OBSERVATIONS ON THE SURVIVAL OF *LEPTOSPIRA AUSTRALIS* A IN SOIL AND WATER

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It has been recognized for many years that water is a common agency by which pathogenic leptospirae are disseminated, and infection transferred from animal carriers to human beings. Very little detailed knowledge has been accumulated, however, on the various factors affecting the survival of pathogenic leptospirae in water or in wet or moist environments, and on the differences in behaviour exhibited in this regard by different serotypes.

Leptospira icterohaemorrhagiae, upon which most observations have been made, appears to be markedly sensitive to changes in pH away from neutrality. It may survive in river water pH 7·0–7·1 for 5 or 6 days at 27° C., but is adversely affected by higher temperatures (Chang, Buckingham & Taylor, 1948). However, some observers have suggested that other pathogenic leptospirae, perhaps less sensitive to environmental conditions, may survive for longer periods and even multiply in natural waters or mud. Babudieri (1953) considers that the highly infective nature of the water of certain Italian rice fields can only be explained by such means.

The increased incidence of leptospirosis in coastal North Queensland following rain, and the preponderance of cases in close proximity to rivers and on low-lying river flats noted by Derrick and his colleagues (Derrick, Gordon, Ross, Doherty, Sinnamon, MacDonald & Kennedy, 1954), clearly indicates the importance of contact with water in the transmission of the disease in this area. These natural waters may be infected directly with leptospirae from the urine of carrier animals, or possibly in an indirect manner if the leptospirae can survive in the soil after being excreted, until surface pools and streams are formed after rain.

This paper records the results of an investigation designed to test the ability of *L. australis* A, a common cane-field serotype, to survive in moist earth and in water.

MATERIALS AND METHODS

The experiments were designed to simulate within certain limits a sequence of events which might occur naturally, namely, the infection of soil with urine from a rat excreting leptospirae, and the formation of an overlying pool of water during heavy rain. In the first instance the soil was infected by the addition of a leptospiral culture. In later tests the cosmopolitan house-rat *Rattus rattus*, experimentally infected with a newly isolated strain of leptospirae, was used as the infecting agent.

Origin and nature of the soil

The soil was obtained from a sugar-cane farm on an alluvial flat bordering the South Johnstone river, North Queensland. The average annual rainfall for the district is 142 in.

Blocks of earth were carefully removed with a minimum of disturbance to the surface debris. Their moisture content when received in Brisbane was approximately 30 % by weight. They were cut to a thickness of about 10 cm., shaped, and packed in a single layer in the bottom of cylindrical glass vessels 23 cm. in diameter and 38 cm. deep. A small amount of rain water was added in the process to consolidate the soil and restore it to a fully moist condition. The jars were then covered with sheets of plate glass.

A report on the soil profile at its place of origin states *inter alia*: 'The profile to a depth of 120 cm. is distinctly demarcated into two horizons; an upper horizon of 50 cm. and a lower horizon of undetermined depth. The upper horizon represents recent flood deposits and the soil here may be classed as a good silt loam. It exhibits a rather mealy structure with a soft consistency. Colour varies with moisture from a light grey (dry) to a light brown (wet).' The soils in the coastal districts of North Queensland are in general of an acid character. This particular soil had a reaction of pH 6·1–6·2.

Selection of experimental serotype

L. australis A was chosen for use in these experiments because of its striking association with the canefields (Derrick *et al.* 1954). As animals were used for testing the viability of the organisms in the soil, virulent cultures were of necessity employed. Two strains, 'Gauci' and 'Brilli', were used within 1 month of their isolation from cane-workers. The former strain was employed when cultures were added directly to the soil, and the latter was used to infect the experimental rats, the source of infection in the other tests.

Selection of experimental carrier

Three rats (*Rattus rattus*) trapped in Brisbane were inoculated intraperitoneally with 0·5 ml. of a culture of the Brilli strain. Their freedom from natural infection had previously been demonstrated by negative serum and urine tests.

Leptospirae were first seen in their urine on the tenth day after inoculation, and samples collected subsequently produced typical infections in guinea-pigs into which they were inoculated. Leptospirae were seen in abundance in the urine of two rats up to their accidental death under anaesthesia on the eighteenth and fortieth days. The third rat also excreted many active leptospirae in its urine up to the seventy-seventh day when observations were discontinued.

