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An Investigation of Rats for Spirochaetes in Colombo

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Occasional cases of jaundice in Colombo suggested that leptospira infection of rats may be the cause of some cases of jaundice. Although a leptospira could not be found in 212 tested rats it may be however that a positive result may be forthcoming with a bigger sample. At any rate only a small number could be infected and the danger to human beings is very limited. Not only must the infection of the rats be considered but also other conditions, such as the PH of the soil and the water, both of which have been tested in Colombo. Before considering the present investigation, I wish to explain the problem of the leptospira infection by referring briefly to some previous investigations.

The technique used includes also an investigation for other spirochaetes. There is no uniform nomenclature. Different authors use different names for the same organism ; for instance treponema recurrentis is called borrelia recurrentis as well. Spirochaetes with hooked ends are named leptospiras by all modern authors.

Inada (1) and his collaborators were the first to implicate a spirochaeta as the cause of the Weil's disease. The pathological changes in the guinea-pig were described. A study of the characters of the spirochaete was made and these workers were successful in the cultivation of the spirochaete. They have also seen the spirochaete in the urine of patients. The infection of coalminers and farmers by the urine of rats was detected. The authors adduce reasons for the rather belated discovery of the spirochaete and these reasons were : (1) All investigators searched for bacteria. (2) The spirochaete resides in the blood only during the early stages of the disease and is present only in small numbers. (3) At autopsy the spirochaete is difficult to identify in the liver. But Ido (2) was the first to find the leptospira in the rats and this fact has been confirmed by numerous workers in different parts of the world.

Inada and others showed that there were : 40·2 per cent. carriers of leptospiras out of 149 mus decumanus and 0·8 out of 22 mus alexandrinus. Darkground examinations and injections of guinea-pigs were carried out. Ido was able to prove the identity of spirochaetes of house rats and wild rats with the human leptospira icterohaemorrhagica by morphological examination and by testing with immune serum. In experiments with kidneys he used 92 animals. In 26 cases he was able to find the leptospiras in the kidneys. Afterwards he injected 8 of these kidney emulsions into guinea-pigs and as a result 7 of them died. Furthermore guinea-pigs were injected with kidney emulsion of 59 animals which microscopically didn't show any organism and 5 of them died. He also examined the urine of 71 rats and in 22 of

them spirochaetes were present ; in 19 rats he found these organisms in the kidneys as well as in the urine. In 3 rats out of 52 he could not see the spirochaetes in the kidneys, but he discovered them in the urine. The urine sediment of 2 rats in which no spirochaetes were found by any other method was injected interperitoneally into guinea-pigs.

I have quoted the figures in some detail in order to compare them with the results in other countries. The following references illustrate the conditions obtaining in temperate climates and may enable us to compare these figures with those in hot climates provided the same method has been used by the different workers. As far as possible only comparable works are quoted.

Middleton (3) in Oxford examined 235 local rats during an epidemic of jaundice among Oxford school children. The children had been playing on refuse dumps associated with standing water. Such places must be considered as rat infested areas. The rats were obtained dead, but were examined usually within 6 hours of death. An incision was made in the abdomen exposing the kidneys. The kidney-membrane was torn with a pair of forceps and a platinium loop thrust in the body of the kidney to get the material for the microscopical examination. Each specimen was examined by means of the darkground microscope for a period of 15 minutes. The urine was tested too. As the bladder was usually empty, the examinations were discontinued. The blood and other organs revealed negative results. It seemed essential that the weight of the rats should be considered as well. There is a steady increase of infection until maturity is reached, whereupon the percentage ceases to rise and then appears to decline slightly in the older animals. Only rats over 150 g. had frequent infections. Out of 235 rats tested with the darkground microscope 41·7 per cent. were found to carry leptospira icterohaemorrhagica in their kidneys. In a set of samples of water leptospiras were detected but not classified further by inoculations and serological tests.

Balfour (4) in London examined rats bacteriologically and also for leptospiras, ecto- and endoparasites. When the rats had urine in the bladders he examined it for leptospiras by means of the darkground microscope. In addition to this he prepared smears from the kidneys and stained these smears by Giemsa. Also he injected urine as well as kidney- and liver-emulsions into the guinea-pigs. Of the 2 emulsion cases one was positive and here again 2 guinea-pigs were used, one of which produced a negative result. There may be an insusceptibility for infection in guinea-pigs. If only positive infections with guinea-pigs are accepted as a criterion of infection there can be no doubt that some cases will be missed. The examination of kidney-smears and the darkground examination, however, are sufficient to reveal the presence of leptospira. Finally the guinea-pigs were injected only in positive cases.

