EXPERIMENTAL LEPTOSPIRA POMONA INFECTIONS IN DOGS

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EXPERIMENTAL *LEPTOSPIRA POMONA* INFECTIONS IN DOGS

by

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ABSTRACT

Dogs have been found to be susceptible to experimental infection with Leptospira pomona. Leptospirosis occurred following either subcutaneous or oral exposure. There were no observable differences in the course of the infection in the orally exposed and subcutaneously exposed subjects. Live leptospirae were detected in the blood, urine and kidneys of infected dogs. Antibody response, both to L. pomona and to heterologous serotypes, L. icterohemorrhagiae and L. canicola, were followed using an agglutination-lysis test. Pathological alterations were observed only in the kidneys. Symptoms of leptospirosis in the infected dogs were not observed.

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INTRODUCTION

Leptospira pomona was first recognized in 1937 by Clayton *et al.* (10). It was isolated in Pomona, southern Queensland, Australia, from the blood of a patient suffering from an acute febrile disease. In 1938 Johnson and Brown (17) further reported on 8 cases of leptospirosis and indicated that the Pomona type differed in agglutination reactions from 11 serotypes of *Leptospira* from other parts of the world. That same year Terskikh (40) in Russia and Babudieri and Bianchi (2) in Italy investigated outbreaks of human leptospirosis. In both instances the organism causing the infection was found to have the same serological reactions as strain Pomona (42). In 1942 Derrick (11) suggested that the species name *pomona* be given to this *Leptospira* since it proved to be serologically different from other known serotypes.

Gochenour *et al.* (13) in 1950 were the first to incriminate *L. pomona* in bovine leptospirosis in the United States. They reported that the *Leptospira* causing mastitis in dairy cattle, detected by Baker and Little (3) in 1946, was identical with *L. pomona* in serological reactions.

Leptospirosis in man and animals is a worldwide problem at the present time. It is the cause of great economic losses to livestock owners here in the United States.

Although *L. pomona* chiefly causes infection in cattle

and swine, naturally occurring infections have been reported in man (39), sheep (15), goats (16), horses (7), dogs (22, 33) and opossums (37).

Leptospirosis in dogs in the United States is usually due to either of two serotypes, L. canicola or L. icterohemorrhagiae, the former being responsible for the majority of infections. Rats are the main reservoir host for L. icterohemorrhagiae and are the common source of infection, either directly when eaten by dogs or indirectly by urine contamination of food and water. L. canicola does not usually infect rats; dogs are considered to be the natural or predominate host for this serotype. Leptospiruria occurs with infections due to either organism, and leptospira-laden urine is the common infective material. Renal tubular and interstitial damage are found, and infected dogs may show icterus and widespread hemorrhages throughout the body.

Both the severity and the course of the disease vary considerably. As a rule, the slower the onset of leptospirosis in a dog, the less severe is the course of the infection. Three general types are described by Bloom (5). The "hemorrhagic type" is characterized by sudden onset and an acute or peracute course, with submucous and subcutaneous hemorrhages. The second or "icteric type", follows an acute or subacute course. Liver damage often accompanies renal impairment. Icterus and symptoms of uremia may be observed. The third type, the "uremic", is the most common. The course varies from acute to chronic, depending on the extent of

kidney damage. Acute fatal cases of leptospiral nephritis are sometimes seen, but more commonly dogs survive. However, the kidney damage incurred is permanent and ensuing attacks of interstitial nephritis throughout life may impair renal function to the degree that a fatal uremia develops.

L. pomona, the principal etiological agent of leptospirosis in cattle, swine, horses and sheep, has been isolated from dogs. Mochtar (22) indicated that an isolation of L. pomona had been made from a dog in Indonesia. Murphy et al. (33) reported the isolation of this organism from the urine of a dog from the Pennsylvania-Maryland area. The animal was one of 357 examined serologically for several species of Leptospira. Although this dog's serum showed a predominant titer for L. autumnalis, the agent isolated was shown by agglutinin-absorption procedures to be L. pomona.

