# *LEPTOSPIRA ICTEROHAEMORRHAGIAE* IN OXFORD RATS.

By A. D. MIDDLETON.

(*From the Department of Zoology and Comparative Anatomy, University Museum, Oxford.*)

(With 3 Graphs.)

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## 1. Introduction.

During June and July, 1928, an epidemic of jaundice among Oxford school-children drew attention to the possibility of a spirochaetal infection in these cases. The common brown rat (*Rattus norvegicus*) being known to be the principal reservoir in this country for *Leptospira icterohaemorrhagiae*, the causal organism of spirochaetal jaundice (Buchanan, 1927), the writer undertook the examination of a number of local rats. As an inquiry by the Ministry of Health into the epidemic among the children reached no definite conclusions regarding the cause of the disease, it was decided to publish this work on the infection of the rats independently of its possible connection with jaundice in the outbreak referred to.

During the course of an extensive research by a team of workers in Oxford into the epidemics of wild rodents, the long-tailed field mouse (*Apodemus sylvaticus*) was also found to be infected with a strain of *Leptospira*, but there is evidence that this is a much milder type than that infecting the rats. A detailed account of this work is being published elsewhere.

I wish to thank Prof. E. S. Goodrich for allowing me the facilities of the Department of Zoology and Comparative Anatomy, and Mr C. S. Elton and

Dr A. D. Gardner for their continual advice and assistance throughout the work. My thanks are also due to the Medical Research Council for expenses, and to the many people who have assisted in obtaining the rats for this investigation.

## 2. Technique.

A total of 235 rats was examined during June–July, 1928, and January–March, 1929. The rats were all obtained from the City of Oxford or its immediate environs: 193 from the Iffley Road refuse dump—the property of the City Corporation.

The rats were obtained dead, but in no case were they examined more than 24 hours after death, and usually within 6 hours. Each rat was weighed and sexed, and its kidneys were examined by means of a $\frac{1}{12}$ in. oil immersion, with dark field illumination (Pointolite). In a number of cases both kidneys were examined; but, as in no case was one kidney alone found to be infected, the routine was shortened by examining the left kidney only. The technique employed for the examination of each rat is as follows:

An incision is made in the left side of the abdomen, exposing the left kidney. A small drop of sterile isotonic saline is placed on a glass slide by means of a platinum loop. The kidney membrane is then torn with forceps and a platinum loop thrust into the body of the kidney and worked vigorously about inside. The loop, thus bearing a representative sample of kidney tissue, is quickly removed to the saline drop and thoroughly rubbed up into an emulsion. The material is covered and examined with the dark field microscope. In order to keep the technique as constant as possible, each specimen is examined for a period of 15 minutes unless spirochaetes are found in a shorter period, in which case the time of examination is recorded. As will be seen from Table I, when spirochaetes were present they were in the majority of cases found within the first two minutes, so that it seems unlikely that many infections could have been missed by this technique.

Table I. *Time taken to find* Leptospira *in infected rats.*

| Time in minutes | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of cases | 33 | 18 | 9 | 3 | 8 | 1 | 0 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |

Three degrees of infection were recorded, signified by +, + +, + + +, and as the size of drop and loop and all details of the technique were kept as constant as possible throughout, this crude relative classification should be of some value. The examinations were all carried out by the writer, so that any great variation in the personal factor was obviated.

A number of examinations of the urine of infected rats was also carried out, and the urine was found to contain *Leptospira*, but, as the bladder was usually empty, rendering urine examinations insufficiently reliable for statistical purposes, these were discontinued. Blood and other organs were also examined, but with negative results.

## 3. RESULTS.

*Percentage of rats infected.*

Table II shows the numbers and percentages of rats infected with *Leptospira*, and the number of males and females infected.

Table II. *Rats infected with* Leptospira.

| | Number examined | Number infected | Percentage infected |
|---|---|---|---|
| ♂ | 97 | 44 | 45·5 |
| ♀ | 138 | 54 | 39·1 |
| Totals | 235 | 98 | 41·7 |

Fig. 1 shows the percentage infection with increasing age. The rats are divided into 7 weight groups, increasing in increments of 75 grm., and the points of the graph represent the percentage infected in each group. In the case of pregnant females the weight recorded is the body weight without

