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Serological studies on British leptospiral isolates of the Sejroe serogroup

I. The identification of British isolates of the Sejroe serogroup by the cross agglutinin absorption test

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SUMMARY

Using the cross agglutinin absorption test 12 British leptospiral isolates of the Sejroe serogroup were identified to serovar level. Six strains isolated from cattle, two from pigs and one from a human were identified as *Leptospira interrogans* serovar *hardjo*. Two isolates from wildlife were identified as *Leptospira interrogans* serovar *saxkoebing*. One further strain isolated from wildlife closely resembled serovar *saxkoebing*, but specific identification was not possible. These are the first reported isolations of serovar *saxkoebing* in the United Kingdom. The problems associated with the cross agglutinin absorption test, and possible alternative typing procedures are discussed.

INTRODUCTION

The classification of leptospira is based on serological criteria and the definitive method for the identification of leptospires to serovar level remains the cross agglutinin absorption test (CAAT) (Dikken & Kmety, 1978). Using this method ' two strains are considered to belong to different serotypes (serovars) if after cross agglutination absorption with adequate amounts of heterologous antigen, 10 % or more of the homologous titre regularly remains in at least one of the two antisera in repeated tests ' (World Health Organization, 1967).

For convenience, serovars whose antisera cross agglutinate to high titre are placed in serogroups. Although serogroups have no official status and cannot be accurately circumscribed (Turner, 1967) they have considerable value in determining which serovars and antisera should be used in serological tests (currently more than 140 serovars have been described and these have been placed in 16 serogroups (Dikken & Kmety, 1978)).

The Hebdomadis serogroup contains 29 serovars (Dikken & Kmety, 1978) and this large number of reference strains poses problems for those engaged in the identification of isolates. However, Kmety (1977) has performed a detailed antigenic analysis of the Hebdomadis serogroup and has proposed that it should be divided into three new serogroups, namely, Hebdomadis (9 serovars), Sejroe (14 serovars) and Mini (6 serovars). This proposal has been followed in this paper.

Sejroe serogroup infection is widespread in cattle in the British Isles and is a cause of outbreaks of mastitis, abortion and premature calving (Higgins *et al.* 1980; Hathaway & Little, 1983; Little & Hathaway, 1983; Ellis *et al.* 1985). Leptospires of the Sejroe serogroup are also increasingly recognized as a major cause of leptospirosis in man in Great Britain (Coghlan, 1979). However, Turner (1967) has stated that serological findings can be regarded as serogroup indicative only, and the infecting organism must be isolated and typed by CAAT to determine its serovar identity. This is especially important in epidemiological investigations where isolates are obtained from a number of different host species.

The original British Sejroe serogroup isolates were from field voles (*Microtus agrestis*) and bank voles (*Clethrinomys glareolus*) caught in Scotland (Broom & Coghlan, 1958) and these strains were found to be related to *Leptospira interrogans* serovars *sejroe* and *saxkoebing*. Michna & Campbell, (1969) isolated a number of Sejroe serogroup strains in Scotland from the kidneys of cows which had recently aborted. These strains were also found to be related to *sejroe*, and a later study (Michna & Campbell, 1970) suggested that wildlife may play a part in the transmission of Sejroe serogroup infection to domestic animals. However in none of these studies were the leptospiral isolates fully identified by CAAT. In more recent studies, a number of bovine isolates have been identified as serovar *hardjo* using the CAAT. (Orr & Little, 1979; Hathaway & Little, 1983; Michna, Ellis & Dikken, 1984).

The purpose of this study was to identify the serovar spectrum of a number of Sejroe serogroup strains isolated from different host species in Great Britain using the CAAT.

MATERIALS AND METHODS

Leptospiral strains

All reference strains were obtained from the Leptospirosis Reference Laboratory, Hereford, UK. Kmety (1977) has described their origin and identification. The reference strains used were: *sejroe* M84, *balcanica* 1627 Burgas, *polonica* 493 Poland, *istrica* Bratislava, *saxkoebing* Mus 24, *haemolytica* Marsh, *ricardi* Richardson, *medanensis* Hond HC, *wolffi* 3705, *hardjo* Hardjoprajitno, *recreo* LT 957, *trinidad* strain LT 1098, *gorgas* LT 829, and *roumanica* LT 294.

