

**INCIDENCE OF BLOOD PARASITES OF SHEEP AT
ALEXANDRIA PROVINCE
BY**

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ABSTRACT

This study was conducted in Alexandria province in Rural areas on 381 sheep of different ages distributed in four different regions (Amryia, Borg El-Arab, Bangar El-Sokkar and Abis). In addition to the routine parasitological examination of Gimsa stained blood film which revealed 20.9% of *B. ovis* infection, two serological techniques were applied.

The first was the indirect fluorescent antibody test (IFA) which revealed an incidence of 82.1% positive cases throughout the total screened sheep confirming the above mentioned routine parasitological Giemsa stained blood films.

The second seriological test was the enzyme linked immunosorbent assay (ELISA) which revealed 86.8% positive cases. The study revealed that the peak of incidence of *B. ovis* in sheep was achieved in summer (87.5 and 90.6) by using IFAT and ELISA tests. From the obtained data, it was clear that the ELISA was more sensitive than both IFA and blood film examination.

INTRODUCTION

Sheep has been known as one of the most important livestock in various countries because it is considered as a good source of meat, wool and milk. In addition, it takes part in some medical purposes such as seriological preparations and manufactured surgical threads. Sheep are hosts to both large and small species of *Babesia*. Both *Babesia motasi*, the large species, and *Babesia ovis*, the small one, contribute to a syndrome causing disease and death of sheep in Egypt (Nagaty, 1947; Abo El-Khair, 1980 and Mahmoud, 1992).

Large *Babesia* spp. infective for sheep and goat in Easter part of Gansu province, China (Qi Bai. et al 2002).

Blood parasites of small ruminants are known to occur in the Mediterranean basin as well as in other areas where the tick vector is present (Yeruham et al., 1992) so, ticks are *Undoubtly* the most dangerous ectoparasites infecting sheep.

Perfect diagnosis of babesiasis is essential for successful control measures.

This study was done on sheep to illustrate. The more specific

methods for diagnosis of Babesia which help in accurate detection of the parasite, therefore, the aim of the present study was:

- 1- To evaluate the efficacy of different techniques used for diagnosis of Babesia infection in sheep (blood film examination, Indirect fluorescent antibody (IFA) and enzyme linked immunosorbent assay (ELISA).
- 2- Seasonal incidence of B.ovis among sheep in Alexandria province.

MATERIAL AND METHODS

1. Animals:

The present study was conducted in Alexandria province on 381 sheep of both sexes randomly selected from different regions of the province as follows Amyria 93 sheep, Borg El-Arab 92 Banger El Sokker 90 and Abis 106 sheep.

2. Samples:

Diagnosis of Babesia ovis.

A. Blood film examination:

- Staining according to Kruse and Pritchard (1982).
- Microscopical Identification of Babesia ovis was made according to the Keys of Levine (1971).

B. Serological Tests:

Two serological tests were applied.

1. Indirect fluorescent antibody test (IFAT) it was carried out according to Leeflang and Perie (1972), Goff et al. (1982) and Tenter and Friedhoff (1988).

a- Preparation of B.ovis antigen for IFA

The antigen was prepared according to Leeflang and Perie 1972 .

- b- Supplementary reagents for TFA test
 - Antigen.
 - Control sera.

It was kindly provided from the department of parasitology and animal diseases National Research center.

The positive control serum was obtained from experimentally splenectomized sheep infected with B.ovis 21-28 days post infection. The negative control serum was obtained from the control negative non-infected animals.

The tested sera

All 381 serum samples out from deep freezer (-20 °c).

Conjugate.

Rabbit anti-sheep IgG (whole molecule) fluorescent isothiocyanate (FITC) conjugate was obtained in liquid form from sigma chemical company, Saint Louis, USA.

Reading the test:

The test was read according to Gray et al (1980).

By using fluorescent microscope (olympus fluorescent Microscope model BHF AX 5075).

