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Detection and Ecology of Leptospirosis in Iowa Wildlife ¹

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Abstract

To gain additional information on the extent of leptospirosis in wildlife following a human outbreak in Iowa, wild mammals and lower forms of life were collected. Isolation, darkfield microscopic, serologic and pathologic procedures were used to identify past or present evidence of leptospiral infection.

Leptospire was isolated from 7 of 75 (9%) mammals. Serotype *grippityphosa* was isolated from three raccoons (*procyon lotor*) and one Western Harvest Mouse (*Reithrodontomys megalotis*). Serotype *ballum* was isolated from three opossums (*Didelphis marsupialis*). Leptospire, unidentified to date, were isolated from frog (*Rana pipiens*) kidneys. Other positive serologic and pathologic tests gave evidence of infection or previous infection. Utilization of Darkfield microscopic and silver staining techniques did not detect all cases of leptospiral infection. Macroscopic and microscopic serologic methods failed to identify evidence of leptospirosis in all mammals from which leptospire were isolated. Pathologic lesions could only be considered presumptive evidence for leptospirosis.

These findings indicate that detection of leptospirosis in wildlife cannot be limited to a single diagnostic test. A combination of diagnostic procedures and clinical evaluation is necessary.

Although serotype *pomona* was implicated as the predominant infecting leptospire in the human cases and domestic animals and was isolated from water at a swimming site, only serotypes *grippityphosa*, *ballum* and ICF (frog isolate) were isolated from wild mammals and lower forms of life in the same vicinity.

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Introduction

During October, 1964, 75 wild mammals, 21 fish, 6 frogs, 5 turtles and 2 crayfish were collected from a creek area where in July and August of 1964, 15 human cases of leptospirosis, with predominant *pomona* titers, occurred following swimming.¹⁴ In 1959, two of 40 human cases of leptospirosis were associated with swimming in the same creek.^{2,20} Serotype *pomona* was isolated from water collected from the swimming site on August 19, 1964, 12 days after the onset of the last human case.⁸ Immediately upstream 19 of 21 beef cattle with access to the creek were found serologically positive, with 14 having predominant titers against *pomona*. Serologic evidence of *grippotyphosa*, *hardjo*

and *pomona* infections were found in 30.5% of 744 bovine serums tested in the township where the stream was located.⁹ During examination of the swimming sites, numerous wildlife signs were observed.

On October 6, 1964, the wildlife trapping project was begun in proximity to the swimming sites. This area included one half mile upstream and downstream (Fig. 1).

In May, 1965, 3 wild mammals, 2 toads, and a turtle were collected in the area. In June, 1965, tadpoles were collected from the swimming site. Wildlife were collected in an attempt to further define the ecology of leptospirosis in this endemic area.

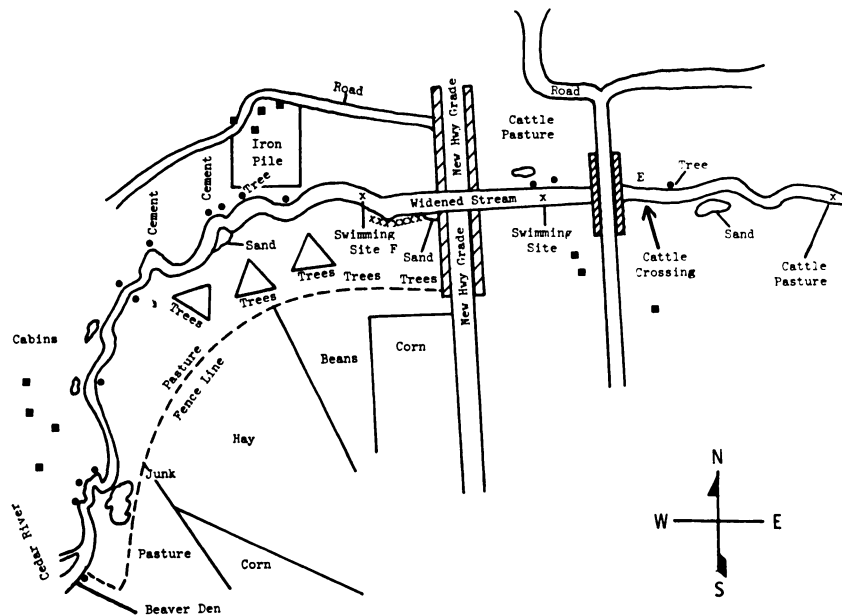


FIGURE 1. Stream, swimming sites, pastures, fields, and wildlife trapping areas.

