EXPERIMENTAL LEPTOSPIRA POMONA INFECTIONS IN DEER

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ABSTRACT

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by James Alton Ray

Leptospirosis is a major disease problem in domestic animals and is of importance as a disease of man. Wild animals have also been established as hosts for the disease and have many times been incriminated as the source of infection for both domestic animals and man. Serum agglutinins against Leptospira pomona have been demonstrated in the sera of deer in several states in the United States. In order to learn more about L. pomona infection in deer, this study was undertaken.

Six apparently normal female deer (Odocoileus virginianus) were infected with L. pomona strain Ohio by subcutaneous inoculation with infected guinea pig blood. The animals were studied to determine the clinical signs, the antibody response both in the serum and urine, the duration of leptospiruria, the existence of leptospirae in body tissues and fluids, and the gross and microscopic pathology of the disease.

The only clinical sign observed was a slight depression during the acute phase of the disease. The infection did not cause abortions in these animals.

Maximum serum antibody titers of 10^7 through 10^{10} were found. These titers gradually declined until postinoculation day 319, when they ranged from 10^2 through 10^4 in the surviving animals. Urine antibody levels were considerably lower than those of the serum. The maximum titer demonstrated was 10^4, which was found in one animal on postinoculation day 56.

The duration of leptospiruria varied considerably from animal to animal, ranging from 27 to 56 days following inoculation.

Leptospirae were demonstrated in the blood during the acute phase of the disease and from the kidneys of the one animal sacrificed during the urinary shedding phase of the disease.

Pathologic changes were limited to the kidneys and were less extensive than those found in other species infected with L. pomona. The microscopic alterations were occasional areas of leukocytic infiltration, primarily lymphocytes. These were located both periglomerularly and in the interstitial tissue surrounding the tubules. Varying degrees of tubular degeneration were also found, mainly in the proximal convoluted tubules.

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by

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INTRODUCTION

Because of its importance in veterinary medicine and public health, leptospirosis has been studied extensively during the last few years. Morse (1955) estimated the losses in the livestock industry due to this disease at more than $112,000,000 annually. Other reports establish its veterinary significance and high incidence in animals (York, 1951; Reinhard, 1953; Galton et al., 1958).

Leptospirosis is also a frequent disease of man throughout the world (Buckland and Stuart, 1945; Kirschner, 1954; Dvoskin and Hook, 1956; Seiler et al., 1956; Murphy and Alexander, 1957; Jellison et al., 1958; Hale and Cathey, 1958). Since it is a zoonosis (Gsell, 1952; Steele, 1960) and transmission from man to man occurs infrequently if ever, the occurrence of this disease in the livestock population is also significant from the public health standpoint. Galton (1959) reported a study on the source of 146 cases of human leptospirosis. Her findings were as follows:

1) 56 (38 per cent) had contact with infected cattle or swine.
2) 39 (26 per cent) had contact with presumably infected water.
3) 21 (14 per cent) had contact with dogs.
4) 19 (13 per cent) had contact with rats.
5) 6 (4 per cent) had contact with wild animals.
6) 5 (3 per cent) had contact with other animals or possibly contaminated environment.

Epidemiologically, rats, dogs and swine have for years been considered the primary animal carriers of leptospirosis.

Recent studies have shown various species of wildlife frequently to be infected. In many cases they have been directly incriminated as the source of human and domestic animal infections.

Deer have been suspected of contributing to the spread of leptospirosis. Various workers have found serologic evidence of leptospirosis in deer in the United States. More specifically, Fay and Youatt (1958) demonstrated antibody titers of 1:100 or higher for Leptospira pomona in 20 per cent of randomly selected deer sera. Since leptospirosis often causes abortions in domestic species, it was postulated that the disease could adversely affect reproduction in deer herds. In order to obtain information regarding the effect of leptospirosis in deer and the potential epidemiologic importance of the infection in this species, a cooperative study between the Michigan Department of Conservation and Michigan State University was undertaken. In this study six presumably pregnant does were artificially infected with L. pomona (Ohio). Studies were made of clinical signs, antibody content of both the urine and serum, duration of leptospiruria, existence of leptospirae in the body tissues and fluids and gross and microscopic pathology of the disease.

LITERATURE REVIEW

The first recorded observation of pathogenic leptospirae was that of Stimson (1907) working in New Orleans. He observed the organisms in human kidney sections which were stained by Levadidi's silver impregnation method. His descriptions of the organisms closely fit those of leptospirae. Stimson named the organisms Spirochaeta interrogans and thought they were the cause of yellow fever since yellow fever had been diagnosed as the cause of the patient's death. When his sections were reviewed by Sellards (1940), the photomicrographs showed organisms which are undoubtedly leptospirae. The first isolation of leptospirae from a human in the United States was reported by Wadsworth et al. (1922). This infection was due to the accidental inoculation of a laboratory worker.

Inada and Ido (1915) working in Japan, Hubener Reiter (1915) and Uhlenhuth and Fromme (1915) working independently in Germany are credited with the first isolations of pathogenic leptospirae from cases of Weil's disease. Noguchi (1917) described the morphology and studied the immunologic and serologic properties of strains of leptospirae isolated in Belgium, America, and Japan. He concluded that the strains were identical and named the organisms Leptospira icteroides. Noguchi (1919) isolated L. icteroides from a man suffering from "yellow fever" and also concluded that it was the cause of this disease.

During the next 20 years, serotypes of leptospirae were

isolated in various areas of the world. Most significant to the study presented here was the isolation by Clayton et al. (1937) of a leptospiral species from the blood of a man suffering with an acute febrile condition. Serologic comparison of this strain to others isolated throughout the world proved that it was antigenically distinct (Lumley, 1937). Derrick (1942) suggested the name L. pomona for this organism, since it was initially recovered from a man in Pomona township, Queensland, Australia.

Until 1944 only L. icterohaemorrhagiae and L. canicola were thought to be present in North America. The only animals known to be infected on this continent were man, rat and dog (Reinhard, 1953). Junherr (1944), using silver impregnation techniques, observed leptospirae in sections of kidney, liver, and mesenteric lymph nodes of an ox. Leptospirae had previously been reported only once in the bovine. This report was made by the Russian workers, Muchin and Azinov (1935).

Baker and Little (1946) studied an atypical mastitis in cattle. After considerable study, including guinea pig infections and neutralization studies with convalescent sera, they decided that the condition was caused by a virus. Later (Baker and Little, 1948), when studying the same condition in other cattle, they found a leptospiral species to be the etiologic agent. Gochenour et al. (1950) found this strain to be serologically indistinguishable from L. pomona. They thereby became the first to identify L. pomona as an

infecting serotype in the United States.

Since 1950, naturally occurring L. pomona infections have been reported in the United States in the porcine (Gouchenour et al., 1952; Bryan et al., 1953; Baker, 1954; Bohl et al., 1954; Ferguson et al., 1955), ovine (Beamer et al., 1953), equine (Roberts, 1958a) and canine (Murphy et al., 1958; Morter et al., 1959), L. pomona is considered to be the major infecting leptospiral serotype of the bovine, porcine, ovine and equine in the United States.

Until 1953, rats, dogs (Steel, 1960) and pigs (Gsell, 1952; Morse and Langham 1958) were considered the primary animal carriers of leptospirosis. Yager et al. (1953), while investigating an enzootic area of bovine leptospirosis due to L. pomona, isolated L. ballum from one of two opossums and nine of 27 house mice (Mus Musculus) trapped in Virginia. These findings stimulated a renewed interest in the role of wild animal species other than rats as carriers of pathogenic leptospirae.

Van der Hoeden (1957) reported a vole (Microtus arvalis) to be the main natural reservoir for L. grippotyphosa in Israel. Previously cattle were incriminated in the role. He pointed out that the human case incidence rose when the vole population increased. He also isolated L. ballum from voles and reported the jackal to be a natural host for L. canicola in Israel.

In studies of leptospirosis in Denmark, Borg-Peterson and Fennestad (1956) isolated L. pomona from 3 of 14 field

mice (Adonemus agararius). This rodent is found only on Lolland Island and Falster Island. L. pomona agglutinins were found in 13 of 200 cattle sera and 5 of 138 swine sera from the animals of Lolland-Falster Islands, while none were found in sera from other parts of Denmark. They concluded that this incriminated this mouse as the principle carrier of L. pomona in Denmark.