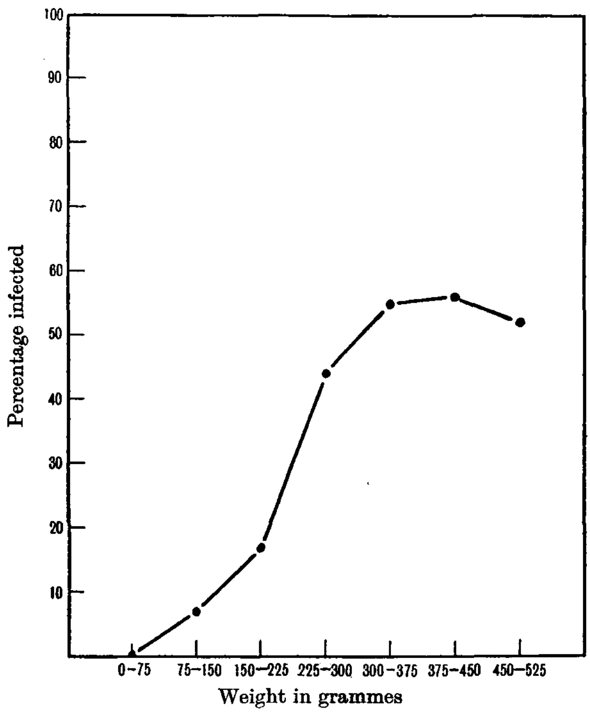


Fig. 1. Percentage of rats of different weights infected with *Leptospira icterohaemorrhagiae.*

embryos. Two very large rats, which would have fallen into an 8th group, have been omitted for the purposes of this graph. The figures on which this graph is based are given in Table III.

Table III. *Percentage of rats, in weight groups, infected with* Leptospira.

| Group | 0–75 | 75–150 | 150–225 | 225–300 | 300–375 | 375–450 | 450–525 |
|---|---|---|---|---|---|---|---|
| Number examined | 10 | 14 | 36 | 48 | 65 | 39 | 21 |
| Number infected | 0 | 1 | 6 | 21 | 36 | 22 | 11 |
| Percentage infected | 0 | 7 | 17 | 44 | 55 | 56 | 52 |

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As will be seen from the graph, there is a steady increase in infections until maturity is reached, after which the percentage ceases to increase, and appears to decline slightly in the older animals.

Fig. 2 shows the percentage infection in weight groups of the two sexes. As the figures are too small to allow of dividing into 7 groups as in Fig. 1, increments of 150 grm. are taken after the first group of very young (below 75 grm.). The males appear to be more infected than the females, apart from

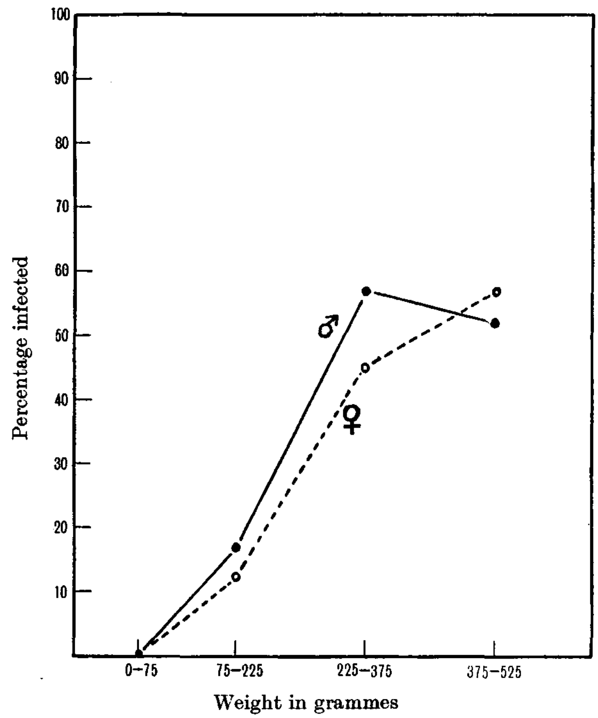


Fig. 2. Percentage of infected males and females of different weights.

the sudden drop in old age, and, as the growth-curve of male rats is steeper than that of females (Donaldson, 1924), the true curves of infection with age in the sexes would be more widely separated than on this graph, *i.e.* there is really a greater difference in the percentage infection with increasing age of the sexes than appears in the graph given. Presumably the male, being the more active, would stand a better chance of becoming infected with *Leptospira*.

