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LEPTOSPIROSIS IN DOMESTIC ANIMALS IN EGYPT

BY

A. Farid, M.E.Hatem and M.Refai

SUMMARY

Blood and tissue (renal cortex) samples were collected from 300 cows, 175 buffaloes, 400 pigs and 112 dogs. The blood samples were examined serologically for leptospiral agglutinins and the tissue samples were cultured into Ellinghausen's Fletcher's media. Antibodies against 13 leptospiral serotypes were found in 20.1% of the cases. The highest rate of positive reactors was found in dogs 34.5%, followed by pigs 31.3%, buffaloes 10.8%, and cows 5.3%, L. canicola was predominant in dogs, L. pomona in pigs, L. javanica in buffaloes and L. turassovi in cows.

L. canicola could be isolated from 10.9% of dogs and L. pomona from 2.5% of pigs. No isolates could be obtained from cows or buffaloes.

All isolates proved to be pathogenic to hamsters. Leptospires could be reisolated from the blood and demonstrated in the kidneys by means of the fluorescent microscope.

Leptospires were found to be sensitive to thionin, methyl, violet, crystal violet, brilliant green, malachite green, safranin, methylene blue and basic fuchsin. The minimal inhibitory concentrations

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From the Department of Microbiology, Faculty of Veterinary Medicine Cairo University.

of these dyes varied between 1.25 and 10 ug/ml. On the other hand, leptospires were resistant to 800 ug. rose-bengal per ml., while anthracoides and E. coli, the common contaminant of leptospiral cultures, were completely inhibited at this concentration. Rose-bengal may thus have a promising role as a selective agent for the isolated of leptospires.

INTRODUCTION

In Egypt, few reports dealing with leptospirosis in animals were published since 1957. Most of these reports serological positivities, while isolation trials were for the most part unsuccessful. McGuire and Myers (1957) detected complement fixing antibodies to leptospiral antigens in 5 out of 30 dog-sera. Hamdy et al. (1962) reported titres against L. autumnalis, L. bataiae, L. canicoa, L. icterohaemorrhagia and L. malaya in buffalo and cattle sera. Hamed (1968) reported leptospira incidence of 15.3%, 24%, and 1.7%, in dogs, swine and buffaloes, respectively L. canicola was the predominant reactor serotype with dog sera, while swine sera reacted with L. icterohaemorrhagia only. Marompot et al. (1971) detected leptospiral antibodies in 25% of stray dogs in Cairo. They could isolated two strains of L. canicola from the urine of the dogs.

The present work is dealing with serological examination of cows, buffaloes, pigs, and dogs for leptospiral antibodies, isolation of leptospires from pigs and dogs and sensitivity of leptospires against different dyes.

MATERIALS AND METHODS

Blood samples were collected from 300 cows, 175 buffaloes, 400 pigs and 110 dogs just before or during slaughtering in the first 3 species of animals and by vein puncture in dogs. All sera were examined by the microscopic agglutination test (Alexander et al., 1970) using actively growing cultures of 16 leptospiral strains (Table 1).

Isolation of leptospires was tried in all cases mentioned. For this purpose small pieces of both kidneys, just after slaughtering or killing were taken by a sterile tube (140 X 6 mm) with sharp edge which was inserted in a rotating manner in the renal cortex. The samples were inoculated into Ellinghausen's and Fletcher's media (Difco) and the tube were incubated at 37°C for the first 24 hours. Subcultures made on fresh media and incubated at 30°C for 4-7 weeks. The isolated leptospires were identified on the base of their motility, morphological characteristics. Serological typing was carried out by the microscopic agglutination test (Galton et al., 1962) using 18 different standard leptospira hyperimmunosera (Difco).

The sensitivity of L. ictohaemorrhagia, L. canicola and L.pomona as well as two commonly contaminating bacteria, namely E. coli and B. anthracoides, was tested against various concentrations of 13 different dyes (Table 2). Serial two-folds dilutions of each dye were made in Ellinghausen's and Stuat's media.

RESULTS

Serological examination:

Antibodies against 13 serotypes of leptospires were demonstrated in titres of 1 : 50 and more in 198 out of the 985 (20.1%) sera examined. 69.7% of the positive cases had significant titres (1 : 200).

The highest rate of positive reactors (34.5%) was found in dogs. More than half of the positive cases had antibodies against L. canicola, The remaining cases had antibodies against L. icterohaemorrhagia, L. pyrogenes and L. javanica (Table 1).

Antibodies against 6 serotypes of leptospires could be detected in 125 out of the 400 (31.3%) swine sera examined. L. pomona was the most predominant sero-type (58.4%), followed by L. canicola, L. tarassovi, L. petoc, L. icterohaemorrhagia and L. butembo.

In buffaloes, agglutinins against 7 serotypes were demonstrated in 19 out of the 175 (10.8%) sera examined. L. javanica was the predominant reactor serotype.

Cows showed the lowest (5.3%) rate of sero-positivity L. tarassovi was the most common.

Isolation of leptospires:

From the kidney of 985 animals examined, 22 isolates of leptospires could be recovered, of which 12 were obtained from 110 dogs (10.9%) and 10 from 400 pigs (2.5%). No isolates could be obtained from bovine

kidney.

All dogs isolates were found to be members of the canicola serogroup, while all swine isolates were identified as L. pomona. All the 22 isolates were obtained with the help of Ellinghausen's media, while only 17 isolates could be isolated by Flecher's medium.

All isolates caused death of hamsters 6-9 days after intraperitoneal injection of 0.5 ml of broth cultures of leptospires. Leptospires could be easily isolated from hamster's blood and demonstrated in impression smears from the kidneys by means of fluorescent microscopy using fluorescein labelled anti L. canicola and L. pomona sera.