Isolations of L. pomona from naturally infected dogs have been few. Several workers, however, have found antibodies present in canine sera during the course of serological surveys for the leptospiroses. Morse et al. (25) reported a serum titer of 1:1000 for this serotype in a farm dog known to have mingled with and consumed aborted materials and milk from infected cattle. Hamsters and guinea pigs inoculated with kidney tissue from this dog did not develop L. pomona antibodies or show evidence of infection. Alexander et al. (1) found antibodies for L. pomona in the serums from 3 of 1117 dogs. Murphy et al. (22) observed that 2.2 per cent or 8 of 357 dogs had positive serological reactions for this

serotype. Reports from Europe indicate similar findings also based on serological surveys conducted among canine populations (6, 38). Brede (6) recorded a clinical case of human L. pomona infection in which the patient's serum was positive at the 1:8000 level while the man's dog had a titer of 1:500.

Cases of clinical canine leptospirosis have occurred, and L. pomona has been incriminated as the cause based upon serological evidence. Newman (34) studied such a case in which the serum titer was 1:80,000. The author (8) has examined other dogs showing symptoms of leptospirosis and on the basis of serological tests concluded that L. pomona was probably involved.

This thesis reports on experimental L. pomona infections in dogs produced by subcutaneous and oral routes of exposure. The pathogenesis of the disease was delineated through serological, hematological, bacteriological and histopathological techniques.

MATERIALS AND METHODS

Twelve apparently normal dogs served as experimental animals. Group 1 consisted of 4 which were exposed subcutaneously. These were obtained from a city pound and the ages were estimated to be from 1 to 4 years. The sera from 3 dogs did not contain agglutinin-lysins for L. pomona, L. icterohemorrhagiae AB, or L. canicola. The serum from one, L24, had an antibody titer of 1:1000 for L. icterohemorrhagiae AB.

Group 2 was composed of 8 young dogs from 2 different litters whelped in laboratory kennels. Vaccination against canine distemper and infectious canine hepatitis was accomplished prior to experimentation. None of the sera contained agglutinin-lysins for L. pomona, L. icterohemorrhagiae AB, or L. canicola. These animals were exposed either orally or by the subcutaneous route.

Two dogs, L26 and L28, of Group 2, did not become infected following oral exposure, as indicated by the failure to develop serum agglutinin-lysins during the 6 week period after exposure and the absence of positive blood cultures during the 4 to 8 day period following exposure. These 2 were then designated Group 3, and inoculated subcutaneously.

Following exposure, the dogs were housed two to a cage, to minimize cross infection. Exceptions were L26 and L28 of Group 3, and L33, the unexposed control. These were kenneled individually. The daily routines for examinations, care and

feeding were also conducted to avoid contact between dogs of different groupings; i.e. the unexposed control dog for Group 2 was handled first each day, before the infected dogs.

Three strains of L. pomona were used to infect the dogs. The first, strain Wickard, was of bovine origin. It was isolated from the urine of a naturally infected Wisconsin dairy cow in 1953 (26) and had been maintained in continuous guinea pig passage. Two dogs of Group 1 and all dogs of Group 2 were infected with strain Wickard. The second L. pomona strain, designated Ohio, was recovered from infected hog urine at the Ohio Agricultural Experiment Station at Wooster during 1956. It had been maintained in either continuous hamster or guinea pig passage since isolation. This agent was employed for subcutaneous inoculation of 2 dogs in Group 1. The third organism was a variant of strain Wickard which was lethal for hamsters (4). The original Wickard strain did not kill hamsters. The lethal characteristic appeared following passage through sheep and subsequent serial transfers in modified Chang's fluid medium (26). Both dogs of Group 3 were inoculated subcutaneously with the variant.

The inocula which were administered subcutaneously consisted of 5 cc. of pooled heparinized, guinea pig blood collected at the height of febrile response (105-106° F.), the leptospiremic phase of infection. Preceding subcutaneous exposure, 1 cc. of 1:1000 solution of epinephrine was administered subcutaneously to counteract possible anaphylactoid

reactions. The approximate numbers of leptospirae contained in the inocula were ascertained using the hamster inoculation technique (30).

In Group 2, 4 littermates were exposed to strain Wickard by feeding the blood, organs and carcasses of guinea pigs sacrificed during leptospiremia. Food and water were withheld from the dogs for 24 hours prior to feeding. Three of the 4 remaining littermates were inoculated subcutaneously with the Wickard strain, while the fourth dog, L33, was not exposed to L. pomona and served as a control for the group. Table 1 gives the scheme of the experiments.

Blood cultures were made at appropriate intervals by inoculating 1 cc. of blood into 10 ml. of modified fluid Chang's medium. These were incubated at 30° C., and examined by darkfield microscopy (590X) at approximately the 14th and 30th days.