The constant excretion of large numbers of active leptospirae by these rats contrasted strongly with the behaviour of four albino rats of a strain of *R. norvegicus*, bred in the laboratory. These rats were inoculated with the Gauci strain and all became infected. Their serum after 3 months contained antibodies against *L. australis* A in titres ranging from 1–300 to 1–3000. However, leptospirae were seen in the urine of only one of them, and then only in scanty numbers on the fifteenth and twenty-sixth days. They were never seen in the urine of the other three rats, although a sample taken from one of them on the thirtieth day produced a typical infection in a guinea-pig into which it was inoculated. In the

laboratory both species of rat were fed on a vegetable diet and their urine was invariably alkaline in reaction (pH 7·7).

From these observations *R. rattus* appears capable of acting as a carrier of *L. australis* A under laboratory conditions. It was used in this role in our experiments.

Isolation of leptospirae from the soil

Leptospirae were recovered from the soil by flooding it with rainwater and subsequently examining the water by the 'subcutaneous stream' method of Van Theil & Veer (1941).

Guinea-pigs of 200–250 g. weight were considered most suitable. The hair was clipped from the abdomen and the skin was perforated on one side to admit a glass cannula. An opening 1 cm. in length was made in the skin on the opposite side and the skin freed from the abdominal wall by blunt dissection to form a subcutaneous tunnel connecting the cannula and the point of exit. With the animal tied on its back, under light anaesthesia, the water was introduced from a funnel via a 'transfusion drip' delivering 100 ml. in 30 min.

A daily record was made of the temperatures of the experimental guinea-pigs. Animals which remained afebrile for 14 days were sacrificed and a saline suspension of liver and kidney was injected into a second animal, which was also retained for 14 days and examined serologically before a negative result was recorded.

Proof of leptospirosis was based on the occurrence of a febrile reaction, classical post-mortem appearances, the demonstration of active leptospirae in wet preparations of the liver examined with dark-field illumination and on the culture of the infecting organism from the heart blood. In all recorded infections the first two and at least one, if not both, of the latter criteria were satisfied. Several blood cultures failed owing to contamination. The leptospirae recovered from the soil in each experiment were typed serologically and found to be of the serotype *L. australis* A.

A control experiment in which no leptospirae were added to the soil demonstrated their absence from the soil and water used in the investigation.

EXPERIMENTAL RESULTS

Soil infected with cultures

Twenty ml. of a 7-day culture in Fletcher's medium were added to the soil in each of three jars A, B and C. The culture was poured into several shallow furrows in the soil and the surface was then levelled. The jars were held in the laboratory away from the direct rays of the sun and covered with a sheet of glass.

After 8 days rain water, collected in Brisbane, was poured into jar A, saturating the soil and covering it to a depth of 3–5 cm. The soil in jars B and C was treated in a similar manner after intervals of 15 and 43 days respectively from the time of adding the cultures. About 3 l. of water were added in each instance.

Water samples were removed from the jars at intervals ranging from 3 to 24 days after the flooding of the soil and examined for leptospirae by the 'subcutaneous stream' technique. Details of these experiments are summarized in Table 1.

Table 1. *The survival of* Leptospira australis *A in soil infected with leptospiral cultures*

Soil in jar	Soil moisture (%)	Days in soil pH 6·2	Days after addition of water	pH of water added	pH of water removed	'Subcutaneous stream' test	No. of test animals	No. of test animals infected	Incubation period (days)
A	Not determined	8	3	6·5	6·6	100 ml. in 30 min., 1 150 ml. in 60 min., 1 250 ml. in 60 min., 1	3	3	5
A	Not determined	8	17	6·5	6·6	100 ml. in 30 min., 1 100 ml. in 30 min., 1 1 ml. inoculated, 1 2 ml. subcutaneously, 1	4	4	6 7 (3 animals)
A	Not determined	8	24	6·5	6·6	100 ml. in 30 min., 1 100 ml. in 30 min., 1	2	2	7 8
B	Not determined	15	3	6·5	6·6	250 ml. in 60 min., 1	1*	1	5
B	Not determined	15	10	6·5	6·6	100 ml. in 30 min., 1 120 ml. in 30 min., 1	2	2	8 7
C	34	43	3	6·9	6·9	200 ml. in 60 min., 1 300 ml. in 60 min., 1 3 ml. inoculated subcutaneously, 1	3*	1	7 — —
C	34	43	10	6·9	Not determined	100 ml. in 30 min., 1 200 ml. in 60 min., 1	2*	—	—
C	34	43	17	6·9	Not determined	100 ml. in 30 min., 2	2	—	—

* A total of five animals which died within 48 hr. of the experimental procedure from intercurrent infections are excluded from these figures.