Altogether 478 wild rats were examined, of which 34 were Rattus rattus and 444 Rattus norvegicus. Leptospiras were found in the kidneys of 22·6 per cent. Rattus norvegicus, but none in the black rats. The urine of 13·6 per cent. of 154 brown rats was positive, but it is not stated whether they were positive in the kidneys as well. Cultures were prepared in Venyon's modified Noguchi medium, but no figures were given for the comparison of this method with the microscopical examination of the kidneys.

Lewis (5) in Philadelphia examined 100 Rattus norvegicus. The rats were captured alive and taken to the laboratory where they were chloroformed. In his results there were comparable figures to show the value of the methods and it appears that in temperate climates low findings are possible. Only one out of 100 rats was positive in the urine in the darkground ; 4 were positive in the culture of the urine ; 2 in the darkground of the kidney-emulsion ; 10 in the darkground of the kidney-culture and 11 proved positive after inoculation into guinea-pigs. The microscopical examination compares favourably with the former investigations and furthermore the results of the different methods in this work can be regarded as comparable.

Among the quoted examinations a comparison of the percentage is possible only to a limited extent because of the different methods. The microscopical results of the kidney examinations, however, are contained in all the experiments and can be compared. Their comparison with the following figures is carried out with the standard error calculated in the usual way.

1. Comparison of kidney examinations.
   Ido (26 out of 92) 28·2 per cent. $\pm$ 4, 7.
   Middleton 41·7 per cent. $\pm$ 3, 2.
   Balfour 22·6 per cent. $\pm$ 1, 98.
   Lewis 2·0 per cent. $\pm$ 1, 4.
   Lahiri 8·8 per cent. $\pm$ 2, 5.
   Knowles and Das Gupta 1 per cent. $\pm$ 1.
   Das Gupta 1·6 per cent. $\pm$ 0, 7.
2. Comparison of urine examinations.
   Ido 31 per cent. $\pm$ 5, 5.
   Balfour 13 per cent. $\pm$ 2, 7.
3. Comparison of the sensitivity of guinea-pigs and the culture.
   Lewis culture 10 per cent. $\pm$ 3.
   Lewis guinea-pig 11 per cent. $\pm$ 3, 12.

Using the threefold standard error the results of Ido and Middleton, Ido and Balfour, Lewis and Lahiri can be regarded as equivalent. The urine examination of Ido and Balfour are equivalent as well. The cultures and the guinea-pig injections are also equivalent methods.

In the hot countries positive as well as negative results are obtained. Kirk (11) investigated 259 rats in North Sudan. The distribution of the species was as follows : Rattus rattus 55 per cent., Rattus norvegicus 42 per cent., Arvicanthus testicularis 3 per cent. All these examinations have consistently failed to show any evidence of leptospiral infection. It seems to be remarkable that he was not able to detect the infection in Rattus norvegicus. Kirk thinks that the strong sunlight in this land may be an important factor, as it is well known that bright light has a highly lethal action on leptospiras and therefore other rats cannot be infected by them.

Knowles and Das Gupta detected leptospiras for the first time in rats in India in 1932. Later on in 1937 leptospiras were found for the first time in the urine of a patient between the 19th and 23rd day of the illness. Further cases of Weil's disease are reported by Gupta (7) during 1938. The patients were a carpenter, a water carrier, a cook, a grocer, a private servant and a porter. As all these people were living in rat infested houses, the infection might have been acquired by contact

with the urine of infected rats. There is no evidence of having contracted the infection by river bathing or by contact with polluted water. These cases occurred sporadically in different parts of the city and no common source of infected water could be detected.

In this connection a systematic examination carried out by Colonel Taylor is mentioned. He tested 162 rats, chiefly Nesokia bengalensis, with negative result too.

