Original Articles

LEPTOSPIROSIS IN INDIA

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In a previous communication, Das Gupta and Chopra (1937) reported the occurrence of a case of leptospirosis in Calcutta with recovery of the causal organism (*Leptospira icterohæmorrhagiæ*). Five more cases have since come under our observation. It has been possible to isolate the leptospira from one of these five cases and the diagnosis of the remaining four has been established by agglutination reaction and other tests.

Epidemiology

With regard to the epidemiology of the disease, as it occurs in Calcutta, there is no evidence of contracting the infection by river-bathing or by contact with polluted water. The theory of water-borne infection, especially in sporadic cases, generally accepted in Europe is not, therefore, established in our cases. As all these cases were living in rat-infested houses (there is hardly any rat-free house in Calcutta), the infection might have been acquired by contact with the urine of infected rats. It is a curious fact that the presence of natural leptospiral infection in the rat population of Calcutta has been found to be of rare occurrence. Knowles and Das Gupta (Knowles, 1932) found only two rats infected out of 193. Last year, at the suggestion of Colonel Taylor, a systematic examination of rats was undertaken. The kidneys and liver of each rat were examined by the microscopical and cultural methods. Guinea-pigs were inoculated with the kidney emulsions, one animal being used for inoculation of materials from three or four rats.

The total number of rats (chiefly *Nesokia bengalensis*) examined was 162. No leptospira was found in any of them. Twenty specimens of rats' sera were tested for agglutination reaction with the local human strains, but the results were invariably negative, there being not the slightest evidence of agglutination or lysis, even in a low dilution of 1:10.

The disease in Calcutta is not confined to the individuals engaged in any particular occupation. The first case was a carpenter, the second a water-carrier, the third a cook, the fourth a grocer, the fifth a private servant, and the sixth was a porter.

It has occurred sporadically in different parts of the city. No two cases were seen in the same house nor in the vicinity.

The six cases which have, so far, come under our observation had the following seasonal incidence :—Two cases occurred in August 1937, 2 in December 1937, 1 in January 1938 and 1 in April 1938.

Clinical features

Three cases had a fatal termination and all became delirious before death. Acute onset, severe headache, agonizing pain all over the body, specially in the lumbar region, cramps in the calf muscles, conjunctival congestion, and marked jaundice were constant features. Four cases had bleeding from the gums and one of these also had epistaxis, melæna and patchy hæmorrhages under the skin. There was suppression of urine in two cases. The urine of three patients was examined, this showed evidence of marked renal irritation, albumen, urinary deposits, casts (chiefly hyaline and granular), leucocytes and red blood cells. All the specimens were very highly coloured on account of the presence of bile. One of the patients developed lobar pneumonia but in no other case was there any evidence of respiratory complication. As none of our patients were seen before the sixth day of illness, the degree of pyrexia at the early stage could not be determined. Low-grade fever persisted for two weeks or more in all the three cases that recovered. There was no relapse of the pyrexia in any case. Two fatal cases had a sub-normal temperature before death.

LABORATORY NOTES ON INDIVIDUAL CASES

Case 1

S. K. B. The patient was seen on the 9th day of illness. Blood examinations by microscopical and cultural methods and by inoculation into guinea-pigs were immediately undertaken.

Direct examination of blood.—Several smears were carefully searched under the darkground illumination, and some films were stained by Tribondeau's modification of Fontana's method and examined, but no leptospira could be found in any specimen.

Culture.—(*a*) On glucose broth—sterile.

(*b*) On Fletcher's medium—of the six tubes inoculated with varying amounts of blood from 0.2 c.cm. to 1.5 c.cm. two were found contaminated and the remaining ones gave scanty growth of leptospira from the 13th to the 15th day.

Animal inoculation.—As young guinea-pigs were not available at the time, two fairly large animals, weighing 400 and 442 grammes respectively, were inoculated with 3.5 c.cm. of blood intraperitoneally. The blood of these animals was examined daily from the 3rd to the 28th day of inoculation, far beyond the usual fatal period, with negative results. On the 29th day the peritoneal fluid of the bigger animal showed scanty leptospiræ. Four days later, this guinea-pig was sacrificed. On opening the abdomen no signs of leptospiral infection were apparent. The section of the liver and kidneys, however, showed the presence of leptospiræ. Apparently this animal passed into a 'carrier' condition. Unfortunately, the tissues were prepared only for the detection of leptospiræ by the silver-impregnation method, so that histological changes could not be studied in them.