Materials and Methods

In October, 1964, the following 75 mammals were collected: 5 raccoon, (*P. lotor*); 12 opossum (*D. marsupialis*); 24 mice — 5 Western Harvest mice, (*R.*

megalotis); 13 house mice, (*M. musculus*); 4 whitefoot mice, (*Peromyscus leucopus*); 1 Eastern Jumping Meadow mouse, (*Zapus hudsonicus*); 1 Northern grasshopper

mouse, (*Onychomys leucogaster*); 6 foxes — 2 gray fox, (*Urocyon cinereoargenteus*) and 4 red fox, (*Vulpes fulva*); 5 fox squirrels (*Sciurus niger*); 14 muskrats, (*Onadatra zibethicus*); 8 Eastern cotton tail rabbits, (*Sylvilagus floridanus*); and 1 feral house cat, (*Felis domestica*). With cooperation from the State Conservation Commission the following species were seined from the *pomona* isolation area; 21 fish including 14 quillbacks, (*Carpiodes* sp.); 4 bullheads, (*Ictalurus* sp.); 2 sunfish (*Lepomis* sp.); and 1 sucker (*Cotostomus* sp.); 6 frogs, (*R. pipiens*); 5 turtles, 4 map turtles; (*Graptemys geographica*) and 1 softshelled turtle, (*Amyda spinifera*) and 2 crayfish (*Cambarus* sp.).

In May, 1965, 1 opossum, (*D. marsupialis*), 2 skunks *Mephitis mephitis*, 1 turtle, (*G. geographica*), 2 toads, (*Bufo americana*) were collected from the same area. In June, tadpoles (*R. pipiens*) were collected from the swimming site.

Havahart and snap traps were used to collect the larger mammals and automatic traps for the mice. Each morning the traps were examined, mammals removed and placed in the cages and returned (24 miles) to a Field Station near Iowa City. Mammals were aged, sexed and immediately processed. Blood specimens were collected by cardiac puncture. The skin and muscles were incised and reflected laterally from sternum to pelvis. Kidneys were examined for gross lesions, aseptically removed and placed in sterile containers. Available urine was aseptically collected from the bladder. Specimens were taken to the Medical Laboratories, College of Medicine, Iowa City.

Selected blocks of kidney tissue were removed from each animal and placed in 10% formalin for histopathologic studies. Stuart's liquid medium was added to fresh kidney during grinding by mortar and pestle to make a 10% kidney suspension. Kidney and urine specimens were examined for leptospires by darkfield microscopy. These suspensions were diluted with Stuart's liquid medium (1:10 to 1:100,000) and 2-3 drops from each dilution inoculated into semisolid media:

bovine albumin-Tween 80¹¹ and Fletcher's.²¹ Inoculated media were incubated at 28 to 30°C for up to 75 days and periodically examined for leptospires. Ten percent kidney tissue suspensions from selected mammals were inoculated intraperitoneally into weanling hamsters and guinea pigs. Kidney tissue from the lower forms of life, gills from fish, and frog eggs collected were inoculated into culture media. Woodticks from the opossum collected in June (1965) were ground and inoculated into hamsters.

Blood specimens were centrifuged and sera were initially tested by the rapid macroscopic slide agglutination test using four pooled Bacto-Leptospira antigens*. The mammal sera were initially screened at a 1:20 dilution by the microscopic agglutination (MA) test using 12 single live antigens. Sera with detectable agglutinins were tested to endpoint using 4-fold serial dilutions starting at a 1:25 dilution. Turtle and fish sera were screened by the macroscopic slide test and by MA against selected antigens. All isolates were definitely identified by the Leptospirosis Reference Laboratory at NCDC, Atlanta, Georgia.

The formalin fixed kidney tissues were embedded in paraffin, sectioned at 6-7 microns, and stained with Harris hematoxylin-eosin, Warthin-Starry silver stain technique, and occasionally Gomori's trichrome technique for connective tissue elements. The renal features evaluated included number and structure of glomeruli, size and status of tubular epithelial cells (normal, degenerative, or regenerative), and luminal casts and debris in tubules; condition of the interstitium (hemorrhage, fibrosis, cellular infiltrates); and condition of the vasculature. Tubular and interstitial lesions were further characterized according to level of the lesion.

The microscopic renal anatomic alterations were reviewed independently of the microbiological and serological studies. Sections of kidney were grouped by species, retaining the accession number for animal identification. After histopathological examinations were completed the observations were correlated with the additional laboratory data.

* Difco and Company.

Results

Clinical

Of the 75 mammals trapped in October, 58 were collected alive. Fifteen mice and 2 muskrats were found dead in traps. Of the 5 raccoons, W6 and W8 and of 12 opossums, W4 showed signs of depression, emaciation and rough hair coat and appeared underweight.

Bacteriology

Leptospires were observed on darkfield examination of kidney suspensions in 2 of 5 raccoons and 2 of 12 opossums. Leptospires were isolated in cultural media from the kidney suspensions 7 of 75 (9%) mammals. Isolations were made from 3 of 5 raccoons in both types of semisolid media. All isolates were identified as serotype *grippotyphosa*. Leptospires were observed in culture media inoculated with hamster heart-blood (W75) raccoon but failed to propagate on subculturing.