Knowles and Das Gupta (7) have found only 2 infected rats out of 193. The same author (12) reports upon further investigations on 310 rats. 3 mus decumanus (Rattus norvegicus), 1 Rattus rufescens and 1 Bandicoota elliotiana have been found positive, that is 1·6 per cent. $\pm$ 0·7. No attempts have been made to explain these low figures. Despite these low percentages of infected animals, there are reports of cases of leptospira disease among human beings in India. Surprisingly an investigation in Rangoon showed a high figure of infected animals : Nesokia bengalensis 43 per cent., Rattus rattus plus Rattus concolor 21 per cent. (13). There can be found low and also high percentages in hot climates. The action of hot climates cannot be adduced as a reason for the low percentage or the absence of leptospira infections in animals.

A low percentage is reported by Lahiri in Bombay too (8). The examination of the kidneys in the darkground microscope has demonstrated 11 positive results out of 124 Rattus norvegicus or 8·8 per cent. 10 guinea-pigs have been inoculated with the positive kidney material. 4 of these were alive and did not show any infection of leptospira.

Kidney substance of 78 rats was cultured. The cultures were not satisfactory due to contamination by bacterium pseudoasiaticum owing to an infection of the kidneys of the rats. 27 out of them were free of bacteria, but only in 2 of them was there growth of leptospiras. The same 2 samples were positive in the darkground examination of the kidney. The cultures have been prepared with small pieces of kidney cortex in Verwoort's medium. The author concludes that the darkground examination is the best method to demonstrate the infection. To isolate the leptospiras either the kidney cortex can be cultured or an emulsion can be injected into guinea-pigs.

There are different possibilities of transferring the leptospiras from the rats to human beings. Rats may enter dwellings and infect people by contamination of food and pottery with their urine. The cases described by Gupta (7) may be of this kind. People of different occupation and living in different parts of the town have been affected without any contact with polluted water. A particular danger exists for workers in sewers and polluted water, as fishermen, coal-miners, etc.

It is frequently reported that after bathing in polluted water numerous cases of jaundice occurred. An epidemic described by Buzzard (10) may be cited as an instance. In a short time 5 patients came to the infirmary, 3 of them had been bathing in a river recently, 2 were occupied in such a manner as to come into contact with the urine of rats. Furthermore Jorge (14) describes the occurrence of the disease in at least 126 persons in the space of a month and this example of a water borne outbreak is very instructive.

The reaction of the water and the soil is of great importance for the spreading out of leptospira. If the PH is lower than 6·8 the pathogenic leptospira ceases growing (13). In 10 water samples of PH higher than 6·9 water leptospira were found, in 6 samples of water of PH lower than 6·6 no leptospira were detected. Limestone areas with alkaline waters and alkaline soils yield a greater possibility of infection than primitive rock areas, having an acid soil and acid water.

As mentioned previously water may transfer the leptospira to human beings. Therefore no more cases of leptospiroses occurred when the water of the coal-mines was pumped out. Moreover water may infect the Rattus norvegicus living mostly near polluted water. Acid soil and acid water which soon destroy the leptospiras are less effective in conveying the leptospiras to rats and field mice. Ido (2) found no leptospirosis in human beings in acid coal-mines, but a great number of people was infected in alkaline soil areas. Rats harbouring leptospiras, however, could be detected in acid mines.

In the following investigation rats caught in the Colombo area have been tested for leptospiras and other spirochaetes. An enquiry was conducted with a view to ascertaining the danger of infection to human beings. The rats have been caught by the Municipality and brought alive to the Institute. They were killed with coal-gas, afterwards dipped in a Lysol solution to destroy the fleas and opened immediately. The kidneys were taken out and cut in 2 pieces with a pair of scissors. One half was cultured in Korthoff's medium, the other half was examined in the darkground in all cases. Material was scratched off the cut surface with a loop, diluted with saline and tested immediately. Giemsa preparations were also made.

Identification of the Rats

Altogether 212 rats were examined. Out of them there were 101 Rattus rattus alexandrinus, 65 Rattus rattus rufescens, 42 Rattus rattus frugivorus, 3 Rattus rattus Kandiyanus with short ears and 1 Rattus rattus Kandiyanus with long ears. All the trapped rats were subspecies of Rattus rattus. Rattus norvegicus was not present.

The identification of the rats was carried out according Manson's Tropical Diseases (15) and is briefly outlined. Rattus rattus can be recognised by the large translucent ears covering the eyes, if they are folded down. The tail is never considerably shorter than head plus body. Four subspecies were observed.