The origin of isolates used in the study are described in Table 1.

Antisera

Antisera were prepared as described by Sulzer & Jones (1974) except that EMJH liquid medium (Difco) was used and the fourth (and fifth if necessary) inoculum was not killed. Specific antisera from paired rabbits were pooled before use.

Serogrouping

The serogrouping of isolates was performed as described by Dikken & Kmety (1978) using the microscopic agglutination test (MAT) and a doubling dilution series with an initial serum dilution of 1/100. Hond Utrecht IV and Vleermuis 90C were both used as the Canicola group antisera.

Cross agglutinin absorption test

Each isolate was tested with all reference antisera using the MAT. Reference strains whose antisera reacted with the isolates to more than 6·3% of the

Table 1. *Origin of strains used in this study*

Strain	Host and site	Location	Source and/or reference
M204	Bovine kidney	Scotland	Michna, Ellis & Dikken (1974) *hardjo*
L43	Bovine kidney	Scotland	Central Veterinary Laboratory (identified by Kmety using factor analysis as *hardjo*)
K1	Bovine kidney	England	Orr & Little (1976) *hardjo*
D38	Badger (*Meles meles*) kidney	England	Salt & Little (1977)
OW 305/4	Field vole (*Microtus agrestis*) kidney	England	Veterinary Investigation Centre, Gloucester
776V	Vole (Species not known) kidney	Wales	Veterinary Investigation Centre, Carmarthen
12/5	Bovine milk	England	Central Veterinary Laboratory, Weybridge
44/471	Bovine milk	England	Higgins *et al.* (1980)
S76	Pig kidney	Northern Ireland	W. A. Ellis, Veterinary Research Laboratory, Stormont
P442	Pig kidney	England	Hathaway, Little & Stevens (1981)
S1201	Human urine	Northern Ireland	W. A. Ellis (Anonymous, 1981)
B215	Bovine urine	England	Hathaway & Little (1983)

Table 2. *Agglutination of strains D38 and M204 with reference antisera of the* Sejroe *serogroup*

Serovar	Antiserum of strain	Reciprocal titre with homologous strain	Reciprocal titre with strain D38	Reciprocal titre with strain M204
sejroe	M84	12800	1600 (12·5)*	3200 (25)
balcanica	1627 Burgas	12800	1600 (12·5)	3200 (25)
polonica	493 Poland	25600	800 (3·1)	3200 (12·5)
istrica	Bratislava	25600	1600 (6·3)	3200 (12·5)
saxkoebing	Mus 24	6400	3200 (50)	200 (3·1)
haemolytica	Marsh	25600	3200 (12·5)	6400 (25)
ricardi	Richardson	25600	6400 (25)	3200 (12·5)
medanensis	Hond H C	12800	800 (6·3)	6400 (50)
wolffi	3705	25600	800 (3·1)	25600 (100)
hardjo	Hardjoprajitno	12800	1600 (12·5)	12800 (100)
recreo	LT957	12800	400 (3·1)	12800 (100)
trinidad	LT1098	51200	800 (1·6)	1600 (3·1)
gorgas	LT829	12800	200 (1·6)	6400 (50)
roumanica	LT294	12800	100 (0·8)	3200 (25)

* Percentage of homologous titre.

homologous titre were used for the CAAT. This was carried out using the Bratislava technique described by Dikken & Kmety (1978) with the following exceptions: (*a*) 24 parts antigen were used for all absorptions; (*b*) the absorption was considered satisfactory if the residual titre was 200 or less. Occasionally difficulty was experienced in reducing this titre to below 400. When this occurred the absorption was repeated. If the titre was still 400 the result was used, but on no occasion did this affect the conclusion reached since the reciprocal absorption was satisfactory and indicated non-identity of the isolate with the reference strain. Each absorbed serum was tested using the MAT on two separate occasions. Where identity was indicated the absorption was repeated to confirm the result.

RESULTS

All 12 isolates gave a high cross-agglutination titre only with those antisera representing the proposed Sejroe serogroup. A representative agglutination test for bovine strain M204 and wildlife strain D38 is presented in Table 2.

