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The coypu as a rodent reservoir of leptospira infection in Great Britain

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SUMMARY

The coypu (*Myocastor coypus* Molina) is an aquatic rodent that has become a widespread pest in the south-east of England. Since the natural habitat of this animal is aquatic, the possibility of infection with leptospires was investigated. Twenty-nine coypu were trapped and examined by serological, histological and cultural methods. Of these, there was serological evidence of infection in seven coypus and *Leptospira interrogans* var. *Wolffii* was isolated from a further animal. This appears to be the first report of the isolation of leptospira from a coypu in Great Britain.

INTRODUCTION

The coypu (*Myocastor coypus* Molina) is an aquatic rodent, native of South America, that was exported to Europe, including Great Britain, for fur farming. It was first introduced into Great Britain in 1929 and later escaped into the wild. In Britain the coypu is classified as a pest because of its destructive activities on crops, mainly root crops, and on dykes and river banks.

Leptospires were first isolated from the coypu by Anchezar and his colleagues in 1949 in Argentina. (Anchezar, Illa & Vivoli, 1949). A more recent study by Twigg & Cuerden in 1966 showed that there was serological evidence of leptospires in wild coypu found in Great Britain but they failed to isolate the organism.

Since the coypu is one of the few members of the rodent family in the United Kingdom in which leptospires have not been isolated and, furthermore, it is known that their natural aquatic habitat should predispose them to casual environmental contamination with leptospires, we decided to study in detail the incidence of leptospiral carriage and their possible isolation in the coypu. This paper reports the successful isolation of leptospires from the coypu and their possible significance in the spread of leptospirosis to domestic animals.

MATERIALS AND METHODS

(A) *Materials required for field project*

(i) Experimental animals: the coypu (*Myocastor coypus* Molina).
(ii) Traps and bait: cage traps with carrot or apples as bait.

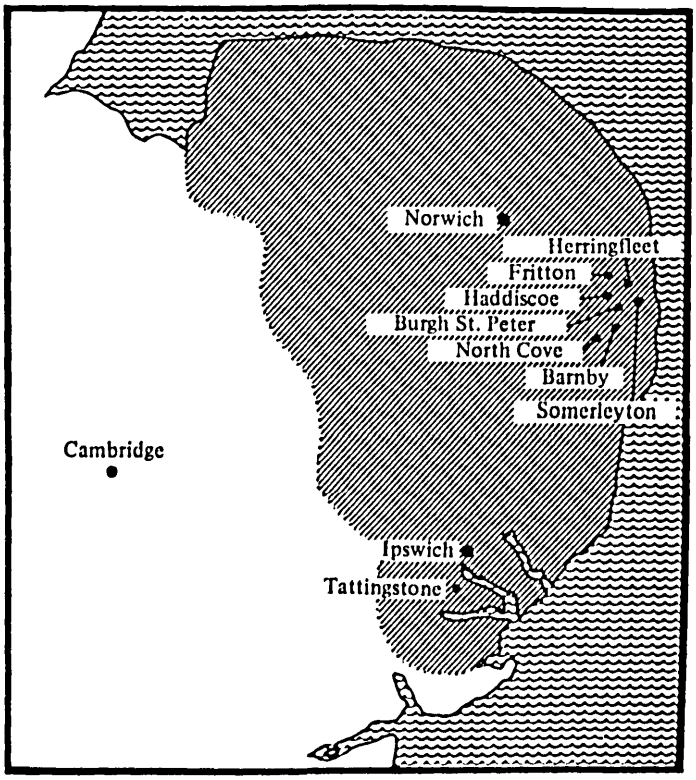

Fig. 1. Shows the sites where the coypu were caught and examined in this study.

(B) *Media for isolation and identification*

(i) *Enrichment media.* Ellinghausen & McCullough media (1965) with added rabbit serum (2%) and 0·1% agar.

(ii) *Selective media.* Ellinghausen & McCullough media with 2% rabbit serum, 0·1% agar and 0·02% 5-fluorouracil (200 μg/ml) (W.H.O., 1982).

(iii) *Differential media.* Ellinghausen & McCullough media with 8-azaguanine (Johnson & Rogers, 1964*a*, *b*).

(C) *Darkfield microscopy*

Leptospires from cultures were examined using a small drop of inoculum on to a glass slide covered with a cover-slip, this was initially screened at low magnification (× 100) and then at high dry magnification (× 400). Leptospires were identified on the basis of their typical morphology and characteristic motility which consists of alternate rotation along their long axis, moving backwards and forwards.