Leptospires were isolated in culture media from the kidney tissue of W4, W34 & W56 of the 12 opossums. Isolations from W4 and W34 were made in both types of semisolid media. Media inoculated with W4 contained leptospires

at 7 days; W34 at 11 days; the W56 isolation was made in bovine albumin-Tween 80 medium at 11 days. Leptospires were isolated from opossum W4 inoculated hamster blood and kidney tissue. All isolates were identified as *ballum*.

Leptospires were observed at 27 days in Fletcher's semi-solid medium inoculated with kidney tissue of W10 of 24 mice collected. The isolate was identified as *grippotyphosa*.^a Leptospires (unidentified) were isolated from pooled kidney tissue of frogs.^a

Serology

On the macroscopic slide agglutination test, 8 of 58 (14%) mammal sera tested were positive in 1 or more of the 4 pools. On the microscopic agglutination test, 8 of 58 (14%) or 3 of 6 (50% from which isolations were made) exhibited antibodies against one or more serotypes. Refer to Table II for species specific information.

Turtle sera were positive with Difco pooled antigens, and MA titers from 1:10 to 1:50 were exhibited against *ballum*, *canicola*, *icterohaemorrhagiae*,

TABLE I. Total number of leptospira positive samples found in wild mammals based on serologic, darkfield microscopic, isolation, pathologic and Warthin-Starry results.

Species	Population	Serology		Darkfield Microscopy	Isolation	Pathologic Lesion		Warthin- Starry
		Macro- scopic Slide Aggl.	Micro- scopic Aggl.			Macroscopic	Microscopic	
Mouse	24	—*	—	—	1	—	—	—
Rabbit	8	—	1	—	—	—	—	—
Squirrel	5	2	—	—	—	—	—	—
Fox	6	1	2	—	—	—	—	—
F. cat	1	—	—	—	—	—	—	—
Muskrat	14	—**	—	—	—	—	1	—
Opossum	12	3	—	2	3	1	4	1
Raccoon	5	2	5	2	3	2	4	3
Total	75	8	8	4	7	3	9	4

* Blood samples collected from 9 of 24 mice.

** Blood samples collected from 12 of 14 muskrats.

— indicates negative.

and *patoc*. Positive serologic results were obtained from fish sera screened with Difco pooled antigens (Table IV). One turtle collected in May 1965 exhibited a 1:50 MA titer against ICF isolate.

Pathology

Gross lesions: Focal, mottled gray-white patches were noted in the kidney cortices of 3 animals (4%): raccoons W6 and W8, and opossum W4. All other kidneys appeared normal.

Varying degrees of glomerular, tubular and interstitial alterations were observed in the kidneys of 4 of 5 raccoons (Table I). (The other raccoon W70 had mild focal cortical mononuclear infiltration). In the 4 mammals, alterations were those of patchy sclerosis and atrophy of glomeruli adjacent to zones of tubular epithelial damage (usually identifiable as distal convoluted tubule), and interstitial fibrosis and mononuclear infiltration (Fig. 2). In W6, of the 4 raccoons,

glomerular involvement included proliferative glomerulitis with hypercellularity of the tufts. The patches of tubular degeneration, interstitial fibrosis and cellular infiltration constituted macroscopic streaked lesions which radiated from the outer cortex into the columns of Bertin (Fig. 2). The medulla proper was not significantly involved. No vascular change was noted.

In 3 of the 4 mammals with renal structural alterations, spirochetes were demonstrated in silver stained sections. The organisms were present in the lumina of degenerative tubules, but occasionally occurred in intact, undamaged tubules. Clusters of spirochetes could be readily observed at low power magnification (40x), but oil immersion (960x) was necessary to establish spirochete morphology (Fig. 3). Spirochetes had several gentle undulations in these preparations and were positioned at the surface of tubular epithelial cells. No organisms could be identified intracellularly.

TABLE II. Summary of individual test results of data used for detection of evidence of infection in wild mammals.

Species and Number	Age	Sex	Clinical Signs	Microscopic DKF Kidney	Urine	Bacteriologic (Isolation)			Isolate Identification Serotype
						Artificial media		Lab Animals	
Raccoon (W6)	J	F	+	+	—	+	—	+ hamst. & guinea pig	<i>grippotyphosa</i>
Raccoon (W8)	J	M	+	+	+	Neg.	<i>grippotyphosa</i>
Raccoon (W24)	J	M	—	—	+
Raccoon (W70)	A	F	—	—	—	<i>grippotyphosa</i>
Raccoon (W75)	A	F	—	—	—	+ hamst.*
Opossum (W4)	A	F	+	+	—	+	—	+ hamst.	<i>ballum</i>
Opossum (W7)	J	M	—	—	—	—	—
Opossum (W34)	J	M	—	—	+	<i>ballum</i>
Opossum (W56)	A	F	—	—	—	+	—	<i>ballum</i>
Opossum (W60)	J	M	—	+	—	—	—
Mouse (W10)	F	—	+	<i>grippotyphosa</i>
Gray Fox (W33)	A	M	—	—	—	—	—
Gray Fox (73)	A	F	—	—	—	—	—
Squirrel (W42)	A	M	—	—	—
Squirrel (W53)	A	M	—	—	—
Rabbit (W35)	F	—	—	—

