

**EPIZOTIOLOGICAL STUDIES ON
LEPTOSPIROSIS IN THE SUDAN WITH
ESPECIAL
EMPHASIS TO BOVINE LEPTOSPIROSIS.**

BY

A.A. EL Wali
Department of Bacteriology
Veterinary Research Administration
P.O. Box 8067 - Khartoum.

Introduction

Leptospirosis has been reported from various parts of the World, Weil (1886), Vasilyev (1888), Bernkopf (1946), Fennestad & Berg-Petersen (1956), Burdin (1957) & Broom (1958). However, epidemiological studies and diagnostic methods of the disease were unsatisfactory.

Knowledge of the epizootiology and etiology of the disease in the Sudan is far from being complete. Observations on *L. canicola* in dogs (Awad, 1960) and on five serotypes in both domestic and wild animals (Shigidi, 1974) were reported. In a previous work, EL Wali, (1978), observed that 34.7% of cattle investigated were positive to nine serotypes. The most frequent were *L. pyrogenes* (32%), and *L. cynopteri* (27%).

This work was undertaken to study the etiology and the epizootiology of bovine leptospirosis in all provinces of the Sudan.

Materials and Methods

In this study 1142 serum samples from all the provinces were collected from cattle, 30 samples from goats & 56 samples from rats. All the sera were subjected to serological examination using Microagglutination test (MAT) tested from various reservoirs.

Moreover, 235 kidney, and liver tissues and 207 urine samples were collected from cattle slaughtered at Omdurman slaughter-house. The pathogenic characteristics of isolates obtained from water samples were studied on 10 Guinea pigs, 10 young hamsters & 6 calves 4-6 months of age free from anti-leptospiral antibodies. The Guinea pigs & hamsters were inoculated intraperitoneally with 2, 3, 4, & 5 ml. of native culture and put under observation for one month after which the animals were killed and microagglutination tests (MAT) using standard strains antigens obtained from USSR Research

Control Institute, were made. Four of the 6 calves were also inoculated subcutaneously in doses of 10-15 ml. of the isolate which contains 200-250 microorganism 400 magnification. Two were left as control.

Histological sections from kidney and liver of specimens were made and stained by levaditis method.

Kidney & liver suspensions were bacteriologically tested by culturing in Kortthof's media. Fluorescent antibody technique was also used. The urine samples were microscopically tested.

The air temperatures was recorded during the survey, Statistical analysis was done according to Snedecor, (1975).

Results

The serological tests showed that 175 out of 1142 cattle sera (15.3%) reacted positively to leptospirosis. While the highest percentage (38.3%) and (30%) were encountered in Upper Nile and Equatoria provinces respectively, the lowest percentage were found in Northern Darfur, Northern Kordofan, El Nil and Northern provinces as shown in Table 1.

Table (2) records the serotypes of cattle leptospirosis found in the Sudan. It is noticed that *L. hebdomadis* (45.9%) and *L. tarassovi* (27.5%) were most frequent. All serogroups gave positive results in different proportions with the exception of *L. celledoni*.

Statistical analyses showed a highly significant positive correlation between climatic conditions (temperature, humidity & rainfall) and the prevalence of leptospirosis (Table 3).

Out of the 56 rat sera examined, 13 (23.3%) were positive reactors. Four goats (13.3%) were found positive. The prevalent serotypes in rats were *L. hebdomadis* (53.8%) and *L. icterohaemorrhagiae* (15.3%) while in case of goats only *L. australis* (13.3%) gave positive results, Table 4.

Bacteriological tests in cattle :—

Microscopic examination of 207 cattle urine samples were negative for leptospire. Using Fluorescent antibody technique (Fig 1) for 235 kidney suspensions, only 6 were positive (2.5%) The same suspensions were cultured in Kortthof's media for one month proved to be negative.

Bacteriological tests on water samples :—

Darkfield Microscopy as well as culturing in Kortthof's media were performed.

Leptospire of short & medium length were observed in water samples taken from irrigating canals in Gezira.

MAT of leptospira cultures with hyperimmune sera compared to 19 serotypes received from all union SRCI proved that it did not belong to any of these serotypes. However, the anti-sera reacted positively with serogroup hebdomadis, cynopteri and grippotyphosa in titres of 1:100 & 1:800.

Histopathology :—

Using levaditi's method, histological sections of kidneys, & livers of 235 cattle revealed five positive cases (2.1%). The histological changes included fatty degeneration of livers and cloudy swelling of kidney with necrotic foci.

Pathogenicity :—

The inoculated Guinea pigs & hamsters with water isolates gave negative serological results.