(D) *Field methods*

(i) *Area studied*

The coypus were collected from nine parishes in Norfolk and Suffolk between November 1983 and January 1984 (Fig. 1). The terrain of the land on which the coypu were caught was agricultural land used as pasture for cattle as well as crop farming. Marshland was also present in the area.

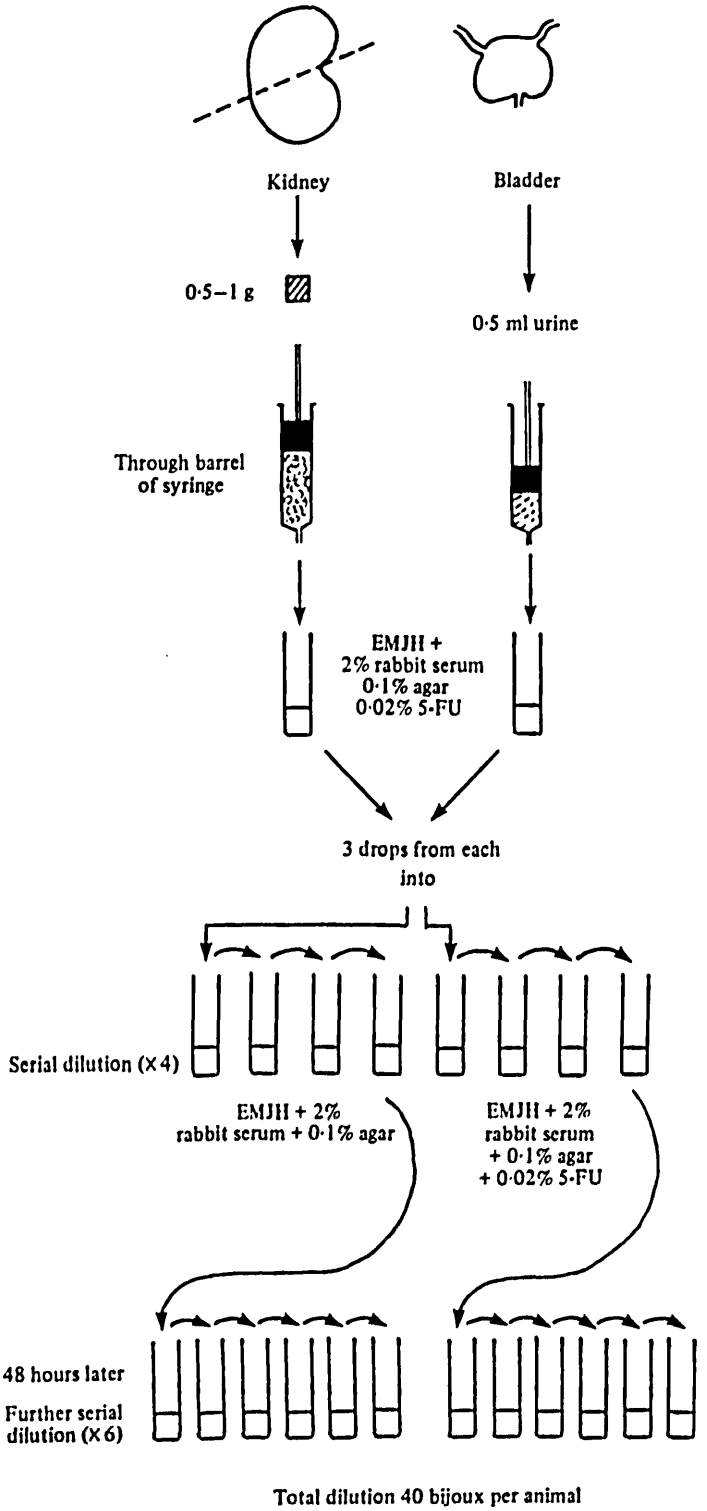

Fig. 2. Illustrates a flow diagram of how the specimens were processed in the field and then in the laboratory.

