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STUDIES ON THE IDENTIFICATION OF LEPTOSPIRAL ISOLATES RECOVERED FROM KIDNEYS OF DOGS AND PIGS IN EGYPT

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SUMMARY: Twenty two leptospiral isolates recovered from kidneys of 522 pigs and stray dogs in Cairo city were subjected to bacteriological and serological identification. Using the microscopic agglutination (M.A.) test and 18 standard leptospira hyperimmune sera. It was shown that all leptospiral isolates recovered from dogs belonged to serogroup Canicola and all isolates recovered from pigs belonged to serogroup Pomona. Both canine and swine isolates were sent to the WHO/FAO Leptospirosis Reference Center, Rome, Italy for confirmation and complete identification. Using the cross - agglutinin - absorption test, it was proven that all of the 12 canine isolates related to serogroup Canicola servar *canicola*. The 10 swine isolates were all belonging to serogroup Pomona serovar *pomona*.

INTRODUCTION

The importance of leptospirosis in man and domestic animals is increasingly realized throughout the world. It is considered as one of the world's most widespread zoonosis affecting man and animals (Van der Hoeden, 1964). In Africa, leptospirosis has been reported in several countries e.g. Sudan, Egypt, Kenya, Uganda, Rhodesia, Cape Town and Ethiopia (Szatalowica et al., 1969, Moch et al., 1975, Hatem, 1976 and 1979).

In Egypt, re ently Farid et al. (1982) reported the isolation of 22 leptospiral isolates from kidneys of pigs and stray dogs in Cairo. These isolates were sent to the WHO/FAO Leptospirosis Reference Center, Rome, Italy for confirmation and complete identification. The present study reports

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the bacteriological and serological idnetification of the recovered leptospires.

MATERIALS AND METHODS

1. Isolated leptospiral strains

22 leptospiral isolates were obtained from the renal cortex of dogs (12 isolates) and pigs (10 isolates) using the method described by Hatem (1979). These were originally isolated in Ellinghausen's liquid medium (EMJH , Difco) and Fletcher's semi-solid medium (Difco) plus 10 % normal rabbit serum. Subcultures were made weekly in EMJH medium. These leptospiral isolates were preliminary identified according to their cultural and morphological characteristics. Each isolate was also subjected to preliminary serogroup typing using the microscopic agglutination (M.A.) test (Galton et al., 1962) using 18 different standard leptospira hyperimmune sera (Difco). All isolates were then sent to the WHO/FAO Leptospirosis Reference Ceneter, Rome, Italy for confirmation and further identification.

2. Agglutinin-absorption test

This was the main test used for leptospiral serovar-identification. The method proposed by Kmety et al. (1970) for the agglutinin-absorption test was adopted.

3. Standard leptospiral strains used in the agglutinin-absorption test

The following leptospira reference strains were obtained from the National Collection of Leptospira (Babudieri, 1972): Bianchi 1, Wijnberg, Alarik, Mezzano I, Pavia 1, Moskova V, Riccio, 37, Ballico, Zanoni, Mus 24, Topo 1, Sari, Castellon 3, Mitis Johnson, Poi and Riccio 2. These standard strains, as well as the Egyptian leptospiral isolates were propagated and maintained in Korthoff's modified medium (Babudieri, 1961) containing 5 % normal rabbit serum. Cultures were incubated at 32 °C and were transferred every 10-15 days using 0.3 ml inoculum per 5 ml fresh medium V/V.

4. Preparation of immune sera

Two rabbits were immunized against each strain with three intravenous injections of 2 ml live well grown leptospiral culture prepared from each individual strain or isolate. 3 injections were given at days 1, 5 and 8, with the sera being collected 5 to 10 days after the third inoculation. The titres of the immune sera collected to perform the agglutinin-absorption test were between 12.800 and 102,400 (reciprocal of the highest dilution showing 50 % or more agglutination of the homologous strain) as measured by the microscopic agglutination (M.A.) test of Galton et al. (1962).

RESULTS

Isolated leptospires showed typical growth in Ellinghausen's medium with slight turbidity starting 3 - 7 days after inoculation. Each individual tube showed typical leptospiral morphology, motility and density when examined microscopically under the dark ground microscope. In Fletcher's semi-solid medium a linear white disk (ring) was noticed 1-2 cm below the medium surface. Leptospires were detected microscopically when a drop from this ring was examined.