Serum antibody response was followed using a modified microscopic agglutination-lysis test (30), employing 10 fold serial dilutions. Living L. pomona cells, strain Johnson, were used as antigen, as well as L. icterohemorrhagiae AB and L. canicola. The presence of leptospiral antibodies in urine was detected by the same procedure. However, the urine dilutions employed were 1:5, 1:10, 1:100 and 1:1000 only.

At necropsy specimens of liver, spleen, kidney, pancreas, thyroid gland, adrenal gland, renal lymph node, tonsil and brain were obtained and fixed in either 10 per cent formalin or Zenker's solution. Staining procedures employed were

hematoxylin and eosin, Verhoeff's stain for elastic tissue, Heidenhain's aniline blue stain for connective tissue, Sudan IV for fat and the Bauer-Feulgen reaction for glycogen.

Animal inoculation techniques were used to ascertain the presence of leptospirae in urine and tissues (30). Approximately 10 per cent tissue homogenates were prepared in sterile 0.85 per cent sodium chloride solution. One to 3 cc. of brain, kidney, liver, spleen, or combined liver and spleen homogenates were inoculated intraperitoneally into 2 to 8 weanling guinea pigs or 3 to 5, 4 to 6 week old hamsters. Urine was taken periodically from each dog following exposure and 0.5 to 2 cc. were similarly inoculated into laboratory animals. Presence of serum antibodies in laboratory animals at approximately 21 days after inoculation indicated that leptospirae were present in the original inocula.

Prior to and following exposure, standard clinical hematological techniques (41) were employed to ascertain levels for the various blood cells and blood hemoglobin values (oxyhemoglobin method) of the inoculated dogs and of the control. Each dog was given a daily physical examination for symptoms of leptospirosis.

EXPERIMENTAL RESULTS

Leptospiremia was demonstrable in all dogs which were exposed *via* the subcutaneous route. Leptospirae were present in the blood during the third to seventh day following inoculation. The organisms were not isolated from the blood of the 4 dogs which were exposed orally.

Leptospiruria was demonstrable for 6 of 12 dogs. Thirty-five urine samples were examined by darkfield microscopy (590X) and leptospirae were observed in only 3. Seventeen of these samples, including the 3, were found to be positive by animal inoculation techniques. Urinary shedding of the agent occurred as early as 15 days after inoculation for 2 dogs. However, most of the positive urine samples were found during the third through the seventh week of infection. Leptospirae were not found in the urine specimens of any of the dogs 47 days after inoculation. One dog, L29, which was exposed orally, shed leptospirae for 32 days following exposure and the organism was present in kidney tissue at 54 days. The other 3 dogs exposed orally did not become infected; unfortunately, 1 died 7 days after infection due to coronary damage at the time blood was drawn. Leptospirae were also present in kidney tissue of 2 dogs at 21 and 26 days following subcutaneous exposure. However, the organism was not present in 8 other infected dogs at necropsy at 7, 10, 13, 14, 31, 49,

54 and 55 days. Table 2 summarizes the bacteriological data for these dogs.

Serum antibodies for L. pomona appeared on the seventh or eighth day following infection. The response was maximal at 2 to 3 weeks and declined slowly thereafter. All dogs, except 3 which were exposed orally, developed demonstrable agglutinin titers.

Serological cross reactions occurred with L. icterohemorrhagiae AB, and L. canicola antigens. L. icterohemorrhagiae AB reactions were higher than those for L. canicola with 7 exceptions for sera from 3 dogs. Reactions for the two heterologous serotypes never exceeded those for L.pomona. See table 3. Antibodies were present in the urine of 9 of the infected dogs at levels as high as 1:1000 and titers persisted for 56 days.

Symptoms attributable to leptospirosis were not observed in any of the dogs. Significant changes in total erythrocytes, leucocytes or in hemoglobin levels were not observed when values were compared with those prior to exposure and those for the uninoculated dog (Table 4).

Gross and microscopic changes appeared only in the kidneys and were present in all the dogs in which serum antibody response was manifest. Scattered white foci, 1 to 5 mm. in diameter, were present in the renal cortex (Fig. 1) and extended into the medulla (Fig. 2). These, individually, were typical of the lesions described in cattle (14, 36), swine (20), sheep (19) and horses (7) infected with L. pomona.