2. Enzyme linked immunosorbent assay(ELISA)it was carried according to Voller et al (1976b).

Statistical Analysis

Differences among localities Amryia, Borg El-Arab, Bangar El - Sokkar and Abis) and seasons summer, autumn, winter, and spring) were assessed using the chi-square test of the Epi info computer software (Epi Info, 1994).

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RESULTS

Table (1): Incidence of *Babesia ovis* in sheep at Alexandria province using **BLOOD FILM**.

Location	No. of examined Sample	No. of positive Sample	Infection %
Amryia	93	18	19.3
Borg-El Arab	92	21	22.8
Bangar El Sokkar	90	20	22.2
Abis	106	22	20.7
Total	381	80	20.9

Table (2): Seasonal incidence of *Babesia ovis* among sheep by **BLOOD FILM** examination.

Seasons	No. of examined sample	No. of positive sample	Infection %
Summer	160	38	23.7
Autumn	65	15	23.07
Winter	45	9	20.0
Spring	111	18	16.2
Total	381	80	20.9

Table (3): Incidence of *B. ovis* in sheep at Alexandria province using **IFA** technique.

Localities	No. of examined Sample	No. of positive Sample	Infection %
Amryia	93	70	75.2
Borg-El Arab	92	81	88.1
Banger El Sokkar	90	72	80.0
Abis	106	90	84.0
Total	381	313	82.1

Table (4): Seasonal incidence of *B. ovis* among sheep by IFA test.

Seasons	No. of examined sample	No. of positive sample	Infection %
Summer	160	140	87.5
Autumn	65	49	75.3
Winter	45	31	68.8
Spring	111	83	74.7
Total	381	313	82.1

Table (5): Quantitative results of IFAT for positive sera samples.

Province	Positive sample	Titers									
		1/40		1/80		1/160		1/320		1/640	
		No.	%	No.	%	No.	%	No.	%	No.	%
Alexandria	313	43	13.1	62	19.8	90	28.7	64	21.7	50	15.9

Table (6): Incidence of *B. ovis* in sheep at Alexandria province using ELISA test.

Localities	No. of examined samples	No. of positive samples	Infection %
Amryia	93	85	91.3
Borg El- Arab	92	86	93.4
Bangar El-Sokker	90	79	87.7
Abis	106	81	76.4
Total	381	331	86.8

Table (7): Seasonal incidence of *B. ovis* among sheep by ELISA test.

Season	No. of examined samples	No. of positive sample	Infection %
Summer	160	145	90.6
Autumn	65	55	84.6
Winter	45	36	80.0
Spring	111	95	85.5
Total	381	331	86.8

Table (8): Incidence of B. ovis infection in sheep using ELISA Method.

Localities	-ve	%	+ve	%	++ve	%	+++ve	%	++++ve	%	No
Amryia	8	8.6	12	12.9	18	19.3	36	38.7	13	13.9	93
Borg El	6	6.5	2	2.1	24	26.0	30	32.6	20	21.7	92
Arab	11	12.2	10	11.1	25	27.7	24	26.6	18	20.0	90
Bangar El	25	23.5	13	12.2	27	25.4	40	37.7	19	17.4	106
Sokker Abis											
Total	50	13.1	37	9.7	94	24.6	130	34.1	70	18.3	381

- ve: Negative < 0.17 OD
 +ve: Weak positive 0.17 – 0.20 OD
 ++ ve: Moderate positive 0.2 – 0.3 OD
 +++ ve: Strong positive 0.3-0.4 OD
 ++++ ve: Very strong positive > 0.4 OD.
 OD = Optical density.

Table (9): Incidence of B. ovis infection in sheep at Alexandria province using different Methods of diagnosis.