The CAAT was able to identify 11 of the 12 Sejroe serogroup isolates, all of which were either *hardjo* or *saxkoebing*. Representative CAAT results for strains M204 and D38 are shown in Tables 3 and 4.

The results for the other strains in this study are summarized in Table 5. Nine strains from several different host species were serovar *hardjo*, two were *saxkoebing* and one, OW 305/4, was very closely related to *saxkoebing*.

DISCUSSION

The results demonstrate the presence of two serovars of the Sejroe serogroups in Britain, *hardjo* and *saxkoebing*. *Hardjo* has been recognized for several years in cattle, which act as the maintenance host for this serovar, but it also has been

Table 3. *Serovar identification of isolate M204 by cross agglutinin absorption*

Antiserum	Absorbed with strain	Reciprocal of Titre: Before absorption — Homologous strain	Before absorption — Absorbing strain	After absorption — Homologous strain	After absorption — Absorbing strain	Percentage of homologous titre remaining after absorption
M204	*sejroe*	1600	3200	1600	0	100
sejroe	M204	12800	1600	12800	0	100
M204	*balcanica*	3200	1600	1600	0	50
balcanica	M204	6400	3200	3200	0	50
M204	*polonica*	1600	6400	800	200	50
polonica	M204	6400	1600	3200	100	50
M204	*istrica*	3200	1600	1600	0	50
istrica	M204	6400	1600	3200	0	50
M204	*haemolytica*	3200	1600	1600	0	50
haemolytica	M204	51200	1600	51200	0	100
M204	*ricardi*	3200	3200	3200	0	100
ricardi	M204	12800	800	6400	0	50
M204	*medanensis*	3200	3200	3200	0	100
medanensis	M204	25600	3200	25600	0	100
M204	*wolffi*	3200	1600	1600	0	50
wolffi	M204	12800	12800	800	100	6·3
M204	*hardjo*	3200	3200	200	200	6·3
hardjo	M204	6400	12800	200	200	3·1
M204	*recreo*	3200	1600	1600	0	50
recreo	M204	6400	3200	1600	100	25
M204	*gorgas*	3200	800	3200	0	100
gorgas	M204	1600	1600	1600	0	100
M204	*roumanica*	3200	3200	3200	0	100
roumanica	M204	12800	1600	12800	0	100

Table 4. *Serovar identification of isolate D38 by cross agglutinin absorption*

Antiserum	Absorbed with strain	Reciprocal of titre: Before absorption — Homologous strain	Before absorption — Absorbing strain	After absorption — Homologous strain	After absorption — Absorbing strain	Percentage of homologous titre remaining after absorption
D38	*sejroe*	3200	3200	1600	0	50
sejroe	D38	12800	1600	6400	0	50
D38	*balcanica*	3200	800	1600	0	50
balcanica	D38	6400	800	1600	0	25
D38	*istrica*	3200	6400	1600	0	50
istrica	D38	25600	1600	6400	0	25
D38	*saxkoebing*	3200	6400	100	200	3·1
saxkoebing	D38	6400	1600	200	100	3·1
D38	*haemolytica*	1600	1600	1600	0	100
haemolytica	D38	25600	3200	6400	0	25
D38	*ricardi*	3200	3200	1600	0	50
ricardi	D38	25600	6400	6400	100	25
D38	*medanensis*	1600	1600	1600	0	100
medanensis	D38	25600	1600	12800	0	50
D38	*hardjo*	3200	200	1600	0	50
hardjo	D38	12800	1600	6400	0	50

Table 5. *Serovar identification of leptospires of the* Sejroe *serogroup isolated in the United Kingdom*

