Isolation of Leptospira canicola From Skunks in Louisiana

EARL E. ROTH, D.V.M., W. V. ADAMS, B.S., and DONNA LINDER, B.A.

LEPTOSPIROSIS caused by *Leptospira canicola* was first recognized in dogs in the Netherlands by Klarenbeek and Schuffner in 1933 (*1*) and has since been found to be widespread among dogs throughout the world. Human infections with *L. canicola* have been reported from various parts of the globe (*2*). Van der Hoeden isolated the organism from cattle and swine during outbreaks of leptospirosis in these species in Israel (*3–5*). Since dogs and jackals in that area were found to be carriers of *L. canicola*, they were considered as possible sources of infection. Jackals were considered to be more important than dogs. In Israel, serologic evidence of infection was found also in horses, mules, and donkeys. Van der Hoeden (*6*) also recovered *L. canicola* from two species of hedgehog, *Hemiechinus auritus* and *Erinaceus europaeus transcaucosicus*, which are related members of the family Erinaceidae. Kmety (*7*) recovered *L. canicola* from swine at an abattoir in Czechoslovakia. In Russia, *L. canicola* was recovered from the brown rat, *Rattus norvegicus* (*8*). In a personal communication from London in 1960, C. E. Smith and co-workers reported isolation of *L. canicola* from the small rat, *Rattus exulans*, in Malaya.

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In this country, *L. canicola* was first isolated from dogs by Meyer and co-workers (*9,10*); however, *L. canicola* infection has since been found to be widespread among dogs (*11,12*). Human infections have been reported by a number of workers. More recently, during epidemiological studies of an outbreak of canicola fever in man by Ward and associates (*13*) and Williams and associates (*14*), *L. canicola* was isolated from human beings, dogs, and a sow. This was the first time *L. canicola* had been recovered from swine. The human epidemic was associated with bathing in a creek accessible to dogs, swine, cattle, and wild animals. The cattle in that area showed serologic evidence of past infection with *L. canicola*, but the organism was not isolated from them. Turner and co-workers (*15*) recently reported the isolation of *L. canicola* from a 2-day-old sick calf. Serologic evidence of leptospirosis in raccoons caused by *L. canicola* was reported by Reilly (*16*).

This report describes the isolation and identification of five strains of leptospires recovered from striped skunks, *Mephitis mephitis*, collected in Louisiana. These strains were homologous with *L. canicola*, strain Hond Utrecht, and represent a new host-serotype relationship. This is the first bacteriologically verified report of *L. canicola* infection in a wild animal host in the United States.

Materials and Methods

Collection and processing of animals. Most of the skunks in the study were collected in south central Louisiana between the Mississippi and Atchafalaya Rivers. A few were collected

west of the Atchafalaya River. This area is primarily agricultural and is moderately populated with cattle and dogs. It is heavily populated with striped skunks. The methods employed in processing the animals are described in detail elsewhere (*17*), except for dark-ground examination of the 10 percent kidney suspensions. These were made by using the same dark-field system for examining semisolid cultures as has been described previously (*17*). If the first preparation proved negative, a second preparation was examined.

Cultural procedures. Five types of semisolid mediums were employed: Fletcher's (*18*) basal medium containing rabbit serum, Fletcher's basal medium containing horse serum, modified Stuart's (*19*) medium containing rabbit serum, modified Stuart's medium containing horse serum, and Chang's (*18*) basal medium containing rabbit serum. Not all five mediums were employed for each specimen. Semisolid mediums were inoculated with 3 to 5 drops of 10 percent kidney suspension. Three plates of solid medium prepared according to the method of Cox and Larson (*20*) were inoculated with 0.1 ml. of 1:10, 1:100, and 1:1,000 dilutions of 10 percent kidney suspension. Remaining details pertaining to cultural procedures have previously been described (*17*).

Serologic procedures. All antiserums were prepared as described by Alexander and coworkers (*21*), except that 10-day-old cultures were used. Antigens employed in the microscopic agglutination test and in the agglutinin-absorption procedure were prepared and were employed in these procedures as previously described (*17*), except that a dilution scheme providing for final tenfold dilutions ranging from 1:100 to 1:100,000 was used in the initial screening studies of the five canicola isolates. In all other studies, the interlocking tenfold scheme of Wolff (*18*) was used. A 1+ reaction was considered positive. The antigens employed are indicated in tables 1 and 2.