Urine.—Direct examination of the centrifuged deposits showed a fair number of leptospiræ from the 19th to the 24th day. Two young guinea-pigs, weighing 150 and 162 grammes respectively, were each inoculated intraperitoneally with 2.5 c.cm. of urine collected aseptically on the 19th day. Both the animals died of leptospiral infection.

Protection experiments.—Two guinea-pigs each weighing 150 grammes (approximately) were taken. One was inoculated intraperitoneally with 0.25 c.cm. of the patient's serum taken on the 25th day of the disease

and the other with the same amount of normal rabbit serum. Next day both the guinea-pigs were given intraperitoneal injections of 0.2 c.cm. of the liver emulsion of a guinea-pig infected with the leptospira recovered from the patient himself. The one receiving the patient's serum previous to inoculation with the infecting organism remained alive and well, and developed immunity against further inoculation with the same strain. But the other animal which had normal rabbit serum succumbed to leptospiral infection on the 6th day of inoculation. As in the preceding experiment two guinea-pigs were inoculated with the patient's serum and normal rabbit's serum, respectively. They were then given injections of 0.2 c.cm. of the liver emulsion of a guinea-pig infected with the strain isolated from case 4. The patient's serum afforded protection against this strain as well, but the control animal (which received normal rabbit's serum) died of leptospiral infection. The above tests were repeated using the patient's serum obtained on the 209th day of illness and the results were exactly the same as before.

Agglutination tests.—

Strains	Patient's serum (collected on the 25th day of illness)	Patient's serum (209th day of illness)
* *L. icterohæmorrhagiæ* (Wijnberg).	1/10,000	1/3,000
* Hond Utrecht IV (canicola)	1/1,000	1/100
* Rachmat ('Indian' strain)	1/1,000	..
* Salinem ('Indian' strain)	0	..
* Swart V. Tienen ('Indian' strain).	0	..
* Andaman strain CH31 ..	0	..
L. icterohæmorrh. (London)	1/3,000	..

* The tests were carried out against these strains by Professor Schüffner and Dr. Sorgdrager.

Case 2

K. B. (fatal). He was seen on the 13th day of illness in a semi-conscious state.

Direct examination of blood.—Microscopical examination of blood by the darkground illumination and by means of stained films was carried out. The results were consistently negative.

Culture.—(*a*) On Fletcher's medium—two tubes remained sterile for a considerable period and the remaining two showed scanty coccoid organisms.

(*b*) On glucose broth (blood was taken half an hour before death)—there was a rich growth of a virulent streptococcus which killed white mice in less than 48 hours, the streptococcus being recovered from the heart blood of the mice.

Animal inoculation.—Two guinea-pigs were inoculated with 3 c.cm. of blood each. None showed any evidence of leptospiral infection, but both these animals became absolutely refractory to the strain of leptospira recovered from case 1, and also the sera of these animals protected guinea-pigs against the above strain but not against the strain isolated from case 4. The guinea-pig used as control was extremely susceptible to the infection.

Examination of post-mortem material.—Fragments of the liver and kidney were available within half an hour of death. No leptospiræ could be demonstrated in the liver or kidney emulsions or in sections stained by Levaditi's method.

Case 3

T. This patient came under our observation on the 10th day of illness.

Direct examination of blood (on the same day)—negative.

Blood culture (10th day).—Two young guinea-pigs, weighing 150 and 155 grammes respectively, were inoculated with the patient's blood. The peritoneal fluid of these animals was systematically examined for a period of three weeks, but no leptospira could ever be detected, nor did the serum of these animals develop any protective antibody, as in the case of the guinea-pigs inoculated with the blood of case 2.

Urine.—During the 3rd week of the disease eight specimens of urine were centrifuged as soon as they were voided and examined both by darkground illumination and by means of films stained by Fontana's method. Five of these samples were also inoculated into guinea-pigs. The results were negative in every case.

Protection experiments.—Patient's serum afforded protection against the strain isolated from case 1, but the guinea-pigs succumbed to the infection following an inoculation with the strain recovered from case 4, although they had been treated with the patient's serum previously. To avoid any experimental flaw these tests were repeated, and the results were identical.

Agglutination tests.—

Strains	Patient's serum (10th day)	Patient's serum (45th day)
* Andaman CH11 ..	1/1,000	1/30
* Moscou V (*L. grippotyph.*)	1/1,000	0
* Wijnberg (*L. ictero.*) ..	1/300	1/3,000
* H. Utrecht IV (*L. canicola*)	1/100	1/10
Andaman CH31 ..	1/100	0
* *L. hebdomadis* ..	1/30	0
* Rachmat	0	0
* Salinem	0	1/30
* Swart V. Tienen ..	0	..
* Hond HC	0	..
* Ballico	0	..
* Pomona	0	..

