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# Serological studies of British leptospiral isolates of the Sejroe serogroup

## III. The distribution of leptospires of the Sejroe serogroup in the British Isles

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SUMMARY

Some 94 strains of leptospires belonging to the Sejroe serogroup isolated in the British Isles were identified to the serovar level using specific factor sera.

Seventy strains were identified as *Leptospira interrogans* serovar *hardjo*, 66 from cattle, 2 from pigs and 1 each from a sheep foetus and a human.

Twenty-four strains were identified as *L. interrogans* serovar *saxkoebing*, most strains were isolated from either wood mice, bank or field voles but strains were also isolated from badgers, a fox and a dog.

## INTRODUCTION

The Hebdomadis serogroup of *Leptospira interrogans* is the largest and geographically most widespread of the serogroups. In 1958 it contained only 6 serovars (Alston & Broom, 1958); the number has now risen to over 30 (Faine 1982). A considerable serological diversity of strains exists within the Hebdomadis serogroup and Kmety (1977) proposed that 3 separate serogroups be recognized: Hebdomadis containing 9 serovars, Sejroe with 14 serovars and Mini with 9.

The serovars isolated in Europe all belong to the Sejroe serogroup (Anon, 1966; Anon, 1975) apart from serovar *mini* of the Mini serogroup, which has been isolated from humans and wildlife in Bulgaria and from humans in Italy (Babudieri, 1972; Mateva & Christov, 1974).

The first of the Sejroe serogroup strains to be isolated in Europe was serovar *sejroe* which Borg Petersen & Christensen (1939) isolated from a Danish fisherman. These workers subsequently described many human cases in Denmark amongst country dwellers and found evidence of widespread infection in the house mouse (*Mus musculus*). Similar findings have been reported in Czechoslovakia (Sebek, 1965; Jindrichova & Sebek, 1974). Isolations from domestic animals have been rare (Gonye & Halmos, 1983; Kmety, Plesko & Chylo, 1956).

Borg-Petersen (1944) also first isolated serovar *saxkoebing* of the Sejroe serogroup in Denmark from *Apodemus flavicollus* the yellow-necked mouse and infection has been reported on rare occasions in man (Sebek, 1965; Mateva & Christov, 1974). Several other serovars belonging to the Sejroe serogroup have been

isolated on rare occasions in Europe; these are *polonica*, *roumanica*, *istrica* and *balcanica* (Parnas & Cybulska, 1965; Kmety, 1977; Babudieri & Mateva, 1961). Little is known of the epidemiology of these organisms.

The most important Sejroe serogroup organism to emerge in Europe in recent years has been *hardjo*, a serovar which causes atypical mastitis and abortion in cattle. First isolated in Italy (Farina, Babudieri & Andreani, 1968), *hardjo* is now thought to be widespread (Trap & Gaumont, 1983; Gonye & Halmos, 1983). *Hardjo* has also emerged as a disease of some importance in cattle in the United Kingdom (Little & Hathaway, 1983). Serological evidence of infection with the Sejroe serogroup was first reported in Scottish cattle and this was associated with abortion and atypical mastitis and with illness in dairymen (Coghlan & Norval, 1967; Michna, 1967; Michna & Campbell, 1969; Sakula & Moore, 1969). Subsequently serological surveys have shown prevalence of 30–40% in cattle in Scotland, South-West England and Northern Ireland (Ellis & Michna, 1976; Little *et al.* 1980; Ellis, O'Brien & Cassells, 1981) and *hardjo* has been isolated from milk samples and aborted foetuses (Ellis *et al.* 1976*a*, *b*) as well as bovine kidney (Orr & Little, 1979; Ellis, O'Brien & Cassells, 1981). Much lower prevalence of serological titres have been reported in other domestic animals, 6·4% in sheep (Hathaway, Little & Stevens, 1982a), 4% in pigs (Hathaway & Little, 1981) and 4% in horses (Hathaway *et al.* 1981).

Britain shares a similar wildlife fauna with Western Europe, however the spectrum of leptospiral infection is poorly understood. This is especially the case with the new subdivision of the Hebdomadis serogroup.

Leptospires of the original Hebdomadis serogroup were demonstrated in wood mice and badgers by Salt & Little (1977) and strains closely related to *sejroe* and *saxkoebing* were isolated from two species of voles (*Microtus agrestis* and *Clethrionomys glareolus*) in Scotland by Broom & Coghlan (1958). An isolate belonging to the original Hebdomadis serogroup was also isolated from a vole (*M. agrestis*) by Michna & Campbell (1970). The serovar identity of these strains has not been determined. Recently isolates from voles have been identified as serovar *saxkoebing* by cross agglutination abortion tests (Little, Stevens & Hathaway, 1986) and factor analysis (Little, Stevens & Hathaway, 1987).

