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Note

A Brief Report on the Survey of Leptospirosis in Iran (*)

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Leptosiprosis in Iran and many other countries is an important zoonosis from public health point of view, and its significant economic losses to livestock owners is evident.

During the past 10 years, considerable attention has been directed to the study of leptospiral infection by many workers in various parts of the world. Our knowledge regarding the existence of leptospirosis in Iran has been obtained during the past decade by research works carried out in the Razi Institute.

We have been able to find rather important foci of this disease among cattle and sheep by serological studies as well as by isolation of Leptospira grippotyphosa from cattle, sheep and man (1, 2, 3).

This study has been limited to area around the Razi Institute, Tehran and some parts of Mazanderan, but it is obvious that the disease is more important in northern part and other regions of Iran where rice is cultivated.

MATERIALS AND METHODS

SEROLOGICAL TEST — Tests designed to detect specific leptospiral antibodies in blood sera have helped us to show the existence and prevalence of leptospirosis in animals. The microscopic agglutination -lysis test with 7-12 day-old living cultures in Korthof's medium has been employed as the method of choice.

SEROTYPES — Blood serum collected from animals which did not show any apparent symptoms of disease were examined for antibodies against 5 serotypes of leptospirae: L. pomona, L. grippotyphosa, L. hyos, L. canicola, L. icterohaemorrhagiae.

TEST SERA — All sera were inactivated at 56°C. for 30 minutes and were initially screened at 1:100 dilution (mixing 0.1 ml amounts of serum diluted 1:50 in saline, and 0.1 ml amounts of 7-12 days old living cultures of different serotypes of leptospirae).

READING — The reactions were read microscopically by dark-field illumination after incubation of one hour in 37°C. water bath. A serum was considered positive if 50% agglutination or lysis occured with serotypes used in the test. Those that showed positive reactions were then titrated using higher dilutions to determine the end point.

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ISOLATION — The isolation of leptospirae was attempted by injecting intraperitoneally into young guinea-pigs, immediately after collecting materials from fresh samples of blood, milk, organs and urine, or by direct culture of blood from sick animals and organs from dead bodies into Fletcher's semi-solid medium. For each sample we used 4 tubes containing 4-5 ml of culture medium.

RESULTS:

A — The result of serological survey is summarized in the table below.

Results of serological survey

Animal	NO. of serum examined	Antigen of Leptosp.	Titres of positive serums									Total positives
			1/100	1/300	1/1000	1/3000	1/10000	1/20000	1/40000	1/60000	1/100000	
Cattle	2708	pomona	29	–	12	–	3	–	–	–	–	44
		g.typho.	234	3	180	5	76	54	8	14	3	577
		hyos	120	12	46	–	3	–	–	–	–	181
		canicola	3	–	–	–	–	–	–	–	–	3
		ict.haem.	5	–	–	–	1	–	–	–	–	6
												811
Sheep	889	pomona	18	6	33	11	5	–	–	–	–	73
		g.typho.	4	–	1	1	–	–	–	–	–	6
		hyos	2	3	–	–	–	–	–	–	–	5
		ict.haem.	2	–	–	–	–	–	–	–	–	2
												86
Camel	5	ict.haem.	–	–	1	–	–	–	–	–	–	1

As shown in the table, 811 out of 2708 sera of cattle(around Tehran, Hessarak and Khorassan)reacted particularly against L.grippotyphosa(29%)

As shown in the table ,811 out of 2708 sera of cattle (around Tehran, Hessarak and Khorassan) reacted particularly against L. grippotyphosa (29%) of which 80% of reactors were from the herds around Tehran. Among 889 sera of sheep examined (around Mazanderan. Karadj and Khorassan) 86 sera (9.7%) were positive particularly with L. pomona in which 95% of reactors were from Mazanderan area.

By this investigation we have found a high titre of leptospiral antibodies in livestock population around Tehran (with L. grippotyphosa, L. hyos and L. pomona respectively) and Caspian Sea area (mostly with L. pomona).

One of 5 camels around Tehran which had aborted 15 days before serum examination and was showing haematuria, showed reaction with L. icterohaemorrhagiae.

None of the sera collected from 149 goats, 148 wild rodents (Mus musculus, Cricetus sp., Nesokia sp., Meriones sp., and Citellus sp.), 6 dogs, 3 jackals, 4 hedgehogs, 9 snakes (cobra), 18 men (16 from Langroud, 1 from Shiraz, 1 from Pahlavi hospital of Tehran) showed any leptospiral antibodies against 5 serotypes used in the test.

By our investigation and examining cloaca content of 11 water turtles from Ramsar (north part of Iran) and around the Razi Institute, numerous unknown leptospira could be detected. 6 out of 11 sera were positive to L. hyos with a 1:200 titre,

but attempts failed to isolate the organisms by culture and inoculation of their blood and homogenate kidneys into laboratory animals.

B — Isolation of Leptospira grippotyphosa:

1) Cattle — It was in June 1959 that we had the opportunity to study an outbreak of leptospirosis among a herd containing 50 cows and 30 calves in the village of Hessarak (near the Razi Institute).

During 3 weeks, 10 calves and 11 cows became sick with symptoms of hyperthermia, icterus, haemoglobinuria, alteration of milk appearance from white to a yellowish colour. Five cows aborted and 7 calves died.

We examined the blood smears from sick animals as well as the smears made from internal organs, but no blood parasites could be detected.