* Carried out by Dr. Sorgdrager.

Case 4

H. C. B. As the patient was seen during the first week of illness (6th day), particular attention was paid to the discovery of the parasite by direct examination of the blood. Accordingly several smears were prepared and very carefully examined under the darkground illumination. Besides, half a dozen films were stained by Tribondeau's modification of Fontana's method and thoroughly searched for leptospiræ; none could be found.

Blood culture (6th day).—(*a*) On glucose broth—sterile.

(*b*) On Fletcher's medium—the culture was examined every other day from the 7th to the 30th day, when it showed a scanty growth of leptospiræ. As it was not examined on the 29th day, leptospiræ might have been detected on that day also. It is important to note that the organism took an unusually long time to grow.

Animal inoculation (6th day).—Two young guinea-pigs were given intraperitoneal injections of the patient's blood. Both the animals developed typical signs of leptospiral infection and died on the 7th and 8th day respectively, although the liver and kidney emulsion of these animals failed to show leptospira in them.

Urine.—Centrifuged deposits of five specimens of freshly-voided urine obtained between the 15th and 25th day were examined under the darkground illumination with negative results.

Protection experiments.—The patient's serum protected guinea-pigs against the strain isolated from the patient himself, but failed to do so in the case

of the guinea-pig inoculated with the strain recovered from case 1. As in the previous cases, the tests, repeated a number of times to ensure accuracy, always yielded the same results.

Agglutination tests.—

Strains	Patient's serum (11th day)	Patient's serum (26th day)
*Ballico	1/300	0
*Andaman CH11 ..	1/300	1/100
*Moscou V.	1/300	1/300
L. icterohæmorrh. (classical strain).	1/100	1/10,000
**L. canicola* (dog strain) ..	1/100	1/3,000
*Rachmat (Human strains from the East Indies.)	0	1/30
*Salinem (Human strains from the East Indies.)	0	1/100
*Swart V. T. (Human strains from the East Indies.)	0	0
*Hond HC (Dog strains from the East Indies.)	0	0
*Hd. 7 (Dog strains from the East Indies.)	0	0
Andaman CH31 ..	0	0
*Pomona (Australia) ..	0	0
**L. hebdomadis* (Japan) ..	0	0

*Carried out by Dr. Sorgdrager.

Case 5

J. K. R. (fatal). Seen on the 8th day of the disease, died within 4 hours of admission.

Direct examination of the blood—negative.

Blood culture.—(*a*) On glucose broth—sterile.

(*b*) On Fletcher's medium—no growth for five weeks; the tubes were then discarded.

Animal inoculation.—A four-day old guinea-pig was inoculated with 1 c.cm. of the patient's blood. The peritoneal fluid of this animal showed a few leptospiræ 48 hours after inoculation and the guinea-pig died two days later of leptospiral infection.

Post-mortem examination.—As autopsy was not permitted a small fragment of the liver was removed through a small incision in the abdominal wall within three hours of death. Direct examination of the liver emulsion and section of this organ stained by the silver-impregnation method showed no leptospiræ, although marked degeneration of the parenchymal cells and cellular infiltration in the portal areas were seen in sections stained by the iron-hæmotoxylin method.

Case 6

C. J. (fatal). Came under our observation on the 8th day of illness and died on the same day.

Blood smears—negative.

Blood culture—no growth.

Animal inoculation.—Three young guinea-pigs were each inoculated with 1 c.cm. of blood. None of them showed any evidence of leptospiral infection for three weeks, peritoneal fluid being negative throughout.

The serum of these guinea-pigs failed to afford protection against the two local strains.

Post-mortem examination.—Portions of the liver and kidneys were available about 48 hours after death. The sections were found crammed with long filamentous bacilli evidently of post-mortem origin; and no leptospira were found.

Agglutination test.—

	Patient's serum (8th day)	Remarks
**L. icterohæmorrhagiæ*	0	
**L. canicola* ..	0	
**L. hebdomadis* ..	0	
*Rachmat ..	0	
*Salinem ..	0	
De (Calcutta) ..	1/20	As the serum was available only on the 8th day of illness, it was not likely to obtain more definite serological evidence of leptospiral infection.

*Carried out by Dr. Sorgdrager.

Discussion

Very recently Gaines and Johnson (1937) have made an extraordinary observation regarding the detection of leptospira by microscopical examination of blood. They observed :—'In our experience examination of the blood by darkfield examination was the most satisfactory method of diagnosis and gave positive results for all patients. Some had as high as 25 organisms per high power field and in none the search was time-consuming'. These workers also pointed out that '*leptospira persisted in the blood stream months after the onset of illness*' (italics ours).

