INFECTION RATES OF *RATTUS NORVEGICUS* WITH *LEPTOSPIRA ICTEROHAEMORRHAGIAE* IN GREAT BRITAIN

I. A RURAL AREA IN CARMARTHENSHIRE, WALES

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(With Plate 9 and 2 Maps in the Text)

INTRODUCTION

An analysis of the occupational incidence of Weil's disease in England and Wales between 1947 and 1950 showed that 30% of the patients worked on the land, and that cases of the disease occurred mainly in four areas—South Wales, Somerset and Devon, Hampshire, and East Anglia (Broom, 1951). Although a few surveys of the infection-rate of rats with *Leptospira icterohaemorrhagiae* have been made in this country, it will be seen from Table 1 that they do not provide a comprehensive picture. It seemed advisable therefore to attempt to determine carrier-rates both in the localities where the relative incidence is high, and also in other parts of the country. Only one district, a rural area in Carmarthenshire, South Wales, has been dealt with so far, but the survey will be extended as opportunity affords.

Table 1. *Infection rates of* Rattus norvegicus *in Britain with* Leptospira icterohaemorrhagiae

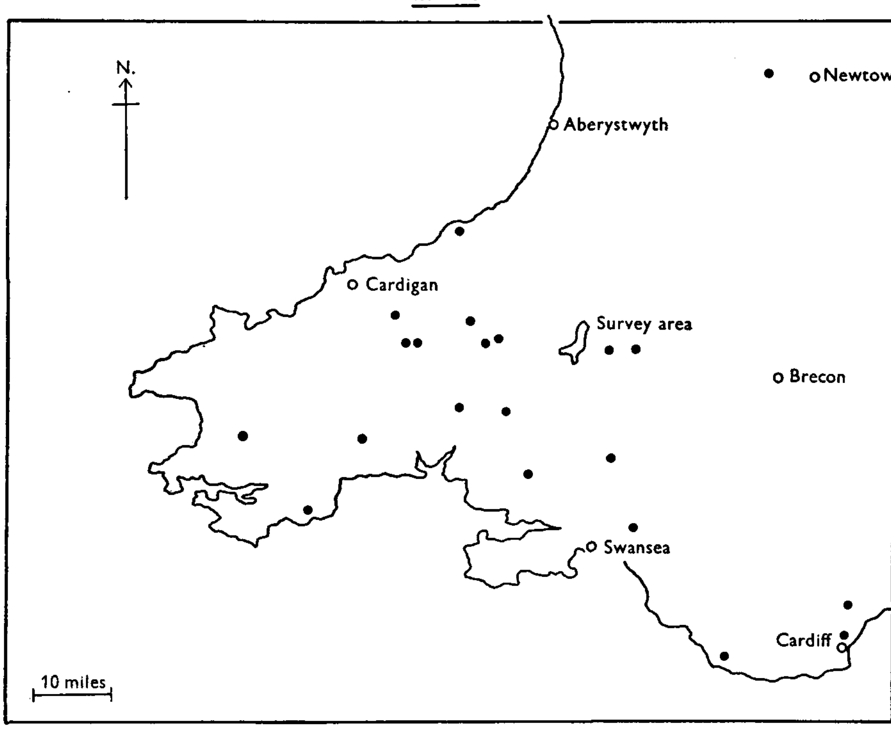
Date	Author	District	No. examined	Percentage infected
1918	Coles	Bournemouth	100	9
1919	Foulerton	London	98	4
1922	Stevenson	London	96	30
1929	Middleton	Oxford	237	41
1936	Coppinger	Aldershot	28	45
1938	Mason	Liverpool	250	26
1938	Smith	Aberdeen	117	27

The chance to obtain material in the present instance arose because one of us (E. A. G.) was engaged in investigating outbreaks of salmonellosis in cattle, and it was thought possible that, in certain circumstances, rats might be responsible for transmitting the disease. Rats collected for bacteriological examination were used to determine leptospiral infection-rates also.

TERRAIN

The area in which the survey was made, and also the places where cases of Weil's disease occurred in man, are shown on Map 1. No case had been reported from the particular district we investigated and, in view of our findings in the rats, this absence of human infection raises an interesting epidemiological problem which will be mentioned later. It is also of interest to note that, up to September 1952, no cases of leptospiral infection had been diagnosed in farm animals in the survey area.