The purpose of this study was to identify a large number of strains of the Sejroe serogroup isolated in the United Kingdom using specific factor sera (Little, Stevens & Hathaway, 1987). This would allow a better understanding of the range of serovars present in free living and domestic species and help define maintenance host and accidental host relationships for individual serovars.

## MATERIAL AND METHODS

A collection of 94 isolates was available for this study and a description of their origin is given in Tables 1 and 2. The majority of the isolates from free-living species were from field surveys conducted between 1976 and 1981 in Surrey, Sussex and Dorset with a small number of strains submitted for identification by Veterinary Investigation Centres. The strains isolated from cattle were from individual herd studies in Sussex or Argyll, abattoir surveys conducted in the south east of England, or investigations into clinical disease outbreaks carried out

Table 1. *Leptospiral Isolates of the Sejroe serogroup identified by specific factor analysis as serovar* hardjo

| Strain ref. | Host | Site | Location | Field report |
|---|---|---|---|---|
| D2, D5, F10, G1, G2, G4, G9, G11, K1*, N7 | Cattle | Kidney | S.E. England | Orr & Little (1979) |
| B33, B58 | Cattle | Milk | E. Sussex | Hathaway & Little (1983) |
| B44, B55, B56, B60, B61, B69, B125, B221, B243 | Cattle | Urine | E. Sussex | Hathaway & Little (1983) |
| F1, F2 | Cattle | Foetal kidney | | Hathaway, Little & Stevens (1982*c*) |
| 191 | Cattle | Milk | Sussex | |
| C30 | Cattle | Kidney | Hampshire | |
| 12/5* | Cattle | Milk | Berks. | |
| C103/65, C103/67 | Cattle | Urine | Berks. | |
| C118/5 | Cattle | Urine | Bucks. | |
| C256 | Cattle | Blood | Dorset | |
| C48/7 | Cattle | Urine | Devon | |
| 97 | Cattle | Milk | Herts | |
| 130 | Cattle | Blood | Herts | |
| C249 | Cattle | Premature calf Kidney | Leics. | Giles, Hathaway & Stevens (1983) |
| C271 | Cattle | Urine | Leics. | Giles, Hathaway & Stevens (1983) |
| 41/15U | Cattle | Urine | N. Yorks. | Higgins *et al.* (1980) |
| 44/194, 44/471* | Cattle | Milk | N. Yorks. | Higgins *et al.* (1980) |
| 103 | Cattle | Urine | N. Yorks | |
| 18, 90, 131 | Cattle | Urine | Cleveland | |
| 40/4, 40/10, 40/15, C573/17, C800/3, C800/4, C800/7, C800/12, C828/2, C828/3, C828/5, C999/3, C997/4, C999/6 | Cattle | Urine | Dyfed | |
| M204* | Cattle | Kidney | Ayrshire | Michna, Ellis & Dikken (1974) |
| L43* | Cattle | Kidney | Argyll | |
| 4A34, 4Y18, 6Y70, 8W75, CNTC | Cattle | Urine | Argyll | |
| AB56 | Cattle | Foetus | N. Ireland | Ellis *et al.* (1982) |
| K13 | Cattle | Kidney | N. Ireland | |
| S117/81 | Cattle | | N. Ireland | |
| P442* | Pig | Kidney | Southern England | Hathaway, Little & Stevens (1981) |
| S76* | Pig | | N. Ireland | |
| S267/81 | Sheep | Foetus | N. Ireland | |
| S1201* | Human | Urine | N. Ireland | |

* Strains identified by CAAT (Little, Stevens & Hathaway, 1986).

Table 2. *Leptospiral isolates of the Sejroe serogroup identified by specific factor analysis as serovar* saxkoebing

| Strain ref. | Host | Location | Reference |
|---|---|---|---|
| 2737 | *Apodemus flavicollis* | Sussex | |
| 3199 | *A. sylvaticus* | Sussex | |
| M3, R3, R18, R43, R53, R89, | *A. sylvaticus* | Surrey | |
| 0W303/12 | *A. sylvaticus* | Glos. | |
| R28, R91, R47, R87, R88 | *Clethrionomys glareolus* | Surrey | |
| 3009, 3027, 3031 | *Microtus agrestis* | Sussex | |
| 0W305/4* | *M. agrestis* | Glos. | |
| 753B, 766V* | Vole sp. unknown | Dyfed | |
| D3, D38* | *Meles meles* | Dorset | Salt & Little (1977) |
| 21/1 | *Vulpes vulpes* | Surrey | |
| 48/1 | Dog | Herts. | |

* Strains identified by CAAT (Little, Stevens & Hathaway, 1986).

either by the authors or Veterinary Investigation Centres in Thirsk, Carmarthen or Sutton Bonington. Strains from Northern Ireland were kindly provided by Dr W. A. Ellis, Veterinary Research Laboratory, Stormont, Belfast.