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Table 1. *Sex, approximate age and weight of coypu examined*

Coypu	Date collected	Sex	Approximate weight (kg)	Approximate age (days)
1	15 Nov.	F	1	46
2	16 Nov.	M	5	317
3	16 Nov.	F	2	113
4	16 Nov.	M	3	310
5	16 Nov.	M	3	201
6	16 Nov.	M	1	n.d.
7	16 Nov.	F	2	254
8	16 Nov.	M	7	449
9	16 Nov.	F	7	388
10	17 Nov.	M	6	450
11	17 Nov.	M	5	117
12	10 Jan.	F	7	735
13	10 Jan.	M	4	1333
14	10 Jan.	M	1	77
15	10 Jan.	M	3	352
16	10 Jan.	F	1	63
17	10 Jan.	F	1	63
18	10 Jan.	M	1	77
19	10 Jan.	Not known	1	86
20	11 Jan.	M	2	172
21	11 Jan.	M	1	104
22	11 Jan.	F	2	188
23	11 Jan.	M	2	158
24	11 Jan.	M	4	188
25	12 Jan.	F	3	167
26	12 Jan.	F	8	553
27	13 Jan.	F	3	559
28	13 Jan.	F	4	531
29	13 Jan.	M	4	680

(ii) *Methods for trapping and collection of specimens*

A total of 29 coypus were caught using cage traps set on their runs. Either carrots or apples were used as bait. The traps were checked 1 day after setting them and the coypus caught and killed with a 0·22 pistol by shooting in the head or just above the centre of the eye level while still in the cage (it is unlawful to transport live coypu outside East Anglia). Blood was collected immediately from the bullet wound into 10 ml plastic tubes with beads coated with kaolin (the blood clotting time in coypu is very short). The tubes were well shaken to let the beads aid clotting.

At post-mortem about 1 ml of urine was withdrawn from the urinary bladder and 0·5 ml put into 10 ml of Ellinghausen & McCullough media (EM) with 2% rabbit serum 0·1% agar and 0·02% 5-FU. The kidneys were located and 0·5–1 g of kidney tissue obtained from the cortico-medulla area of each kidney was put in a 5 ml syringe whose plunger had been removed. The plunger was put back and the tissue was gently squashed into EM with 2% rabbit serum, 0·1% agar and 0·02% 5-FU. Eyes were removed and together with the other halves of kidneys were put in plastic bottles with 10% neutral formalin.

The specimens were processed as outlined in Figure 2.

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Table 2. *Antigen pools for microscopic agglutination test (MAT)*

Serogroup	Pools
I: *Icterohaemorrhagiae*	RGA, Wijnberg, Mankarso
II: *Javanica*	Javanica
III: *Celledoni*	Whitcombi
IV: *Canicola*	Canicola, Schueffneri
V: *Pyrogenes*	Pyrogenes, Robinsoni, Biggis
VI: *Ballum*	Mus, 127, Castellonis
VII: *Autumnalis*	Rachmati, A. akiyami
VIII: *Australis*	Ballico, Lora, Jalna
IX: *Grippotyphosa*	CH 31, Moskova V.
X: *Hebdomadis hebdomadis*	Hebdomadis, Kambale
XI: *Hebdomadis sejroe*	Sejroe
XII: *Hebdomadis saxkoebing*	Ricardi, Saxkoebing
XIII: *Wolffii wolffii*	Wolffii, Hardjo (cow 204)
XIV: *Hebdomadis mini*	Mini, Georgia
XV: *Bataviae*	Poidjan, Djatzi
XVI: *Tarassovi*	Panama, G 120
XVII: *Pomona*	Mitis, Perepelicin, Rama LT 955
XVIII: *Cynopteri*	Butembo
XIX: *Semaranga*	Patoc

The eyes were weighed and the age of the coypus computed (Gosling, Huston & Addison, 1980). Eyes for coypu number 6 were not collected (see Table 1).

(E) *Laboratory methods*

(i) *Serological tests*

The following tests were performed on the sera obtained: (*a*) slide agglutination test (SAT) (W.H.O., 1982), (*b*) complement fixation test (CFT) (W.H.O., 1982), (c) microscopic agglutination test (MAT) (Turner, 1968).

The sera were tested by the MAT at a dilution of 1:100 against 19 pools of formalized antigens. These antigens represented a total of 19 serogroups (see Table 2). Sera with titres to pooled antigens were in a twofold dilution system beginning at 1:80 to 1:20480 titrated against each antigen within the positively reacting pool.

(ii) *Culture examination*

Urine and kidney samples in both EMJH and EMJH+5-FU were examined immediately on return to the laboratory for leptospiral growth by dark-field microscopy and thereafter every 2 weeks for 16 weeks or until live leptospira were seen. The isolated strains of leptospira were classified as either pathogenic or saprophytic strains according to the criteria laid down by the W.H.O. (1982) and Johnson & Harris (1967). Eight-azaguanine was used as the differential medium (Johnson & Rogers, 1964*b*).