Serological examination of liquid cultures prepared from canine isolates proved that all of them belonged to serogroup Canicola. In the meantime,all of the swine isolates were belonging to serogroup Pomona. These results were confirmed at the WHO/FAO Leptospirosis Reference Center, Rome Italy.

Table 1 shows the results of agglutinin-absorption test performed on leptospiral isolates recovered from dogs. It is evidnet that all of the 12 recovered canine leptospiral cultures are related or identical to strain Alarik serogroup Canicola serovar *canicola* . On the other hand,Table 2 reveals that the recovered swine leptospiral isolates are all related to strain Mezzano I, serogroup Pomona serovar *pomona*.

Table 1: Agglutinin-absorption test amongst reference strains and isolated canine strains.

Immune serum against	Absorped with	Agglutinin titres[1] with strain: Alarik	D_1@	D_2	D_3	D_4	D_5	D_6	D_7	D_8	D_9	D_{10}	D_{11}	D_{12}
Alarik[2]	-	102400	25600	51200	26500	51200	25600	25600	25600	51200	51200	51200	51200	51200
Alarik	D_1	1600	100	100	100	100	-	100	100	100	100	100	100	100
D_1	-	409600	102400	102400	102400	102400	102400	120400	102400	102400	102400	102400	102400	10240
D_1	Alarik	200	-	100	200	100	-	-	100	100	100	-	-	100

@ D = Dog - isolate

(1) = Reciprocal value of the highest serum dilution showing 50 % or more agglutination of leptospires. Dashes represent negative reactions at 1:100 dilution.

(2) = Strain Alarik, serogroup Canicola, serovar

Table 2: Agglutinin-absorption test amongest reference strains and isolated swine strains.

Immune serum against	Absorbed with	Mezzano I	S_1@	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}
Mezzano I(2)	-	25600	51200	25600	51200	51200	51200	12800	51200	25600	25600	25600
Mezzano I	S_1	12800	100	-	100	-	-	100	-	100	200	-
S_1	-	102400	51200	25600	102400	51200	51200	25600	102400	51200	12800	12800
S_1	Mezzano I	-	200	200	200	200	200	200	200	200	200	200

@ S = Swine - isolate

(1) = Reciprocal value of the highest serum dilution showing 50 % or more agglutination of leptospires. Dashes represnet negative reactions at 1 : 100 dilution.

(2) = Strain Mezzano I, serogroup Pomona, serovar

DISCUSSION

The recovery of *Leptospira canicola* from stray dogs in Cairo, documented in the present study, is the second report of isolation of *L. canicola* in Egypt. The first report was published by Maronpot et al. (1971) reporting the isolation of 2 leptospiral agents from the urine of 68 stray dogs. They identified these two isolates to be *L. canicola*. The present identification of the 12 isolates recovered from kidneys of apparently healthy stray dogs to be belonging to serogroup Canicola serovar *canicola* could be of great value in the epizootology of canine leptospirosis in Egypt. This also indicates the presence of serovar *canicola* as the most common etiology of canine leptospirosis in this area. Moreover, this clearly reflects the dangerous role played by these uncontrolled animals as carriers of *L. canicola* which is known to be pathogenic to other animals and man. This finding is parallel with that of Alston and Broom (1958) who stated that 30 - 40 % of apparently healthy dogs may show serological evidence indicative of former active infection or of a symptomless carrier state.

On the other hand, the isolation of *Leptospira pomona* and the identification of the recovered isolates to be belonging to serogroup Pomona serovar *pomona*, reported in the present study, is the first report of recovery of a member of this serogroup in Egypt. Pigs are considered as one of the msot important domestic host-reservoirs for the dissemination of leptospirosis to man and animals (Myers et al.,1973). However, swines are generally ignored in the overall scheme of animal disease-control in Egypt (Maronpot and Barsoum, 1972). The present isolation of *L. pomona* from pigs reflects the importance of considering pigs as current reservoir of this organism. Similar findings were previously reported by Kirschner et al. (1952) who suggested that pigs should be kept completely separate from cattle to avoid leptospiral infection. Moreover, Michna (1970) reported that the subclinical infection with leptospirosis was commonly seen in pigs which remain as healthy carriers.

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