The blood of one sick cow showing high temperature (41, 2°C.) was inoculated into Fletcher's semi-solid medium and also 1 ml of blood was injected intraperitoneally into 2 young guinea-pigs.

After 6 days incubation of the cultures at 28°C., leptospirae were detected in the cultures.

The temperature of the inoculated guinea-pigs rose to 41.2°C. and 40.2°C. on the 5th and 6th day post-inoculation respectively, which lasted 3 days in the first animal and 4 days in the second one. By direct cultures of the blood of these guinea-pigs, when showing high temperature, into Fletcher's semi-solid medium leptospirae were detected after 9 and 12 days incubation at 28°C.

Other cases of isolations of leptospirae from sick and dead calves of this herd were performed and after typing with different immune sera, we have found that the isolated strains were L. grippotyphosa.

In July 1959, one sick cow from village of Heydar-abad (near Hessarak) which was showing anorexia, icterus, reduction of milk production which was yellowish, and rectal temperature was 37.6°C., was brought to the Razi Institute for diagnosis of the disease. In blood smear no Anaplasma, Babesia or other parasites could be detected. By injecting intraperitoneally 5 ml of milk, collected freshly, into each of 2 guinea-pigs, we were albe to isolate leptospirae in the cultures of Fletcher's medium inoculated with the blood of these guinea-pigs when showing high temperature.

In another case, in September 1959, a bull aged 5 years, from village of Sofi-abad (3 km. north-east Hessarak), was brought to the Razi Institute for diagnosis of the disease. This animal was not showing icterus but, it was presenting high temperature (40.2°C.) and severe haemoglobinuria. The smears made from blood did not show any parasites, but in the urine freshly collected and examined under dark-field illumination, a few organisms resembling leptospirae were detected. By injecting the blood into 2 guinea-pigs and Fletcher's medium we were not able to isolate leptospirae. But injection of fresh urine into 2 guinea-pigs and direct cultures of their blood, when showing high temperature, in Fletcher's medium resulted in isolation of leptospirae.

2) Sheep — In August 1959 an outbreak of leptospirosis occured among a flock containing 50 sheep and 13 goats in the village of Sofi-abad. During one week of this outbreak 9 sheep died, with the symptoms of haemoglobinuria, in 24 hours from the onset of the disease. After autopsy of one sheep we made smears from blood and internal organs, but no blood parasites or anthrax bacillus were detected.

By injecting ground liver and spleen into 2 guinea-pigs we succeeded to isolate

leptospirae from these guinea-pigs by culturing their blood into Fletcher's medium when showing high temperature.

Four sick animals as well as the rest of this flock were treated successfully by injecting a mixture of Penicilline and Streptomycine. After this treatment, no more sick animals were seen in this flock.

In another outbreak, in September 1959, by direct culture of bon-marrow from one sick sheep showing icterus and haemoglobinuria (from the village of Hessarak which had been sacrificed by the owner) into Fletchers' medium the leptospirae were isolated. 3) Human —In Iran, to our knowledge, not any case of human leptospirosis had been recorded. In the first outbreak of bovine leptospirosis from the village of Hessarak we had the opportunity to study one case of human leptospirosis.

Seifollah Kamal-rousta, 40 years old, who was the owner of a herd of cattle (in the village of Hessarak) became sick after the outbreak of leptospirosis in his herd. During this period, he had skinned 3 calves that had died from this herd. At first he was showing inappetence which lasted for 4 days, and then he showed sudden onset of chills ,fever (40°C.), severe headache, myalgia, vomiting and upper abdominal pain. His urine was dark yellowish in colour. Blood obtained on the 3rd day after onset of illness was inoculated immediately into 2 guinea-pigs each with 2 ml, and 3 drops of blood also was inoculated into each of 4 tubes containing Fletcher's medium.

On the 6th day of inoculation we examined the cultures microscopically by dark-field illumination and leptospirae were detected in the cultures. The 2 inoculated guinea-pigs showed high temperature on 5th and 6th day postinoculation. By culturing their blood into Fletcher's medium, when showing high temperature, leptospirae were found in the cultures.

The paitent recovered after the treatment rendered to him by his physician.
TYPING OF THE STRAINS -Typing of all the isolated strains was carried out by using different immune sera and agglutination-lysis test, and all of them found to be L. grippotyphosa. Sera from man and animals (sheep cattle) recovered from the disease were tested against 5 serotypes of leptospires (L. pomona, L. grippotyphosa, L. canicola, L. hyos, L. icterohaemorrhagiae). All showed reactions with L. grippotyphosa.

In order to confirm the identity of strains identified by us, they were sent to Professor Broom (The Welcome Laboratories of Tropical Medicine, London), and were proved to be correctly identified.

As shown in this report, economic and public health aspect of leptospirosis have created problems which demand cooperation amongst farmers, veterinarians, public health workers, and physicians to make plan for studying the disease from epidemiologisal, epizootological and wild-life vectors points of view, and the effort to control and study this disease should be extended to various parts of the country.

SUMMARY

Leptospirosis in Iran has been investigated for the first time at the Razi Institute. Serological tests in animals showed prevalence of L. grippotyphosa, L. hyos, and L. pomona around Tehran, and L. pomona in the northern part (Mazanderan) of the country.

L. grippotyphosa is isolated from sheep, cattle and man around the Razi Institute

(48 km. north-west Tehran). Suggestions have been made to more comprehensive study of the disease in all parts of Iran in future.

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