We carried out direct examination of the blood both under the darkground illumination and by means of stained films using the silver impregnation method. On no occasion did we succeed in detecting the organism, although inoculation of blood into Fletcher's medium at the same time gave positive cultures in two cases.

Fletcher (1927) reported the detection of scanty leptospiræ in the blood films stained by Fontana's method in only 3 out of 32.

Taylor and Goyle (1931) examined the blood of several cases under the darkground illumination, but no spirochætes were ever found. It is difficult to reconcile these observations with the findings of Gaines and Johnson (1937), and one cannot help suspecting that they may have been dealing not with the true organism but with artifacts which arise from the disintegration of red cells and platelets, and may bear a striking resemblance to spirochætes. These peculiar structures were described by Balfour (1911) and also by Knowles and Das Gupta (1924). In spite of the emphasis placed on the nature of these pseudo-organisms, several observers have mistaken them for true spirochætes. Other workers who have studied this disease are inclined to the view that leptospiræ disappear from the blood stream by the end of the first week of illness and on no occasion could they be demonstrated after the 9th day.

As regards the susceptibility of guinea-pigs to leptospiral infection, Taylor and Goyle (1931) reported that in the Andamans out of 22 guinea-pigs inoculated with the blood of the infectious jaundice cases only one died of leptospiral infection, although positive cultures were obtained from many of the bloods used. Even in this case they were unable to demonstrate the organism in the kidneys and liver, though the animal showed typical signs of leptospiral infection. Later work of these observers, however, shows that they succeeded in infecting young guinea-pigs with the Andaman strains. In our experience guinea-pig inoculation was invariably successful when suitable materials were used. One animal, rather large, weighing 442 grammes, inoculated with a patient's blood obtained on the 9th day of illness, passed into a 'carrier' state. It is not unlikely that some of the guinea-pigs used by Taylor and Goyle for diagnostic inoculations behaved in the same way and that the infection remained undetected, as the peritoneal fluid of the inoculated animals was not examined.

In our series of animal inoculations, one very young guinea-pig just weaned, showed leptospiræ in the peritoneal fluid as early as the 2nd day of inoculation, although the blood of this animal was not positive till two days later. This is an interesting observation in that such an early detection of the causal organism in the guinea-pig (inoculated with the blood of a human case) is, as far as we are aware, recorded for the first time. It has been noted, however, that during a serial passage of the virus through guinea-pigs, the leptospiræ frequently appear in the peritoneal fluid of the 'subpassage' animal inoculated with the infected blood or liver emulsion as early as the 2nd day of inoculation, by whichever route the injection is given.

It will be seen, from the results of the agglutination tests recorded above, that the two strains isolated in Calcutta belong to the same serological type as the classical *L. icterohæmorrhagiæ* strain of Europe and differ from many Eastern strains, including a number of the so-called Indian strains. Strains Rachmat, Salinem, Swart V. Tienen and probably others have been referred to as Indian strains in literature, but to my knowledge this is the first time that a real Indian strain has been isolated and typed. The so-called Indian strains listed above were isolated in the Netherlands Indies and the Dutch workers call them Indian strains. Therefore, in the interest of clarity and in honour of Colonel R. N. Chopra, C.I.E., K.H.P., I.M.S., we should prefer to call the strain first isolated in Calcutta 'Strain Chopra, Calcutta'.

Sera of cases 3 and 4, taken on the 10th and 11th day of the disease respectively, gave an agglutination reaction markedly different from that obtained later during the convalescence. Dr. Walch-Sorgdrager of the Institute of Tropical Hygiene at Amsterdam, who very kindly carried out most of the agglutination tests referred to in this paper, informed me that he had also sometimes noticed pradoxical reaction in his cases, but could not quite explain this anomaly.

If we analyse the results of the protection experiments performed with the sera of 4 convalescents against the two strains isolated from cases 1 and 4, we notice that there are at least three different strains of the causative organism in our series of six cases. For purposes of convenience the results of these tests are shown in the following table :—

Serum collected during convalescence from	Strains recovered from	Results.
Case 1	Case 1	Protection.
„ 1	„ 4	„
„ 3	„ 1	„
„ 3	„ 4	Death.
„ 4	„ 1	„
„ 4	„ 4	Protection.
Serum of the guinea-pig which acquired immunity following inoculation of blood of case 2.	„ 1	„
	„ 4	Death.