	Cross agglutinin absorption test			
Strain	Antiserum	Absorbed with	Percentage of homologous titre remaining after absorption	Serovar
M204	M204	*hardjo*	6·3	*Hardjo*
	hardjo	M204	3·1	
L43	L43	*hardjo*	6·3	*Hardjo*
	hardjo	L43	6·3	
K1	L1	*hardjo*	3·1	*Hardjo*
	hardjo	K1	3·0	
D38	D38	*saxkoebing*	3·1	*Saxkoebing*
	saxkoebing	D38	3·1	
OW 305/4	OW 305/4	*saxkoebing*	3·1	Probably
	saxkoebing	OW 305/4	12·5	*Saxkoebing*
766V	766V	*saxkoebing*	0·8	*Saxkoebing*
	saxkoebing	766V	0·8	
12/5	12/5	*hardjo*	3·1	*Hardjo*
	hardjo	12/5	6·3	
44/471	44/471	*hardjo*	0·8	*Hardjo*
	hardjo	44/471	1·6	
S76	S76	*hardjo*	6·3	*Hardjo*
	hardjo	S76	6·3	
442	442	*hardjo*	3·1	*Hardjo*
	hardjo	442	6·3	
S1201	S1201	*hardjo*	3·1	*Hardjo*
	hardjo	*S*1201	6·3	
*B*215	*B*215	*hardjo*	3·1	*Hardjo*
	hardjo	B215	3·1	

isolated from pigs and man which may have become accidentally infected by contact with cattle. Broom & Coghlan (1958) isolated strains from both bank and field voles which they found to be related to *sejroe* and *saxkoebing* but these strains were never fully identified. Thus, this paper records for the first time the identification of *saxkoebing* in British wild mammals. *Saxkoebing* was first isolated from yellow necked mice (*Apodemus flavicollus*) in Denmark by Borg-Peterson (1944) and has also been isolated from wood mice (*Apodemus sylvaticus*) and house mice (*Mus musculus*) in Europe (Anon. 1966). Many more strains need to be examined before the host range of *saxkoebing* can be defined.

The CAAT indicated that there was very little resemblance between strain OW 305/4 and all the reference strains other than *saxkoebing*. This suggests that there is a small but recognizable difference between OW 305/4 and the reference serovar *saxkoebing*. One of the drawbacks of the CAAT is the freshly isolated strains have to be compared with reference strains which have been in the laboratory for many years and which often come from different geographical areas. It is not surprising, therefore, if on occasions slight differences between isolates and reference serovars

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are detected, and OW 305/4 may in fact be a *saxkoebing* strain. There are also problems associated with the exact and arbitrary determination of the 10% limit. These problems have been discussed by Kmety (1974).

Whilst the CAAT is the only recognized method for identifying leptospira (Dikken & Kmety, 1978), it is also time consuming and costly to perform. To identify the 12 strains in this study required the production of 94 antisera, 288 absorptions and over 2300 MAT's. The CAAT is thus not a suitable test for examining large numbers of strains which may be isolated in a long-term epidemiological study.

A number of other methods for the identification of leptospira have been proposed such as polyacrylamide gel electrophoresis (Vassilevska, Jankov & Atanasov, 1974) gas liquid chromatography (Bisso, Silva & Merli, 1978) and restriction endonuclease analysis (Marshall, Wilton & Robinson, 1981) but none have been sufficiently widely developed and evaluated to be of general application at this time.

Kmety (1966) has proposed a detailed serological approach to the classification of leptospires based on their main antigens. Reviewing the problems of the CAAT and the discrepancies in results obtained from different laboratories further emphasises the advantages of his factor analysis method become apparent (Kmety, 1974). The strains used in this study have been subjected to factor analysis and the results will be reported separately.

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REFERENCES

Anonymous (1966). Leptospiral serotype distribution list according to host and geographical area. July 1966, USDHEW, Atlanta, Georgia (Communicable Diseases Centre Zoonoses Surveillance).

Anonymous (1981). Leptospirosis in man, British Isles, 1979–80. *British Medical Journal* **282**, 2066.

Bisso, G. M., Silva, I. & Merli, F. (1978). Gas, liquid-chromatography of trimethylsilyl derivatives from whole cell methanolysates of *Leptospira*: Preliminary evaluation of its applicability to the taxonomy of the genus. *Annales de Microbiologie* (*Institut Pasteur*) **129A**. 195–311.

Borg-Peterson, C. H. (1944). *L. Saxkoebing*, ein neuer serologischer Leptospiratyp. *Acta Pathologica et Microbiologica Scandinavica* **21**, 165–180.

Broom, J. C. & Coghlan, J. D. (1958). *Leptospira ballum* in small rodents in Scotland. *Lancet* i, 1041–1042.

