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Prevalence of *Leptospira* spp. in wild brown rats (*Rattus norvegicus*) on UK farms

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SUMMARY

Wild brown rats (*Rattus norvegicus*) are frequently implicated in the carriage and spread of *Leptospira* spp. Wild brown rats ($n = 259$) were trapped from 11 UK farms and tested for *Leptospira* spp. using a number of diagnostic tests. The prevalence of leptospiral infection was low, but there was variation in the results obtained with the different diagnostic tests. Estimates of prevalence ranged between 0% by silver-staining of tissues, 1% by the microscopic agglutination test, 4% by the enzyme-linked immunosorbent assay, 4% by culture, and 8% by fluorescent antibody technique. In total, 37 (14%) rats were positive by at least one of the tests, which contrasts with the frequently reported prevalences of 50–70% for wild rats in the UK. Serovar *bratislava* was as prevalent as *icterohaemorrhagiae*, although it was present only on farms with larger rat populations.

INTRODUCTION

Leptospirosis has been stated to be the zoonosis which causes the greatest problems to humans and livestock in the UK today [1]. The type organism, *icterohaemorrhagiae*, was first reported from a human with Weil's disease in 1916 [2] and then from wild brown rats (*Rattus norvegicus*, Berkenhout) in 1917 [3]. Wildlife, and brown rats in particular, were thus immediately implicated as important factors in the epidemiology of leptospirosis. By 1930 the brown rat was considered to be a world-wide carrier of *icterohaemorrhagiae*, and current literature states (or assumes) that most, if not all, rat populations are infected with *Leptospira icterohaemorrhagiae* [4] at a prevalence of 50–70% [1, 5]. However, there are few data to validate these generalizations. Epidemiological conclusions have often been based on small numbers of wild rats [6, 7]. Moreover, the majority of publications date back to the 1960s or earlier, which predates radical shifts in the agro-ecosystem, and when diagnostic techniques were generally unable to achieve serovar or serogroup specificity.

The aim of this study was to survey a large number of wild brown rats to determine current leptospira status, both in terms of its prevalence and the range of serogroups and serovars carried.

MATERIALS AND METHODS

Rats ($n = 259$) were trapped between 1991 and 1993 from 11 farms (9 were in Oxfordshire, 1 in Hampshire, and 1 in North Wales; all farmers had responded to an advertisement requesting access to farms with large rat infestations). Every 2 months, 40 'Bledorberry' live-traps were pre-baited with whole wheat for 7 nights prior to 7 nights of trapping. Trap-night averages and census-baiting [4] were used to estimate the relative population density of rats on the farms. The trap-night average is the mean number of rats trapped per night from 40 live-traps placed at each farm. Census-baiting involves placing 1000 g of wholewheat into each of 22 covered bait trays at the farms and measuring the nightly grain consumption. Rat population density is estimated by dividing the total grain consumption by 28 g, which is the mean nightly grain consumption of an adult wild rat [4]. Rats were categorized by weight as juveniles (< 100 g), sub-adults (100–200 g), and adults (> 200 g). Forty-five woodmice (*Apodemus sylvaticus*), housemice (*Mus musculus*), bank voles (*Clethrionomys glareolus*), and common shrews (*Sorex araneus*) were also trapped in Longworth live-traps from two farms. Animals were taken to the laboratory, killed with CO_2 and bled by cardiac puncture. The following diagnostic tests were performed.

Serological examination

The microscopic agglutination test (MAT) [8] was performed using eight antigens, *autumnalis*, *ballum*, *bratislava*, *canicola*, *hardjo*, *icterohaemorrhagiae*, *pomona* and *tarassovi*. Live antigens were used because they are more sensitive than formalized antigens [9]. Titres of $\geqslant 30$ were considered positive.

The enzyme-linked immunosorbent assay (ELISA) [10] was performed using *icterohaemorrhagiae* and *bratislava* antigens. Two ELISAs were performed upon each sample, one using a carbohydrate antigen [11] and the other a protein antigen [12]. Titres of $\geqslant 80$ were considered as positive.