(a) Survival in soil for 8 *days*

Water was removed from jar A, 3, 17 and 24 days after flooding. Nine guinea-pigs were exposed to infection by this water and all of them became infected. Three animals were infected with the first sample removed on the third day, four animals were infected with the 17-day sample and two animals with the 24-day sample. In two instances the infecting doses from the 17-day sample were only 1 ml. and 2 ml. respectively, inoculated subcutaneously.

(b) Survival in soil for 15 *days*

The water in jar B was examined on the third and tenth days. One of two animals treated with the first sample became infected with leptospirae. The second animal died of pneumonia within 48 hr. of the experimental procedure. Both animals treated with the 10-day sample contracted leptospirosis.

(c) Survival in soil for 43 *days*

The water in jar C was examined on the third, tenth and seventeenth days. Two of four animals treated with the first sample died of pneumonia within 48 hr., but one of the remaining two contracted leptospirosis. A fifth animal inoculated subcutaneously with 3 ml. of the water did not become infected.

Two of four animals treated with the 10-day sample died within 48 hr. from an intercurrent infection. The remaining two failed to contract leptospirosis. Two animals treated with the 17-day sample likewise showed no evidence of infection.

Soil infected with rats' urine

The soil in four jars D, F, G and H was infected with the urine of one or more rats (*Rattus rattus*) which were caged on a wire grid 2·5 cm. above the surface of the soil for several days.

After the removal of the rats, the jars were covered and held in the laboratory for periods ranging from 8 to 28 days before rain water was added, and the soil flooded. Water was removed and examined for leptospirae at intervals ranging from 3 to 66 days after flooding of the soil. Details of these experiments are summarized in Table 2.

(a) Survival in soil for 8 *days*

The water in jar D was examined on the third and sixty-sixth days. Six animals treated with the first sample of water became infected. However, eight animals exposed to the 66-day-old speciman failed to become infected.

(b) Survival in soil for 15 *days*

A total of ten animals were treated with water from jar G on the third and fourth days. Five animals died within 48 hr., exhibiting an intense inflammatory reaction with sloughing and gas formation over the abdomen. The remaining five animals which were affected to a much lesser degree all became infected with leptospirosis.

Table 2. *The survival of* Leptospira australis *A in soil infected with rats' urine*

Soil in jar	Origin of infecting leptospirae	Soil moisture (%)	Days in soil pH 6·1	Days after addition of water	pH of water added	pH of water removed	'Subcutaneous stream' test	No. of test animals	No. of test animals infected	Incubation period (days)
D	Urine of 3 rats for 3 days	Not determined	8	3	6·9	7·6	100 ml. in 30 min., 4 100 ml. in 30 min., 2	6	6	6 7
D	Urine of 3 rats for 3 days	Not determined	8	66	6·9	6·7	100 ml. in 30 min.	8	—	—
G	Urine of 2 rats for 3 days	37	15	3	6·7	6·6	100 ml. in 30 min., 1 100 ml. in 30 min., 1 100 ml. in 30 min., 1	3*	3	7 8 9
G	Urine of 2 rats for 3 days	37	15	4	6·7	6·6	100 ml. in 30 min., 1 100 ml. in 30 min., 1	2*	2	7 9
H	Urine of 1 rat for 6 days	45	22	3	6·7	7·0	100 ml. in 30 min.	7	—	—
F	Urine of 2 rats for 3 days	41	28	3	6·7	6·7	100 ml. in 30 min.	8*	—	—

* A total of seven animals which died within 48 hr. of the experimental procedure from intercurrent infections are excluded from these figures.

(*c*) *Negative findings after* 22 *and* 28 *days*

Water was added to jar H and jar F, 22 and 28 days respectively after removal of the rats. The infectivity of the water was tested on the third day in each instance.

Seven animals treated with water from jar H failed to become infected. Two of ten animals used to test the water in jar F died from an intercurrent infection within 48 hr. and none of the remaining eight became infected with leptospirae.

Moisture content of the soil

The moisture content of the soil was determined in several cases just before flooding with water: in two jars, C and G, it was 34 and 37% by weight respectively. Leptospirae were isolated from both of these soils.

The soil in two of the jars, F and H, in which infected rats were placed showed values of 41 and 45%. When the rats were removed, the soil was found to be 'waterlogged' by the large amount of urine excreted, a condition brought about by feeding the rats overmuch pumpkin. These were the only jars from which leptospirae were not recovered, and the possibility that the excessive amount of urine present had a deleterious effect is one to be considered.