Methods of diagnosis	No. of examined samples	No. of positive samples	Infection %
Blood film	381	80	20.9
IFAT	381	313	82.1
ELISA	381	331	86.8

DISCUSSION

In Egypt Nagaty (1947) mentioned that babesiosis is one of the most important tick born diseases which cause severe economic losses to sheep industry. In present study, parasitological examination of stained thin blood films prepared from 381 sheep of various ages and sexes in Alexandria province found that only 80 sheep harboured natural infection with B. ovis (20.9%) it was found to be in agreement with Mandusic (1953) in YUGOSLAVIA Petrovic (1953) in south east Serbia, Abo-ElKhair

(1980), and Mahmoud (1992), but higher than those obtained by Ramadan and Al-Akabawy (2000), Maronpot et al (1974) Sherkov et al in Jordan (1977), Charyev (1977) in Turkemina, Rodriguez et al (1989) in Cuba, Savini et al. (1999) in Italy; in Nigeria, Adeoye (1985). In Israel, Yeruham et al. (1992).

Concerning the seasonal incidence of B. ovis, the obtained results revealed. That the high rate of infection was recorded during spring followed by summer, autumn

and finally winter, these results are in agreement with those given by Simic and Petrovic (1953), Georgescu and Stoicea (1961), Sherkov et al. (1977) Abo-Shehada et al (1988), Trifanov and Ruseve (1989), Pipano (1991) and Yeruham et al. (1995) in ISREAL reported that the peak of *B. ovis* in spring months .

IFA and ELISA were recorded to be the most specific and sensitive tests in detection of *Babesia* species antibodies Weiland and Reiter, (1988).

The high percentage which has been recorded by seriological tests than that recorded by blood film examination was due to the direct method could detect only the acute cases of *Babesia* infection but not sub clinical (*Babesiosis*) (Callow, 1977).

Concerning the sensitivity of the serological test ELISA revealed the highest value 86.8% followed by IFAT 82.1% these result were in agreement with Bidwell et al (1978), Hussein (1997), Hassan (2000), and Reda (2001).

REFERENCES

- Abo-El-Khair S.A. (1980): Some studies on morphology and biology of ovine blood parasites. M.V. Sc. Thesis, Cairo University.
- Abo-Shehada, M. N.; MuWalla, M.M. and Tawfeek, F (1988): Ovine babesiosis in Jordanian sheep mistakenly diagnosed as plant poisoning. *Prev.Vet.Med.*,6: 235-238.
- Adeoye, S.A.O. (1985): Disease profiles of sheep and goat in two groups of villages in south west Nigeria. Proceedings of the work shop on small ruminants production system Ibadan (Nigeria) 1984. 13-16.
- Bidwell, D.E., Turp, P., Joyner, L.P., Payne, R.C. and Purnell, R.E. (1978); Comparison of seriological tests for *Babesia* n British Cattle. *Vet. Rec.*, 103: 446-449.
- Callow, L.L. (1977): Vaccination against bovine Babesiosis in immunity to blood parasites of animal and man. Miller, L.H., Pino, J.A. and McKelvey. J.J. (eds). Plenum, New York. 121-149.
- Charyev. O. Ch. (1977): Piroplasmid fauna of sheep and diseases caused by piroplasmas. *Seriya Biologicheshinkh Nauki*, 6: 70-71.
- EPI INFO (1994): A word – processing, database and statistics program for public Health Designed by A.G. Dean, J.A. Dean, D. Coulombier, A.H. Burton, K.A. Brendel, D.C. Smith, R.C. Dicker, K.M. Sullivan, and R.F. Fagan.
- Version 6. Produced by. The division of surveillance and Epidemiology, program office, centers, for disease control and pre vention (CDC) Atlanta, Georgia 30333 in Collaboration with Global programme on AIDS, (WHO) Geneva, Swizerland.
- GeorgeScu, L. and Stoicea, V. (1961): Epidemiological data on *Babesia ovis* infection in Rumania. *Probl. Parasit. Vet. Bucurest*, 9: 69-72.
- Goff, W.L.; Wager, G.G.; Cialc, T.M. and Long, R.F. (1982): The bovine immuno response to tick derived *Babesia bovis* infection seriological