Sensitivity of leptospires to dyes:

All of the three leptospiral serotypes behaved the same towards alldays tested and no differences were observed when different media were used. Moreover, the inhibitory effect of the dyes as not influenced by autoclaving or filtration with millipore filter.

From Table (2), it is evident that leptospires are much sensitive than E. coli and B. antaracoides to safranin, basic-fuchsin, methylene blue, crystal-violet, brilliant green and malachite green. On the other hand, all tested organisms were almost resistant to orange G., nigrosin and trypan-blue.

Of all dyes tested, rose bengal showed the most interesting results asleptospires seem to be fairly resistant to it while both types of contaminating bacteria were inhibited by lower concentrations than those which inhibited leptospires.

Table 1: Serological survey of leptospiral agglutinins in the sera of animals

Serotypes	Dogs		Pigs		Buffaloes		Cows		Total	
	P.R.	S.T.	P.R.	S.T.	P.R.	S.T.	P.R.	S.T.	P.R.	S.T.
L.canicola	22	16	20	14	2	1	-	-	44	31
L.pomona	-	-	73	53	-	-	-	-	73	53
L.ictero-haemorrhagiae	7	6	8	5	-	-	-	-	15	11
L.tarassovi	-	-	10	7	-	-	6	5	16	12
L.javanica	5	3	-	-	5	4	4	3	14	10
L.grippo-typhosa	-	-	-	-	2	2	3	2	5	4
L.pyrogenes	4	3	-	-	1	-	2	2	7	5
L.wolffi	-	-	-	-	3	2	-	-	3	2
L.butembo	-	-	5	3	-	-	-	-	5	3
L.hardjo	-	-	-	-	3	2	-	-	3	2
L.borincana	-	-	-	-	3	2	-	-	3	2
L.bratislava	-	-	-	-	-	-	1	-	1	-
L.patoc	-	-	9	3	-	-	-	-	9	3
L.autumnalis	-	-	-	-	-	-	-	-	-	-
L.castellonis	-	-	-	-	-	-	-	-	-	-
Total	38	28	125	84	19	14	16	12	198	138
%	34.5	25.4	31.3	21.3	10.8	7.4	5.7	4.0	20.1	14.0

P.R. = Positive reactors, titres of 1:50 and more.

S.T. = Significant titres, titres of 1:100 and more.

Table 2: [illegible] growth inhibitory concentration of dyes (µg/ml) for leptospires, B. anthracoides and E. coli

Dyes	Minimal growth inhibitory concentrations µg/ml		
	Leptospires	B. anthracoides	E. coli
Crystal violet	1.25	200	50
Methyl violet	2.5	400	200
Brilliant green	2.5	400	200
Malachite green	2.5	400	200
Methylene blue	2.5	400	200
Thionin	2.5	400	400
Basic fuchsin	10.0	800	200
Safranin	10.0	1600	800
Orange G	800	1600	1600
Erythrocin	800	1600	800
Trypan blue	3200	3200	3200
Nigrosin	3200	3200	3200
Rose bengal	1600	800	400

The repeated subculturing (6 times) of the three leptospires in Ellinghausen's medium containing 800 microgram rose-benal per ml revealed no change in the density or motality of leptospires.

The subculturing of experimentally contaminated cultures of L. canicola with B. anthracoides, E. coli or both showed that L. canicola could be purified by means of rose-bengal containing medium.

DISCUSSION

The demonstration of leptospiral antibodies in 34.5% and the isolation of L. canicola from 10.9% of the examined dogs indicate that stray dogs in Egypt are carriers of leptospires and they can dissiminate these organisms through the urine. Such dogs represent an actual hazard to man and animals. It is interesting to note that antibodies against L. canicola, the most common canine leptospira were found, not only in the sera of swine and buffaloes in the present study, but also in human sera (Barsoum et al., 1972; Marenpot and Barsoum, 1972 and Hatem,1976).

L. pomona was the predominant reactor serotype in pigs. This serotype could be isolated from pigs for the first time in Egypt. This is of particular interest as man and several animals are fully susceptible to infection with L. pomona. Blood and Henderson (1974) considered L. pomona as the most common cause of leptospirosis among animals. Unfortunately, pigs are generally ignored in the overall scheme of animal disease control in Egypt. However, the high frequency of sero-positivity

to a wide range of serotypes may signify the importance of these animals as possible source and reservoirs of different leptespires.

The reactivity of swine sera to L. patoc needs further study, as this serotype is considered non-pathogenic, therefore, it cannot be decided whether the antibodies against L. patoc demonstrated in this study at titres of 1 : 50 to 1 : 800 are specific; i.e., produced by L. patoc or non-specific due to cross-reaction with other pathogenic leptospires.

The incidence in cows and buffaloes was comparatively lower than in dogs and pigs. Buffaloes showed, however, the widest spectrum of serotypes, as agglutinins could be detected against 7 leptospires. It should be, however, noted that L. wolffi and L. berlinensis are antigenically cross-reacting. It is therefore difficult to decide whether one of them is the actual cause of these agglutinins or all are incriminated.

From the results obtained in this work, the dyes can be classified according to their effect into 3 groups. The first group comprising thionin, methyl violet, brilliant green, malachite green, safranin, methylene blue and basic-fuchsin inhibited leptespires in low concentrations and may be of interest in the treatment or disinfection of leptospires. The second group, of orange G., erythrocin, trypan blue and nigrosin is of no significance ofor leptospires or the contaminating bacteria. The third group, consists only of rose bengal which is of particular importance as all three serotypes of leptospires tested tolerated 800 microgram per ml. while the anthracoides and E. coli were completely inhibited. Accordingly, rose-bengal may have a premising role as

a selective agent for the isolation of leptospires.

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