MAP 1



Key: ● Cases. ○ Towns.

The distribution in South Wales of cases of Weil's disease in man.

The farms from which rats were obtained are indicated by circles on Map 2, but three farms which lie a short distance farther west are omitted. The farms are situated on both sides of a valley which extends for about 5 miles in a roughly north-south direction from Crugybar in the north, through Talley to the hamlet of Halfway in the south.

The floor of the valley is from 250 to 400 ft. above sea level, and the containing hills rise to 850–1100 ft. At Talley the hills form a narrow gap, less than half a mile wide. Here too the floor of the valley is at its highest, so the valley is divided into two drainage areas. The southern part of the valley drains south, into the River Dulais. The northern part is occupied by the River Cothi, which first flows south from Crugybar towards Talley, and then swings westwards into an adjoining valley.

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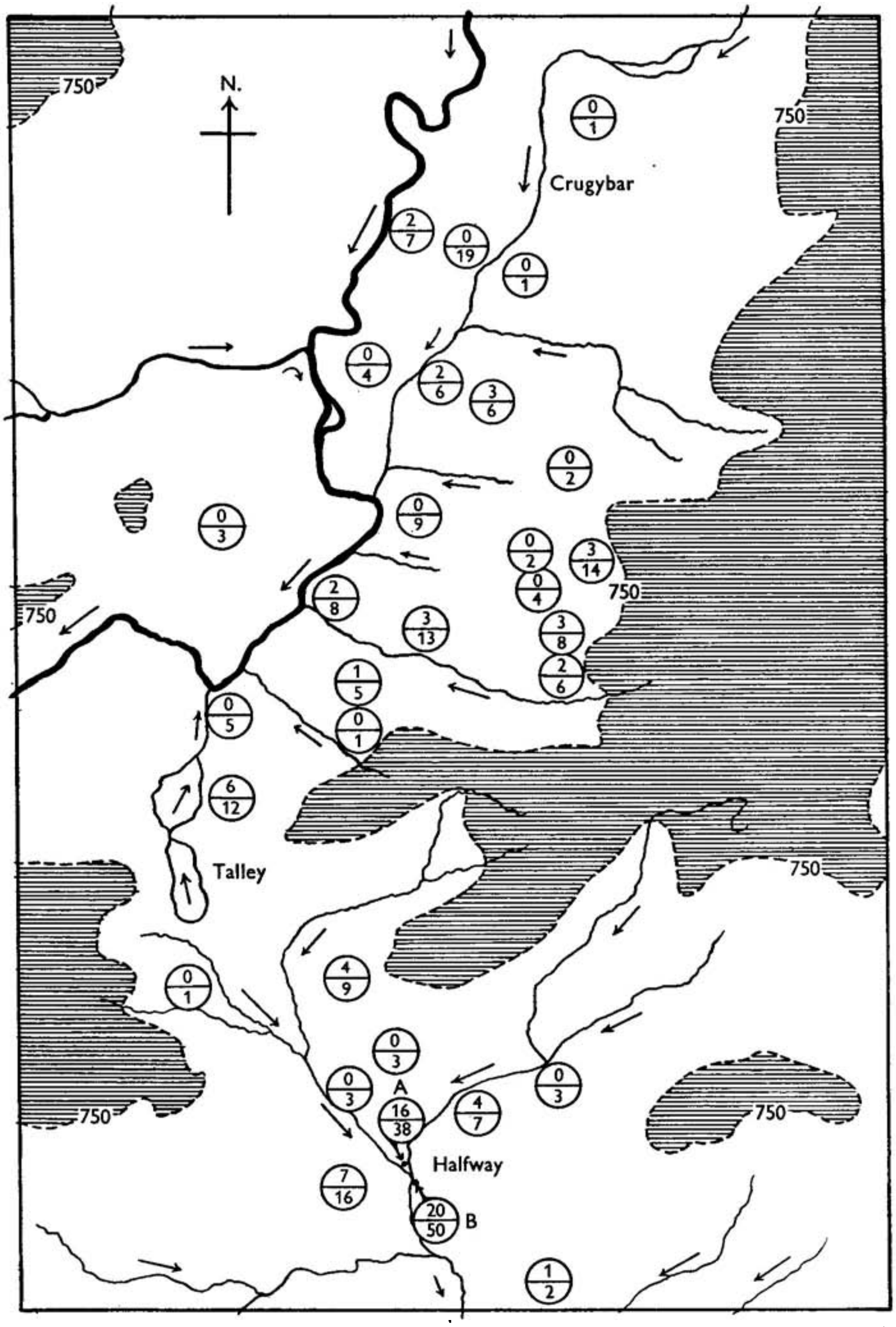
MAP 2



Sketch map of the survey area.