In a two-year study of leptospirosis in rodents completed in 1955, Brown and Gorman (1960) reported L. ballum to frequently infect the house mouse (Mus muculus) in southwestern Georgia. In the same area this serotype was isolated occasionally from the roof rat (Rattus rattus), cotton rat (Sigmodon hispidis), and the field mouse (Peromyscus polionotis). No other serotype of leptospira was found.

Galton et al. (1956) isolated L. grippotyphosa from racoons. These animals were trapped within 50 miles of a Florida farm on which the cattle had serum agglutinins for L. grippotyphosa.

From New Zealand, Webster (1957) reported the natural infection of the hedgehog (Erinaceus europaeus) with L. pomona. These animals were trapped on farms where leptospirosis had been diagnosed in cattle. He experimentally infected hedgehogs with the L. pomona strain isolated from this species and produced classical signs of leptospirosis. Death was observed in the young and half-grown animals, while pregnant animals aborted. Infected animals developed high serum titers, and leptospiruria was demonstrated on the 16th to 18th

day of infection.

Van der Hoeden (1957) reported natural infections in hedgehogs in Israel. Serologic evidence of leptospirosis was found in 21 (33.3 per cent) of 63 animals tested. L. canicola was isolated nine times from ten animals serologically positive for this serotype. He also isolated L. grippotyphosa once from five L. grippotyphosa serologically positive animals. He reported the isolation of L. canicola and L. grippotyphosa from goats. He found a significant serum titer for L. sejroe in 1 of 59 bats (Rossetus aegytiacus).

From a study of various wild animal species, Galton et al. (1957) reported the isolation of two leptospiral serotypes new to the United States. They isolated leptospirae of the L. mitis-hyos serogroup from four opossums, and L. australis from two raccoons. Similarly, Roth and Knieriem (1958) reported finding natural L. pomona infection of one opossum. This animal was trapped in Louisiana.

Leptospiral isolations from wild animal species in the United States were again reported by McKeever et al. (1958). In the six month study ending March, 1956, kidneys from 846 wild animals (14 species) trapped in southwestern Georgia were studied bacteriologically. The incidence of infection was 5.4 per cent. L. pomona was isolated once from raccoons (Procyon lotor), ten times from striped skunks (Mephitis mephitis), and two times from wildcats (Felis rafa). The incidence of leptospiral infection and the serotypes of the strains isolated from the various wild animal species studied were as follows:

1) Opossum 6.1 per cent; L. ballum, L. mitis-hyos group

2) Gray Fox 3.9 per cent; L. ballum*

3) Raccoon 3.8 per cent; L. ballum*, L. pomona*, L. australis A, L. grippotyphosa*, L. hebdomadis*

4) Striped skunk 13.6 per cent; L. pomona*, L. ballum*

5) Wildcat 7.5 per cent; L. pomona*, L. ballum*

*New host records.

Tissues from the cottontail rabbit, marsh rabbit, fox squirrel, red squirrel, feral dog, spotted skunk, otter and feral house cat were negative when cultured.

In a survey of leptospirosis, the Ohio Department of Agriculture (1958) found serum agglutinins for L. pomona in Ohio wildlife. The agglutinins were found in the sera of 43 (19 per cent) of 224 deer, 16 (22 per cent) of 70 raccoons, 14 (25 per cent) of 55 foxes, and 6 (54 per cent) of 11 skunks.

In contrast to these studies, Menges and Galton (1958) reported only six serologic indications of leptospiral infection and no isolations from 131 wild animals studied in Oregon. Significant titers for L. sejroe were found in 12 per cent of 173 cattle studied in this area.

Baduieri (1958) reported the findings of studies of wild birds in northern Italy. Leptospirae were observed in the tissues of 12 of "hundreds" of wading birds obtained from Italian rice fields. He was able to isolate L. bataviae from these tissues five times. These birds migrate from northern Italy to central Africa where L. bataviae infections have

also been found. He experimentally infected these wild birds with L. pomona, L. icterohaemorrhagiae, and L. bataviae and was able to reisolate the organisms for ten days from the blood and after 26 days from the feces. He was able to reisolate leptospirae from the feces of artificially infected domestic ducks for 38 days. The serum antibody titers produced by these birds were low and were present for a short period of time, sometimes shorter than the period of organism shedding.

Chalquest (1937) induced serum antibody production in chickens, Hungarian partridges, Pekin ducks, and ringneck pheasants with L. pomona by contaminating their drinking water with urine from a cow shedding leptospirae. He was unable to isolate the organism from the tissues of these birds or their excreta by injecting emulsions of tissue or feces intraperitoneally into two week old chicks.

Gillespie et al. (1957) were able to detect serum antibodies for L. pomona in chickens. These chickens were obtained from a ranch where L. pomona had been isolated from cattle.

L. hyos was isolated by Ferris et al. (1959) from a deer mouse. Agglutinins for L. grippotyposa were found in the sera of cattle from the same area.

Reports of leptospirosis in deer have been few in number and limited to serologic studies. This is probably due to the difficulties encountered in obtaining fresh urine or kidney tissue from these animals. Experimental infections

are also difficult to study due to the wild nature of this species; the animals often injure themselves during restraint and confinement.

Wedman and Driver (1957) reported finding serum agglutinins for L. pomona in deer in Minnesota. They found an incidence of 16 per cent in 187 samples tested.

In a study of Illinois deer, Ferris et al. (1958) reported finding serum agglutinins for L. pomona in 10.2 per cent of 343 samples tested. They also found titers for L. grippotyphosa in 9.8 per cent of the samples.

As indicated previously the Ohio Department of Agriculture (1958) reported finding serum antibodies for leptospirae in deer. Using an L. pomona antigen, they demonstrated antibodies in 43 (19 per cent) of 224 serum samples tested.

The incidence of L. pomona antibodies found in the sera of Ohio deer is similar to that found in Michigan deer by Fay and Youatt (1958). In a survey of deer killed in 1957 and 1958 they found that 20 per cent (558) of the sera showed agglutination-lysis reactions at dilutions of 1:100 or higher.

In contrast to these reports, Richardson (1958) stated that Delaware workers had failed to demonstrate evidence of leptospirosis in the deer of that state. In a survey of southwestern deer, Shotts et al. (1958) found significant titers in only 1.73 per cent of 403 sera processed. Reynolds and Smith (1958) failed to demonstrate L. pomona antibodies in deer from Massachusetts. It should be noted that Shotts et al. and Reynolds and Smith used the Stoener plate test

and the Stoener capillary tube test respectively. These tests are considered less sensitive than the agglutination-lysis test.

MATERIALS AND METHODS

Experimental animals. Eight apparently normal, adult, deer (Odocoileus virginianus) served as the experimental animals for this study. Six of the animals, females bearing Game Division ear tag numbers 129, 132, 147, 160, 853 and 864, were obtained from the deer herd at the Houghton Lake Wildlife Experiment Station. These animals had been naturally bred and were assumed to be pregnant. The seventh animal, number 870, was a nonpregnant doe that had been held at the Rose Lake Wildlife Experiment Station for two years. The eighth animal, a male number 37101, served as a negative control. All animals were negative by serologic tests for L. pomona (table 4). During this study, the animals were maintained by the Department of Conservation at the Rose Lake Wildlife Experiment Station in pens constructed for the confinement of wild animals. These pens were made of eight-foot wire fence and were approximately 20 feet wide and 40 feet long. All animals were kept in individual pens excepting animals number 147 and 870, which shared a pen 40 feet by 40 feet in size. Each pen had a small shed which provided shelter. The animals were fed a ration of commercial deer pellets and shelled corn supplemented with hay.

Inoculum. Leptospira pomona, strain Ohio, was used to infect does number 129, 132, 147, 160, 853 and 864 while animal 870 was not infected. The leptospiral strain used was isolated from hog urine at the Ohio Agricultural Experiment Station in 1956. Following isolation, it was maintained by

transfer every six months in Chang's semi-solid medium (Chang, 1947) containing 15 per cent sterile, normal rabbit serum. Before the deer inoculations were made, the strain was passed four times in guinea-pigs by the intraperitoneal injection of guinea pig blood obtained at the height of febrile response (105-106F).

