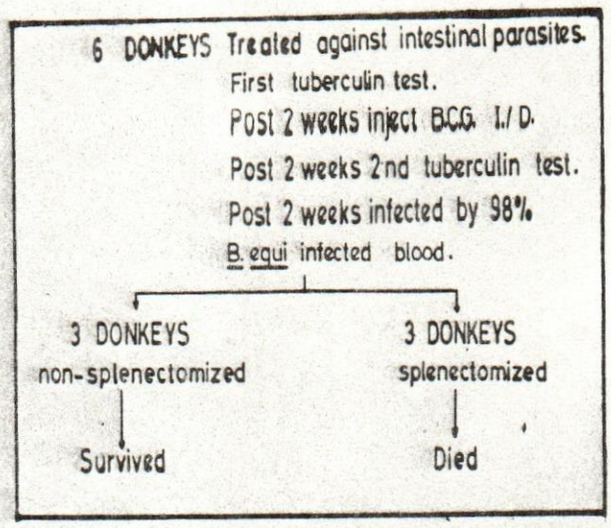
Time of sample	Statistical data	Total count of leukocyte X 10^3/ul	Absolute differential lenkocytic count X 10^3/ul					
			Seg. neut	non seg. N.	Eosino	Basoph	Lympho.	Monocytes
Before BCG injection	Range	6 - 9.4	2.320-4.420	3.00-1.128	0.70-0.940	-	2.04-4.560)	0 - 0.940
	x	7.8 ±	3.107 ±	0.699.7 ±	0.491 ±		3.474	0.168.3
	S.E.	0.5	0.180	0.157	0.146		0.427	±0.169.5
Two weeks post BCG	Range	11-33.4	4.07-12.20	0,99-4,9	0.69-1.3	0 -	4.84-17.7	0.0-0.78
	x	19.2±**	6.83 ±*	2.3 ±*	0.93 ±*		8.76 *	0.327 ±
	S.E.	3.4	1.33	0.59	0.10	-	±2.1	1.3
4 weeks post BCG inject	Range	7.8-19.4	2.1-8.14	0.39-1.1	0.35-1.6	-	2.86-9.89	0.2-0.5
	x	13.97±**	5.3±	0.66±	0.78	-	6.22±*	0.2 ±
	S.E.	1.8	1.18	0.1	0.24	-	0.3	0.160
2 weeks post infection	Range	8.8-19.4	2.82-10.88	0.39-4.8	0.13-1.6	-	0.77-9.9	0.13-0.6
	x	14.6±**	6.95±**	2.3±	0.78±	-	4.23±	0.33±
	S.E.	1.9	1.3	0.85	0.24	-	1.4	0.07
4 weeks post infection	Range	12.0-19.0	2.1-7.8	0.9-3.8	0.3-1.04	-	5.2-6.14	0.13-0.6
	x	14.3±**	5.03±*	2.2±	0.501±	-	6.31±**	0.29±
	S.E	1.1	0.9	0.5	0.12	-	0.585	0.1
6 weeks post infection	Range	12-822.0	3.7-10.9	0.9-5.5	0.13-1.54	-	0.8-11.4	0.0-0.552
	x	16.7±*	6.3±*	2.4±	1.01	-	6.74±	0.279
	S.E.	1.6	1.2	0.7	0.2	-	1.9	0.09

* P $\angle$ 0.05 significant.

** P $\angle$ 0.001 Highly Significant.

Table (2): Total and absolute differential leukocytic count of: BCG splenectomized group

Animal No.	Time of sample	total count of leukocyte X 10^3/ul	differential leukocytic count x 10^3 /ul					
			Sig. neut	non Sig N.	Eosino.	Basoph.	Lympho.	Monocytes
Animal No. 1								
	Before	6.1	5.60	0.43	0.062	-	0.06	0.06
	Days post 1	6.15	4.24	0.68	-	-	0.98	0.18
	Splenect. 2	6.65	4.01	0.85	-	-	0.62	0.69
	3	27.6	18.80	1.38	-	-	7.45	0.74
	4	37.2	27.90	2.98	1.12	-	4.46	1.83
	5	36.6	23.06	4.39	0.73	-	6.59	2.94
	6	42.0	23.10	10.92	1.68	-	3.36	0.45
	7	45.2	23.96	17.18	1.36	-	2.26	0.54
	8	27.2	15.78	6.53	0.54	-	3.81	0.56
	9	32.6	3.91	22.82	-	-	5.22	
	Before	6.00	5.10	0.36	-	-	0.54	-
Animal No. 2	Days post 1	5.75	3.62	1.49	0.1150	-	0.46	0.057
	splenec. 2	5.75	3.74	1.49	0.288	-	0.23	-
	3	4.85	1.60	2.09	0.242	-	0.78	0.145
	4	2.70	0.70	1.62	0.81	-	0.22	0.081
Animal No. 3	Before	16.10	6.44	1.93	484	161	7.08	-
	1	17.90	6.44	9.31	179	-	1.79	0.0199
	Days post 2	35.80	20.76	12.53	-	-	2.51	-
	splenec 3	46.00	20.70	16.56	-	-	8.74	-
	4	47.00	26.79	18.33	-	-	0.47	0.1410



Outlines of the experimental work.