Denominator = Rats examined. Numerator = Number positive.

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As regards the geological formation, the Director of the Geological Survey and Museum, London, informs us that the district has not yet been surveyed on a 6 in. scale, and that no analyses of the river waters appear to exist. He has however supplied us with the following information:

'The strata in the valley range in age from Upper Ordovician to Lower Silurian. They comprise a great thickness of mudstones and shales with frequent thick intercalations of massive grits, sandstones and conglomerates. The rivers concerned, the Cothi and Dulais, are, in all probability, fed by surface run-off and by springs emerging from the beds of coarser lithology. Water from such sources might be expected to be neutral in reaction.'

The rainfall is moderately heavy. Thus, at Llandilo, some seven miles to the south, the average annual rainfall over the years 1945–1951 was 57 in., the range being from 46 to 67·5 in. The soil tends to be shallow, especially on the slopes of the hills. Much of it is poorly drained, so that sedges and coarse tussocky grass are common. There are some neglected woods on the lower slopes of the hills, and parts of the upper slopes are covered with bracken.

TYPE OF FARM-HOLDING AND HUSBANDRY

Nearly all the land in this district is farmed by tenant farmers who employ little or no labour outside their own families. Most of the farm buildings are constructed of local stone. Few of them are rat-proof, and the general standard of hygiene tends to be low.

On most farms the dairy herd is the chief source of income; the herds vary in size from a few beasts to some 70 head of cattle, a common size being about 30 head. The rearing of dairy heifers or young beef cattle for sale plays an important part in the economy of some farms. Others, especially those with grazing-rights on the hills, maintain flocks of sheep. A few pigs are kept 'for the house', and every farm has its 'barn-door' flock of poultry, but neither pigs nor hens are kept in large numbers.

The winter feeding of the cattle depends largely on the hay crop. Some root and green crops are grown, but little corn. Much of the grassland is permanent pasture of poor quality.

There are no sewerage or electricity services in the valley, and practically no mains-water supply. Almost every farm is traversed by one or more streams, and has a number of springs rising on its own land. Usually one of these springs is piped to supply the farm house. One small group of farms in the north of the valley shares a piped supply derived from springs, and the village of Talley also has a piped supply. On about two-thirds of the farms the cattle can drink from bowls or troughs during the period they are kept inside the cow-sheds. There are however no troughs in the fields, so the cattle drink from streams or springs, at least for that part of the year when they are at grass.

The streams receive both domestic sewage and the drainage from the farm buildings, even though they are often partly dried up during the summer months.

PREVALENT ANIMAL DISEASES

Infestation with the liver fluke *Fasciola hepatica* is widespread throughout the valley, so most of the cattle show chronic biliary cirrhosis at slaughter. The tick *Ixodes ricinus* is present on farms with rough pastures or hill grazings; it transmits the piroplasm *Babesia bovis*, giving rise to cases of 'redwater'. Bovine salmonellosis is also enzootic in this district.

RAT INFESTATION

Taking the valley as a whole, the level of rat infestation at the time of this survey, namely, April to November 1951, was lower than it had been in previous years. We were told by Mr J. T. Jenkins, Pests Officer for Carmarthenshire, that the number of rats in the Talley area reached a peak in 1941, and became a serious menace. At that time, the chief method of destruction was gassing, with some ferreting and trapping. The introduction of the use of rat poisons in the spring of 1942, and the employment of a larger staff, brought about a great reduction of the infestation.

The Prevention of Damage by Pests Act, 1949, consolidated the previous legislation on rodent control, and placed the responsibility for such control on the local authorities. This encourages the treatment of infestation on *groups* of farms, whereas previously only the more progressive farmers had undertaken rat destruction. The infestation in the valley is now described as having been reduced to a minimum.

