LEPTOSPIROSIS IN DANISH WILD MAMMALS 1

Authors: FENNESTAD, K. L., and BORG-PETERSEN, C.

Source: Journal of Wildlife Diseases, 8(4) : 343-351

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-8.4.343

LEPTOSPIROSIS IN DANISH WILD MAMMALS [1]

K. L. FENNESTAD [2] and C. BORG-PETERSEN, Statens Seruminstitut, Copenhagen, Denmark

Abstract: Leptospirosis in man and animals has been studied in Denmark since 1934. Strains of the following ten serotypes have been isolated: *icterohaemorrhagiae, poi, canicola, ballum, bratislava, pomono, grippotyphosa, sejroe, saxkoebing,* and *bataviae.* Twenty-eight of the 44 species of wild land mammals living in Denmark have been examined culturally. Leptospires of eight serotypes were isolated from 14 species. Leptospires were demonstrated microscopically in the urine and/or kidneys of 31 bats belonging to four species. These leptospires could not be cultured *in vitro* or transmitted to animals other than bats. Serological evidence of present or past leptospirosis was found in four species of *Lagomorpha, Carnivora* and *Ungulata* not examined culturally.

Our findings indicate that the following species are maintaining hosts for leptospires of identified serotypes in Denmark: *Sorex araneus* (poi), *Erinaceus europaeus* (bratislava), *Microtus arvalis* (grippotyphosa), *Rattus norvegicus* (icterohaemorrhagiae), *Mus musculus* (sejroe), *Apodemus agrarius* (pomona), and *Apodemus flavicollis* (saxkoebing). *Mus musculus* is probably also a maintenance host for leptospires of the *ballum* serotype. Three bat species, *Myotis daubentonii, Pipistrellus pipistrellus,* and *Nyctalus noctula,* with a high carrier rate (15-20%), may be maintenance hosts for unknown serotypes of a unique group of leptospires. *Bataviae* and *canicola* leptospires have not yet been isolated from wild animals in Denmark.

The significance of leptospirosis in wild mammals for the epidemiology of leptospirosis in man and domestic animals is discussed.

INTRODUCTION

The present review of leptospirosis in Danish wild mammals leaves much to be desired with respect to completeness. First, the mammals in Greenland and the Faroe Islands have not been examined. Secondly, the sea mammals and 12 of the 44 wild land mammal species occurring in the Scandinavian part of Denmark are not included in the review, because either no or only very few (less than four) specimens of any of these species have been examined. Admittedly, also some of the species included have been only inadequately studied. Thirdly, the main purpose of the studies performed has been to detect host animals in order to get a better understanding of the epidemiology of leptospirosis in man and domestic animals. Consequently, we have been interested primarily in the isolation and identification of leptospires, and less inteersted in purely serological examinations of wild animals and in their leptospiral diseases as such.

The first study of leptospirosis in Danish wild mammals was made in 1934 by Zuelzer.[11] She found *Leptospira icterohaemorrhagiae* in about 25% of 91 brown rats. When these findings were reported and adequate diagnostic facilities were established, it was soon recognized that human infections by *icterohaemorrhagiae* and *canicola* leptospires were in no way uncommon in our country, and that the incidence of these infections in the Danish dog population was high.

[1] Paper presented at the 2nd International Conference on Wildlife Diseases, Brighton, England, 1971.

[2] Author's address: Statens Seruminstitut, Amager Boulevard 80, DK-2300 Copenhagen S., Denmark.

Examination of wild animals other than rats began in 1939, after a third serotype of leptospires, the *sejroe* type, had been discovered in man,[2] and the epidemiological evidence pointed to mice, rather than to rats, as the most probable source of the *sejroe* infections. Eventually, leptospires of the *sejroe* serotype were found to be carried by the house mouse *(Mus musculus)*,[4] whereas the serologically closely related *saxkoebing* leptospires were isolated from the yellow-necked field mouse *(Apodemus flavicollis)*.[3] The common vole, *Microtus arvalis*, which Schüffner and Bohlander[13] had found to be a carrier of *grippotyphosa* leptospires in Holland, was found to carry this serotype also in Denmark;[8] and in 1943 leptospires of the *ballum* serotype were isolated from a house mouse in Southern Jutland.[5]