- studies of isolated immunoglobulins. *Vet. Parasitol*, 11 (2-3): 109-120.
- GRAY . M .A ., LUCHINS . P. F and BROWEN C .G. D(1980) :** Evaluation of an enzyme immunoassay for serodiagnosis of infection with theileria annulata *Res. Vet. Sci.*, 29: 360-366.
- Hassan, T.R.H. (2000):** Studies on some diseases transmitted by ticks in small ruminants. Ph. D. Thesis, Fac. Vet. Med., Cairo Univ.
- Hussein, Mervate, M.A. (1997):** Studies on vaccine preparation for Babesia species infection Egyptian Sheep. M.V. Sc. Thesis. Fac. Vet. Med. Cairo Univ.
- Kruse, G.O.W. and Pritchard, M.H. (1982):** The collection and preservation of animal parasites. HW MI, University of Nebraska Press, Lincoln, USA.
- Lee Flang, P. and Perie, N.M. (1972):** Comparative immunofluorescent studies on 4 Babesia species of Cattle. *Res. Vet. Sci.*, 13: 342-346.
- Levine, N.D. (1971):** Taxonomy of Piroplasmosis. *Trans. Amer. Micros. Soc.*, 90(1): 2-33.
- Mahmoud, Mona, S. (1992):** Some seriological studies on Babesia species infecting sheep in Egypt. M.V. Sc. Thesis, Fac. Vet. Med. Cairo University.
- Mandusic, D. (1953):** Piroplasmosis in sheep in the district of Rogatica, Yugoslavia. *Veterinarian, Sarajevo*, 2: 401-402.
- Maronpot, R.R., Michael, S.A. and Botros, B.A.M. (1974):** Blood parasites in Egyptian domestic animals. *J. Egypt. Vet. Med. Ass.*, 34: No. 3-4, 301-308.
- Nagaty, H.F. (1947):** Some new and rare records of pipoplasmosis with a list of species of Babesia and Theileria so far recorded from Egypt. *Vet. Res.* 59(11): 145-147.
- Qibai, Guangyuangliu, Dekaoliu, Jiaxing Ren and Xing LT (2002):** Isolation and preliminary characterization of a large Babesia sp. From sheep and goats in the eastern part of Gansu Province, China. *Parasitology Res. Vol 88, Mr. Sl, P. s16-s21.*
- Petrovic, K. (1953):** Babesia infection in sheep in south East Serbia. *Vet. Glasn*, 7: 23-32.
- Pipano, E. (1991):** Observation on the seasonal distribution of blood parasites in sheep in Israel. *Israel. Vet. Med.*, 46 (1): 37-38.
- Ramadan, M.Y. and El-Akabawy L.M. (2000):** Studies on sheep Babesiosis in Kalubya Governorate, Egypt. *Minufiya Vet. J.*, 1(1): 147-155.
- Red. S.F. (2001):** Blood parasites of sheep at Behera province. Ph. D. Thesis. Fac. Vet. Med. Alexandria University.
- Rodriguez, O.N., Rodriguez, P. Espaine, L. and Rivas, A. (1989):** Frequency of Haemoparasites in sheep in Cuba. *Revista Cuba Na de Ciencias. Veterinarias*, 20 (1): 70-75.
- Savini G.; Conte, A.; Semproni, Gand Scaramozzino, P. (1999):** Tick-borne diseases in ruminants of central and southern Italy. *Epidemiology and case reports. Parasitologia*, 41 (1): 95-100.
- Sherkov, S.N., El-Rabie, Y. and Kokash, L. (1977):** A survey of parasitic blood diseases tick borne Fever in domestic animals in Jordan. *Egypt. Vet. Sci.*, 13(1): 29-35.
- Simic,C. and Petrovic.Z.(1954):**Ovine piroplasmosis in YugoSlavia. *Acta. Vet. Bull. Grade*,4:3-6.
- Tenter, A.M. and Friedhoff, K.T. (1988):** Serodiagnosis of experimental and natural Babesia equi and B.