The rat population of the valley was studied during the two-year period ending November 1951 by one of use (E. A. G.) who noted that the level of infestation varied greatly from farm to farm. Marked differences were often observed on adjacent farms, because the degree of infestation depended largely on the vigour of the measures taken against rats. Thus if extensive campaigns of trapping or poisoning were undertaken, or good working cats introduced, the surviving rats would migrate *en masse* to less protected farms. For this reason the degree of infestation on certain farms was found to vary greatly at different times.

Apart from these variations, changes in the level of infestation were caused by the usual seasonal migrations. After passing the winter in the farm buildings the great majority of the rats went into the fields in the spring, and lived in hedgerows and the banks of streams. In the late autumn the lack of food out of doors, or the onset of cold wet weather drove them back to the buildings. Thus, the rats in the farm buildings and those found out of doors appeared to be interchangeable members of the same community.

A number of the rats examined in this survey were trapped in hedgerows, while others were caught in runways between streams and nearby farm buildings. This was especially the case in farms A and B (see Map 2) which were close to a stream with rat-infested banks.

DETERMINATION OF INFECTION IN RATS

Various methods can be used to ascertain whether rats are carriers of leptospires, because the micro-organisms form dense colonies in the kidney tubules, and they also stimulate the production of specific antibodies. Evidence of infection can therefore be obtained by either: (1) determining the presence of specific agglutinins in the blood; (2) isolating strains from the kidneys, by direct culture or by animal inoculation; or (3) demonstrating leptospires microscopically, in fresh kidney tissue or in stained sections. No single method will detect every infection and, when comparative tests have been made, the method giving the highest proportion of positives has varied in different investigations. This diversity is illustrated by the examples shown in Table 2, in which the number of positives found in each case is expressed as a percentage of the total number of rats examined: the highest proportion is shown in bold type in each instance.

Table 2. *Comparison of efficiency of different methods of estimating infection rates of rats with leptospires*

Author	No. of rats examined	Percentage infected when examined by				
		Agglutination	Direct culture	Animal inoculation	Dark-field microscopy	Stained sections
Robinson (1924)	100	—	—	4	**7**	—
Langworthy & Moore (1927)	69	—	—	**30**	15	—
Aoki, Kaneko & Morimoto (1935)	50	72	**80**	—	56	—
Lahiri (1941)	125	5	3	5	**9**	—
Larson (1943)	27	**55**	30	35	40	45
Kalfayan (1947)	65	—	6	8	9	**12**
Monlux (1948)	100	—	**45**	29	37	41
Guida & Monici (1949)	57*	—	**55**	50	40	45
Pumarola Busquets, & Gallego Berenguer (1950)	76	—	35	**45**	40	—

* 34 (60%) positive by all methods combined.

From an extensive review of the literature Walch-Sorgdrager (1939) came to the conclusion that serological tests and cultures of kidney tissue detect the highest proportions of infections. To obtain satisfactory results with these methods, however, the rats should be trapped alive, and sacrificed immediately before autopsy. It is impossible to obtain blood samples from rats which have been dead for any length of time, and the *post-mortem* bacterial invasion of the kidneys precludes the culture of leptospires, and often makes the material useless for animal inoculation. For example, Smith (1938) found that, whereas thirty-two strains of *L. icterohaemorrhagiae* were isolated from 117 rats caught alive, not a single strain was obtained from forty-nine rats which had been dead for 24 hr.

MATERIAL AND METHODS

The rats in this series were all *Rattus norvegicus*, the common brown rat, and it appeared that the black rat, *R. rattus*, is absent from the survey area. It was noted also that the term 'water rat', which was used in everyday speech, referred as a rule to *R. norvegicus*, and only occasionally to the much rarer water vole, *Arvicola amphibius*.

Most of the rats were obtained by trapping, but a certain number had been poisoned with zinc phosphide. No live rats were received at the laboratory: indeed any period from a few hours up to 3 days elapsed after death before the animal could be investigated. Cultures were made from the kidneys of the more recently dead animals, but no leptospires were isolated. Agglutination tests were carried out in a few cases also but, for the purposes of this article, infection-rates are based entirely on the results of the microscopical examination of sections of kidney, stained by Levaditi's method of silver-impregnation.

The different species of leptospires cannot be identified by their morphological appearances, so there is no absolute proof that all the infected rats were carriers of *L. icterohaemorrhagiae*. In this particular instance, however, it seems valid to assume so, because (1) all rats examined were *R. norvegicus* which is the host of election of *L. icterohaemorrhagiae*, and (2) *L. icterohaemorrhagiae* and *L. canicola* are the only species known to be present in Britain, and *L. canicola* has never been found in rats.