TABLE II—continued

Species and Number	Serology				Pathology			
	Macroscopic Slide Aggl. Test (Pools)				Microscopic Agglutination Test	Macroscopic Lesions	Microscopic Lesions	Warthin Starry Stain
	I	II	III	IV				
Raccoon (W6)	+	—	—	—	grippe. 1:100 pyrogenes 1:25 australis 1:25	+	+	+
Raccoon (W8)	+	+	—	—	grippe 1:6400, pyrogenes 1:25, australis 1:25	+	+	+
Raccoon (W24)	—	—	—	—	grippe 1:100 australis 1:25	—	+	+
Raccoon (W70)	—	—	—	—	grippe 1:20	—	—	—
Raccoon (W75)	—	—	—	—	grippe 1:20 pyrogenes 1:20	—	+	—
Opossum (W4)	+	—	—	—	—	+	+	+
Opossum (W7)	+	—	—	—	—	—	+	—
Opossum (W34)	—	—	—	—	—	—	+	—
Opossum (W56)	+	—	—	—	—	—	+	—
Opossum (W60)	—	—	—	—	—	—	—	—
Mouse (W10)	—	—	—	—
Gray Fox (W33)	—	—	—	—	australis 1:25	—	—	—
Gray Fox (W73)	+	—	+	—	grippe 1:100 Icter. australis. pomona 1:25	—	—	—
Squirrel (W42)	+	—	—	—	—	—	+	—
Squirrel (W53)	+	—	—	—	—	—	—	—
Rabbit (W35)	—	—	—	—	canicola 1:20 australis 1:20	—	—	—

* *Leptospire*s observed in culture media inoculated with 8 day hamster heart blood (hamsters previously inoc.) with raccoon kidney suspension.

** J—indicates less than 1 year age.

A—indicates more than 1 year age.

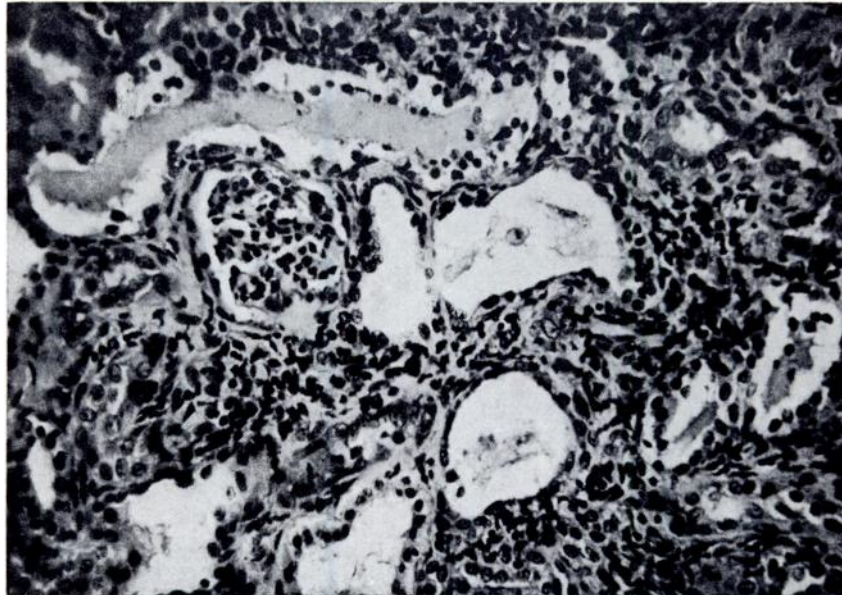
.... indicates not done.

Microscopically, W4, W34, W69 of the 12 opossums had patchy sclerosis and atrophy of glomeruli. In W4 all levels of tubules showed severe but patchy degenerative changes, regeneration of epithelium, and interstitial cellular infiltration. The patchy, intense granulocytic and mononuclear infiltrate at the levels of tubule damage produced macroscopic streaks in cortical zones. Distal convoluted tubules were most severely damaged, although proximal tubule segments were also affected. Leukocytic casts were present in tubular lumina. Kidneys of opossum W4 exhibited glomerular scler-