1. Rattus rattus frugivorus with grey-brown back, pure white or pale yellow abdomen bordered with a sharp line of demarcation.
2. Rattus rattus rufescens with grey-brown back and without line of demarcation between the colours of the back and the abdomen. The ventral hairs have rusty tips. It is the usual Indian house rat.
3. Rattus rattus alexandrinus is similar to Rattus rattus rufescens, but the ventral hairs have no rusty tips. Whitish and light yellowish tips were observed here. The line of demarcation is missing.
4. Rattus rattus Kandiyanus. The identification of the 3 previously mentioned rats is easy, whilst it presents some difficulties with Rattus rattus Kandiyanus, the descriptions in the literature being diverging.

Martin A. C. Hinton (18) describes the Rattus rattus kandiyanus as dorsal bright rufous brown and also with dark back, separated by a sharp flank line of demarcation from the pure white or cream belly. But some specimens have soiled bellies and darker feet. He remarks that Rattus rattus kandiyanus cannot be strictly differentiated from Rattus rattus wroughtony. He also observes that Rattus rattus wroughtony has relatively shorter ears than other rats.

W. A. Phillips (18) describes Rattus rattus kandiyanus of very different colours and with a sharp flank line. Light grey underpart is possible too. The seminaked ears are dusky. The description is very exhaustive, but the length of the ears and the differentiation from the white bellied Rattus rattus frugivorus is not mentioned.

E. Schwartz (5) describes the Rattus rattus kandiyanus as follows : Rattus rattus kandiyanus Kelaart (syn. wroughtony Hinton) is more reddish than alexandrinus, has a whitish buff or very light greyish belly and pale feet. The ears are short and the bullae very small.

The last description could be used to identify 3 Rattus rattus kandiyanus, namely 2 rats with short ears and grey and light grey belly, a third rat with short ears, more brownish back than alexandrinus and mixed yellow-brownish and black hairs on the back, but yellow-brown belly. A forth rat had mostly grey hairs mixed with buff ones on the back and mostly white hairs mixed with buff ones on the belly, but the ear of this rat covered the eye easily. It is considered as Rattus rattus kandiyanus but with a different length of the ears. It could also be a crossbreeding of Rattus rattus frugivorus and Rattus rattus kandiyanus with brown belly. A proof could only be furnished by interbreeding such rats. All 4 rats showed the line of demarcation.

Rattus rattus norvegicus was not found. It has small and opaque ears, the tail is noticeably shorter than head plus body.

Habits of Rats

It has been already stated that sometimes Rattus rattus is not infected with leptospiras (11, 20) and that it is less infected than Rattus norvegicus (14). It is suggested that these differences are due to the dissimilar habits of these animals as described by M. A. C. Hinton as follows (17) : Rattus rattus is essentially an arboreal and climbing animal and it rarely burrows ; therefore when infesting buildings or huts it is usually found in the walls, ceilings or roofs, not in cellars or drains. Rattus norvegicus is essentially a water loving and burrowing animal. The only factor indispensable for the success for this species is the presence of water ; it drinks freely and displays great skill as a swimmer and a diver. Therefore it naturally abounds along the banks of all water courses. It infests sewers and drains not only for the sake of the water flowing through them, but because ordure serves it quite well as food.

Comparing the habits of Rattus rattus and Rattus norvegicus it can be stated that Rattus rattus lives in cleaner and dryer surroundings than Rattus norvegicus. It does not inhabit and infest the sewers and therefore it cannot be infected from there. It urinates on the ground where the leptospiras soon die by desiccation. The contamination therefore is not spread by water, while in fresh lake water the pathogenic leptospiras survived for more than 10 days (16).

According to these conditions the possibility of spread from rat to rat is considerably reduced with Rattus rattus. The possibility of conveying the disease from rat to human being is reduced too, as extended epidemics of Weil's disease are caused by polluted bathing water and Rattus rattus does not live near the water.

PH determination of Water and Soil

In Colombo there are two lakes connected by canals. These values were determined because of the importance of the PH for the conveyance of leptospiras. These lakes are used as bathing places. They are also used for the cleaning of cattle and for the washing of clothes. People therefore are in contact with these waters frequently and for long periods.