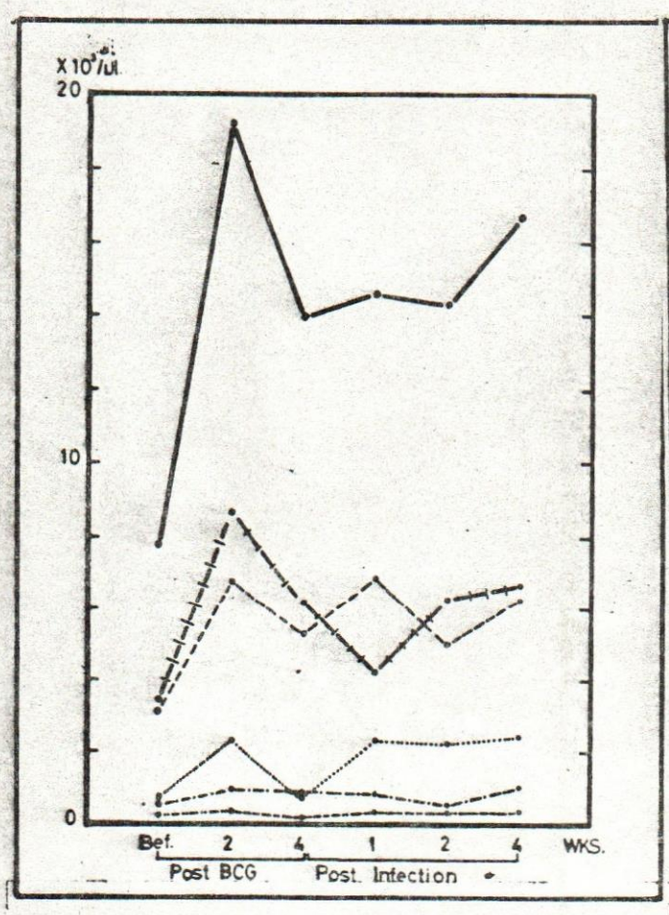


Fig.(1)- Leukogram of BCG Immunized group.
TLC. SN. NSN. EOS. L. MON.

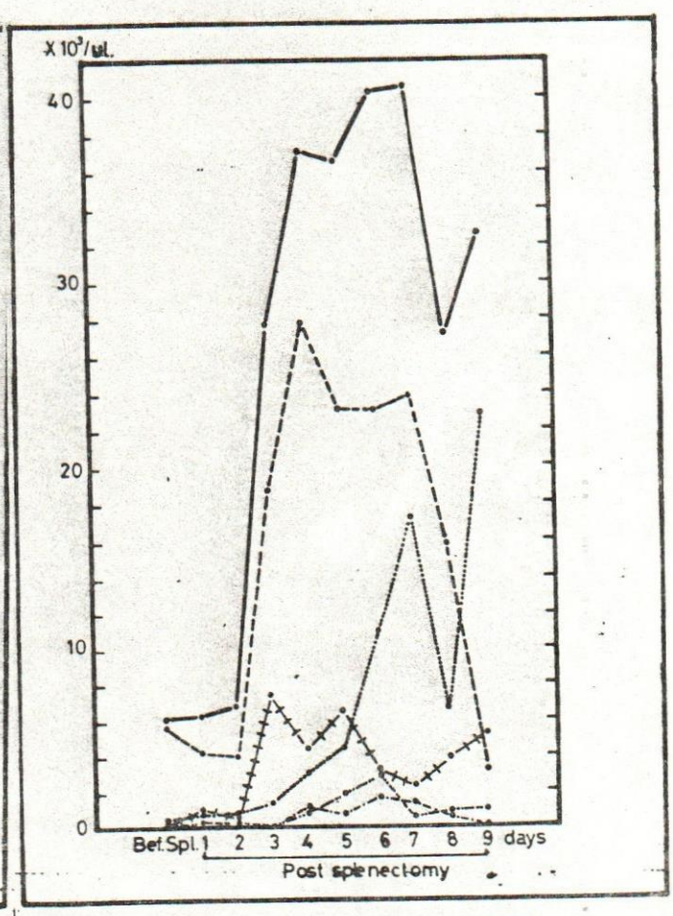


Fig.(2)- Leukogram of splenectomized donkey NO.(1).
TLC. SN. NSN. EOS. L. MON.