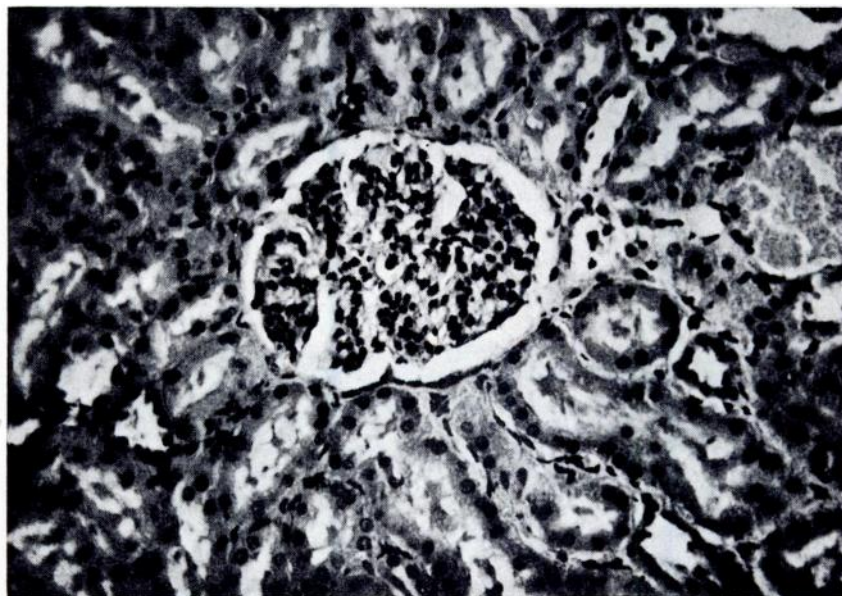
osis, tubular damage, and interstitial infiltration and fibrosis similar to the raccoon renal lesions, and was the only opossum in which spirochetes were demonstrated in tissue by silver stain (Table II).

In opossums W7, W34, W56, W60 and W69 minor patches of mononuclear infiltration occurred in the cortex (predominantly) or medulla. Mild and patchy interstitial fibrosis with or without cellular infiltration occurred in W7, W13, W14, W34, and W56 in addition to W4. In W7, W34, and W56 there was mild interstitial fibrosis with mononuclear cell

FIGURE 2. *Raccoon kidney*:

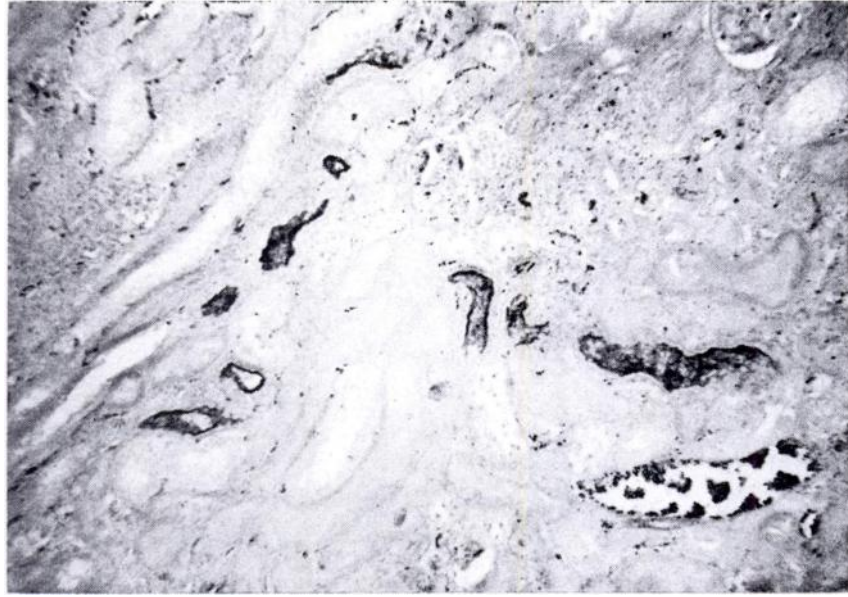


A: Characteristic lesion showing dilated degenerative tubules, sclerotic glomerulus, mild interstitial fibrosis, and mononuclear cellular infiltrate.