- Caballi infections. *Vet. Parasitol.*, 20 (1/3): 49-61.
- Trifanov, T. and Ruseve, V. (1989): Epizootiological study of piroplasmosis of cattle sheep and its ticks vectors in Atangia region of Bulgaria. *Veterinarya Sbirka*, 87 (4): 43-46.
- Voller, A.; Bidwell, D.E. and Bartlett, A. (1976 b): Enzyme immunoassay in diagnostic medicine: Theory and practice. *Bull. World Health Organization*, 53: 56-63.
- Weiland, G. and Reiter, I. (1988): Methods for the measurement of the serological response to *Babesia*, in *Babesiosis of Domestic animals and man*. CRC Press: 143-162.
- Yeruham, L.; Hadani, A.; Gofker, F. and Rosen, S.H. (1995): A study of an enzootic focus of sheep babesiosis (*Babesia ovis*, Babes 1892). *Vet. Parasitol.*, 60: 349-354.
- Yeruham, L.; Hadani, A.; Gofker, F., Rosen, S.H and Schlien, J. (1992): A field study of Haemoparasities in two flocks of sheep in Israel. *Isr. J. Vet. Med.*, 47: 107-111.

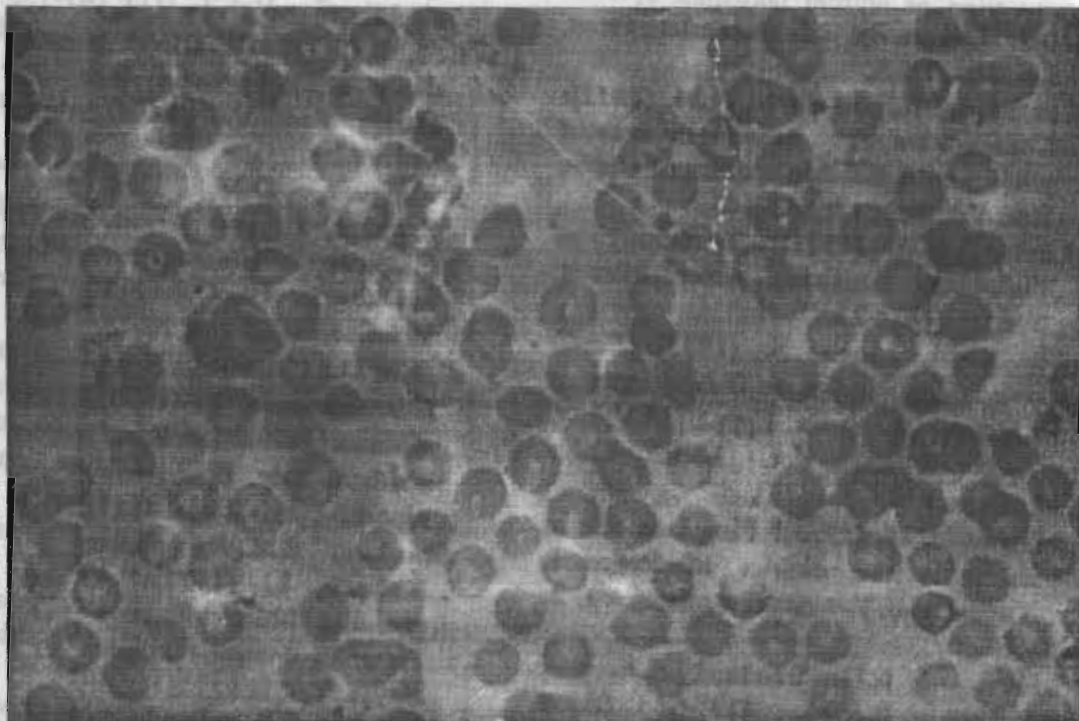


Photo 1 : Giemsa stained blood smear showing dot and rounded form of *Babesia ovis*. X100