On the bank of the first lake in a place freshly polluted with faeces of cattle (Lake Road) a sample of water was taken out and the PH obtained was 7·6. In another place only polluted with mud (Gordon Road) the value was 7·2 ; on the western bank (Alvis Place) it was 7·1. The water of this small lake is therefore alkaline enough to keep alive introduced leptospiras for a longer time. As far as I have found Rattus rattus has proved not to be infected, he does not live on the water and therefore is not dangerous. There would be danger if infected rats, especially Rattus norvegicus, were living in the surroundings of this lake.

Alkaline values were also found in the water of the larger part of the Beira Lake, namely : Vauxhall Street 7·6 ; bank of MacCallum Road 7·8 ; neighbourhood of the Secretariat 7·8. There are the same conditions as on the first small lake. The PH value in the Dehiwala Canal is extraordinarily high, namely 8·4. The only favourable value found (PH, 6·4) was the Kirillapone Canal. Leptospiras have not been found in waters with such an acidity (13). Such waters are regarded as unsuitable for the transmission of leptospiras. The same conditions prevail in the following waters : Kelani Ganga, tested near Peliyagoda on the Negombo Road 6·5 ; paddy field nearby 5·0 ; paddy field at Kelaniya 6·2.

The acidity of the soil is also an important factor for the viability of the leptospiras of the rats urine (2). Here too PH values below 6·8 destroy the leptospiras. These values have been tested, because there exists the possibility of infection by means of the soil from rat to rat and from rat to human being. The PH values were : Kollupitiya, Galle Road 7·2 ; Cinnamon Gardens 7·0 ; Slave Island, Ramsay Road 7·4 ; Campbell Park 6·6 ; Baseline Road 6·2 ; Kent Road 6·7. Here too there are suitable as well as unsuitable places for the transmittance of leptospiras.

Results

The following results have been obtained during the investigations of the rats :

All the 212 examined rats were negative for leptospiras in the darkground examination. 188 kidney cultures were negative and not sterile, 24 were negative and sterile. 64 smears of blood, peritoneal fluid and kidney were stained by Giemsa and were negative for leptospira and spirillum minus. Only one old Rattus rattus kandiyanus showed spirochaetes in the darkground preparation of the kidney and in the Giemsa stain. It was the fourth observed Rattus rattus kandiyanus. The kidneys of this rat were enlarged, brown and soft.

I wish to describe again shortly this positive Rattus rattus kandiyanus as these rats are of a different colour. It had short opaque ears which hardly reached the

eyes. The back had grey hairs mixed with buff ones. The belly was light grey with some buff hairs. The back and belly were bordered by a line of demarcation.

The kidneys showed straight spirochaetes in the darkground. These began to bend after 2 hours and stopped moving. They were 22-24 μ long. The kidneys were ground with saline and injected immediately into 2 guinea-pigs. After six days blood specimens were taken from the guinea-pig and tested under a phase microscope. Numerous spirochaetes could be seen. Most of them were straight, resting and fixed on red blood cells. Only a few were moving. None of the spirochaetes had a hook on the end. Their length was about three times the diameter of a red blood cell.

One guinea-pig died on the 7th day, the other on the 10th day. The autopsy revealed haemorrhagic peritonitis and light jaundice. Liver and kidneys were positive for spirochaetes in the darkground. Liver, kidneys and spleen were fixed in formalin. The sections were impregnated with silver and all three organs contained numerous spirochaetes with not more than 4 or 5 waves and sometimes with filamentous ends.

The identification of these spirochaetes was not very difficult as the hooks on the ends were absent ; only some of them developed a bend on the end when dying and therefore a leptospira can be excluded. The length of the organism and the number of the waves correspond to those of treponema. As only treponema persicum (syn. Treponema usbekistanica, Treponema sogdiana) is known to cause the death of guinea-pigs by haemorrhagic peritonitis (21), it is regarded as Treponema persicum.

The search for spirochaetes in rats causing jaundice in human beings led to the detection of a spirochaeta of relapsing type instead of a leptospira as supposed at the beginning. This spirochaeta is also capable of producing jaundice as a complication and a high mortality in human beings results (22).

I am thankful to the Parasitology Department of this institute for the sections of the organs and for the examinations under phase microscope.

Summary

212 rats were tested for leptospiras with negative result.

65 Giemsa stains of blood, kidney and peritoneal fluid were negative for leptospira and spirillum minus as well. Only one rat was infected by a spirochaete, identified as Treponema persicum.

All the rats could be identified.

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