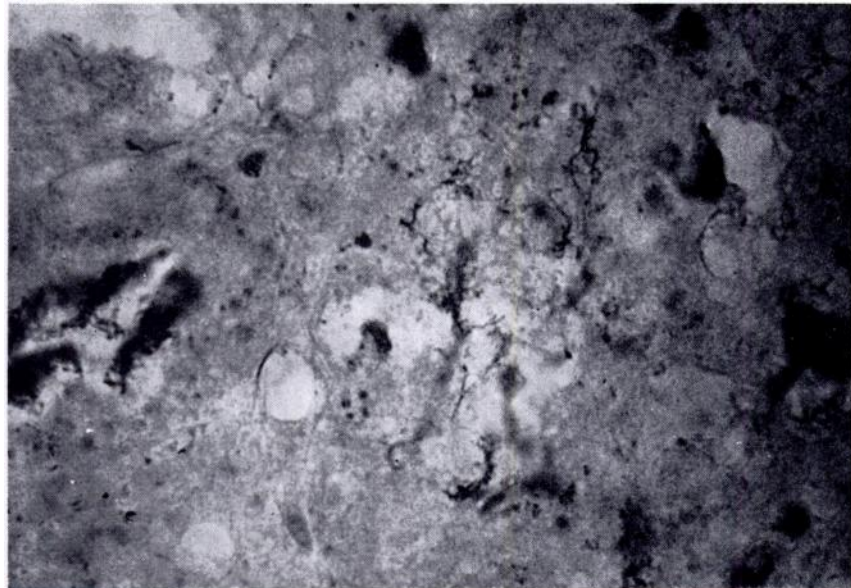


B: normal control 400x.

FIGURE 3. *Leptospires* in raccoon kidney, Warthin-Starry silver stain:



A: Tangled masses of spirochetes outline the margins of tubular lumina, 125x.



B: Single and intertwined leptospires show spiral pattern and terminal hook, 1080x.

TABLE III. Percentage comparison of leptospiral detection methods made to actual isolations from animal kidney tissue.

Species	Number	Clinical		Isolation			Serology		Pathology		
		Signs	DF	Kidney	Urine	Lab. Animal	MSA Screening	MA Screening	Macro.	Micro.	W-S
Raccoon	W6	+	+	+	—	+	+	+	+	+	+
Raccoon	W8	+	+	+	—	+	+	+	+	+
Raccoon	W24	—	—	+	—	—	+	—	+	+
Opossum	W4	+	+	+	—	+	+	—	+	+	+
Opossum	W34	—	—	+	—	—	—	+	—
Opossum	W56	—	—	+	—	+	—	—	+	—
Mouse	W10	—	+	—	—	—
Percentage Positive		50%	43%	100%	0	67%	67%	50%	43%	86%	57%

+ indicates positive test.

— indicates negative test.

.... indicates not done.

TABLE IV. Summary of serologic testing of turtle and fish sera.

Species	Macroscopic slide agglutination pools				Microscopic agglutination test serotype						
	1	2	3	4	ballum	canicola	ictero-haemorrhagiae	grippotyphosa	ICF*	patoc	pomona**
Turtle 1	+	—	—	—	1:25	1:10	1:10	—	—	1:10	—
Turtle 2	+	—	—	—	1:10	1:10	1:25	—	—	—	—
Turtle 3	—	—	—	—	—	—	—	N.D.	N.D.	N.D.	—
Turtle 4	+	—	—	—	—	1:10	1:50	—	—	1:10	—
Turtle 5	+	—	—	—	1:10	—	—	—	—	—	—
2 quillbacks	+	—	—	—	Microscopic agglutination test was not conducted due to insufficient sera.						
2 quillbacks	+	—	—	—							
2 quillbacks	+	+	+	+							
2 quillbacks	+	—	+	+							
2 quillbacks	+	+	+	+							
2 quillbacks	—	—	—	—							
2 quillbacks	—	—	—	—							
1 sucker	—	—	—	—							
4 bullheads	—	—	—	—							

* ICF—Iowa City Frog Isolate.

** pomona—Stream Isolate.

infiltration. In W34 mild glomerular sclerosis occurred in patchy fashion. Tubular epithelial degeneration occurred only in W4.

Microscopically, in addition to several instances of post mortem autolysis of

tubules, mononuclear cell infiltration occurred in 9 of the 24 mice. The infiltrate was equally distributed between cortex and medulla, focal to diffuse, and ranged in intensity from slight to moderate. No fibrosis occurred, and no spiro-

chetes were found in tissue sections. The kidneys of four foxes showed autolysis of a minor degree. Squirrel kidneys, W42 and W61 exhibited light and focal mononuclear infiltrations in cortical interstitium.

Microscopically, in muskrat W31 kidney, a few glomeruli were shrunken and sclerotic, renal tubules were slightly dilated, and there was considerable autolysis of the epithelium. Minor autolysis occurred in 12 other mammals in the group. Seven animals had focal, mild

mononuclear cell infiltrates in cortex and medulla, and in one (W31) the infiltrate occurred in association with minimal interstitial fibrosis. No spirochetes were demonstrated in tissue.

Except for mild mononuclear infiltration in cortex or medulla in rabbits W58, W62 and W63, there were no microscopic alterations. Spirochetes were not observed in sectioned kidneys. Microscopic pathologic alterations were not present in the cat kidneys. All other isolation attempts from May and June (1965) trappings were negative.

Discussion

In addition to using isolation and identification techniques, other laboratory methods were used to determine evidence of past or present leptospiral infection in the wildlife collected.

Clinical

Although acute clinical illness was observed in 3 of 6 mammals from which leptospires were isolated, signs of clinical disease were difficult to detect in captured wild mammals.