Incubation periods in experimental infections

Two control guinea-pigs each inoculated intraperitoneally with 1 ml. of the Gauci culture used to infect the soil, reacted with fever after an incubation period of 4 days. It is noteworthy that the incubation periods exhibited by many of the water-infected animals are not greatly removed from this figure. The longest incubation period recorded was 9 days.

pH *and survival in water*

The pH of the water used to flood the soil ranged in value from pH 6·5 to 6·9, and that of the infective water removed from the jars ranged from pH 6·6 to 7·6. The single specimen of pH 7·6 was the only one which had an alkaline reaction (Tables 1 and 2).

Leptospirae were isolated from the artificial pools at various intervals ranging from 3 to 24 days after their formation. Only one later test was made, at 66 days, and this was unsuccessful. The water found to be infective after 24 days had a reaction of pH 6·6.

The minimum and maximum air temperatures recorded in the laboratory during the course of the investigation were 20 and 29° C. respectively. The temperature for the most part remained stable about the middle of the range.

DISCUSSION

The nature of these experiments was such that it does not appear unreasonable to relate the findings to field conditions. It would appear that *L. australis* A excreted by a rodent carrier may survive in an acid soil characteristic of many North Queensland cane-fields for at least 15 days; the upper limit of its viability has yet

to be established. It might be considered that its survival for 43 days in soil infected by a culture was influenced favourably by the medium added; on the other hand, it can be said that the excessive amount of rats' urine present in the soil in two of our tests may have had a deleterious effect on the leptospirae and contributed to our failure to recover them after 22 and 28 days respectively.

The leptospirae migrating from infected soil survived in water, pH 6·6, for at least 24 days. The majority of natural waters in North Queensland are acid in character, but a substantial proportion of them fall within a pH range of 6·5–7·0 (Derrick, 1953), and would at least on this account be capable of harbouring *L. australis* A.

A statistical analysis of human cases and rainfall data reported by Derrick *et al.* (1954) revealed that many human infections in this area must have occurred almost immediately after heavy rain, as a highly significant correlation existed between rainfall and clinical cases observed 12 days later. In view of our findings it is suggested that the surface waters in some situations are more likely to become infected by the migration of leptospirae from previously contaminated soil than by direct infection by animal carriers after the rain has actually fallen. In this regard it is of particular interest to note that *L. australis* A infections not uncommonly occur during the cutting of well and effectively burnt cane, when rain follows the burning and the ground is wet and boggy underfoot. Sawers (1938) demonstrated that during the burning process, sufficient heat was generated to destroy leptospirae on the cane and the surface of the soil, but it did not appreciably penetrate either the soil or water. The maximum temperature recorded at a depth of 2·5 cm. below the surface of the ground was 31·7° C. If our submissions are valid it would appear that the burning of cane before cutting, a widespread practice favoured for both economic and health reasons, may reduce but by no means eliminate the risk of infection, as it will not destroy the leptospirae in contaminated soil.

The character of the soil and its ability to harbour leptospirae may be significant factors influencing the relative incidence and geographical distribution of human infections in North Queensland. This aspect appears to be worthy of further investigation because of its bearing on the effectiveness of current control measures.

SUMMARY

Soil from a sugar-cane farm in North Queensland was infected with leptospirae. In some instances a culture of *Leptospira australis* A was added to the soil; in others it was infected by rats, *Rattus rattus*, known to be excreting leptospirae in their urine.

After periods ranging from 8 to 43 days, the soil was flooded with rain water which was subsequently examined for leptospirae by treating guinea-pigs according to the 'subcutaneous stream' technique.

Leptospirae survived in culture-infected soil for 43 days and in urine-infected soil for 15 days prior to the addition of the rain water. They were recovered from the water at intervals ranging up to 24 days after the flooding of the soil.

The infected soil was of pH 6·1–6·2, its moisture content where determined was 34 and 37 %, and the prevailing temperature 20–29° C. The reaction of the infected water samples ranged from pH 6·6 to 7·6.

The possible significance of these findings in relation to the epidemiology of leptospirosis in North Queensland is briefly discussed.

We are indebted to Mr J. M. Kennedy, of Innisfail, for collecting the soil; to Mr T. G. Graham, of the Bureau of Tropical Agriculture, for his description of the soil profile; to Mr T. J. Beckmann of the Agricultural Chemical Laboratory, for the soil pH determination, and to Dr J. I Tonge for his interest and criticism throughout the investigation.

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(*MS. received for publication* 19. v. 55)