However, the major part of our material has been collected since 1954 when a collaborative study by the State Serum Institute and the Royal Veterinary and Agricultural University in Copenhagen was initiated. The primary purpose of that study was to investigate whether leptospires were a major cause of bovine abortion in Denmark. The incidence of leptospirosis in cattle was found to be about 8%, but these infections apparently only rarely resulted in abortion,[9,10] except in a very restricted part of the country, the islands of Lolland and Falster. On these two islands a considerable number of bovine abortions were caused by *pomona* leptospires,[11,12] and the striped field mouse *(Apodemus agrarius)*, which in our country occurs only on these islands, was found to harbour leptospires of this serotype.[7]

Fig. 1 shows that Scandinavian Denmark consists of the Jutland peninsula, which accounts for about two thirds of the total area of our country (16,500 square miles), and a multitude of islands, the larger of which are Zealand, Funen, Bornholm, and the twin islands Lolland-Falster. The surface of the country is a low rolling plain, most of which is farmland. About 10% is covered by forest and 1.5% is fresh-water lakes or small streams.

RESULTS

Insectivora

This order is represented in Denmark by five species. So far, no specimens of the pigmy shrew *(Sorex minutus)* have been examined. Table 1 shows that no leptospires were isolated from nine moles *(Talpa europaea)*. All three identified strains from the common shrew *(S. araneus)* belonged to the *poi* serotype, and a strain of the same type was isolated from one of five water shrews *(Neomys fodiens)*. The majority of the 43 strains isolated from 193 hedgehogs *(Erinaceus europaeus)* belonged to the *bratislava* serotype, but leptospires of no less than five other serotypes were also isolated from this species. Only one strain was isolated from 19 hedgehogs weighing less than 500 g and, therefore, registered as immature. The carrier rate was considerably higher in male than in female hedgehogs: 32 strains were from 113 males (28%) and 11 from 80 females (14%).

Three *bratislava* strains were isolated from hedgehogs in which no antibodies against the infecting strain could be demonstrated; these animals may have been examined in the acute phase of infection. The remaining 40 culturally positive animals showed a positive seroreaction, *i.e.* a microscopic agglutinin titer of 1:100 or more with live leptospires of the infecting serotype. Positive seroreactions were found also in 44 culturally negative animals, with a relative serotype distribution similar to that in culturally positive animals. Thus, by the combined cultural and serological examination 45% of the hedgehogs showed evidence of present or past leptospirosis.

The kidneys of the first 93 mature hedgehogs trapped were examined histologically for interstitial nephritis. Kidney lesions were demonstrated in 33 of 44 animals which were culturally and/or serologically positive for leptospirosis, but only in 2 of 49 negative animals. In mature hedgehogs there was a positive correlation between interstitial nephritis and evidence of leptospirosis.