Darkfield Microscopic Examination

In 3 acutely ill mammals, leptospires were observed in kidneys from which isolations were made. Two mammals, opossum W60, and raccoon W70 were found positive by direct darkfield examination of kidney tissue only. This observation was either a false positive or the organism failed to propagate. False negatives are indicated in 4 animals from which leptospires were isolated. The false negatives were probably due to very few leptospires being present and the inability to detect by direct microscopic examination.

Cultural Isolations

Leptospires were isolated in artificial culture media inoculated with kidney tissue suspensions from 7 of 75 (9%) of the mammals (Table I, III). Attempted urine isolations failed from 3 of 3 mammals from which kidney isolations were made. In opossum W4 and raccoon W6

leptospires were isolated by inoculation of kidney tissue into laboratory animals as well as cultural media.

In raccoons W6 and W8, and opossum W4, all clinically ill mammals, leptospires were observed one week after inoculation in culture media. Gross pathologic lesions of the kidneys and large numbers of leptospires were observed on darkfield examination of kidney suspensions and on Warthin-Starry stained slides. The raccoon W6, W8, and W24 *grippotyphosa* isolates were made from juvenile animals. The opossum W4, W34 *ballum* isolates were adult and W56 was juvenile. Slower growth correlated with fewer other positive diagnostic tests. (Table II). In the mouse (W10 *grippotyphosa* isolate), no other evidence of leptospirosis infection was observed which could indicate an inapparent infection. In mammals other than the mouse there was an association between leptospiral isolation and other positive diagnostic tests.

Unfortunately, cultured procedures do not as yet provide a rapid diagnostic test.

Serology

The screening procedure (macroscopic slide agglutination) was found more sensitive test for the squirrel and opossum sera, while the MA test was found more sensitive for the fox and raccoon sera. However, the stage of infection may have influenced these tests. Blood serums for serologic testing were not available from 15 mice and two muskrats found dead in the traps. Evidence of infection

or previous infection (positive serologic tests) was found in one rabbit, two squirrels and two gray fox.

Positive serologic tests found in 5 raccoons and 3 opossums, coincided with other positive diagnostic tests including the isolation of leptospire. In the raccoons, all 5 sera exhibited MA titers against *grippityphosa* and 2 of 5 had macroscopic slide agglutination positives in one or more pools. Evidence of negative serology, macroscopic slide agglutination and/or MA with other positive diagnostic evaluation, (clinical illness, isolation, presence of lesions) were found. An opossum W34 with negative serology had microscopic kidney lesions and an isolation was made from it. Raccoon W24, (positive MA test) had kidney lesions with a positive isolation. Opossum W34 indicates that the screening macroscopic slide agglutination and MA tests used in combination or separately may not detect infection or a carrier mammal. Results of tests in opossum W34 may indicate a false negative serologic reaction or that serologic procedures used were not adequate to detect antibodies. The opossum was not observed clinically ill, but had kidney lesions and exhibited leptospire.

In limited studies the macroscopic agglutination slide test was found more reliable in detecting *ballum* in opossums, and MA test was more accurate in detecting *grippityphosa* in raccoons. A combination of macroscopic slide agglutination and the MA test methods was necessary to detect leptospiral infections. Serologic testing did not detect evidence of leptospiral infection in one opossum.

According to McKeever, et al.,¹⁰ disagreement between bacteriological and serological tests for leptospirosis may be attributed to one or more of five factors: (a) testing the animal after infection but prior to antibody formation; (b) testing after the disease has terminated but with antibodies persisting; (c) insufficient reaction between antigens being used and antibodies of other serotypes of leptospirosis; (d) antigen — antibody reaction not being the same for all species of hosts; and (e) missing the organism by bacteriological tests, even though it is present.

Pathology

Since independent pathologic evaluation of kidneys was performed before correlation with other biologic tests, opportunity was provided to assess the specificity of renal lesion in leptospirosis. Is there a pathognomonic lesion for leptospirosis in the kidney? Reference to Tables I and II shows that a significant lesion was found 9 times — once in a muskrat in the absence of other positive biologic tests, 6 times (3 raccoons and 3 opossums) associated with leptospire isolations, and twice in a raccoon and an opossum with positive serology only. Pathologic lesions were found in 6 of 7 animals (86%) from which isolations were made (Table III). These findings would indicate that there is not a definitive pathognomonic microscopic renal lesion, but that a highly suggestive lesion consists of a combination of features: (1) patchy glomerular sclerosis, (2) tubular epithelial degeneration and sometimes regeneration, and (3) interstitial fibrosis with moderately intense cellular infiltration (Fig. 2). The infiltrate was often predominantly mononuclear, but granulocytes were numerous in some animals. Combined alterations were found in association with each other, not spatially remote. Hemorrhagic foci were not a feature. In raccoons W4, W6 and W8 the combined alterations produced gross lesions consisting of cortical radiating gray-white streaks. In view of the 8 animals in which other biologic tests were positive among 9 with significant renal lesions, leptospirosis was associated with nephritis among the animals tested. However, other causes of nephritis were not eliminated. The lesion was absent in only mouse W10 from which isolation was obtained.