(iii) *Histology*

Histological examination of kidney and liver specimens for the presence of leptospires was performed using haematoxylin and eosin (HE) and Warthin–Starry silver impregnation stain (Warthin & Starry, 1920). Results were equivocal and leptospires could not be detected with certainty.

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Table 3. *The serological results on sera from coypu*

Coypu no.	Slide agglutination, neat dilution	CFT titre	MAT result (a) Screen at 1:100	(b) Titres against reading antigen pools I	VI	VII	VIII	XI	XIII	XIV	V
1	−	160	−	.	.	.	.	.	.	.	.
2	−	80	+	160	.	.	.	.	.	.	.
3	−	80	+	320	.	.	.	.	.	.	.
4	−	160	−	.	.	.	.	.	.	.	.
5	+	160	+	640	.	320	80	.	.	.	.
6	−	80	−	.	.	.	.	.	.	.	.
7	−	160	+	320	.	.	.	.	.	.	.
8	−	80	−	.	.	.	.	.	.	.	.
9	Trace	80	+	160	.	.	.	.	.	.	.
10	+	80	−	.	.	.	.	.	.	.	.
11	−	80	−	.	.	.	.	.	.	.	.
12	−	20	−	.	.	.	.	.	.	.	.
13	Trace	80	−	.	.	.	.	.	.	.	.
14	−	0	−	.	.	.	.	.	.	.	.
15	+	320	+	.	160	.	.	1280	80	80	80
16	−	40	−	.							
17	−	20	−	.							
18	−	40	−	.							
19	−	40	−	.							
20	−	40	−	.							
21	−	80	−	.							
22	Trace	320	+	160							
23	+	40	−								
24	−	80	−								
25	−	40	−								
26	Trace	40	−								
27	+	80	−								
28	++	40	−								
29	+	40	−								

RESULTS

Serological results

The serological results obtained on the 29 coypu are presented in Table 3. The slide agglutination test (SAT) demonstrated that coypu serum numbers 5, 10, 15, 23, 27, 28 and 29 had low levels of antibody to leptospires when compared to those found in the positive control serum. Tracing reactions were recorded in a further four specimens, coypu numbers 9, 13, 22 and 26.

The complement fixation test (CFT) results presented in the same Table 3 show that all animals except coypu number 14 had antibodies to leptospires. However, if a titre of 100 as recommended by Michna (1967) is considered to be a base minimum positive titre, then only six animals (numbers 1, 4, 5, 7, 15 and 22) are positive.

The microscopic agglutination test (MAT) results are presented in Table 3, columns (*a*) and (*b*). Column (*a*) outlines the results obtained after screening the coypu sera at a dilution of 1:100. Those considered to be positive (i.e. 2, 3, 5, 7, 9, 15 and 22) were then further titrated against their reacting antigen pool. The

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Table 4. *Percentage prevalence by serogroup*

Serogroup	Number positive against serogroups: (*a*) +ve cases at 1:80	(*b*) Total no. of coypu	Percentage: Per +ve case	Percentage: Per population
Icterohaemorrhagiae	6/7	6/29	86%	21%
Ballum	1/7	1/29	14%	4%
Autumnalis	1/7	1/29	14%	4%
Australis	1/7	1/29	14%	4%
*Hebdomadis**	3/7	3/29	43%	10·3%
Bataviae	1/7	1/29	14%	4%

* Including *Sejroe*, *Wolfii* and *Mini* only.

results are shown in Table 3, column (*b*). All the MAT positive sera, with the exception of number 15, had antibodies to *L. interrogans* var. *Icterohaemorrhagiae* (pool 1). Coypu numbers 5 and 15 had antibodies to more than one serogroup (*Icterohaemorrhagiae*, *Autumnalis*, *Australis* and *Autumnalis*, *Sejroe*, *Wolffii*, *Mini* and *Balcanica* respectively). A significant titre of 1280 was recorded for coypu number 15 against *L. interrogans* var. *Hebdomadis sejroe*. Table 4 summarizes the overall serological prevalence of leptospires in the coypu in Great Britain; it shows the two serogroups *Icterohaemorrhagiae* and *Hebdomadis* are clearly the predominant groups found in Great Britain.

Cultural examination results

Leptospires were observed in urine cultures of one coypu (coypu number 13) after 49 days incubation. Growth occurred in only one bijoux with 5-FU. The isolate was then passaged in EMJH+2% rabbit serum and then further identified to be a pathogenic leptospire belonging to the *Wolffii* serogroup by the methods outlined in W.H.O. (1982) and Kmety (1967).