However, the inoculated calves showed a mild positive reaction at titres 1:100. Haematological results revealed a significant ($P < 0.05$) decrease in erythrocytes counts (7.73 ± 2.3 to $4.9 \pm 0.07 \times 10^6$ mm^3). Leucocytes counts increased significantly ($P < 0.01$) (From 8.7 ± 1.12 to $11.9 \pm 0.1 \times 10^3/\text{mm}^3$).

At autopsy three weeks post inoculation, small haemolysis & necrotic foci were observed in the kidneys.

Discussion

This study shows that leptospirosis is prevalent in the Sudan in areas where relative humidity and rainfall are high, namely in Upper Nile, Equatoria, White Nile & Blue Nile and the percentage of positively reacting animals decreases as we proceed to the North until no reactors were encountered. Table 1.

There are 13 serotypes incriminated in causing leptospirosis in the Sudan with the exception of *L. cellidoni*. Table (2). The most prevalent is *L. hebdomadis* & *L. tarassori*. The detection of antibodies in wide spectrum can contribute to the infection of animals with different or many serotypes found in the surrounding environment, or may be due to interspecies reaction. This aspect had been confirmed by Malakhov (1974) Lobashenko et al (1974). They

stated that : the most of the positive cases in higher titres, 10-15% were due to leptospire which were not isolated from animals. And in case of serological reactions using MAT using 2-3 strains of leptospira, should be considered due to interspecies reaction and not mixed reaction.

The source of infection could be a carrier which in this study might be rodents, particularly in case of *L. hebdomadis*, *L. Tarassovi* & *L. Icterohaemorrhagiae* (Table 4). This form of leptospirosis is known as asymptomatic leptospirosis which is characterized by absence of clinical signs & presence of leptospira antibodies among carriers.

Water reservoirs or irrigating canals could also be a source of infection.

In this study leptospirosis was found to cause a remarkable reduction in RBC and a tremendous rise in leukocytes counts.

The leptospiral epidemiological process in the Sudan is characterized by complicated and contradicting factors: From one side the presence of naturally immune animals (15.3%), unfavorable high atmospheric temperature in hot seasons, the difficulty to overcome the contamination in such conditions and the indiscriminate use of antibiotics by cattle owners. From the other side, the extensive type of animal breeding migration of both domestic and wild animals and lack of aspecific veterinary rules made it difficult to give a definite epizootological character for leptospirosis in this country.

Table 1.

Proportion of Positively Reacting Cattle Sera

Blue Nile	19.18 %
Bahr El Gazal	15.43 %
Equatoria	30.0 %
Kassala	18.9 %
Khartoum	16.18 %
Southern Darfur	17.54 %
Southern Kordofan	13.79 %
Northern Darfur	0.0 %
Northern Kordofan	3.5 %
Upper Nile	38.33 %
White Nile	19.23 %
Red Sea	16.67 %
El Nil	4.2 %
Northern	7.9 %

Table II:

Etiologic structure of cattle leptospirosis in sudan

NN	Serogroups	Number of samples total	Number of samples with positive reaction 1:100 and higher % total		Relative prevalence of serogroups of leptospire %
1.	L. hebdomadis	1142	80	7	45.9
2.	L. trassovi	1142	48	2.5	27.5
3.	L. grippotyphosa	1142	8	0.4	4.5
4.	L. bataviae	1142	7	0.35	4.0
5.	L. cynopteri	1142	6	0.30	3.4
6.	L. icterohaemorrhagiae	1142	5	0.26	2.8
7.	L. pyrogenes	1142	5	0.26	2.8
8.	L. semeranga	1142	4	0.20	2.3
9.	L. australis	1142	4	0.20	2.3
10.	L. canicola	1142	3	0.14	1.7
11.	L. pomona	1142	2	0.10	1.1
12.	L. shermani	1142	2	0.10	1.1
13.	L. autumnalis	1142	1	0.06	0.6
14.	L. celledoni	1142	0	0.00	0.0
Total No of test.		/5998	175	11.87	100%

Table III:

The influence of meteorological factors on the seasonal dynamics of serological reactions
at cattle leptospirosis in sudan

Months Indices	III	IV	V	VI	VII	VIII	IX	X	XI	Correlation coefficient	P
Number of cattle reacting by MAT (%)	5.1	9.8	14.7	20.7	21.2	18.9	18.9	13.9	6.8	r = 0.54	P < 0.05
Atmospheric temperature	27.3	29.3	30.7	30.6	28.7	27.7	28.6	29.1	26.6		
Relative atmospheric humidity %	33.1	32.9	42.3	52.7	67.5	73.8	67.1	54.7	45.1	r = 0.82	P < 0.01
Amount of precipitation (rain- fall)	5.8	14.3	35.1	9.99	117.6	61.5	61.5	32.8	23	r = 0.89	P < 0.01