Most of the facets of the renal lesion when encountered individually were not found to be significant. Focal mononuclear cell infiltration was noted in 27 mammals but was not considered significant unless associated with glomerular and tubular changes in addition to interstitial fibrosis. Focal glomerular atrophy alone was not important in suggesting the presence of leptospirosis in minimal and focal interstitial fibrosis alone was not significant. Tubular epithelial degen-

eration and regeneration was present only in the nine instances of significant lesions; it was not encountered as an isolated finding.

Identification of leptospires in kidney tissue (Warthin-Starry stain) was found in 4 of 7 mammals from which leptospires were cultured and which had significant renal lesions; 3 of 4 were observed clinically ill and had leptospires identified on darkfield microscopic examination.

Ecology

In the United States, wildlife leptospirosis has been found widely distributed.^{12,13,15,16,18,19} The ecology of leptospirosis by definitive identification in 7 wild mammals and the positive serologic results found in turtles, fish, and the unidentified leptospiral isolate from frogs indicate that a very complex ecological common habitat exists in the distribution of leptospirosis. In the fall of 1966, serotype *grippityphosa* was also isolated from a fox squirrel collected from a nearby hunting site where a man developed leptospirosis following squirrel hunting.⁴ In Iowa, three other human cases of leptospirosis were associated with squirrel hunting.

Serotype *ballum* had been previously isolated from mice (*M. musculus*) collected from an Iowa farm with leptospirosis in the cattle.

The ecologic role of wildlife in the transmission of leptospirosis such as occurred in the area of a human outbreak is difficult to define. No evidence of serotype *ballum* and *grippityphosa* were identified in the water environment utilized by man and animals. Environmental and water isolation studies have been previously reported.^{3,7} Saphrophytic leptospires were isolated from the swimming site of the stream during each attempt, beginning June 23, 1965 through November 11, 1966. The pH of the stream ranged from 6.9 - 8.7 and soil pH ranged from 7.0 - 8.6. This pH range appears adequate for leptospiral maintenance.

Opossums which were found infected with *ballum* may be found along stream edges and in shallow woods, and occa-

sionally in the farmyard in search of food. Opossums feed on mice, lower forms of life, insects and eggs. Opossum feces were found on leaning limbs over the stream and signs were in the area of the swimming sites. These mammals may range several miles in search of food.

Raccoons which were found infected with *grippityphosa* may be found along the stream and in the timber adjacent to farm lots and buildings. They enter barns in search of food such as corn. Their main activity is along streams where they feed on fish, crayfish and insects. Feces and other signs were observed on trees hanging over the stream, in tree crotches and on logs near the swimming sites. Raccoons often follow streams and range up to 2 or 3 miles. Reilly et al.¹⁷ were able to experimentally infect the skunk, opossum and fox, but not the raccoon by placing *grippityphosa* in the duodenum via enteric coated capsules.

It is likely that both raccoons and opossums have contact with cattle and swine, both in pastures and farm lots and via the creek water. Much of the land along the creek was utilized for pasture. Potential transmission of leptospirosis between species is possible.

Grippityphosa infection was documented in 1 mouse collected. Inter-species transmission of leptospirosis is possible in the ecologic habitat where animal species were found to be infected. In an 1964 Illinois farm study,¹³ *ballum* was the most widespread serotype found on the farm. Infected rodents may have caused some degree of cross infection between cattle and wild mammals. Serotype *grippityphosa* was isolated in both wild mammals and cattle.

The isolation of leptospires from frog kidney tissues and the serologic evidence of leptospirosis found in fish and turtles collected from the area, indicates that the lower forms of life may be involved in the ecology of leptospirosis. In the laboratory, the frog isolate was continually propagated by Ellinghausen¹⁰ at 31.5, 29, 20, 15, and 9C, indicating growth at a wide temperature range.

Man's direct exposure to opossums, raccoons, other mammals and lower

forms of life is through hunting or trapping, or indirect by contact with a common environment. In 1964-1965 report, the Iowa State Conservation Commission indicated that 64,936 raccoon and 2,600 opossum pelts were collected. A postcard survey of hunters indicated that 268,560 raccoon were killed by 27,975 hunters, with a 9.6 raccoon average per hunter that season. There is no

data available on how many raccoons were used for human consumption.

With animals infected with leptospiruses and the proper chain of ecological circumstances, a disease transmission potential to susceptible new hosts will continue to exist. Only by thoroughly understanding the complex ecosystems can we develop a proper understanding of how to prevent leptospirosis in man and animals.

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