Each doe inoculated received 4 ml of heparinized infected guinea pig blood subcutaneously. Each ml of the inoculum contained approximately 5×10^4 guinea pig infective doses ($I.D._{50}$) as determined by the intraperitoneal inoculation of guinea pigs with 10 fold dilutions of the blood in saline. Guinea pigs were considered infected if they developed antibody titers of 10^3 or higher to L. pomona, strain Johnson, within 18 to 21 days following inoculation. Calculations were by the method of Reed and Muench as presented by Cunningham (1956).

Collection of specimens. Blood samples were taken from the external jugular vein, using a California bleeding needle with an attached vial. Urine samples were obtained by catheterization with sterile, stainless steel female canine catheters lubricated with sterile vaseline. A human vaginal speculum was used to retract the vaginal wall and expose the external urethral orifice.

Animal restraint was difficult and required the assistance of five biologists of the Rose Lake Station. Each animal was caught with a large net and manually restrained in lateral recumbancy whenever injections were made or laboratory specimens taken.

To establish the existence of leptospiremia, blood was collected from infected animals on postinoculation days four, six, eight and nine for bacteriologic studies. Serum for serologic studies was also collected on the same days. Serum and urine samples were collected from surviving, inoculated animals for the determination of antibody titers and leptospiruria on days 15, 21, 27, 32, 39, 49, 56, 67, 81 and 95 following inoculation. Serum samples for serologic studies were collected from animal 870 (uninfected penmate) at the same time. No samples were taken between day 95 and day 230 because of possible heat exhaustion of the deer due to restraint in the summer months. Since leptospirae were not present in the urine samples obtained at day 95, only serum was collected at day 230. Serum samples were obtained from all experimental animals at death. Samples for bacteriologic studies were obtained from animals number 129, 160, 853 and 864 when they were sacrificed. Sections for histopathologic examination were saved from animals number 129, 132, 160, 853, 864, 870 and 37101 during necropsy.

Clinical studies. The infected animals were observed for clinical signs of illness such as anorexia, depression, icterus, fever, or lameness. Rectal temperatures were recorded when specimens were obtained until 56 days following inoculation.

Bacteriologic studies. Intraperitoneal inoculation of guinea pigs was used to determine the presence of leptospirae in blood, urine and tissue emulsions. Within five minutes of

the time the samples were obtained, two or three 150-250 gram guinea pigs were inoculated with 0.5- 3 ml urine or blood. To determine the presence of leptospirae in tissues 3 ml of a 10 per cent emulsion of the tissues in sterile saline was injected intraperitoneally into guinea pigs. Leptospirae were considered to be present if the guinea pig serum agglutinated and/or lysed L. pomona (Johnson) at dilutions of 1:1000 or higher 18-21 days following inoculation.

Isolation of leptospirae from blood of each animal was also attempted by inoculating five tubes each containing 10 ml of Stuarts medium (Difco) with 10 per cent normal rabbit serum added. Each tube was inoculated with 3-5 drops of heparinized blood. The tubes of medium were incubated at 30C for 30 days or until growth was detected by the observation of leptospirae at 540x using dark field microscopy.

Antibody detection. A modified agglutination-lysis test was used throughout this study to detect serum and urine antibodies. Living 7-14 day cultures of L. pomona, strain Johnson, grown in Stuarts medium with 10 per cent sterile, normal rabbit serum added, were used as antigen. Starting with serum or urine dilution of 1:5, ten fold serial dilutions were made with sterile saline in Kahn tubes. Two-tenths ml of each serum or urine dilution was then mixed with 0.2 ml of antigen resulting in final ten fold dilutions starting with 1:10. The mixtures were then incubated in a thermostatically controlled water bath for two hours at 37C. Agglutination and/or lysis of the leptospirae was determined microscopic-

cally at 100x magnification using a microscope equipped with an Abbe condenser which was fitted with a dark field stop. The end point was the highest dilution showing agglutination and/or lysis of 50 per cent or more of the leptospirae. Titers are expressed as the number of reacting units per ml of serum or urine.

Pathologic studies. A necropsy was conducted on each experimental animal. Animal number 870 and number 37101 were uninfected and served as controls.

Immediately prior to sacrifice, the animals were anesthetized with pentobarbital sodium injected intravenously. They were exsanguinated by cutting the external jugular vein.

Animals number 129, 160, 864 and 870 were sacrificed at postinoculation day 319. Animals number 132 and 853 were sacrificed at postinoculation days 117 and 22 respectively because of injuries sustained while being caught prior to restraint. Animal 147 died on day 313 of unknown causes. Tissues were not saved from this animal for further studies because of post-mortem decomposition.

Sections of liver, kidneys, adrenals, spleen, brain and cervical spinal cord from animals 129, 160 and 864 were saved for histopathologic examination. In addition, renal lymph nodes were saved from animals 160 and 864. Brain, kidney, liver, spleen and cotyledons were saved from animal 853 and liver, kidney and spleen were saved from her fetus. Only kidneys and cotyledons were saved from animal 132. Specimens for histopathologic examination were preserved in 10 per cent neu-

tral formalin-saline solution, Carnoy's fluid, or Zenker's fluid. Tissues for pathologic examination were embedded in paraffin and cut at a thickness of 6-8 microns. Stains utilized were hemotoxylin and eosin for general histologic structure or Prussian blue for iron pigments.

Clinical findings. Clinical manifestations of the disease were not pronounced even during its acute (leptospiremic) phase. No anorexia, icterus or lameness was noticed. A slight depression was observed when samples were obtained during leptospiremia.

The study of rectal temperatures obtained was inconclusive. Due to the activities of restraint, temperatures as high as 108.8 F were recorded. For this reason the body temperatures are not presented.

No abortions were observed, however, only three of the animals were pregnant. Does number 129 and 147 gave birth to normal fawns with number 129 fawning twins. Number 853 was pregnant with a living, apparently normal fetus when she was sacrificed at day 22.

Bacteriologic findings. Leptospirae were demonstrated bacteriologically in the blood of all infected animals during the acute phase of the disease. The results presented in table 1 indicate that leptospiremia occurred in all animals on postinoculation days four, six and eight. On day nine leptospirae were present in the blood of all animals except animal number 132.

The data concerning leptospiruria are presented in table 2. It should be noted that the duration of leptospiruria varied considerably from animal to animal. Leptospirae were demonstrated in the urine of animals 129 and 147 until day 56. Positive results were obtained from the urine of

TABLE 1. The duration of leptospiremia in deer artificially infected with Leptospira pomona as determined by inoculation of guinea pigs and artificial medium.

Deer Number	Days Following Inoculation of Deer						
	Four		Six		Eight		Nine
	Artificial Medium	Guinea Pigs	Artificial Medium	Guinea Pigs	Artificial Medium	Guinea Pigs	Artificial Medium Only
129	+[1]	+[2]	+	+	+	+	+
132	+	+	+	+	+	+	-
147	+	+	+	+	+	+	+
160	+	+	+	+	+	+	+
853	-	+	+	+	+	+	+
864	-	+	+	+	+	+	+

1. Leptospirae observed microscopically following incubation at 30C.

2. Serologic evidence of L. pomona infection in 18-21 days.

TABLE 2. The duration of leptospiruria in deer artificially infected with Leptospira pomona as determined by the inoculation of guinea pigs.

Days Following Inoculation	Deer Number 129	132	147	160	853	864
15	+[1]	-	Q.N.S.[2]	-	+	+
21	+	+	+	+	+	+
27	+	+	+	+	Animal Sacrificed	+
32	+	+	+	Q.N.S.		-
39	+	-	+	+		-
49	+	-	+	+		-
56	+	-	+	Q.N.S.		-
67	-	-	-	-		Q.N.S.
81	-	-	-	Q.N.S.		-
95	-		-			

1. Serologic evidence of L. pomona infection of guinea pig in 18-21 days.

2. Quantity not sufficient for animal inoculations.

animal 160 on day 49, but an insufficient amount of urine for animal inoculation was obtained on day 56. In contrast, leptospiruria was demonstrated only until days 27 and 32 in animals number 864 and 132 respectively. Leptospirae were present in the urine of animal number 853 until day 22 when she was sacrificed.