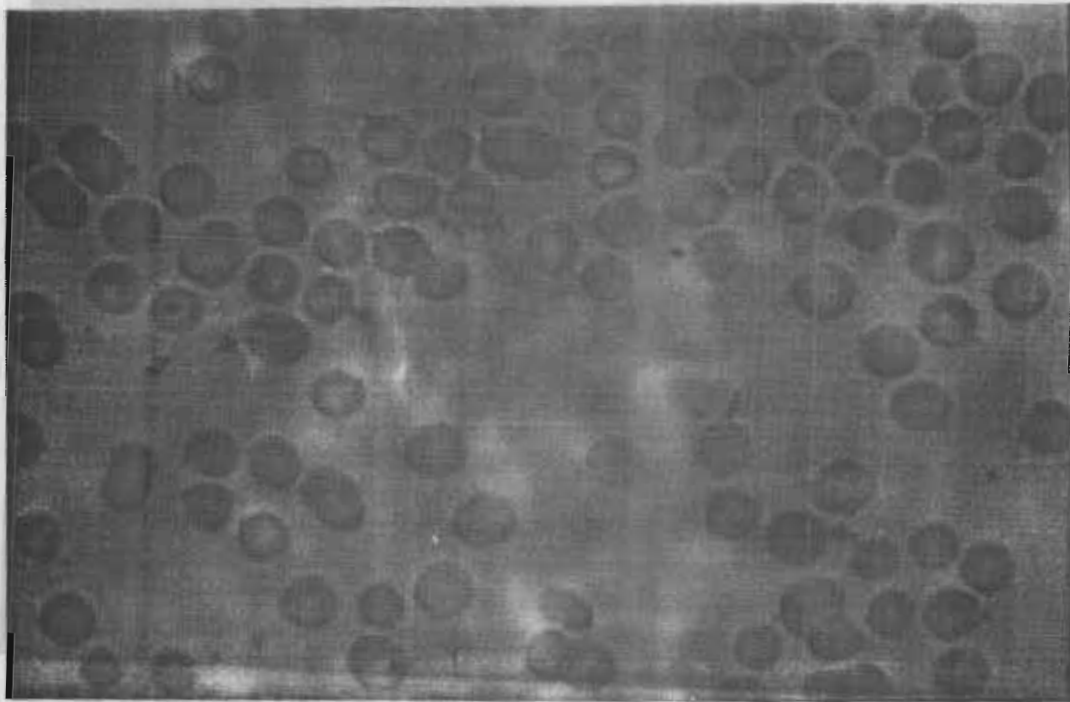


Photo 2 : Giemsa stained blood smear showing different piroplasmic forms of *Babesia ovis* (ring, pyriform, dot and round forms) .