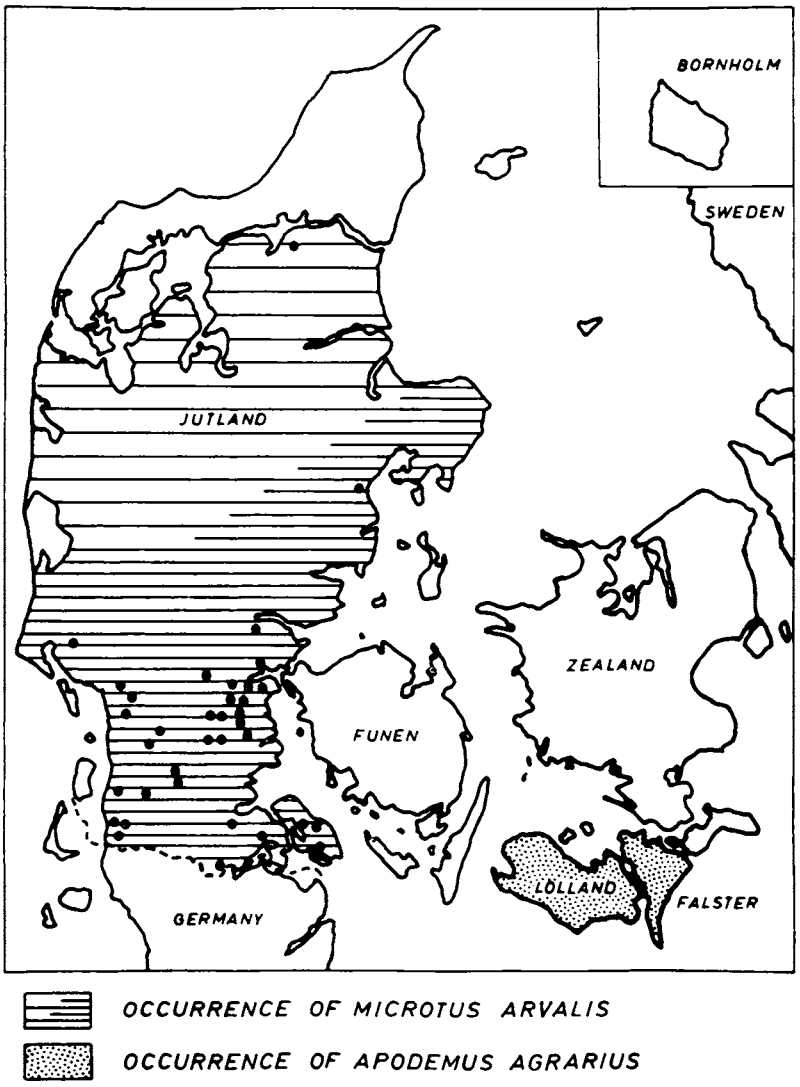

FIGURE 1. Distribution of the common vole **(Microtus arvalis)**, maintaining host for **grippto-typhosa** leptospires, and the striped field mouse **(Apodemus agrarius)**, maintaining host for **pomona** leptospires in Denmark. The dots indicate verified human and bovine **grippotyphosa** infections. **Pomona** infections in domestic and wild animals were found on Lolland-Falster only.

TABLE 1. Demonstration of leptospires in Danish wild mammals.

Order and species*	No. positive / No. examined	Serotype** distribution
Insectivora		
Sorex araneus	4/49	poi 3, undet. 1
Neomys fodiens	1/5	poi 1
Erinaceus europaeus	43/193	brat. 31, pom. 6, poi 2, sej. 2, sax. 1, ict.1
Chiroptera		
Myotis daubentonii	5/26	undet. 5
Pipistrellus pipistrellus	5/34	undet. 5
Nyctalus noctula	20/89	undet. 20
Eptesicus serotinus	1/17	undet. 1
Rodentia		
Clethrionomys glareolus	1/239	poi 1
Microtus arvalis	35§/225	grip. 26§, sax. 1§, undet. 9
Microtus agrestis	3/68	sax. 2, sej. 1
Rattus norvegicus	17/82	ict. 17
Mus musculus	28/220	sej. 25, sax. 2, bal. 1
Micromys minutus	1/64	sej. 1
Apodemus agrarius	39/514	pom. 31, sax. 4, sej. 3, undet. 1
Apodemus sylvaticus	6/201	sax. 4, sej. 1, poi 1
Apodemus flavicollis	31/243	sax. 31
Carnivora		
Vulpes vulpes	1/16	poi 1
Mustela erminea	3/37	poi 1, pom. 1, sej. 1

* No leptospires demonstrated in 9 *Talpa europaea*, 4 *Myotis mlystacinus*, v4 *Myotis nattereri*, 8 *Plecotus auritus*, 9 *Barbastella barbastellus*, 46 *Sciurus vulgaris*, 40 *Arvicola terrestris*, 5 *Rattus rattus*, 16 *Mustela nivalis*, 11 *Mustela putorius*.

** Abbreviations: bal. = ballum, brat. = bratislava, grip. = grippotyphosa, ict. = icterohaemorrhagiae, pom. = pomona, sax. = saxkoebing, sej. = sejroe, undet. = undetermined.

hairline rule = maintaining host for serotype.

§ One double infection.