Table 3 contains the data obtained from studies to determine the presence of leptospirae in samples taken from infected animals when sacrificed. Guinea pig inoculations were made with samples from the kidneys, urine, liver and spleen, cotyledons, amnionic fluid and fetal tissues of animal number 853. This animal was sacrificed 22 days after inoculation. Leptospiruria was still present at day 21 (table 2). Leptospirae were demonstrated only in the kidney tissue. Kidney emulsions from animals 129, 160 and 864 failed to show evidence of leptospirae 319 days after inoculation.

Antibodies detected. The data concerning the antibodies detected in both serum and urine are presented in table 4. Serum samples collected prior to infection contained no antibodies for L. pomona demonstrable by the technicues used.

Positive serologic reactions were not obtained from the serum of any animal during days four, six, eight or nine. Serum samples were next taken on day 15 when serum antibody titers ranging from 10^5 through 10^8 were found. The maximum serum antibody titers ranged from 10^7 through 10^{10}. By day 95, serum antibody levels had gradually fallen to a range of 10^3-10^5. Serum antibody titers of the three infected ani-

TABLE 3. The presence of leptospirae in various tissues and body fluids of deer artificially infected with Leptospira pomona as determined by the inoculation of guinea pigs.

Deer Number	Days Post-inoculation	Specimen					
		Kidney	Urine	Amnionic Fluid	Liver & Spleen	Cotyledon	Fetal Tissues
129	319	-					
160	319	-					
853	22	+[1]	-	-	-	-	-
864	319	-					

1. Serologic evidence of L. pomona infection of guinea pig in 18-21 days.

TABLE 4. Antibodies detected in serum and urine samples from deer artificially infected with Leptospira pomona as determined by the agglutination and/or lysis of living Leptospira pomona.

Days Pre-inoculation	Deer Numbers					
	129		132		147	
	Serum	Urine	Serum	Urine	Serum	Urine
40	-		-		-	
6	-		-		-	
Days Post-inoculation						
0	-		-		-	
4	-		-		-	
6	-		-		-	
8	-		-		-	
9	-		-		-	
15	8[1]	-	7	-	7	Q.N.S.[3]
21	8	-	6	-	5	Q.N.S.
27	10	-	7	-	7	Q.N.S.
32	10	Sl.[2]	8	1	7	Sl.
39	7	Sl.	8	2	6	2
49	7	Sl.	8	2	7	Q.N.S.
56	4	Sl.	8	3	7	4
67	5	Sl.	5	2	7	2
81	5	2	4	2	5	1
95	3		3		4	
117			2			
230	3				3	
319	2					

1. Numbers indicate the logarithm of the reciprocal of the highest dilution in which 50% or more of the antigen leptospirae were agglutinated and/or lysed.
2. Some reaction observed but less than 50% of the antigen leptospirae agglutinated and/or lysed.
3. Quantity not sufficient for tests.

TABLE 4. Cont. Antibodies detected in serum and urine samples from deer artificially infected with Leptospira pomona as determined by the agglutination and/or lysis of living Leptospira pomona.

Days Pre-inoculation	Deer Numbers						
	160		853		864		870
	Serum	Urine	Serum	Urine	Serum	Urine	Serum
40	-		-		-		
6	-		-		-		-
Days Post-inoculation							
0	-		-		-		
4	-		-		-		
6	-		-		-		
8	-		-		2		
9	-		-		-		
15	6[1]	Q.N.S.[3]	8	-	5	-	-
21	7	-	7	-	5	-	-
27	7	-			7	2	-
32	7	Q.N.S.			9	Q.N.S.	-
39	7	2			9	Sl.[2]	-
49	8	Q.N.S.			8	3	-
56	7	Q.N.S.			8	2	-
67	5	2			6	Q.N.S.	-
81	6	Q.N.S.			5	-	-
95	5				5		-
117							
230	4				3		
319	3				4		-

1. Numbers indicate the logarithm of the reciprocal of the highest dilution in which 50% or more of the antigen leptospirae were agglutinated and/or lysed.
2. Some reaction observed but less than 50% of the antigen leptospirae agglutinated and/or lysed.
3. Quantity not sufficient for tests.

mals sacrificed at day 319 were 10^2, 10^3, and 10^4. Deer 870 and 37101 had no serum antibodies for L. pomona.

Urine antibody titers for L. pomona were determined when a sufficient volume of urine was collected. They were low when compared to serum antibody levels. In many instances a 1:10 dilution was too great to cause the agglutination and/or lysis of 50 per cent of the leptospirae in the antigen. The highest titer recorded was 10^4 (animal 147 on day 56). This antibody did not adversely affect the survival of leptospirae in this urine sample under the conditions of this experiment (table 2).

Pathologic findings. Significant lesions were present only in the kidneys. Grossly one or two grayish-white foci 1-7 mm in diameter were found in the renal cortex of animals number 129, 160 and 864 (all infected animals held until day 319). Some of the grayish-white areas extended into the medulla of the kidneys in animals 129 and 160.

In animal number 132, lesions of leptospirosis if present were masked by multiple abscesses. These abscesses were apparently due to pyemia resulting from infected wounds sustained during catching prior to restraint.

Animal 853, sacrificed at day 22 because of posterior paralysis showed no gross lesions attributable to leptospirosis. A fracture of a thoracic vertebra was observed. Since one of the bone fragments was exerting pressure on the spinal cord, this was thought to be the cause of the paralysis. The fracture probably occurred during restraint of the animal on

day 21.

Microscopically, occasional areas of leukocytic infiltration, consisting mainly of lymphocytes were observed in the kidney tissue. These lesions were more numerous than the grayish-white areas observed grossly. Leukocytic infiltrations were periglomerular (figure 1) and in the interstitial tissue (figure 2). Some proximal convoluted tubules in the areas of leukocytic infiltration were degenerate and necrotic. Hyaline casts were present in some of the renal tubules. Iron deposits as shown by Prussian blue stain were also present, particularly in the epithelium of the proximal convoluted tubules (figure 3).