Culture

One kidney, the brain, one side of the genital tract (in males and females) and the embryos of pregnant females were homogenized by forcing them through the nozzle of a sterile disposable 5 ml syringe into a gamma-ray-sterilized stomacher plastic bag containing 5 ml of EMJH base medium (Difco). The mixture was then placed in a Colworth Stomacher 400 (Steward & Co Ltd, London) for approximately 5 min. Then 0·1–0·2 ml of each homogenate were inoculated into each of the following (7·5 ml) semi-solid media. Media A: EMJH base (Difco, ref. 0794-01-9), EMJH supplement (Difco, ref. 0795-73-1), agar (0·15%; BBL®). Media B: media A, rabbit serum (6%; Sigma), 5-fluorouracil (200 μg/ml; Calbiochem). Media C: media A, rabbit serum (6%), 5-fluorouracil (400 μg/ml), rifampicin (1000 μg/ml; Sigma), amphotericin B (100 μg/ml; Sigma).

The cultures were incubated at 30 °C for 12 weeks and examined by dark-field microscopy at 2-week intervals. Whenever leptospires were observed the sample was passaged into its appropriate fresh media.

Staining techniques

One-half of one kidney from each mouse, shrew, vole and 100 randomly selected rats, was fixed in formalin and embedded in paraffin. Serial sections were stained by the Leviditi method [13] and Elliott's modification of Young's stain [14]. The other kidney half was fixed in acetone and serial sections stained with fluorescent antibody (FAT) [15].

RESULTS

Leptospira-positive rats were detected from 9 out of the 11 farms (Table 1). Estimates of prevalence ranged between 0% by silver-staining, 1% by MAT, 4% by ELISA, 4% by culture, and 8% by FAT. A total of 37 (14%) rats were positive by at least one test. There was no overall significant effect of sex or age (Fisher's exact test, $P = 0{\cdot}60$ and $P = 0{\cdot}40$ respectively; Table 2).

Serology

The serum of 3 of 259 rats was seropositive for *icterohaemorrhagiae* by the MAT. ELISAs, in contrast, revealed 10 rats (including those 3 MAT-seropositive) to be seropositive to *icterohaemorrhagiae*. ELISAs also revealed the sera of nine rats to be seropositive to *bratislava* (one rat was seropositive to both *icterohaemorrhagiae* and *bratislava*) (Table 2). The ELISA carbohydrate and protein antigens, however, gave different end-point titres and thus only ELISAs for which titres in both assays were $\geqslant 80$ were considered positive.

Icterohaemorrhagiae-seropositive rats were found on 4 farms (3 in Oxfordshire, 1 in Wales), whilst *bratislava* seropositives were present on 2 (both in Oxfordshire). These two farms had the greatest rat populations as estimated using trap-night averages and census-baiting (Table 1). There was no significant effect of sex or age on the distribution of rats seropositive to *icterohaemorrhagiae* (Fisher's exact test, sex: $P = 0{\cdot}52$; age: $P = 0{\cdot}43$) or *bratislava* (sex: $P = 0{\cdot}73$, age: $P = 0{\cdot}54$). Nevertheless, only one juvenile rat was seropositive to either serogroup (*icterohaemorrhagiae* by ELISA) and no subadult rat was seropositive (Table 2).

Culture

Leptospira were cultured from the kidneys of eight rats, with a greater number of positive male rats than females. Leptospira were cultured from only one subadult and one juvenile rat, neither of which was seropositive. Leptospira were cultured from four adult rats which were seropositive by ELISA to *icterohaemorrhagiae*, but none from rats seropositive to *bratislava*.

Leptospira were cultured from the genitalia of one adult female rat and the embryos of another. Leptospira were also cultured from their kidneys. No Leptospira was cultured from the brain or urine of rats.

Contamination of cultures after the fourth passage prevented typing and hence confirmation of Leptospira.

Staining techniques

No leptospire was detected in silver-stained sections of kidney, although three rats were seropositive to *icterohaemorrhagiae* by ELISA.