When the rats were received at the field laboratory, one entire kidney was removed and placed in approximately 15 ml. of normal saline containing 10% of formalin by volume. Batches of kidneys were sent to London; there the kidneys were split in half longitudinally, and the two parts were used for silver and for histological staining respectively. When examining the silver-stained sections, only a single, complete, longitudinal section was scrutinized as a rule. The whole section was first scanned with a 16 mm. objective and $\times 10$ ocular, and any suspicious areas were then studied with a 2 mm. oil-immersion objective. Each section was examined independently by two workers.

As will be seen in Pl. 9, figs. 1 and 2, colonized tubules normally stand out sharply defined with low-power magnification, and individual leptospires are clearly distinguishable with higher powers. Since only a proportion of nephrons are affected, the method we used may have failed to disclose a certain number of light infections. In addition, as was noted above, some of the rats had been dead for considerable periods before the kidneys were fixed. We found some evidence which suggested that, with lapse of time after the death of the host, leptospires tend to break up into a series of amorphous granules which retain the silver stain. These fragments may be similar to the 'coccal' granules which, according to McIntyre & Montgomery (1952), are believed by certain workers to be the result of degeneration and dissolution of the micro-organisms. It would be impossible however to discriminate between such granules and other undifferentiated depositions of reduced silver which are sometimes present in sections stained by this method. A positive diagnosis was made therefore only when leptospires of typical morphological appearance could be distinguished with a 2 mm. objective.

The true carrier-rate may thus be higher than is indicated by our findings, but there is at least no reason to believe that it should be lower.

The kidneys of 357 rats were examined, and leptospires were seen in eighty-two (23 %). Table 3 shows that there is no difference in the sex-incidence, but that the infection rate is very low in young rats as compared with adults.

DISCUSSION

The striking difference in age-incidence is a usual finding, and Ostertag (1950) remarked that the change in carrier-rate seemed to coincide with sexual maturity. He thought this might indicate a venereal route of transmission—a suggestion which had been made earlier by Schüffner & Kuenen (1923).

This hypothesis does not postulate an ascending infection of the urinary tract, because leptospires can penetrate through intact mucous membrane and so reach the blood stream. It is interesting to note that transmission of *L. icterohaemorrhagiae* during sexual intercourse was believed to be the most likely method of infection in a human case of Weil's disease described by Doeleman (1932).

Table 3. *Age and sex-incidence of infection in* 357 *rats*

	Adults		Young	
	Male	Female	Male	Female
No. examined	146	129	48	34
No. infected	46	35	0	1
Percentage infected	30	27	—	—

It may be assumed that leptospires circulate in the blood of carrier-hosts for some period of unknown duration in the early stage of infection, but thereafter the organisms are confined to the urinary tract. There is no means of knowing therefore whether leptospires can pass through the placental barrier in rats. In the case of guinea-pigs Saenz (1929) and Das Gupta (1939) both found leptospires in the foetuses of pregnant females infected experimentally with *L. icterohaemorrhagiae*. In man the position is less clear. Hiyeda (1928) examined the 4-month foetus of a woman who aborted during an attack of Weil's disease. He states that 'pathologico-histological examination was negative, though there were reasons for believing that the fetus had been infected'. Another interesting, though not fully proved, instance is recorded by Lindsay & Luke (1949). In this case the mother, who was not ill, was delivered of a full-term child which appeared normal, although the vernix caseosa and amniotic fluid were stained with bile. The infant became jaundiced 30 hr. after birth, and died in convulsions 14 hr. later. The autopsy, and the histological appearances of the liver and kidney were compatible with leptospirosis and, in sections stained by Levaditi's method, rare leptospires were found in the liver. Several typical organisms were demonstrated microscopically in the renal tubules, but satisfactory photographs could not be obtained. The mother's serum, taken 2 weeks after the death of the infant, had a positive titre of 1/10,000 with both *L. icterohaemorrhagiae* and *L. canicola*, but the reactions became completely negative 2 months later. The authors assume that the mother was suffering from latent leptospirosis during pregnancy, and that the foetal tissues were more susceptible to invasion than the maternal ones.