## *Degree of infection.*

The relative abundance of the *Leptospira* in different rats shows no general correlation with age or sex of the host. These observations are admittedly crude, but it seems safe to assume that no increase in the degree of infection occurs with age, in fact it seems probable that the contrary is the case, as, of infected rats weighing between 300 and 400 grm., 31 per cent. were recorded as either + + or + + +; but of those weighing more than 400 grm. only 21 per cent. were similarly recorded.

One case stands out markedly from the rest: No. 94, a male weighing only 100 grm. (the smallest rat found to be infected), had the heaviest infection of *Leptospira* of any seen throughout the work. The spirochaetes were swarming in enormous numbers, and all were in a state of great activity. They were also abundant and active in the urine, but examinations of the blood and liver were negative. The reaction of the urine was slightly alkaline: it was found that the spirochaetes were quickly killed, and degenerated, under the action of acid urine.

### *Activity of the* Leptospira.

In many of the rats examined within a few hours of death, practically all the spirochaetes were inactive, while in others they remained very active for a long time after death. Two rats which were left outside for 16 hours at a temperature continually below 25° F. were found to contain extremely active *Leptospira*. It is probable that the activity at any time is dependent on the concentration of uric acid in the kidney, for it appears that acid urine is fatal to the organisms. In the case of an animal such as the rat, the urine reaction would presumably vary from day to day, being influenced by the type of food eaten.

### *Measurements.*

The mean of six micrometer measurements of the length of different *Leptospira* was 0·0104 mm., or 10·4 $\mu$.

### *House mice.*

Sixteen house mice (*Mus musculus*) were examined for *Leptospira*, but the results were all negative. Two dormice (*Muscardinus avellanarius*), and one wild rabbit (*Lepus cuniculus*) were also uninfected.

### *Transmission to guinea-pigs.*

Dr A. D. Gardner succeeded in infecting guinea-pigs with the *Leptospira* from one or two rats, and obtained pure cultures. This strain was found by Major H. C. Brown to be serologically similar to *Leptospira icterohaemorrhagiae* of rats from London and elsewhere (Gardner, 1928).

Attempts to infect guinea-pigs with *Leptospira* from slime and dirty water gave negative results.

### *Water* Leptospira.

Several samples of water from rat-infested and other areas were examined, being centrifuged when necessary. *Leptospira* recorded as present in the accompanying table (Table IV) were morphologically indistinguishable from *Leptospira icterohaemorrhagiae*. In rat-infested water it is probable that both non-pathogenic water strains and the *Leptospira* from rat urine would be present, but without a series of inoculations and serological tests it is impossible to differentiate the pathogenic strain.

Table IV. *Examinations of water for* Leptospira.

| Date | Locality | Kind of material examined | *Leptospira* |
|---|---|---|---|
| 15. vi. 28 | Port Meadow | Black slimy water, near dumps | + + |
| ,, | ,, | Green slime, near dumps | + |
| 18. vi. 28 | Pathology Laboratory | Tap water (centrifuged) | + |
| ,, | Zoology Laboratory | ,, | + |
| 25. vi. 28 | City Reservoir, Lake St | Inlet water from river | – |
| ,, | ,, | Bottom water | – |
| ,, | ,, | Surface water | – |
| ,, | ,, | Inlet pipe filter to reservoir | + |
| ,, | Iffley Rd, rubbish dump | Black slime, near dump | – |
| ,, | ,, | Slime on grass, under water | + |
| ,, | ,, | Slimy water, stagnant stream | + |
| ,, | ,, | Wet soil, edge of stream | – |
| ,, | ,, | Wet soil in rat runs | + |
| 27. vi. 28 | Port Meadow | Black ooze, near dumps | + + |
| ,, | ,, | Mud from stagnant stream | + |
| ,, | ,, | Black slime, near dumps | + + |
| ,, | ,, | Slime from stream | + |
| ,, | ,, | Black slime, near dumps | – |
| ,, | Summertown Recreation Ground | Mud from stagnant stream | – |
| ,, | ,, | ,, | – |
| 30. vi. 28 | Oxpens Recreation Ground | Mud from stream | – |
| ,, | Botley Recreation Ground | Muddy stream | – |
| ,, | Hinksey | Mud from bathing place | – |
| ,, | Canal bank | Mud | – |

*Sex ratio of rats.*

The sex ratio of the rats caught for this work is extremely low. Of rats weighing more than 150 grm. there were 88 males and 123 females: a sex ratio of 72 males per 100 females. Most of the rats were ejected from burrows by means of gas and ferrets, and killed with the aid of sticks and dogs; it would, therefore, seem reasonable to assume that those caught represent a random sample of the population.