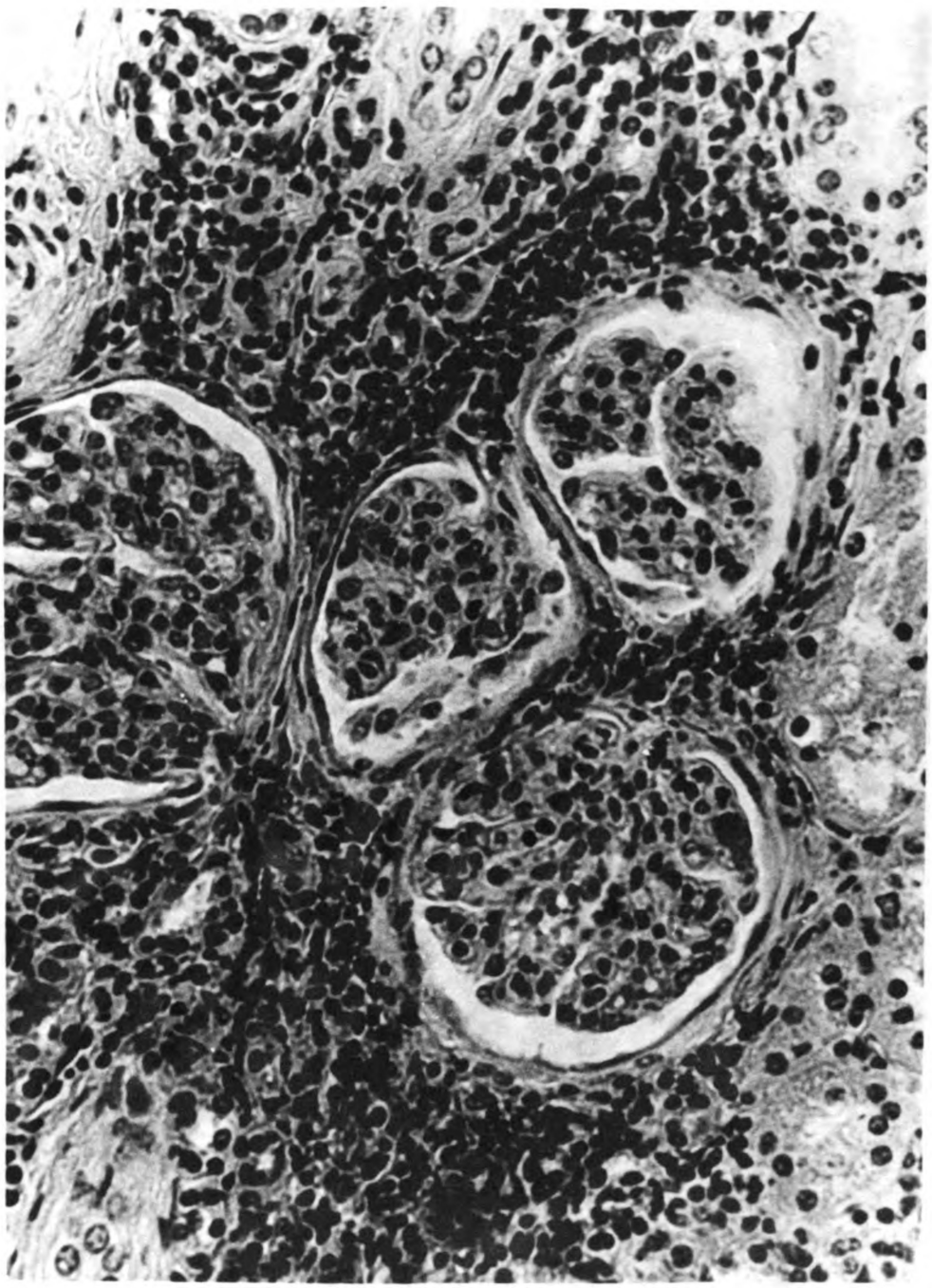

Figure 1. Kidney with periglomerular leukocytic accumulations. Stain hematoxylin and eosin. x 454.

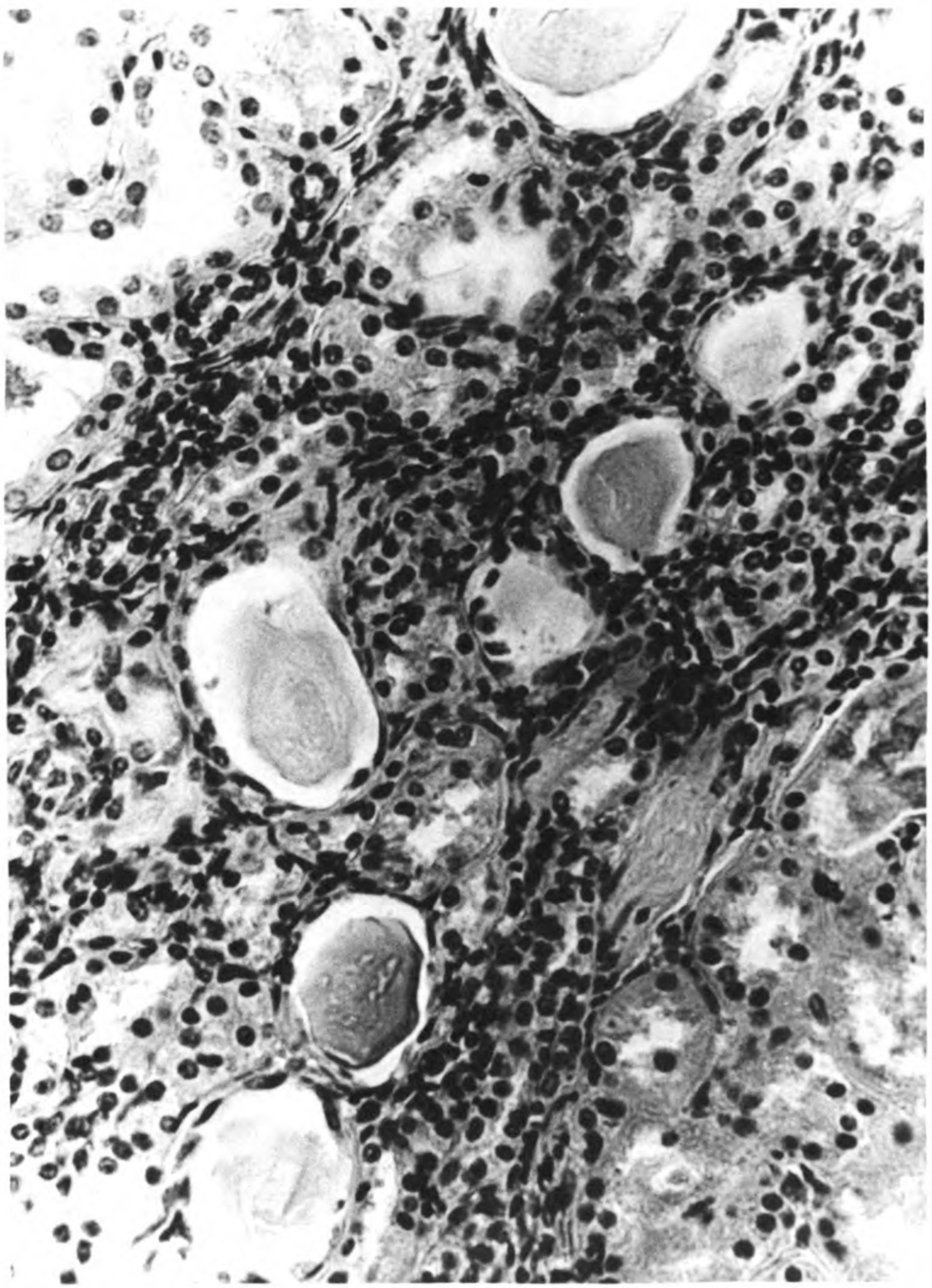

Figure 2. Kidney with leukocytic accumulations in the intertubular areas, degenerate epithelium of the proximal convoluted tubules, and hyaline casts in the lumen of the tubules. Stain hematoxylin and eosin. x 454.