Chiroptera

Twelve species of this order are known in Denmark, but two of them *(Eptesicus nilssonii* and *Pipistrellus nathusii)* are of very rare occurrence. Individuals of eight species have been examined. As seen from Table 1, leptospires were found (by dark-field microscopy of urine and/or kidney suspension) in specimens of four species. Histological examination of silver-impregnated sections of the kidneys from three dark-field positive great bats revealed leptospires in the lumen of the kidney tubules (see Fig. 2). All attempts to isolate leptospires from bat kidneys and urine by cultivation in Korthof's medium with 10 or 20% rabbit serum, in Ellinghausen's medium, and in other media, at temperatures from 20 to 37 C, failed. Likewise, inoculation of newborn guinea-pigs, hamsters, mice and rabbits,

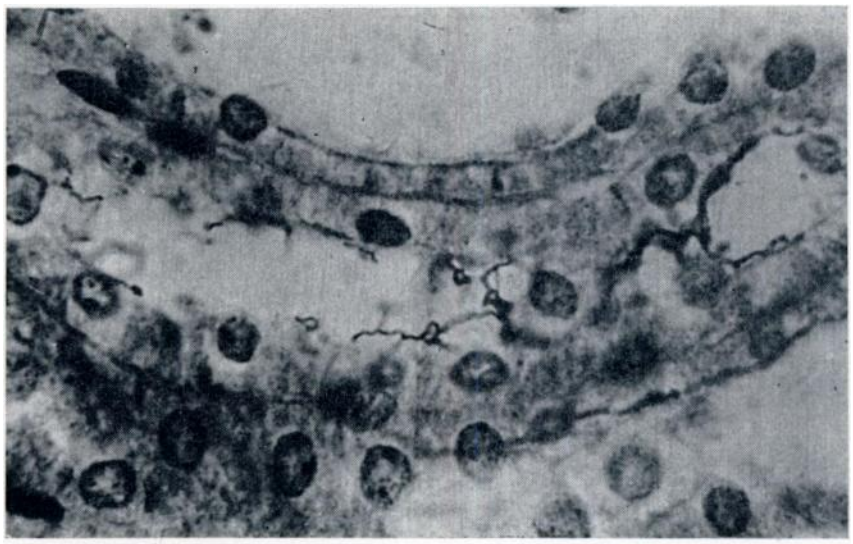

FIGURE 2. Leptospires in the kidney tubules of a great bat (**Nyctalus noctula**). Warthin-Starry.

newly hatched chickens, and chick embryos gave negative results. Three great bats which were kept alive for 6, 11, and 20 weeks excreted leptospires regularly with their urine. Intraperitoneal inoculation of urine from one of these bats into 24 dark-field negative great bats apparently resulted in infection and leptospiruria in three cases .

Sera from 64 bats of six species, including nine dark-field positive animals, were examined for agglutinins against live leptospires of 18 different serotypes. Only three red-grey bats *(Myotis nattereri)* showed a positive reaction (titer 1:100 with the *saxkoebing* serotype). Even though no leptospires were isolated *in vitro* and electron-microscopical examination was not performed, and even though serological evidence of leptospirosis could not be established in the microscopically positive bats, our findings indicate that bats are carriers of microorganisms indistinguishable from leptospires in their 'gross' morphology, movements, and habitat, but differing from known parasitic leptospires in growth requirements, pathogenicity, and probably also in antigenic composition.

Rodentia

This order is represented by 14 species. No specimens of the northern birch mouse *(Sicista betulina)* and the dormouse *(Muscardinus avellanarius)*, both of which are of rare and geographically restricted occurrence, have been examined. Three other species are also of geographically restricted occurrence. The black rat *(Rattus rattus)* occurs only in areas adjoining certain harbours. The common vole occurs only in Jutland south of the Limfjord, with the highest density in the southern part of the peninsula. The striped field mouse is common on the twin islands of Lolland-Falster but does not occur in other parts of Denmark (see Fig. 1).

As shown in Table 1, leptospires were isolated from nine of the 12 species examined. Our failure to isolate leptospires from the red squirrel *(Sciurus vulgarus)* the water vole *(Arvicola terrestris)*, and the black rat may be explained by the relatively small number examined.