The important part played by water contaminated with infective urine is of course well established in the transmission of leptospirosis to man, but its significance in rat to rat transmission is less certain. Walch-Sorgdrager (1939) says that, in the absence of direct observations, it must be assumed that rats are infected either by eating food which has been fouled with urine, or by direct contact with infective material. Fühner (1950), on the other hand, thinks that ready access to water increases the carrier-rate, because few leptospires survive in undiluted rat urine for as long as 5 hr. after the urine is shed. As supporting evidence he states that, whereas leptospires were found in nearly 50 % of rats trapped near water-courses in and around Hamburg, ten old rats caught in fields remote from streams were all free.

The absence of infection in the latter group could occur as the result of chance quite apart from any question of proximity to water. In our series, with an average of 23 % infections, there were eight occasions when more than ten consecutive rats were negative, the highest sequences being 19, 23, 23, and 26. It would be unwise therefore to assume from only ten observations that contaminated water plays an essential part in transmission from rat to rat.

Our own observations indeed suggest that there is some essential difference in the method of the transmission of infection among rats, and from rat to man. For example, our biggest catches—eighty-eight adult rats with twenty-eight (30 %) positive—were made on two farms (A and B on Map 2) which adjoin each other, and which are well watered by the same stream. In spite of these high rates, none of the seven persons living and working on these farms had suffered from clinical Weil's disease. We were able to test serum from six of the seven, and none of the sera contained agglutinins for *L. icterohaemorrhagiae*. The farm houses are within a few yards of the stream, the banks of which were heavily infested with rats during the period of our survey. Rats were found in the outbuildings, cattle-food store and cow-sheds, so it was probable that the farm residents must often have handled materials soiled by rat excreta.

Both farm houses had a supply of well water, but stream water was used for washing down the cow-sheds, and was the only source of drinking water for the dairy cattle. No cases of leptospiral infection had however been diagnosed among the animals either.

It would seem therefore that some unknown factors were acting as a barrier to transmission from rat to man and to domestic animals, without affecting the dissemination of leptospires among the rats themselves.

SUMMARY AND CONCLUSIONS

A description is given of the topography, type of farming carried out, and the living conditions of the agricultural community of a district in South Wales where a survey was made of the infection rate of rats with *Leptospira icterohaemorrhagiae*.

Leptospires were seen in stained sections of the kidneys of eighty-two of the

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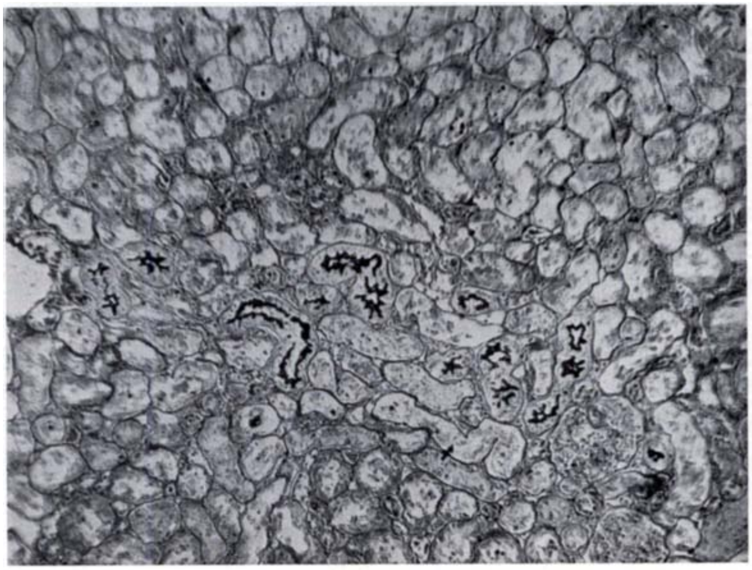

Fig. 1.