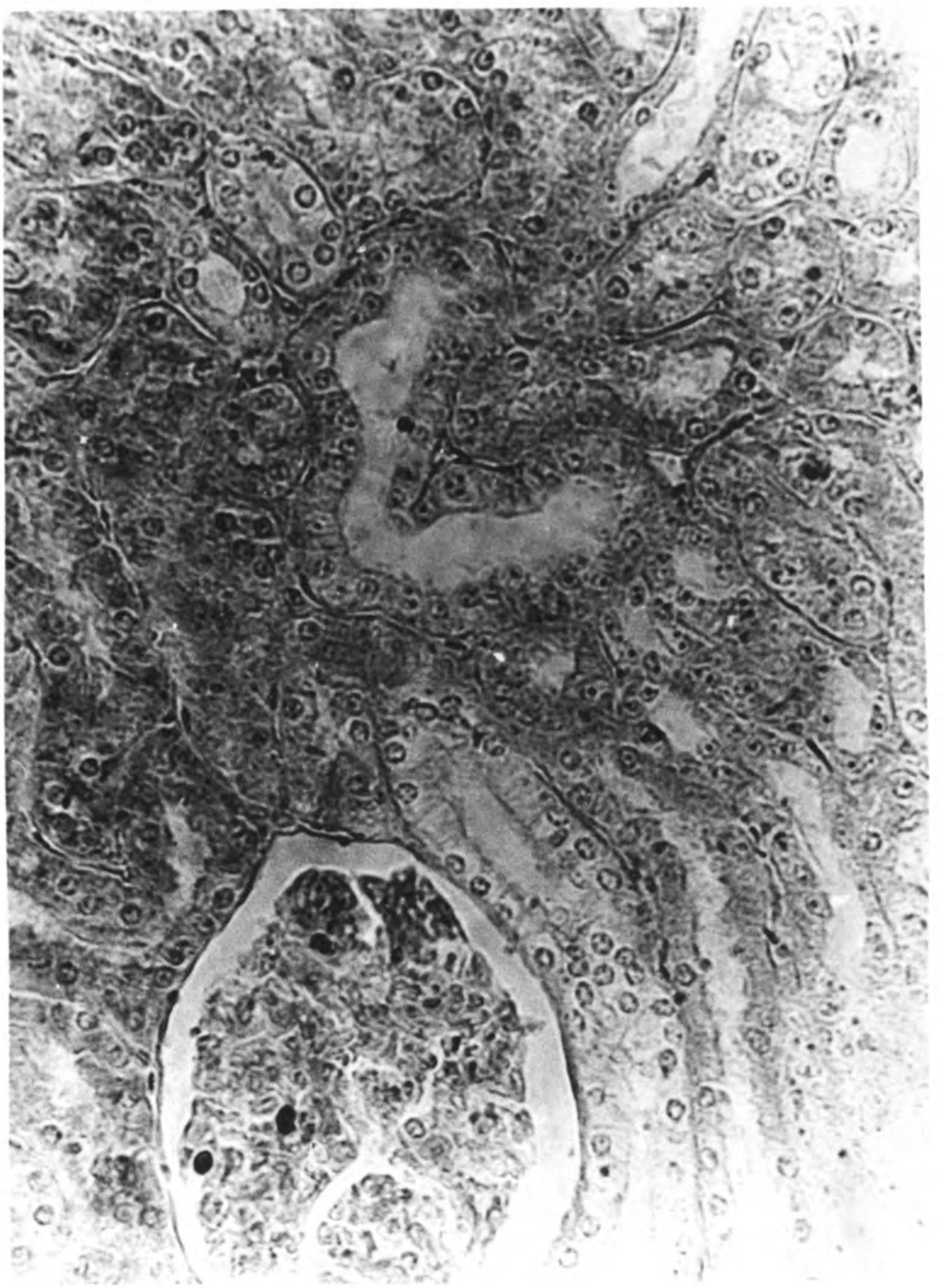

Figure 3. Kidney with iron deposits in the epithelium of the proximal convoluted tubules. Stain Prussian blue. x 454.

DISCUSSION

L. pomona (Ohio) has been proven to be pathogenic for bovine and porcine (Morter and Morse, 1956), ovine (Linquist, 1957), canine (Cholvin, 1958), and caprine (Morse and Langham, 1958). The study reported here establishes the ability of this strain to infect deer, since all animals inoculated developed leptospiremia, leptospiruria, and serum antibodies.

Clinical manifestations of the disease if present were mild. The depression noticed during the acute phase of the disease may have been due to the rigors of repeated restraint. Careful clinical examination of deer is impossible because of the animal's excitability. As pointed out earlier, even the usually simple procedure of determining rectal temperature is difficult and the results are of little value. Severe signs of illness, had they been present, would have been noticed under the conditions of this experiment.

Leptospirosis usually causes abortions in cattle when the infection occurs during the last half of pregnancy (Sippel et al., 1952; Reinhard, 1953; Morse et al., 1955). In contrast to these findings L. pomona infection of deer failed to produce abortion in the test animals. It should be noted, however, that the deer were infected during the first half of gestation and only two pregnant animals were allowed to live until the end of gestation.

Leptospiremia was demonstrated through day nine in five animals and through day eight in the sixth. Bacteremia following artificial L. pomona infection has been reported on

days one through seven in ovine (Lindquist, 1957), and days three through seven in the canine (Cholvin, 1958). Leptospiremia was present slightly longer after inoculation in deer than in the domestic animals studied.

Leptospirae have been cultured frequently from aborted fetuses and the weak newborn of swine (Bryan et al., 1953; Bohl et al., 1954) and occasionally of cattle (Podgwaite et al., 1955; Dacres and Kiesel, 1958). No organisms were demonstrated in the one deer fetus examined.

Leptospirae were demonstrated in the kidney tissue of only one animal (number 853). This animal was sacrificed at postinoculation day 22. Since leptospiruria was demonstrated to be present in this animal on day 21, this finding was not surprising. Failure to find organisms in the other tissues examined compares favorably with the findings in domestic species. Following leptospiremia organisms are usually found in the kidneys for less than six months.

Leptospirosis is commonly spread by the urine of animals during leptospiruria. The hosts usually appear normal during this period. The importance of the animal as a spreader is based upon the duration of shedding and the animal's mobility during this time. Leptospirae have been shown to remain viable in the soil for up to 39 days under proper conditions (Smith and Self, 1955). When contaminated with the organisms, slow moving streams are often incriminated in the spread of leptospirosis. Deer, being unconfined, could easily disseminate the disease over a wide area.

Probably the most important part of this study was that concerning the duration of leptospiruria in deer. Leptospiruria was demonstrated as long as 56 days but less than 67 days following inoculation. Following infection, leptospirae have been found in the urine of the bovine for up to three months (Sutherland et al., 1949), of swine for longer than five months (Baker, 1954) and of sheep up to nine months (usually two-three months) (Webster and Reynolds, 1955). By comparison, the shedding period observed in deer is short. However, because of the freedom of movement, deer could still be important spreaders of leptospirosis.

Leptospiral serum agglutinins were not demonstrated during leptospiremia. Antibody titers rose sharply between the 9th and the 15th days after inoculation. Maximum titers were presented by all animals and ranged between 10^7 and 10^{10}. The antibody levels then fell gradually. Relatively strong reactions (10^2-10^4) were still present 319 days postinoculation. Cattle and horses have been shown to have serum antibodies seven and six years respectively following infection (Roberts, 1958a; Roberts, 1958b). Titers of 10^3-10^4 were found in swine 14 months after artificial infection with L. pomona (Morter et al., 1960). Therefore, as in various domestic species, serum antibodies can be demonstrated long after infection in deer, possibly for life. This is important when evaluating the results of serologic surveys because the presence of serum antibodies does not necessarily indicate current or even recent infection.

The pathologic findings indicate a relatively minor amount of tissue damage. Both gross and microscopic kidney lesions found were similar to those previously found in domestic species (Morter and Morse, 1956; Lindquist, 1957; Cholvin, 1958; Morter et al., 1960). However, the lesions were not as extensive as those found by these workers.

It would appear from the findings of this study that L. pomona infection of deer is of minor importance to this host. This fact does not detract from possible importance of deer as a carrier of this organism.

L. pomona, when used as an antigen, will react with immune rabbit sera of at least seven heterologous serotypes of leptospirae (Wolff, 1952). While L. pomona is considered to be the main serotype infecting domestic animals in the United States, there is no assurance that this serotype is the main cause of leptospirosis in deer. The reactions found in serologic studies of deer sera may be cross reactions. Recent findings indicate that leptospirosis of domestic species may be due to serotypes other than L. pomona (Hale, 1958; Turner et al., 1958; Ferris et al., 1959; Roth and Galton, 1959). Efforts should be made to isolate leptospirae occurring naturally in wildlife. These should be followed by the study of the infection in that host. This information would help determine the importance of wildlife in leptospirosis of animals and man.

SUMMARY

Six female deer (Odocoileus virginianus) were experimentally infected with L. pomona by the subcutaneous route. Clinical, bacteriologic, serologic, gross pathologic and histopathologic studies of the infection were made.

Clinical signs of the disease were not pronounced. The only sign observed was a slight depression during leptospiremia. No abortions were observed.

Bacteriologically, leptospiremia was demonstrated as long as nine days after inoculation. Leptospirae were demonstrated bacteriologically in the urine up to 56 days after inoculation in two animals.

Antibody titers against L. pomona as high as 10^{10} were demonstrated in the serum. Serum antibody titers of 10^{2}-10^{4} were present in three animals on postinoculation day 319. Urine antibody titers were low when compared to those in the serum. However, a urine antibody titer of 10^{4} was demonstrated in one animal 56 days following inoculation.

Gross and microscopic pathologic changes were found only in the kidneys and resembled those found in domestic species infected with L. pomona. They were, however, less extensive in nature. Microscopically, alterations appeared as periglomerular and interstitial leukocytic (primarily lymphocytic) infiltrations with varying degrees of tubular degeneration.