Only one of 239 bank voles was found to harbour leptospires. All but one of the identified strains isolated from the com-

mon vole were of the *grippotyphosa* serotype. Apparently, the common vole is the sole carrier of *grippotyphosa* leptospires in Denmark. Particularly, strains of this serotype were not isolated from 68 field voles which yielded only two *saxkoebing* strains and one *sejroe* strain. The impression that the common vole is the sole carrier of *grippotyphosa* leptospires in Denmark is supported by the fact that all diagnosed human cases of *grippotyphosa* infections occurred in Jutland, and mainly in Southern Jutland; this distribution corresponds very well with the geographical occurrence of the common vole. Serological surveys of cattle have also shown that bovine *grippotyphosa* reactors are found in Jutland only.

All strains isolated from the brown rat *(R. norvegicus)* were of the *icterohaemorrhagiae* serotype. The house mouse *(Mus musculus)* carried leptospires mainly of the *sejroe* serotype, but a few *saxkoebing* and a single *ballum* strain were also isolated from this species. Only one strain, a *sejroe* strain, was isolated from 64 harvest mice. The majority of the strains isolated from the striped field mouse belonged to the *pomona* serotype. As mentioned above, this species occurs only on thei slands of Lolland-Falster. Examination of wild animals and serological surveys of cattle and swine from various parts of Denmark have shown that *Pomona* infections are found only in animals from these two islands. Only six out of 201 long-tailed field mice harboured leptospires, mainly of the *saxkoebing* serotype. All the 31 strains isolated from 243 specimens of the yellow-necked field mouse were of the *saxkoebing* type.

Carnivora

This order is represented by eight species. So far, the pine marten *(Martes martes)* and the stone marten *(Martes foina)* have not been examined, and two other species have been examined only serologically, as will be mentioned later. Table 1 shows that no leptospires were isolated from 16 weasels *(Mustela nivalus)* and 11 polecats *(M. putorius)*. Serological examination of these animals revealed weakly positive reactions in two weasels (titer 1:30 with *poi* or *saxkoebing)* and two polecats (titer 1:30 with *saxkoebing);* one polecat was definitely seropositive (titer 1:300 with *saxkoebing)*. Three strains, of the serotypes *poi, pomona,* and *sejroe,* were isolated from 37 stoats. Positive seroreactions were found only in the culturally positive animals. A *poi* strain was isolated from one of 16 foxes examined culturally. However, eight culturally negative foxes showed serological evidence of past or present infection, predominantly with the serotype *poi.*

Similarly, examination of sera from 55 foxes that were not examined culturally revealed past or present infection with leptospires in more than 50% of the animals (see Table 2).

Three of seven badgers and two of four otters were seropositive.

Lagomorpha and Ungulata

The order *Lagomorpha* is represented in Denmark only by the hare, while the ungulates are represented by the roe deer *(Capreolus capreolus),* the red deer *(Cervus elaphus),* the fallow deer *(Dama dama),* and the sika deer *(Cervus nippon).* Only sera from hare and roe deer have been examined. As seen from Table 2, both of these two species occasionally show evidence of leptospiral infection, in the roe deer most often by the *saxkoebing* serotype.

Maintaining Hosts and Other Hosts of Leptospires

In Table 1 is indicated which species are at present considered maintaining hosts for the leptospiral serotypes isolated in Denmark. All other hosts of identified serotypes are considered incidental hosts. A maintaining host, in the sense of Audy,[1] for a given serotype of leptospires is an animal species in which leptospires of this serotype are carried by the infected individuals for a sufficiently long period of time to ensure a continued transmission of the infection from generation to generation. Individuals of other species may get infected when they inadvertently or deliberately *(e.g.:*

TABLE 2. Serological evidence of leptospirosis in wild mammals not examined culturally.

Order and species	No. seropositive* / No. examined	Serotype** distribution
Lagomorpha		
Lepus europaeus	3/185	poi 1, sax. 1, sej. 1
Carnivora		
Vulpes vulpes	32§/55	poi 24, sax. 14, sej. 5, ict. 3
Meles meles	3§/7	poi 2, sax. 2
Lutra lutra	2§/4	poi 2, sax. 1
Ungulata		
Capreolus capreolus	12/315	sax. 9, sej. 2, pom. 1

* Seropositive = agglutinin itter 1:100 o rmore.
** Abbreviations: see Table 1.
§ Some animals with evidence of double or triple infection.

as predators) intrude on the habitat of the maintaining host — or if the maintaining host intrudes on the habitats of other animal species.