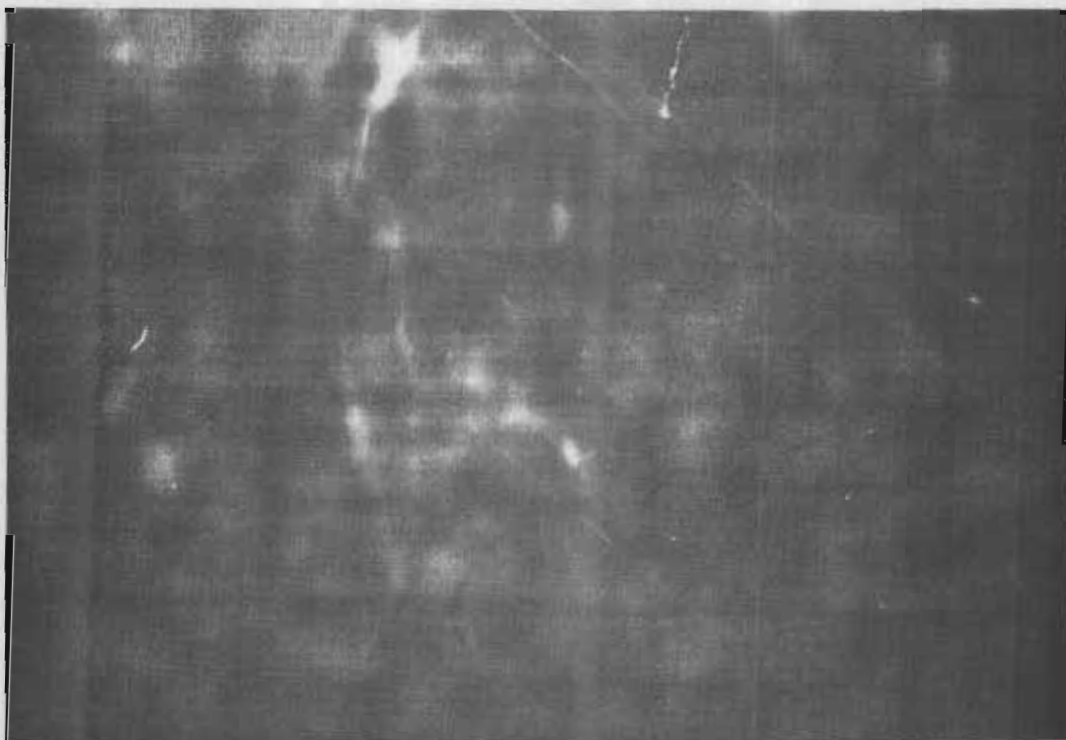


Photo 3 : Positive indirect fluorescent antibody (IFA) reaction of *B. ovis* antigen with serum of sheep naturally infected with *B. ovis*

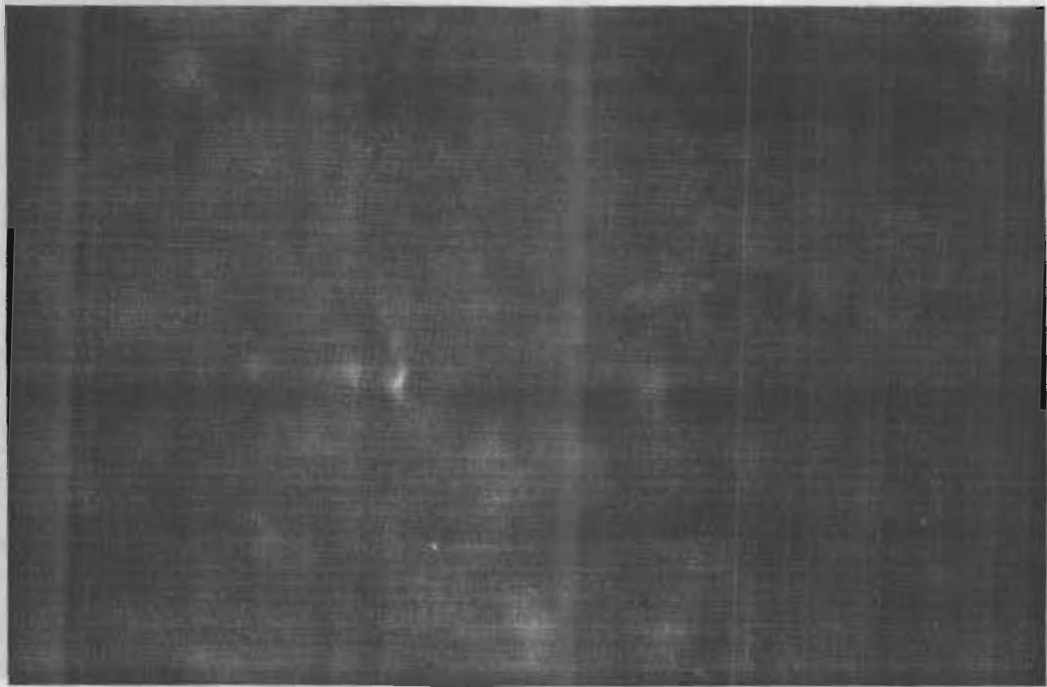


Photo 4 : Negative indirect fluorescent antibody (IFA) reaction of B. ovis antigen serum of non-infected sheep .