REFERENCES

BABUDIERI, B. 1958 Wild birds as carriers of pathogenic leptospires. A symposium on leptospira and leptospirosis in man and animals held on December 5-7, 1958 at the ministry of health, state institute of rural occupational medicine and rural hygiene, Lublin, Poland. Abstracted by Galton, M. M. and J. H. Steele, Leptospirosis, a special report. Department of Health, Education and Welfare, Public Health Service, Bureau of State Services, Communicable Disease Center, Atlanta, Georgia.

BAKER, J. A. 1954 Status of leptospirosis as caused by Leptospira pomona infection. J. Am. Vet. Med. Assoc., 124, 477-478.

BAKER, J. A., AND F. B. LITTLE 1946 A virus causing abnormal milk in cattle. Proc. Soc. Exp. Biol. Med., 63, 406-407.

BAKER, J. A., AND F. B. LITTLE 1948 Leptospirosis in cattle. J. Experimental Medicine, 88, 295-308.

BRAMER, P. D., H. I. HARDENBROOK, JR. AND C. C. MORRILL 1953 Studies on leptospirosis in domestic animals. I. Leptospirosis in sheep. Vet. Med., 48, 365-366.

BOHL, E. H., T. E. POWERS, AND L. C. FERGUSON 1954 Abortion in swine associated with leptospirosis. J. Am. Vet. Med. Assoc., 124, 262-264.

BORG-PETERSON, C., AND K. L. FENNESTAD 1956 A field rodent (Adonemus agararius) as carrier of Leptospira pomona in Denmark. J. Am. Vet. Med. Assoc., 128, 204-205.

BROOM, J. C. 1952 Global significance of the leptospiroses. Army Medical Service Graduate School, Med. Sci. Publ., No. 1, 3-9.

BROWN, R. Z., AND G. W. GORMAN 1960 The occurrence of leptospirosis in feral rodents in southwestern Georgia. Am. J. Public Health cited by Steele, J. H., 1960 Epidemiology of leptospirosis in the United States and Canada. J. Am. Vet. Med. Assoc., 136, 247-252.

BRYAN, H. W., H. E. RHOADES, AND D. A. WILLIGAN 1953 Isolation of Leptospira pomona from aborted swine fetuses. Vet. Med., 48, 438-439.

BUCKLAND, F. E., AND R. D. STUART 1945 Mud fever in the British army in France. The Lancet, Sept. 15, 331-337.

CHALQUEST, R. R. 1957 Experimental infection of birds with Leptospira pomona present in bovine urine and preliminary studies on their role in transmission of leptospirosis. Poultry Sci., 36, 110-113.

CHANG, S. L. 1947 Studies on Leptospira icterohaemorrhagiae. J. Infectious Diseases, 81, 28-34.

CHOLVIN, N. R. 1958 Experimental Leptospira pomona infection in dogs. A Thesis, Michigan State University.

CLAYTON, G. E. B., E. H. DERRICK, AND R. W. CILENTO 1937 The presence of leptospires in a mild type (seven day) fever in Queensland. Med. J. Australia, 1, 647-654.

CUNNINGHAM, C. H. 1956 A laboratory guide to virology. Burgess Publishing Co., Minneapolis, Minnesota.

DACRES, W. G., AND G. K. KIESEL 1958 Isolation of Leptospira pomona from a bovine fetus. J. Am. Vet. Med. Assoc., 132, 525-526.

DERRICK, E. H. 1942 Leptospira pomona. Med. J. Australia, 1, No. 15, 431.

DVOSKIN, S., AND E. W. HOOK 1956 Canicola fever with meningitis. A.M.A. Arch. Internat. Med., 97, 793-797.

FAY, L. D., AND W. G. YOUATT 1959 Results of tests for brucellosis and leptospirosis in deer in 1957-1958. Michigan Department of Conservation, Game Division, Report No. 2241.

FERGUSON, L. C., E. H. BOHL, AND T. E. POWERS 1955 Leptospirosis in swine. Proc. U.S. Livestock Sanitary Assoc., 332-336.

FERRIS, D. H., L.E. HUNSON, J. O. ALBERTS, J. C. CALHOUN, AND R. MARLOW 1958 Epizootiological studies on leptospira in Illinois. Serological tests on Illinois deer in 1957. Presented at the conference for teachers of veterinary public health workers. Communicable Disease Center, Atlanta, Georgia. Cited by Galton, M. M., 1959. The epidemiology of leptospirosis in the United States with special reference to wild animal reservoirs. Southwestern Vet., 13, 37-42.

FERRIS, D. H., H. E. RHOODES, AND L. E. HANSON 1959 The isolation of Leptospira hyos from a new host, Peromycus maniculatus, Cornell Vet., 49, 344-348.

GALTON, M. M. 1959 The epidemiology of leptospirosis in the United States with special reference to wild animal reservoirs. Southwestern Vet., 13, 37-42.

GALTON, M. M., J. A. ACREE, A. LEWIS, AND E. C. PRATHER 1956 Leptospirosis in domestic animals in Florida with reference to cattle. J. Am. Vet. Med. Assoc., 128, 87-91.

GALTON, M. M., R. W. MENGES, AND J. H. STEELE 1958 Epidemiological patterns of leptospirosis. Ann. N. Y. Acad. Sci., 70, 427-444.

GALTON, M. M., D. K. POWERS, S. MCKEEVER, AND G. W. GORMAN 1957 Identification of two leptospiral serotypes new to the United States. U.S. Public Health Repts., 72, 431-435.

GILLESPIE, R. W. H., S. G. KENZY, L. M. RINGEN, AND F. W. BRAKEN 1957 Studies of leptospirosis. III Isolation of Leptospira pomona from surface water. Am. J. Vet. Research, 18, 76-80.

GOUCHENOUR, W. S., R. H. YAGER, AND P. W. METMORE 1950 Antigenic similarity of bovine strains of leptospirae (United States) and Leptospira pomona. Proc. Soc. Exp. Biol. Med., 74, 199-202.

GOUCHENOUR, W. S., JR., R. V. JOHNSON, R. H. YAGER, AND W. S. GOUCHENOUR 1952 Porcine leptospirosis. Am. J. Vet. Research, 13, 158-160.

GSELL, O. 1952 Epidemiology of the leptospiroses. Symposium on the leptospiroses. Army Medical Service Graduate School, Med. Sci. Publ., No. 1, 34-56.

HALE, J. W., AND C. W. CATHEY 1958 Leptospira pomona. J. Oklahoma State Med. Assoc., 51, 470-472.

HALE, M. W. 1958 Laboratory report of leptospirosis in Georgia in 1957. J. Am. Vet. Med. Assoc., 133, 196-197.

HUBENER, E. A., AND H. REITER 1915 Dtsch. Med. Wschr., 41, 1275. Cited by Broom, J. C., 1952. Global significance of the leptospiroses. Army Medical Service Graduate School, Med. Sci. Publ., No. 1, 3-9.

INADA, R., AND Y. IDO 1915 Tokyo Ijinskinshi No. 1908. Cited by Broom, J. C., 1952. Global significance of the leptospiroses. Army Medical Service Graduate School, Med. Sci. Publ., No. 1, 3-9.

JELLISON, W. L., H. G. STOENNER, AND G. M. BERG 1958 Leptospirosis among the indians of the Dakotas. Rocky M. Med. J., December.

JUNHERR, E. 1944 Bovine leptospirosis. J. Am. Vet. Med. Assoc., 105, 276-281.

KIRSCHNER, L. 1954 Recent studies on leptospirosis in New Zealand, New Zealand J. Med., 53, 119-123.

LINDQUIST, K. J. 1957 Experimental infection of pregnant ewes with Leptospira pomona and intestinal absorbtion of leptospiral antibodies in lambs. A Thesis, Michigan State University.

LUMLEY, G. F. 1937 Leptospirosis in Queensland. A serological investigation leading to the discovery of distinct serological groups of leptospirae causing leptospirosis as it occurs in northern Queensland. Med. J. Australia, 1, No. 18, 654-664.

MCKEEVER, S., G. W. GORMAN, J. F. CHAPMAN, M. M. GALTON, AND D. K. POWERS 1958 Incidence of leptospirosis in wild mammals from southwestern Georgia, with a report of new host for six serotypes of leptospires. Am. J. Trop. Med. Hyg., 7, 646-655.