Maintaining hosts for leptospires of the *ballum, bataviae,* and *canicola* serotypes are not indicated. *Ballum* leptospires have been isolated only once from a wild house mouse, but have been isolated repeatedly from pet albino mice, so it is probable that the house mouse is a maintaining host also for the *ballum* leptospires. So far, we have not isolated *bataviae* or *canicola* leptospires from wild mammals; however, the fact that all registered human *bataviae* infections occurred in Jutland suggests that an animal host should be sought in that part of the country. *Canicola* reactors have never been encountered among Danish wild animals.

Wildlife and the Epidemiology of Leptospirosis in Domestic Animals and Man

Table 3 represents a survey of leptospiral infections in domestic animals. The prevalence rates are based on various serological surveys during the last 16 years. Naturally, these rates depend on

TABLE 3. **Leptospira** infection of domestic animals in Denmark.

Species	Prevalence %	Infection with serotype*									
		ict.	poi	can.	bal.	brat.	pom.	grip.	sej.	sax.	bat.
Dog	12-25	+	s	+		s	s		s	s	s
Cat	ca. 5	s	s			s			s	s	s
Swine	5-16	s	s			s	s		s	s	s
Cattle	8-18	s	s	s	s	s	+	s	s	s	s
Horse	ca. 50	s	s	s	s	s	s	s	s	s	s

* Abbreviations: bat. = bataviae, can. = canicola; for other abbreviations, see Table 1.
+ = Leptospires isolated.
s = Serological evidence only.

many different factors such as locality, year of examination, and the selection of animals with regard to age, clinical history, etc. The upper rates of prevalence for swine and cattle are based on surveys carried out in connection with epizootics of bovine leptospirosis *pomona* in 1957 and 1961 on Lolland-Falster. In these two years the striped field mouse was unusually abundant and many clinical cases of bovine leptospirosis *pomona* were diagnosed in the autumns. Serological surveys of mature swine from Lolland-Falster carried out annually in the month of January since 1962 have shown that within a few years the prevalence of *pomona* infections decreased from about 15% to a level of 2-3%.

Failure of *pomona* infection to become permanently established in the swine and cattle population on Lolland-Falster, in spite of heavy exposure in certain years, may be explained by the tradition of husbandry in Denmark, which differs from that in, *e.g.*, the U.S.A. The Danish swine are always raised in small groups (usually less than 10) in indoor pens without direct contact with cattle. The cattle population is almost entirely dairy cattle, with minimal contact between young and adult animals.

Twenty to 30 years ago the dog undoubtedly acted as a maintaining host for the *canicola* leptospires. Today, *canicola* infection apparently has virtually disappeared from Denmark. A serological survey carried out in 1959-60 and comprising almost 900 dogs revealed only a few weak *canicola* reactors, most of which were more than 9 years old.[9]

TABLE 4. Human cases of leptospirosis in Denmark 1934-1970.

Period	Total	Serotype* distribution								
		ict.	poi	can.	bal.	brat.	grip.	sej./sax.	bat.	undet.
1934-38	124	97	—	8	—	—	—	17	—	2
1939-43	408	82	—	39	—	—	11	268	3	5
(1943)	(253)	(17)	—	(24)	—	—	(9)	(198)	(2)	(3)
1944-48	275	77	3	48		—	4	128	13	2
1949-53	140	45		5	1	—	4	78	5	2
1954-58	60	18	1				2	29	8	2
1959-63	49	28						14	3	4
1964-68	34	15						13	2	3
1969-70	13	10						3		
Total	1103	372	4	100	1	1	21	550	34	20
Percent		33.7	0.4	9.1	0.1	0.1	1.9	50.0	3.1	1.8

* Abbreviations: see Tables 1 and 3.
— = Serotype not used in serotest.