Cross-agglutination identification of the isolate indicated that it was *L. interrogans* var. *Wolffi* serovar. hardjo (previously included in the *Hebdomadis* serogroup) (Dikken & Kmety, 1978).

Histological results

Histological examination of haematoxylin- and eosin- (HE) stained kidney sections revealed no pathological evidence of leptospires in any of the coypu examined. The results obtained using the Warthin–Starry technique were equivocable and did not contribute to the diagnostic confirmation of leptospirosis.

DISCUSSION

The results obtained from our study show that the coypu (*Myocastor coypus* Molina) is a reservoir of leptospires in Great Britain. There is both serological and cultural evidence that the prevalent leptospiral serogroups found in the coypu are *Icterohaemorrhagiae* and *Hebdomadis*. A similar study undertaken by Twigg & Cuerden in 1966 demonstrated by serological means only that the coypu had antibodies to leptospires.

The present study confirms these serological findings and reports for the first time the isolation of leptospira from coypu in Great Britain.

Our serological evidence is based mainly on the microscopic agglutination test; this is the reference test for the diagnosis of leptospirosis both in animals and humans (Turner, 1968). We also attempted other serological investigations using both the slide agglutination test and the complement fixation test; neither proved satisfactory for screening large numbers of animal sera. The slide test utilizes the strain *L. biflexa* var. *Semaranga patoc* as an antigen, usually its cross-reactivity with pathogenic leptospira is found useful in human diagnosis. Unfortunately, this non-specificity leads to false positive results in the sera of wild animals and we therefore decided that the test was probably of doubtful value in the veterinary field. Similarly, the CFT was found to be unreliable. However, in this case several factors contributed to its unreliability.

(i) Most animals do not readily fix guinea-pig complement in the presence of antigen–antibody complexes.

(ii) The CFT will detect antibodies about 10 days after the onset of infection and will decline with time. Therefore, unless the coypu was acutely infected within the previous 1–2 months prior to sampling, the CFT would be of little value.

(iii) Individual host variation to antibody production. For example, the failure to detect CFT antibody response to leptospiral infection has been well documented by Sturdza, Elian & Tulpan (1960) and Nicolescu & Lelutiu (1967). Interestingly, these authors concluded that the failure to respond antigenically to infection may be more important in hosts which act as the reservoir of leptospires.

From the three tests used in this study it appears that both slide agglutination and complement fixation techniques are unreliable methods for retrospective screening of animal sera.

Previous attempts to isolate leptospires from British coypu were unsuccessful (Twigg & Cuerden, 1966), only those serovars capable of colonizing the kidney are usually isolated. In the present study we were successful in isolating *L. interrogans* var. *Wolffi* serovar. hardjo from coypu trapped in East Anglia. The success of this attempt may be due to the number of dilutions made on the biological material studied which was cultivated into EMJH and EMJH + 5FU. The coypu from which the leptospires were isolated showed no clinical nor histopathological evidence of infection. The serological results obtained indicated antibody by the CFT method only, MAT antibodies were not detected.

This phenomenon of a culturally positive but serologically negative animal reservoir has been partially explained by Babudieri (1958) in his theory of 'ectoparasitism'. This states that the initial leptospiraemia is followed by an immunized state. The organisms accumulate in the secondary convoluted tubules of the kidney and then cease to be internal parasites. The organisms then stop acting as antigens and do not stimulate further antibody production. The blood antibody control then drops below detectable levels. Leptospiruria may persist, sometimes for life, and these 'carrier' animals become a potentially dangerous source of infection. Therefore the coypu carrying leptospires but having no detectable MAT antibodies may then be a true 'carrier' for the organism.

This study confirms that leptospires may be detected serologically in the coypu but it appears to be the first report of isolation of a leptospire from this animal. Indeed, this may be the first report of a rodent vector as a reservoir for *L. interrogans* var. *Wolffii* serovar. hardjo infection in domestic animals in the East

Anglia region where sporadic outbreaks of the disease in cattle has been thought to be a result of contamination of rivers and canals by infected cattle on distant up-stream farms. The coypu is therefore a reservoir of *L. interrogans* var. *Wolffii* serovar. hardjo infections and may contribute to cattle-associated leptospirosis.

We would like to thank the staff and particularly Dr Gosling of the Coypu Research Laboratory, Ministry of Agriculture, Fisheries and Food, Norwich, for determining the age of the coypus; the Coypu Control Officers and Trappers from Norwich for their help in trapping the coypu, and Mr G. Taylor, Division of Pathology, County Hospital, Hereford, for preparing the illustrations.

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