MENGES, P. W., AND M. M. GALTON 1958 An investigation of wild animals for leptospirosis in Klamath County, Oregon. Microtus studies 1957-1958. Oregon State College Bulletin. Cited by Galton, M. M. 1959. The epidemiology of leptospirosis in the United States with special reference to wild animal reservoirs. Southwestern Vet., 13, 7-42.

MICHIN, N. A., AND S. A. AZINOV 1935 Spirochaetal Jaundice of cattle in North Caucasus. Soviet Vet., 10, 23-27. Abstracted 1937 in Vet. Bulletin, 7, 419.

MORSE, E. V. 1955 The economic significance of leptospirosis in domestic animals. Proc. Am. Vet. Med. Assoc., 162-167.

MORSE, E. V., V. ALLEN, A. KROHN, AND R. HALL 1955 Leptospirosis in Wisconsin. I. Epizootiology and clinical features. J. Am. Vet. Med. Assoc., 127, 417-421.

MORSE, E. V., AND R. F. LANGHAM 1958 Experimental leptospirosis. III. Caprine Leptospira pomona infection. Am. J. Vet. Research, 19, 139-144.

MORTER, R. L., AND E. V. MORSE 1956 Experimental leptospirosis. II. The role of calves in the transmission of Leptospira pomona among cattle, swine, sheep and goats. J. Am. Vet. Med. Assoc., 128, 408-413.

MORTER, R. L., E. V. MORSE, AND R. F. LANGHAM 1960 Experimental leptospirosis. VII. Re-exposure of pregnant sows with Leptospira pomona. Am. J. Vet. Research, 21, 95-98.

MORTER, R. L., J. A. RAY, AND D. F. CHAPEL 1959 Leptospira pomona isolation from naturally occurring canine infections. J. Am. Vet. Med. Assoc., 135, 570-571.

MURPHY, L. C., AND A. D. ALEXANDER 1957 Significance of Leptospirosis in military medicine. Military Med., 121, 1-10.

MURPHY, L. C., P. T. CARDEILHOC, A. D. ALEXANDER, L. B. EVANS, AND R. H. MARCHWICKI 1958 Prevalence of agglutinins in canine serums to serotypes other than Leptospira canicola and Leptospira icterohaemorrhagiae. Report of isolation of Leptospira pomona from a dog. Am. J. Vet. Research, 70, 145-151.

NOGUCHI, J. 1917 Spirochaeta icterohaemorrhagiae in American wild rats and its relationship to the Japanese and European strains. J. Exp. Med. 25, 755-763.

NOGUCHI, J. 1919 Etiology of yellow fever. II. Transmission experiments on yellow fever. J. Exp. Med., 29, 565-584.

OHIO DEPARTMENT OF AGRICULTURE AND OHIO DEPARTMENT OF HEALTH 1958 Leptospirosis survey. Animal Disease Trends, 4, No. 1.

PADGWAITE, G. D., M.E. TOURTELLOTTE, R. E. JACOBS, C. F. HELMBOLDT, H. L. EASTERBROOKS, L. E. WILLIAMS, E. L. JUGHERR, AND W. N. PLASTRIDGE 1955 Isolation of Leptospira pomona from three aborted bovine fetuses. Vet. Med. 50, 164-165.

REINHARD, K. R. 1953 Newer knowledge of leptospirosis in the United States. Exp. Parasitol. 2, 87-115.

REYNOLDS, I. M., AND R. E. SMITH 1958 Serological survey for Leptospira pomona antibodies in cattle and deer in Massachusetts. J. Am. Vet. Med. Assoc., 132, 293-296.

RICHARDSON, J. H. 1958 Personal communication to S. B. Shotts. Cited by Shotts, E. B., W. E. Greer, and F. A. Hayes 1958 A preliminary survey of the incidence of brucellosis and leptospirosis among white-tailed deer (Odocoileus virginianus) of the southeast. J. Am. Vet. Med. Assoc., 133, 359-361.

ROBERTS, J. J. 1958a Sequelae of leptospirosis in horses on a small farm. J. Am. Vet. Med. Assoc., 133, 189-194.

ROBERTS, J. J. 1958b A study of leptospirosis in a large artificial insemination stud. Cornell Vet., 48, 363-371.

ROTH, E. E., AND M. M. GALTON 1959 Isolation and identification of Leptospira hardja from cattle in Louisiana. Cited by Galton, M. M., 1959. The epidemiology of leptospirosis in the United States with special reference to wild animal reservoirs. Southwestern Vet. 13, 37-42.

ROTH, E. E., AND B. B. KNIERIEM 1958 The natural occurrence of Leptospira pomona in an opossum. A preliminary report. J. Am. Vet. Med. Assoc., 132, 97-98.

SEILER, H. E., J. NORVAL, AND J. D. COCHLAN 1956 Leptospirosis in piggery workers. Nature, 177, 1042.

SELLARDS, H. W. 1940 Trans. Roy. Soc. Trop. Med. Hyg. 33, Cited by Broom, J. C., 1952, Army Medical Service Graduate School, Med. Sci. Publ., No. 1, 3-9.

SIPPEL, W. L., C. I. BOYER, AND E. E. CHAMBERS 1952 Bovine leptospirosis in Georgia. J. Am. Vet. Med. Assoc., 120, 278-282.

SMITH, D. J. W. AND H. R. M. SELF 1955 Observations on the survival of Leptospira austrailis A in soil and water. J. Hyg., 53, 436-444.

SHOTTS, E. B., W. E. GREER, AND F. A. HAYES 1958 A preliminary survey of the incidence of brucellosis and leptospirosis among white-tailed deer (Odocoileus virginianus) of the southeast. J. Am. Vet. Med. Assoc., 133, 359-361.

STEELE, J. H. 1960 Epidemiology of leptospirosis in the United States and Canada. J. Am. Vet. Med. Assoc., 136, 247-252.

STIMSON, A. M. 1907 Public Health Repts., 22, 541. Cited by Bloom, J. D. 1952 Global significance of the leptospiroses, Army Medical Service Graduate School, Med. Sci. Publ. No. 1, 3-9.

SUTHERLAND, A. K., G. C. SIMONS, AND G. C. KENNEY 1949 Bovine leptospirosis. Three outbreaks in calves in Queensland. Australian Vet. J. 25, 197-202.

TURNER, L. W., C. S. ROBERTS, A. W. WIGGINS, A. D. ALEXANDER, AND L. C. MURPHY 1958 Leptospira canicola infection in a newborn calf. Am. J. Vet. Research, 19, 780-784.

UHLENHUTH, P. AND W. FROMME 1915 Med. Klink, 44, 1202. Cited by Broom, J. C., 1952. Global significance of the leptospiroses. Army Medical Service Graduate School, Med. Sci. Publ., No. 1, 3-9.

VAN DER HOEDEN, J. 1957 Newer knowledge of leptospira serotypes and their vectors in Israel. Bull. Research Council, Israel, 6E, 27.

WEBSTER, W. M. 1957 Susceptibility of the hedgehog (Erinaceus europaeus) to infection with Leptospira pomona. Nature 180, 1372.

WEBSTER, W. M. AND B. A. REYNOLDS 1955 Immunization against Leptospira pomona. New Zealand Vet. J., 3, 47-59.

WEDMAN, E. E. AND F. C. DRIVER 1957 Leptospirosis and brucellosis titers in deer blood. J. Am. Vet. Med. Assoc., 130, 513-514.

WADSWORTH, A., V. H. LANGWORTHY, C. F. STEWART, A. C. MOORE, AND M. B. COLEMAN 1922 Infectious jaundice occurring in New York State. J. Am. Med. Assoc., 78, 1120-1121.

WOLFF, J. W. 1952 The classification of pathogenic leptospires. Army Medical Service Graduate School, Med. Sci. Publ., No. 1, 174-182.

YAGER, R. H., W. S. GOCHENOUR, JR., A. D. ALEXANDER, AND P. W. WETMORE 1953 Natural occurrence of Leptospira ballum in rural mice and in an opossum. Proc. Soc. Exp. Biol. Med., 84, 589-590.

YORK, C. J. 1951 Aspects of control in bovine leptospirosis. Proc. U. S. Livestock Sanitary Assoc. 295-300.