Table 4 is a chronological presentation of the 5-yearly number of verified human cases of leptospirosis in Denmark since 1934. The incidence of *icterohaemorrhagiae* infections has decreased gradually to an average of some four cases annually. *Canicola* infections were relatively frequent during World War II, but no cases have been diagnosed since 1950. The high number of human cases of leptospirosis during the war—and particularly in 1943 — was predominantly due to *sejroe* and *saxkoebing* infections. Most of these cases occurred in the rural population and coincided with an exceptionally large population of the house mouse, the

maintaining host of the *sejroe* leptospires. The number of diagnosed *sejroe/saxkoebing* infections has now decreased to an average of two to three cases annually. The *grippotyphosa* and *bataviae* infections all occurred in Jutland, as mentioned above. No cases of these infections were diagnosed since 1957 and 1964, respectively. Human *pomona* cases have never been diagnosed in Denmark *(pomona* antigen has been used in serotests since 1941).

The low number of verified human cases in the last 15 years does not reflect a reduction of leptospiral infections in wild animals, but is probably the result of higher living standards and the increased mechanization of agricultural and industrial operations, with the ensuing reduction of the infection risk.

At present, a rather stable equilibrium seems to have been attained between wildlife carriers of leptospires and leptospirosis in man and domestic animals in Denmark. This balance may change in the future, either as a result of changes in human life and the husbandry of domestic animals, and/or as a result of (possibly man-mediated) changes in the wild animal populations harbouring leptospires.

LITERATURE CITED

1. AUDY, J. R. 1958. The localization of disease with special reference to the zoonoses. Trans. roy. Soc. trop. Med. Hyg. 52: 308-334.
2. BORG-PETERSEN, C. 1938. Leptospirenuntersuchungen in Dänemark. Acta conv. III trop. morb., Amsterdam 1: 396-406.
3. BORG-PETERSEN, C. 1943. *Leptospira saxkoebing,* a new serological *Leptospira* type. Acta path. microbiol. scand. 20: 793-794.
4. BORG-PETERSEN, C. 1944. Om Weils Sygdom og andre i Danmark forekommende Leptospiroser. Ugeskr. Læg. 106: 617-629.
5. BORG-PETERSEN, C. 1944. *Leptospira ballum,* a new serological type. Acta path. microbiol. scand. 21: 504-509.
6. BORG-PETERSEN, C. 1949. Experience of leptospirosis in Denmark. Proc. roy. Soc. Med. 42: 714-718.
7. BORG-PETERSEN, C., and K. L. FENNESTAD. 1956. A field rodent *(Apodemus agrarius)* as carrier of *Leptospira pomona* in Denmark. J. Amer. vet. med. Ass. 128: 204-205.
8. BORG-PETERSEN, C., and K. L. FENNESTAD. 1956. Studies on bovine leptospirosis and abortion. I. Serological examination of aborting and normal cattle. Nord. Vet.-Med. 8: 465-480.
9. BORG-PETERSEN, C., and K. L. FENNESTAD. 1962. Incidence of canine leptospirosis in Denmark. Nord. Vet.-Med. 14: 609-619.
10. FENNESTAD, K. L., and C. BORG-PETERSEN. 1956. Studies on bovine leptospirosis and abortion. II. Experimental leptospirosis in pregnant heifers. Nord. Vet.-Med. 8: 815-833.
11. FENNESTAD, K. L., and C. BORG-PETERSEN. 1958. Et udbrud af leptospirosis pomono med hæmoglobinuri og abort hos kvæg pa Lolland-Falster. Medlemsbl. danske Dyrlægeforen. 41: 279-282.
12. FENNESTAD, K. L., and C. BORG-PETERSEN. 1958. Studies on bovine leptospirosis and abortion. IV. Demonstration of *Leptospira* in foetuses from field cases of abortion in cattle. Nord. Vet.-Med. 10: 302-308.
13. SCHUFFNER, W., and H. BOHLANDER. 1942. Schlammfieber in Holland — die Feldmaus als Träger. Zbl. Bakt., I. Abt. Orig. 148: 264-273.
14. ZUELZER, M. 1935. Biologie und Epidemiologie der Weil'schen Krankheit mit besonderer Berücksichtigung von Dänemark. Acta path. microbiol. scand. 12: 511-535.

Received for publication April 24, 1972