Table 1. *Characteristics of farms and rat populations*

	Farms				*Leptospira*		
	Region	Type	Livestock	Census-bait	No.	No. + ve	% + ve
F1	Oxfordshire	Smallholding	Ch.	1	1	0	—
F2	Oxfordshire	Dairy	Ca.Ch.	8	4	0	—
F3	Oxfordshire	Smallholding	Ch.Go.	9	2	0	—
F4	Oxfordshire	Arable	Ca.	9	5	1	20
F5	Oxfordshire	Smallholding	Ch.Sh.Go.	10	30	4	13
F6	Oxfordshire	Arable	Ca.Sh.	15	14	2	14
F7	North Wales	Dairy	Ca.Sh.	—	31	4	13
F8	Oxfordshire	Arable	Ca.Sh.Pi.	44	23	5	22
F9	Hampshire	Dairy	Ca.Sh.	52	34	4	12
F10	Oxfordshire	Arable	Go.Sh.	55	38	5	13
F11	Oxfordshire	Arable	Pi.Ch.	73	77	12	15

Farms are presented in ascending order of estimated rat population density as indicated by trap-night and census-bait averages (see text for details). —, not sampled. Livestock refers to the principal domestic animals present at each farm as follows: Ca, cattle; Ch, chickens; Go, goats; Sh, sheep; Pi, pigs. Leptospira: no., number of rats sampled at each farm; no. + ve and % + ve, number and percentage of rats positive to at least one diagnostic test (see text for further details).

Table 2. *Prevalence of* leptospira *in wild rats by different diagnostic tests*

		Serology			Culture			Staining			
		MAT *ict.*	ELISA *ict.*	ELISA *brat.*	Kid.	Gen.	Bra.	SS.1	SS.2	FAT	Max.
No	T	259	259	219	219	219	212	100	100	219	259
No + ve	T	3	10	9	8	3	0	0	0	18	37
% + ve	T	1	4	4	4	1	0	0	0	8	14
No	M	120	120	97	108	108	101	50	50	111	120
No + ve	M	2	6	3	6	0	0	0	0	5	16
% + ve	M	0·1	5	3	5	0	0	0	0	5	13
No	F	139	139	122	111	111	111	50	50	118	139
No + ve	F	1	4	6	2	3	0	0	0	12	19
% + ve	F	0·7	3	5	2	2·7	0	0	0	10	14
No	J	39	39	17	27	27	20	22	22	22	39
No + ve	J	0	1	0	1	0	0	0	0	4	6
% + ve	J	0	2·5	0	4	0	0	0	0	18	15
No	S	41	41	29	34	34	34	31	31	30	41
No + ve	S	0	0	0	1	0	0	0	0	2	3
% + ve	S	0	0	0	3	0	0	0	0	6	7
No	A	179	179	173	158	158	158	47	47	167	179
No + ve	A	3	9	9	6	3	0	0	0	12	28
% + ve	A	2	5	6	4	2	0	0	0	7	16

T, Total; M, males; F, females; J, juveniles (< 100 g); S, sub-adults (100–200 g); A, adults (> 200 g). ELISA values are for only those samples in which the results of both assays were ⩾ 80 (see text). The results obtained by culture may be underestimates since a proportion of cultures were contaminated with other microorganisms thereby preventing the isolation of the leptospires. Kid., kidney; Gen., genitalia; Bra., brain. SS1 and SS2, two types of silver-staining techniques. Further details of tests are provided in the text. Max., number of rats diagnosed positive by at least one test.

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Leptospira were demonstrated in the kidneys of 18 rats using the FA technique. lthough this figure equals approximately that of the total prevalence obtained y ELISA (*icterohaemorrhagiae* and *bratislava* combined), no rat was positive by oth FAT and serology. Similarly, leptospira were demonstrated in the kidneys f only three rats by both culture and FAT.

Leptospira were demonstrated by FAT in the kidneys of rats from five farms in xfordshire and one in Wales. There was no effect of sex or age (Fisher's exact st, sex: $P = 0{\cdot}13$, age: $P = 0{\cdot}22$), although a greater number of juvenile and sub-dult rats were FAT-positive than obtained by either serological technique.