The isolates were stored in liquid nitrogen and then subcultured in liquid EMJH medium until of sufficient density to be identified to serogroup level using the battery of antisera recommended by Dikken & Kmety (1978). Antisera to Hond Utrecht IV as well as Vleermuis 90C were used to represent the Canicola serogroup.

The isolates were identified to serovar level using the method of specific factor analysis as described by Kmety (1977). The factor sera used were prepared as described by Little, Stevens & Hathaway (1987).

## RESULTS

The results are presented in Tables 1 and 2. *Hardjo* was the only strain to be isolated from cattle but this serovar was also isolated from pigs, sheep and man. The cattle strains were isolated from several locations in England, Wales, Scotland and Northern Ireland. *Hardjo* isolates from other species were also widely distributed. Serovar *saxkoebing* was isolated predominantly from wood mice, field voles (*Microtus agrestis*) and bank voles (*Clethrionomys glareolus*). A single strain was isolated from a yellow necked mouse (*A. flavicollis*) and strains were also isolated from badgers (*Meles meles*), a fox (*Vulpes vulpes*) and a fox hound. The *saxkoebing* isolates were all from the southern parts of England and Wales.

## DISCUSSION

In the study of infectious diseases it is useful to distinguish between certain classes of host (Smith, 1982). In particular it is necessary to determine the maintenance or reservoir host which has been defined by Audy (1958) as a host which ensures the perpetuation of a particular population of parasites without the intervention

of other incidental hosts. The characteristics of a maintenance host in terms of leptospiral infection has been defined by Hathaway (1982) as follows:

(1) high susceptibility of the host to the specific infection;
(2) relatively low pathogenicity of the organism for the host;
(3) long-term kidney infection relative to the systemic phase of infection;
(4) natural transmission within the host species.

A maintenance population is regarded as 'a population of a species of animal which acts as a continuous reservoir of a serovar in a particular ecosystem (habitat)'. In contrast, the characteristics of leptospiral infection in an accidental host (Hathaway, 1982) are:

(1) low susceptibility to infection;
(2) if the infection is established, the pathogenic effect may be severe;
(3) the renal phase is short;
(4) intra-species transmission is inefficient.

Smith (1982) pointed out that the maintenance host may be the same species world wide or the maintenance host may vary within the climatic, geographical and ecological features of each infected area. Quite clearly recognition of maintenance host–parasite relationships and accidental host–parasite relationships allow a better understanding of the epidemiology of leptospires in different mammals sharing a common environment.

The results of this study are a further demonstration of the highly efficient parasitic adaptation of serovar *hardjo* to cattle: all 66 bovine isolates being identified as *hardjo*. The isolates came from a number of locations in England, Scotland, Wales and Northern Ireland demonstrating the widespread nature of *hardjo* infection of cattle which exists throughout the British Isles. This reinforces the conclusion of Ellis, O'Brien & Cassells (1981) that cattle are acting as the maintenance host of *hardjo*, a situation which appears to exist in many parts of the world (Amatredjo & Campbell, 1975).

Hathaway *et al.* (1983) have already reported the identification of two strains of *hardjo* from pigs which were part of this study. As antibody titres to *hardjo* were found in less than 1% of pigs (Hathaway & Little, 1981) and only one strain of *hardjo* was isolated from the kidneys of over 500 pigs in an abattoir survey it appears that the pig is an accidental host for *hardjo*. However the serovar may cause occasional cases of reproduction loss in pigs which have contact with cattle.

*Hardjo* infection was also confirmed in humans in the United Kingdom. Man is an accidental host for *hardjo* and cases most frequently occur in those who have contact with cattle (Anon, 1983; Higgins *et al.* 1980; Hart, Gallagher & Waitkins, 1984). Accidental infection of humans is now accepted as an important occupational hazard for dairymen and control measures in cattle are to some extent aimed at decreasing this risk.

The isolation of *hardjo* from sheep is of interest. In a serological study of 188 flocks in England and Wales, Hathaway, Little & Stevens (1982*a*) found high within flock serological prevalences suggesting in a number of flocks that intra-species transmission may be occurring. Experimental studies in New Zealand (Hathaway & Marshall, 1979) have shown that *hardjo* infection is readily established in sheep and in a study of naturally infected sheep in Australia, Gordon

(1980) demonstrated that leptospiruria may last for over 2 months. He suggested that sheep may be acting as a maintenance host for *hardjo* but this remains to be substantiated. Sporadic cases of reproductive disease in sheep attributable to *hardjo* have been reported in a number of countries (Andreani, Santarelli & Diligenti, 1974; Schmitz *et al.* 1978).