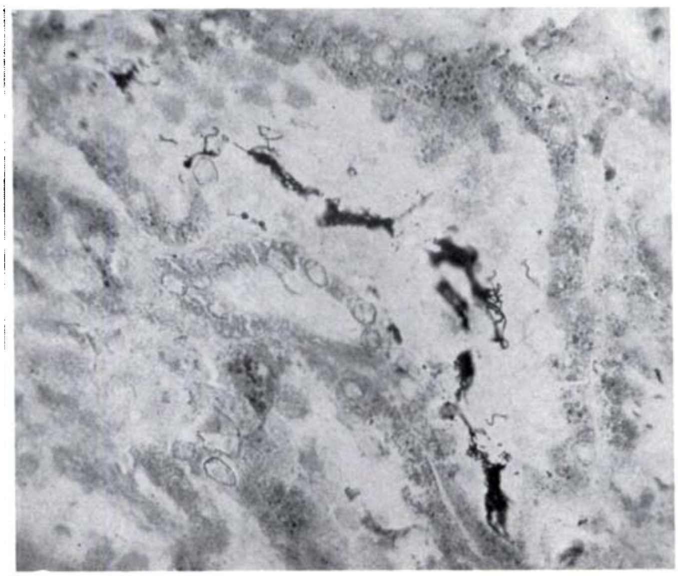

Fig. 2.

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357 rats examined (23 %). With a single exception, all the infected rats were adult, but no difference in sex-incidence was found.

Weil's disease occurs in this area, but no cases in man or cattle had been reported from this particular locality. Blood samples taken from six persons living on two heavily infested farms contained no anti-leptospiral agglutinins.

We should like to record our thanks to the Director of the Geological Survey and Museum for information about the geology of the survey area; to the Director of the Ordnance Survey for permission to publish maps based on the Ordnance Survey Maps; to Mr J. T. Jenkins, Pests Officer for Carmarthenshire for help and advice; and to our staffs at the Llandilo Laboratory, and the Wellcome Research Institution for their technical assistance.

REFERENCES

AOKI, Y., KANEKO, K. & MORIMOTO, T. (1935). *Z. Hyg. InfektKr.* **117**, 208.
BROOM, J. C. (1951). *Brit. med. J.* **2**, 689.
COLES, A. C. (1918). *Lancet*, **1**, 468.
COPPINGER, C. J. (1936). *J. R. Army med. Cps*, **66**, 82.
DAS GUPTA, B. M. (1939). *Indian med. Gaz.* **74**, 28.
DOELEMAN F. P. J. (1932). *Ned. Tijdschr. Geneesk.* **76**, 5057.
FOULERTON, A. G. (1919). *J. Path. Bact.* **23**, 78.
FÜHNER, F. (1950). *Städthyg.* **1**, 218.
GUIDA, V. O. & MONICI, N. (1949). *Bol. Soc. paul. Med. vet.* **8**, 133.
HIYEDA, G. (1928). *Japan med. World*, **8**, 15.
KALFAYAN, B. H. (1947). *Trans. R. Soc. trop. Med. Hyg.* **40**, 895.
LAHIRI, M. N. (1941). *Indian med. Gaz.* **76**, 536.
LANGWORTHY, W. W. & MOORE, A. C. (1927). *J. infect. Dis.* **41**, 70.
LARSON, C. L. (1943). *Publ. Hlth Rep., Wash.*, **58**, 949.
LINDSAY, S. & LUKE, J. W. (1949). *J. Pediat.* **34**, 90.
McINTYRE, W. I. M. & MONTGOMERY, G. L. (1952). *J. Path. Bact.* **44**, 145.
MASON, W. N. M. (1938). *J. Path. Bact.* **46**, 631.
MIDDLETON, A. D. (1929). *J. Hyg., Camb.*, **29**, 219.
MONLUX, W. S. (1948). *Cornell Vet.* **38**, 199.
OSTERTAG, H. (1950). *Z. Hyg. InfektKr.* **131**, 482.
PUMAROLA BUSQUETS, A. & GALLEGO BERENGUER, J. (1950). *Rev. ibér. Parasit.* **10**, 301.
ROBINSON, G. H. (1924). *Amer. J. Hyg.* **4**, 327.
SAENZ, A. (1929). *C. R. Acad. Sci., Paris*, **188**, 1455.
SCHÜFFNER, W. & KUENEN, W. A. (1923). *Ned. Tijdschr. Geneesk.* **67**, 2018.
SMITH, J. (1938). *J. Hyg., Camb.*, **38**, 521.
STEVENSON, A. C. (1922). *Amer. J. trop. Med.* **2**, 77.
WALCH-SORGDRAGER, B. (1939). *Bull. Hlth Org. L. o. N.* **8**, 143.

EXPLANATION OF PLATE 9

Fig. 1. Rat kidney showing colonies of leptospires in tubules. (× 100: Levaditi.)
Fig. 2. Higher magnification of part of fig. 1, showing individual leptospires and clumps. (× 600.)

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