Leptospiral serotypes. Strains of the following serotypes were employed to produce antigens and antiserums:

ballum, MUS 127
canicola, Hond Utrecht
icterohaemorrhagiae, M 20
bataviae, Swart
grippotyphosa, Moskva V
pyrogenes, Salinem

Table 1. Cross agglutination reactions of the isolates and Leptospira canicola, Hond Utrecht, with antileptospire serums

Antileptospire serums	Homologous titers	Reciprocal of titer against antigen	
		L. canicola	Isolates [1]
	Initial screening study [2,3]		
L. canicola	10, 000	10, 000	10, 000
L. pyrogenes	10, 000	(4)	1, 000
L. ballum	100, 000	(4)	100
L. icterohaemorrhagiae	10, 000	(4)	100
	Cross agglutination study [5]		
L. jonsis	100, 000	10, 000	10, 000
L. patane	10, 000	10, 000	10, 000
L. malaya	100, 000	3, 000	3, 000
L. sumneri	100, 000	10, 000	10, 000
L. schueffneri	30, 000	3, 000	3, 000
L. benjamin	100, 000	10, 000	10, 000
L. canicola	10, 000	10, 000	10, 000

[1] Strains: LSU 1113, LSU 1114, LSU 1116, LSU 1346, and LSU 1347.
[2] Tenfold dilution scheme, 1:100 to 1:100,000.
[3] No reactions at 1:100 against *L. bataviae, L. grippotyphosa, L. autumnalis, L. pomona, L. sejroe, L. hyos, L. harjo, L. semaranga, L. djatzi, L. djasiman, L. sentot, L. australis A, L. zanoni, L. alexi, L. medanensis, L. javanica,* and *L. andamana.* Homologous titers were 1:10,000 or 1:100,000.
[4] Not done.
[5] Interlocking tenfold scheme, 1:100, 1:300, 1:1,000, 1:3,000, and so on.

autumnalis, Akiyami A
pomona, Pomona
sejroe, M 84
hyos, Hyos
hardjo, Hardjoprajitno
semaranga, Veldrat S 173
alexi, HS–616
djatzi, HS–26
djasiman, Djasiman
sentot, Sentot
australis, Ballico
zanoni, Zanoni
medanensis, Hond HC
javanica, Veldrat Batavia 46
andamana, CH 11
jonsis, Jones
sumneri, Sumner
schueffneri, Vleermuis 90 C
benjamin, Benjamin
malaya, H–6
patane, Patane

All strains were supplied by the Division of Veterinary Medicine, Walter Reed Army Institute of Research, Washington, D.C.

Results

Since February 1959, 180 isolations of leptospires have been obtained from 310 striped skunks. Identification studies have been completed for 12 strains; 7 were *L. pomona* and 5 were *L. canicola*. This report is concerned with *canicola* strains LSU 1113, LSU 1114, LSU 1116, LSU 1346, and LSU 1347, which were isolated from skunks 17, 18, 19, 41, and 42, respectively.

All five strains were isolated by the agar plate method (*17*) employing the solid medium described by Cox and Larson (*20*). Strain LSU 1113 produced 39 colonies on plate 1, 5 on plate 2, and 1 large spreading colony on plate 3. The growth of strain LSU 1114 was confluent on plate 1, colonies were too numerous to count on plate 2, and a few leptospiral colonies along with some colonies of contaminents were produced on plate 3. Strain LSU 1116 produced colonies too numerous to count on plates 1 and 2 and 39 colonies on plate 3. They eventually covered the entire plate. Plates 1 and 2 were grossly contaminated for LSU 1346; however, areas of leptospiral growth were present between the contaminants on plate 3. Strain LSU 1347 produced several spreading colonies on plate 1, whereas plates 2 and 3 remained negative. Leptospiral growth was noted on the sixth day for strains LSU 1113 and LSU 1114. Strain LSU 1116 was positive on the eighth day, and strains LSU 1346 and LSU 1347 were found positive by the 15th day.

Four of the five *canicola* strains were obtained in pure culture by direct inoculation of

Table 2. Results of cross agglutinin-absorption tests with Leptospira canicola and the canicola isolates

Antiserum against—	Absorbed with—	Reciprocal of titer against antigen [1,2]			
		Homologous strain		Absorbing strain	
		Before	After	Before	After
L. canicola	*L. canicola*	10, 000	Negative	10, 000	Negative.
	LSU 1113	10, 000	do	10, 000	Do.
	LSU 1114	10, 000	do	10, 000	Do.
	LSU 1116	10, 000	do	10, 000	Do.
	LSU 1346	10, 000	do	10, 000	Do.
	LSU 1347	10, 000	do	10, 000	Do.
LSU 1113	LSU 1113	100, 000	do	100, 000	Do.
	L. canicola	100, 000	do	100, 000	Do.
LSU 1114	LSU 1114	30, 000	do	30, 000	Do.
	L. canicola	30, 000	do	30, 000	Do.
LSU 1116	LSU 1116	10, 000	do	10, 000	Do.
	L. canicola	10, 000	do	10, 000	Do.
LSU 1346	LSU 1346	100, 000	do	100, 000	Do.
	L. canicola	100, 000	do	100, 000	Do.
LSU 1347	LSU 1347	100, 000	do	100, 000	Do.
	L. canicola	100, 000	do	100, 000	Do.

[1] Living antigen was used.
[2] Negative indicates no reaction in a 1:100 dilution.