Table IV

Goats & Rodents Positively Reacting In Percentage

Serogroups	Rats	Goats
L. hebdomanis	53.6 %	0.0 %
L. icterohaemorrhagia	15.3	0.0 %
L. Canicola	7.6	0.0
L. tarassovi	7.6	0.0
L. Semerange	7.6	0.0.
L. Autumnals	7.6	0.0
L. Australis	0.0	13.3

Summary

A survey including 1142 cattle sera, 30 goat sera, 56 rodents and 26 water samples was done in all Provinces of the Sudan in an attempt to investigate the epizootiology of bovine leptospirosis in the Sudan. Simultaneously atmospheric temperature, relative humidity & the amount of rainfall in all the zones surveyed were recorded.

Further, 235 kidney & liver tissues and 207 urine samples were taken from cattle slaughtered at Omdurman slaughterhouse.

The survey was supplemented by an experiment using 10 Guinea pigs, 10 young hamsters & 6 calves 4-6 months of age. These animals were inoculated with leptospira isolated from the water samples. The isolate contained 200-250 microorganisms /400 magnification. Guinea pigs & hamsters were inoculated intraperitoneally in doses of 2,3,4, & 5 ml. of the isolate while the calves were given 10-15 mls subcutaneously.

Microagglutination test, dark field microscopy & culturing in Kortthof's media were done.

The serological studies revealed the presence of antileptospiral antibodies to 13 serological groups of leptospira in 15.3% of cattle sera studied. The predominating sera-groups were L. hebdomadis (45.9%) & L. tarassovi (27.5%).

Antibody fluorescent technique, bacterioscopy & histopathology methods applied to cattle sera showed 2.1 - 2.5% were carriers.

A highly significant correlation between the positive reactors and the climatic conditions (temp, humidity & rainfall) justifies the assumption that leptospira epizootics are found in the Southern provinces, they decrease northwards.

The presence of leptospira carriers and the absence of clinical symptoms in the seropositive animals support the assumption of the occurrence of asymptomatic form of cattle leptospirosis in the Sudan.

The strain isolated from water samples collected from irrigating canals of the Gezira induced a significant decrease of erythrocytes counts with a significant increase in leucocytes counts in experimental calves.

REFERENCES

- Amatredjo, A.; Cambell R.S. (1975): The Vet. Bull 42 (12) 875-891.
- Alston, J.M; Broom J.C. (1958): Leptospirosis in Man & animal.
- Anayin, V.V. (1951): Journal of Microb, Epid. Immuning No. 4, 33-36.
- Annual Vet. Report of the Ministry of Agric. Food & and Natural Res. (1902-1973).
- Awad, F.I. (1960): Zentrabl Veterinarmed 7, 420-423
- Bernkopf, (1946): Refuah Vet. 3, 49-54.
- Broom J.C. (1959): Disease due to bacteria Vol. I pp. 346-378.
- Brownlow W.J. (1964) L Am. J. Trop. Ned. Hgg. 13, 311-318.
- Burdin, M.L. (1957): Nature 179 (6) 1140.
- El Wali, A.A. (1978): Sbornik Nauschnik Trudof 108 pp.
- Fennestad.K.C. Berg-Petersen (1956): Vet. Med. 8, 815-833 & 882-886.
- Karasuva E.B, Anaayin V.V. (1956): Journal of Zoology pp 341-349.
- Lobashenka S.Y. (1964): Veterinaria No. 7 pp. 32-42.
- Lobashenka, S.Y. (1974): Veterinaria No. 4. pp. 48-54.
- Malakhov, Y.A. (1974): Veterinaria No. 4 pp. 59-61.
- Shigidi, M.T.A. (1974): Br. Vet. J. 130 (6) 528-531
- Shorokhov, V.V. (1969): Veterinaria No 7, 39-42.
- Sosov, R.F. (1974): Epizootology -Moscow (Kolos)
- Vasilyev, N.P. (1888): Ejenedelynik Klinichisko Gazeti 22, 23, 25, & 26
- Weil A. (1888): Peutsch Arch. F. Klin Med. Bd. 39, 5209



Leptospira in Kidney suspension from cattle
(Magnified-1 × 400)



Leptospira in Liver section from cattle (Magnified-1
× 400)