It is very significant that *hardjo* was not isolated from any of the wildlife species examined, especially as they were captured in areas where *hardjo* is present in cattle. From this study it would appear that small rodents do not constitute a reservoir of *hardjo*. However, unlike Northern Ireland where various studies failed to detect leptospiral infection in small rodents (McCaughey & Fairley, 1971; Ellis, O'Brien & Cassells, 1981) in this study Sejroe serogroup strains were isolated from a number of species. All isolates were identified by factor analysis as *saxkoebing*, and some of these isolates had been identified as *saxkoebing* by cross agglutinin absorption tests (CAAT) in an earlier study (Little, Stevens & Hathaway, 1986). The maintenance host for *saxkoebing* in parts of Europe is the yellow necked mouse (Sebek & Rosicky, 1975). A single isolate from a yellow necked mouse caught in Sussex was identified as *saxkoebing*. This mouse is not present in the western parts of Europe and does not occur in Ireland, much of the north of England or Scotland (Brink, 1967). It occurs in small numbers in the South of England in association with wood mice populations (Twigg, Cuerden & Hughes, 1968). The wood mouse is one of the most common and widely distributed of British small mammals and strains of *saxkoebing* were isolated from this species from several sites. *Saxkoebing* has also been isolated from wood mice in Denmark, Czechoslovakia, Germany, Italy and Bulgaria (Anon, 1966; Anon, 1975).

As with the yellow-necked mouse it appears that the wood mouse can act as a maintenance host for *saxkoebing*, however there may be some geographical variations.

No isolations have been made from wood mice captured in Northern Ireland and Scotland (McCoughey & Fairley, 1971; Ellis, O'Brien & Cassells, 1981), however isolates which may well have been *saxkoebing* were isolated from both bank voles and field voles in Scotland (Broom & Coghlan, 1958); Michna & Campbell, 1970).

In this study, *saxkoebing* was isolated from both bank and field vole isolates in sufficient numbers from one site in Surrey to suggest that voles may have been maintaining this serovar.

The absence of voles from Northern Ireland may be a contributing factor for the absence of *saxkoebing* from wood mice there. The isolation of *saxkoebing* from two badgers, a fox and a fox hound further extends the host range of this serovar. Too few samples from these species of animal have been examined to determine whether they may act as maintenance species. However, predator chain transmission has been postulated as a natural method of acquiring leptospiral infection (Reilly, Hanson & Ferris, 1970) and it is possible that these species have accidentally become infected by eating mice and voles. *Saxkoebing* has been isolated once before from a dog in Germany (Anon, 1966) but this is the first report of this serovar from the badger and the fox.

The failure to identify *sejroe* amongst any of the isolates examined would tend to indicate its absence from the British Isles. The maintenance host for this species

in continental Europe is the house mouse (Sebek & Rosicky, 1975), but a study of 272 house mice trapped in farm buildings at four widely separated locations in south-east England failed to demonstrate serological or bacteriological evidence of *sejroe* infection (Hathaway, Little & Stevens, 1982*b*). Similarly no evidence of *sejroe* infection in house mice was found in smaller studies in Northern Ireland (McCaughey & Fairley, 1971; Ellis, O'Brien & Cassells, 1981) or Scotland (Broom & Coghlan, 1958). The single report by Michna & Campbell (1969) of the isolation of *sejroe* from the kidney of cows which had aborted requires some comment. The strain was not identified by either the CAAT or factor analysis and the small number of reference sera and antigens in agglutination tests did not include *hardjo*. The presence of serovar *sejroe* in Britain therefore remains open to question.

The failure to demonstrate the presence of *hardjo* in populations of voles and mice which had close contact with cattle, and equally the failure to demonstrate the presence of *saxkoebing* in cattle populations is further evidence of the nidality of leptospirosis in an intensively farmed environment (Hathaway, 1982). This study demonstrates the usefulness of the concept of the maintenance and the accidental host in studying the epidemiology of leptospirosis in particular ecosystems. Although in Britain a close ecological association between different hosts exists there is little evidence of interspecies transmission of leptospires of the Sejroe serogroup.

This study has demonstrated the enormous advantage of using specific factor sera for identifying large numbers of leptospiral isolates such a study could not be attempted using the traditional CAAT. Accurate identification of serovars is important for epidemiological purposes especially where serologically related organisms are circulating in the same ecosystem. The development of control strategies also depends heavily on accurate identification of isolates. Whether it will be possible to produce monoclonal antibodies to detect the major antigens instead of using absorbed polyclonal antisera remains to be seen but in the meantime it would seem sensible to use Kmety's extensive work on the antigenic structure of leptospires as the basis for provisional typing. The introduction of bacterial restriction endonuclease DNA analysis as a means of further subdivision of serovars (Robinson *et al.* 1982) offers further exciting possibilities, especially with serogroups where serological analysis has been demonstrated to have limitations.

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