## 4. Discussion.

The fall in percentage infection in old age may not be statistically significant on the figures available. But when it is remembered that each increment of 75 grm. means a much longer period of time among the larger weight groups than in the young and rapidly growing rats, it is obvious that the rate of infection in old age must be considerably lower than that of the immature rats.

Fig. 3 shows this rate of infection, or percentage increment in infections in the weight groups, calculated from the same data as Fig. 1. From this it appears that the percentage increment rapidly declines after the rats reach maturity: this is only to be expected when the older groups are already heavily infected, since the possible rate of infection must then be automatically reduced independently of any other factors. But the sudden fall in the percentage increment after the 225–300 grm. group cannot be entirely accounted for by the percentage of infections in the older groups: it indicates that a very much higher percentage of the population in these groups is in some way unable to become infected. The most reasonable explanation of this would seem to be that a certain percentage of these rats has become immune to the *Leptospira*, and are at that age entirely free from the organism and incapable of being re-infected. This view is supported by the work of Langworthy and Moore (1927), who found that 60 per cent. of the rats in Albany gave positive serological reactions for *Leptospira* although only 40 per cent. were found to be actually infected.

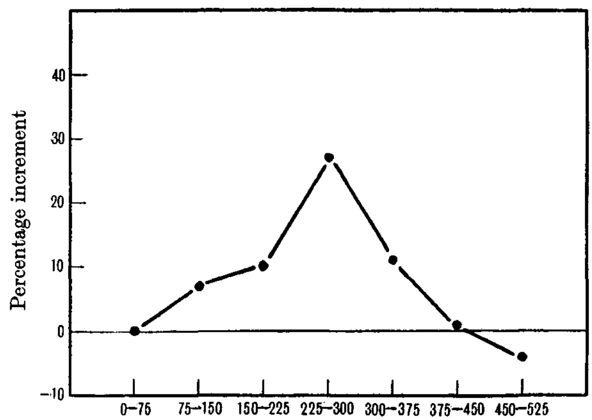

Fig. 3. Variation in the rate of infection at different weights.

Taking into account the remarkably heavy infection of one young animal, and the lack of severe infections in old rats, it seems reasonable to suggest the hypothesis that the rats suffer from a short attack of the disease when they are first infected, which may be at any period during life, but would tend to occur at a comparatively early date after the animals became fully active, and thereafter gradually acquire an immunity to the organisms, which are isolated in the kidneys. If the rat lives long enough, complete freedom and immunity from the parasite might be acquired.

It is, of course, possible that the fall in percentage infection with old age may be due to the infected animals dying off before the healthy ones, on account of slow or accumulating toxic action. However, as far as the writer is aware, there is no evidence that *Leptospira* is pathogenic to rats.

The rats could be infected either from the urine of other rats or, as Uhlenhuth and Zuelzer (1922) suggested (after they had succeeded in making a water strain pathogenic by continual culturing), primary invasion may be by a non-pathogenic water strain, which is rendered pathogenic to guinea-pigs and human beings by passage through the rat.

So far no satisfactory conclusion has been reached regarding the epidemic of jaundice among children: whether it is or is not caused by a *Leptospira* is a point which urgently needs settling. But there are ample facilities for the spread of the organism, among both rats and children, in the habitats which the rats most frequent. Refuse dumps invariably seem to be associated with standing water or stagnant streams, and the fascination of such places as playgrounds for children is only too obvious. Infection through a slight abrasion in the skin from water containing pathogenic *Leptospira* would be an easy matter when children are paddling and playing with sharp-edged tins and other refuse in such localities. When rats are known to be carrying a spirochaete, which in other cases has been amply proved to be pathogenic to human beings, it would seem well worth while

(*a*) To keep rubbish dumps as rat-free as possible.

(*b*) To make rubbish dumps as far from water or water-logged land as possible.

(*c*) To make such dumps inaccessible to children.

## 5. Summary.

41·7 per cent. of the rats in the Oxford district were found to carry *Leptospira icterohaemorrhagiae* in their kidneys. The percentage infection increases until the animals are mature, reaching a maximum of 56 per cent. among one class of the population, but is less among the oldest rats. There is evidence that the organism is acquired comparatively early in life, and that immunity is obtained after a long period of infection.

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