Kidney sections from bank voles (1 male, 15 female; 11 adult, 5 juvenile), oodmice (5 male, 9 female; 8 adult, 6 juvenile) house mice (4 male, 2 female; 5 dult, 1 juvenile) and common shrews (1 male, 4 female; 4 adult, 1 juvenile) ained with FA were all negative.

DISCUSSION

The three major findings of this study were: (1) there was great variation in sults obtained by different diagnostic tests, (2) *bratislava* was as prevalent as *terohaemorrhagiae*, and most importantly, (3) prevalence of leptospiral infection 1 these wild brown rats was low.

iagnostic techniques

The disparity between diagnostic methods, and the controversy over which is ne most reliable, is not a new phenomenon [e.g. 16, 17]. However, this study evealed further differences within, as well as between, serological and bac-eriological tests. The MAT, a frequently used serological test for diagnosing ptospiral infection [16], revealed fewer *icterohaemorrhagiae* seropositives than id the ELISA. The ELISA also detected antibodies to *bratislava* while the MAT iled to do so (Table 2). However, the ELISA failed to detect antibodies in rats 1 which leptospira were demonstrated in their kidneys by the FA technique. ulture proved an unreliable method of detection due to contamination, a roblem encountered by others [e.g. 18]. The failure to demonstrate leptospires by lver-stained tissue sections has also been reported [18], which may be due, in art, to non-specific background staining and spontaneous precipitation of silver ns [18]. The FA technique produced the largest number of leptospira positives lthough the technique cannot differentiate serogroups or serovars and so must be sed in conjunction with other tests, and this may be of doubtful value as no ssociation between ELISA seropositives and FAT positives was found.

ratislava *v.* icterohaemorrhagiae

Bratislava is usually associated with hedgehogs [19], pigs [20] and horses [21], ather than rats. Its presence within rat populations may suggest that it is a ecently acquired leptospira and/or that rats are normally incidental rather than aintenance hosts for this serovar. Wild brown rats in New Zealand, which are aintenance hosts for *copenhageni* are incidental carriers of *ballum* at low opulation densities but become maintenance hosts at high densities [18, 22]. The vo farms with *bratislava* in our study had the largest rat populations (Table 1).

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Another explanation for the findings is that *bratislava* has always been carried by wild brown rats in the UK but the early diagnostic techniques failed to detect it. Indeed, Balfour [23] and Broom and Gibson [24] state that all leptospira detected were automatically assumed to be *icterohaemorrhagiae* as this was the only serogroup believed to be carried by brown rats.

Leptospira prevalence

In contrast to the generalizations frequently cited in both the lay and academic literature, leptospira appears to have a low prevalence within at least some wild rat populations.

Low prevalence might have arisen if the age structure of rats trapped was biased, for example towards juveniles or sub-adults, since a number of workers have found significantly lower prevalences within younger rats [7, 22]. Yet, not only was our sample not biased, the FA technique indicated an approximately equal leptospira prevalence within each age-category. Low prevalence might be an atypical characteristic of Oxfordshire although the two populations from Hampshire and North Wales also had low levels of leptospiral infection. Another alternative may be that leptospira may be more prevalent in rats from urban areas than in the rural populations examined here. However, this also seems unlikely since the opposite pattern, with high prevalences in rural areas and an absence within suburban and urban sites, has been reported [e.g. 25, 26].

Thus, it appears that either the epidemiology of leptospira in rats may have changed since the early studies, or that the status may have been misinterpreted. Indeed, it is noteworthy that several authors who cite 50–70% as the average prevalence of leptospira infection in wild rats [e.g. 1, 5] do not provide data in support of their figures. The absence of leptospira within the other rodents and insectivora examined here may imply freedom from infection in other similar wildlife species also.

To conclude, rat-borne leptospira infection may not be as prevalent, at least on some farms, as was generally believed. Whilst this alone must not rule out the importance of human hygiene and rodent control, it does, together with the variability in diagnostic techniques observed, emphasize the need for caution in the interpretation of generalizations in the literature. It also suggests the need to examine wild rat populations from different parts of the country and re-evaluate their role in the epidemiology of leptospiral infections in mammals including man.

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