It will be seen from the above table that the infecting organism responsible in case 3 is identical with that of case 2. But the strains recovered from cases 1 and 4 are distinct from one another and from case 2 or 3. We may mention here that to ensure accuracy the immunization experiments were carried out many times.

About seven months after recovery the serum of case 1 gave complete protection against the two local strains, but there has been a fall in the agglutination titre.

Erber (1932) reported that wild rats invariably gave positive agglutination reactions in dilutions from 1:50 to 1:2,000. But in Calcutta the sera of 20 rats, including one caught in a patient's house, reacted completely negatively with the two local human strains. The incidence of natural infection with leptospira in wild rats in Calcutta is exceedingly low, as will be seen from the work of Knowles (1928) who found none infected out of 180 rats examined and from that of Knowles and Das Gupta (Knowles, 1932) who recorded the presence of leptospira in 2 out of 193 animals. Besides, in connection with the present investigation 162 rats were very carefully examined, with negative results, although the most recent methods advocated for the detection of leptospira were employed.

The guinea-pigs inoculated with the blood of a patient on the 13th day of illness developed immunity against a local strain (case 2), while those which received inoculations on the 8th and 10th day of illness did not (cases 6 and 3).

The urine of three cases was systematically examined for the presence of leptospira, both by

direct microscopical examination and animal inoculation, but in one case only could it be demonstrated (case 1). This is in accord with the findings of Taylor and Goyle (1931) who record the presence of leptospiræ in 15 out of 48 cases examined. Kouwenaar (1926), however, was able to detect leptospiræ in the urine of about 77 per cent of his cases.

In none of our fatal cases did we succeed in demonstrating leptospiræ in kidney and liver sections. Our experience of the failure to find post-mortem evidence of the presence of leptospira in the fatal case corresponds with the results obtained by Taylor and Goyle (1931) in the Andamans. It has been noted by some observers that the organism disappears from the liver by the 7th day in human cases and this is probably the reason for our negative findings.

Sanarelli (1928) noticed that experimentally-infected animals frequently showed secondary invasion with other organisms, especially the streptococcus and the paratyphoid bacillus. Moreover, in cases of infectious jaundice in human beings, the latter organism is frequently present in the blood. In order to determine the rôle played by these secondary organisms, he carried out some experiments in collaboration with Dr. Pergher. The results seem to indicate that these secondary organisms in themselves are able to produce the characteristic symptoms, but rarely so severe as to cause death.

We cultured the blood of all our cases, both on Fletcher's medium and on glucose broth; on no occasion was any organism of the paratyphoid group recovered, although the blood culture taken from case 2 half an hour before death gave a rich growth of virulent streptococci.

As noted by Taylor and Goyle (1931) the blood cultures usually take seven to ten days to develop, but in some cases did not show till the 21st day. In one of our positive cultures the growth first appeared as late as the 30th day (case 4).

All our cases showed the characteristic symptoms of the severe type of the disease. It is quite likely that, as in other countries where the disease is prevalent, milder forms with or without jaundice also occur in Calcutta, which are yet to be recognized.

Summary

(1) The occurrence of six cases of leptospiral jaundice in Calcutta during the last eight months is recorded. The identity of the disease was established in every case by laboratory examination.

(2) The causal organism has been isolated from two cases. It belongs to the same serological group as the classical *L. icterohæmorrhagiæ* strain of Europe and differs from many Eastern strains, including two from the Andamans (CH31 and CH11).

(3) Although the infecting organisms recovered from the two cases gave almost identical agglutination reactions, yet they could be differentiated from one another by protection tests.

(4) The disease occurred sporadically in different quarters of the city and is not associated with river-bathing or contact with polluted water, nor has it a predilection for any particular occupation.

(5) The incidence of natural infection with leptospira in the rat population of Calcutta is exceedingly low.

I wish to express my indebtedness to Colonel R. N. Chopra, C.I.E., K.H.P., I.M.S., Director of the Calcutta School of Tropical Medicine, for providing every facility for carrying out this investigation and to Colonel J. Taylor, D.S.O., C.I.E., I.M.S., for many helpful suggestions. To Prof. W. Schüffner and to Dr. Walch-Sorgdrager of the Institute for Tropical Hygiene, Amsterdam, I should like to express my grateful thanks for so generously supplying me with cultures and anti-sera and for carrying out the serological tests, as recorded in this paper. My sincere acknowledgments are also to Major H. C. Brown, C.I.E., I.M.S. (retired), of the Wellcome Bureau of Scientific Research, London, for testing the serum of case 1 for agglutination reaction. I also thank Prof. M. N. De and Dr. D. R. Dhar of the Medical College, Calcutta, for drawing my attention to two cases under their care.

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