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semisolid mediums with 10 percent kidney suspension. Semisolid cultures of strain LSU 1346 were positive for leptospires; however, contamination was present in all tubes. Strains LSU 1113 and LSU 1114 were isolated in all semisolid mediums except Chang's. Strains LSU 1116 and LSU 1347 were positive in all semisolid mediums, except that modified Stuart's medium containing horse serum was contaminated in the case of LSU 1116 and was not used in the case of LSU 1347.

Initial screening and additional cross-agglutination studies clearly showed the serologic affinity of the isolates for the *canicola* serogroup (table 1). Since the cross-agglutination pattern of the isolates was found to be the same as for *L. canicola*, reciprocal agglutinin-absorption studies were performed with *L. canicola*, Hond Utrecht (table 2).

The serums of the five skunks from which these isolates were obtained were tested against the first 21 antigens listed. The serums had low agglutinin titers for *L. canicola*. The serum from skunk 18 showed a titer of 1:1,000; titers for the other four were 1:300. Heterologous titers of 1:30 to 1:100 were found for *L. ballum* and *L. pyrogenes*. These are commensurate with infections of *L. canicola*. The serum from skunk 41 agglutinated antigens prepared from *L. hardjo*, *L. sejroe*, and *L. medanensis* to a titer of 1:100 and also agglutinated the antigen of *L. canicola* to a titer of 1:300. The serums were negative for the remaining 21 antigens.

Four of the five 10 percent kidney suspensions from which *L. canicola* was isolated were positive by dark-field examination. The suspension from skunk 41 was negative. Large numbers of leptospires were observed in the positive suspensions from skunks 18 and 19.

Since the preparation of this report was begun, eight additional strains of leptospires isolated from skunks were found to have the same agglutination pattern as the five strains being reported. These are probably strains of *L. canicola*.

Discussion

The demonstration of *L. canicola* infection among skunks directs additional attention to their potential role in the transmission of leptospirosis in this country. The skunk has been shown to be a host for *L. ballum* and *L. pomona* (*22*). The occurrence of a member of the *hyos* serogroup in skunks has been reported (*23*). Galton and associates (*24*) reported the isolation of a new member of the *hebdomadis* serogroup, for which the name *L. mini georgia* was proposed. Serotypes represented among the 180 leptospiral isolates obtained from skunks in Louisiana are *L. pomona*, *L. ballum*, *L. canicola*, and serotypes of the *hyos* and *hebdomadis* serogroups (*17*). From these studies it appears that the skunk is readily infected with a number of diverse serotypes of *Leptospira*. These infections apparently are not fatal. In the majority of instances, even though the skunk proves to be bacteriologically positive, gross lesions of the kidneys are minimal or undetectable.

The discovery of an additional natural host for *L. canicola* further complicates the epizootiological picture, particularly since *L. canicola* has recently been found to cause bovine and porcine leptospirosis. The public health significance of leptospirosis caused by *L. canicola* has already been established. Skunks are sometimes found at night feeding among resting cattle, thereby affording the necessary association for interspecies transmission of leptospires. The question that remains open is whether the skunk becomes infected from cattle, dogs, or other animals, or whether it serves as a reservoir of *L. canicola*. Nevertheless, it could certainly provide an intermediate link in a chain of interspecies transmissions and thereby play an important epizootiological role. Its mode of living is conducive to intraspecies transmission and this may help to explain the high rate of *L. canicola* infection among skunks in Louisiana.

Since skunks are sometimes trapped, descented, and sold as pets, their possible public health significance cannot be overlooked. As pets they are in contact with human beings and other pets and domestic animals.

The low agglutinin titers for *L. canicola* observed in the serums of the five skunks from which *L. canicola* was isolated emphasized the limitations of epizootiological studies based on serology alone. They did, however, elicit a

predominant titer for *L. canicola*. Even though Wolff and Broom (*25*) reported slight cross reactions between *L. canicola* and *L. schueffneri* antiserums with members of the *hebdomadis* serogroup, it appears that the reactions observed suggest that skunk 41 had previously been infected with a serotype of the *hebdomadis* group. Furthermore, *L. hardjo*, a member of the *hebdomadis* group, has been isolated from cattle in Louisiana (*19*).

Summary

Isolation of *Leptospira canicola* from five striped skunks, *Mephitis mephitis*, collected in Louisiana establishes a wildlife source of *L. canicola* in the United States that may infect man and animals. All five strains were isolated by direct inoculation of solid medium with diluted kidney suspension. Four of the five strains were obtained in pure culture by direct inoculation of five types of semisolid mediums with 10 percent kidney suspension. Employing the microscopic agglutination test and the agglutinin-absorption test, all five strains were shown to be homologous with *L. canicola*, Hond Utrecht.

Agglutination tests with serums of the five skunks revealed low but predominant serotiters for *L. canicola*. The serum from one skunk also agglutinated antigens of the *hebdomadis* serogroup.

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