Coghlan, J. D. (1979). Leptospirosis in man, British Isles, 1978. *British Medical Journal* **2**, 872–873.

Dikken, H. & Kmety, E. (1978). Serological Typing Methods of Leptospires. In *Methods in Microbiology*, vol. 11, (eds. T. Bergan and J. R. Norris), pp. 268–307. New York: Academic Press.

Ellis, W. A., O'Brien, J. J., Bryson, D. G. & Mackie, D. P. (1985). Bovine Leptospirosis: Some clinical features of serovar *hardjo* infection. *Veterinary Record* **117**, 101–104.

Hathaway, S. C., Little, T. W. A. & Stevens, A. E. (1981). Serological and bacteriological survey of leptospiral infection in pigs in southern England. *Research in Veterinary Science* **31**, 169–173.

Hathaway, S. C. & Little, T. W. A. (1983). Epidemiological study of *Leptospira hardjo* infection in second calf dairy cows. *Veterinary Record* **112**, 215–218.

HIGGINS, R. J., HARBOURNE, J. F., LITTLE, T. W. A. & STEVENS, A. E. (1980). Mastitis and abortion in dairy cattle associated with Leptospira of the serotype *hardjo*. *Veterinary Record* **107**, 307–310.

KMETY, E. (1966). Main antigens as criterion for differentiating Leptospiral serotypes. *Annales de la Societe Belge de Medecine Tropicale* **46**, 103–108.

KMETY, E. (1974). Problems of the serological classification of pathogenic Leptospires. *Folia Facultatis Medicae Universitatis Comenianae Bratislaviensis* **12**, *Supplementum*, pp. 101–110.

KMETY, E. (1977). Study of antigen structure of Leptospirae classification of the Hebdomadis serological group. *Folia Facultatis Medicae Universitatis Comenianae Bratislaviensis* **15**, 245–309.

LITTLE, T. W. A. & HATHAWAY, S. C. (1983). *Leptospira hardjo* infection in cattle: an emerging problem in the United Kingdom. In *Proceedings 10th Conference of the OIE Regional Commission for Europe, London 1982*, pp. 73–84. *Office International des Epizooties Paris.*

MARSHALL, R. B., WILTON, B. E. & ROBINSON, A. J. (1981). Identification of Leptospira serovars by restriction–endonuclease analysis. *Journal of Medical Microbiology* **14**, 163–166.

MICHNA, S. W. & CAMPBELL, R. S. F. (1970). Leptospirosis in wild animals. *Journal of Comparative Pathology* **80**, 101–106.

MICHNA, S. W. & CAMPBELL, R. S. F. (1969). Isolation of *Leptospira sejroe* from the kidneys of aborting cattle. *Veterinary Record* **84**, 83–86.

MICHNA, S. W., ELLIS, W. A. & DIKKEN, H. (1974). Isolation of *Leptospira hardjo* from an aborting cow. *Research in Veterinary Science* **17**, 133–135.

ORR, H. S. & LITTLE, T. W. A. (1977). Isolation of Leptospira of the serotype *hardjo* from bovine kidneys. *Research in Veterinary Science* **27**, 343–346.

SALT, G. F. H. & LITTLE, T. W. A. (1977). Leptospires isolated from wild mammals caught in the south west of England. *Research in Veterinary Science* **22**, 126–127.

TURNER, L. H. (1967). Leptospirosis. I. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **61**, 842–855.

SULZER, C. R. & JONES, W. L. (1974). Leptospirosis: Methods in laboratory diagnosis. *United States Department of Health Education and Welfare, Center for Disease Control, Atlanta, Georgia.* (HEW Publication No. (CDC) 76-8275, 40.)

VASSILEVSKA, M. D., JANKOV, N. S. & ATANASOV, N. A. (1974). Electrophoretic protein patterns from six Bulgarian strains of saprophytic and pathogenic leptospires. *Folia Facultatis Medicae Universitatis Comenianae Bratislaviensis* **12**, *Supplementum*, 141–145.

WORLD HEALTH ORGANIZATION (1967). Current problems in leptospirosis research. Report of the WHO Expert Group. *World Health Organization Technical Report Series* **380**, 8.