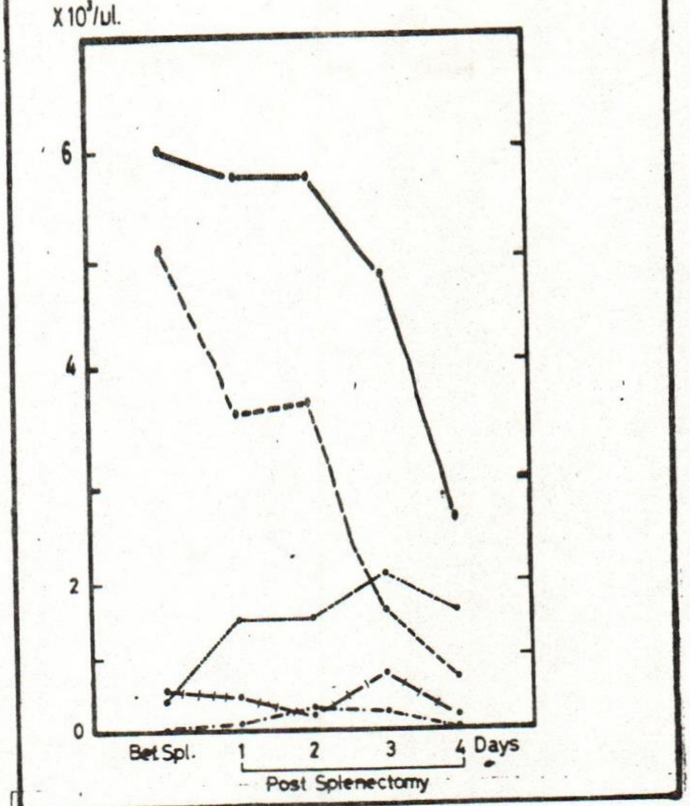


Fig.(3)_ Leukogram of splenectomized donkey NO.(2).
TLC. SN. NSN. EOS. L. MON.

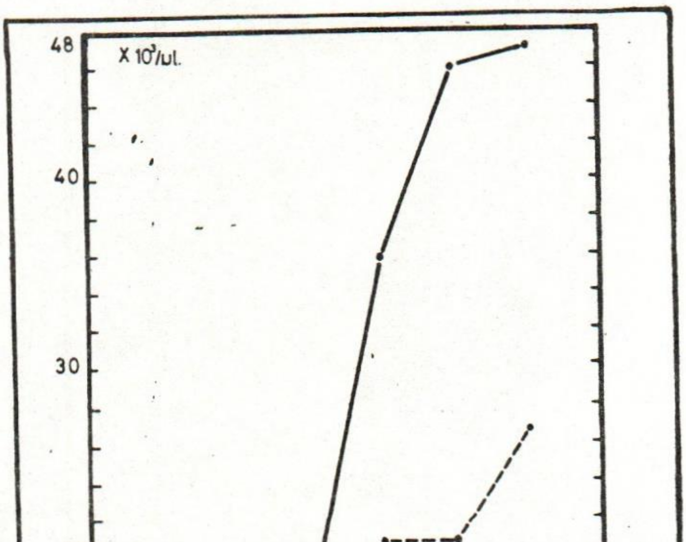
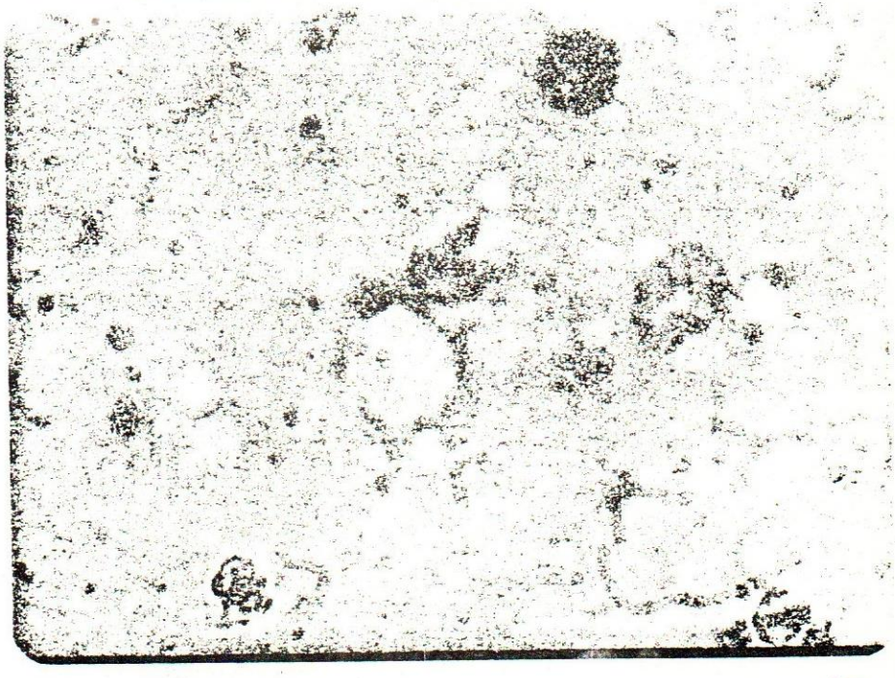


Fig (4) _ Leukogram of splenectomized donkey NO (3).
TLC. SN. NSN. EOS. L. MON.

Pict.(1): Babesia equi within the Red Blood cells and the neutrophil cell.

Pict.(2): Infected Erythrocytes within large monocyte.



Pict.(3): Neutrophil and monocyte performing phagocytic and lysing processes on Babesia equi.