In general, these macroscopic changes were not as severe as those observed in cattle and swine. The exception was L32, in which the kidneys were greatly enlarged and contained numerous scattered focal lesions.

Microscopically the alterations also resembled those observed in bovine and porcine leptospirosis. An interstitial nephritis with an infiltration of lymphocytes, plasma cells and macrophages was present (Figs. 3, 4, 5). Renal tubules underwent degeneration, necrosis and atrophy (Fig. 6), and hemosiderin deposits were evident. Areas in which tubules were nonfunctional contained cells with pyknosis and loss of structure. Glomerular damage consisted of congestion of the capillaries and protein loss within the renal corpuscle (Fig. 7). Connective tissue proliferation was evident in many of the lesions of dog L32 at 54 days. Renal lesions in the other dogs, however, did not show fibrosis.

DISCUSSION

L. pomona infections in the experimental dogs were symptomatically occult. Anorexia and malaise, as commonly reported for naturally and experimentally infected cattle, sheep and swine, did not occur. The fact that none of the dogs showed illness suggests that extreme stress might be a necessary accompanying factor to overcome innate resistance. Experimental caprine infections have been found to run a similar course, although high serum antibody levels for L. pomona developed and leptospirae were isolated from urine and kidney tissue.

The apparent natural resistance of dogs to L. pomona is indicated by the difficulty with which infection is established per os. This is in contrast to the relative ease of establishment of infection among cattle, swine, goats and sheep during pen contact exposure (32).

With several exceptions, most of the dogs were in apparently good health prior to experimentation. L25 had a vaginal discharge suggesting a low grade metritis. The dogs of Groups 2 and 3 had subclinical infections with Toxascaris leonina. At necropsy L24 was found to have extensive hydronephrosis involving one kidney. The functional kidney had only minimal changes attributable to infection, despite the increased physiological function demanded of it. Even these conditions did not influence the susceptibility of the

animals to L. pomona insofar as could be ascertained.

Several factors, which could predispose dogs to infection, were not present in these experiments. Concurrent bacterial and viral infections were not in evidence. Dogs of Groups 2 and 3 were vaccinated several times for canine distemper and infectious canine hepatitis before experimentation. The ration fed before and during the investigation was considered adequate, and hygienic conditions were good.

Several infected dogs developed intermittent pyrexia of 1 - 2° F. during the 10 day period following exposure. However, since the elevated temperatures could not be correlated with periods of demonstrable leptospiremia, and frequently the affected dogs would be highly excited, this symptom could not be related to leptospirosis. A transient conjunctivitis without further involvement of the deep structures of the eye was observed in two dogs on days 1 and 10, but again this symptom was not equivocal. However uveitis is not uncommon in leptospiral infections of man (18).

Newman (34), in 1955, recorded a serum agglutinin-lysin reaction of 1:80,000 for L. pomona for a dog showing classical symptoms of leptospirosis. Serum titers for L. icterohemorrhagiae or L. canicola were not present. The dog was emaciated; pyrexia (103° F.) was present as well as icterus and an ulceration of the tongue. Hematological examination indicated a leucocytosis of 38,100 cells, and kidney impairment was evident (NPN of 95 mg. per cent). The urine contained protein. Liver function was impaired as

based upon the methylene blue reaction. The dog failed to respond to antibiotic and supportive therapy and died. The renal cortex contained numerous white foci which on section extended well into the medulla. The liver showed fine mottling and was greenish in color. The renal lesions in this case were similar to those which are reported for the experimentally infected dogs. Apparently L. pomona under proper circumstances can produce a disease of severity in dogs, but such was not observed in the experimental infections. An analagous paradox has been reported for Brucella abortus infection in dogs (28, 31).

The infective material used for subcutaneous inoculation contained 5×10^2 to 5×10^5 leptospirae, and infection was readily established. This would certainly indicate that adequate exposure was effected. There were no observable differences in the nature or course of infection in the orally exposed and subcutaneously exposed subjects. L29, infected orally, received material similar to the leptospira-laden urine, milk and aborted porcine fetuses and placentas which dogs might contact naturally. Both the Wickard and Ohio strains have been kept in continuous guinea pig passage since isolation, and have the ability to infect several species of animals (29, 32, 21). These strains appear to be comparable in virulence to field strains.

Demonstrable leptospiremia occurred during the third through seventh days following infection, but ceased abruptly when serum antibodies were demonstrable. This supports the

finding that leptospiremia in dogs is of low magnitude, and is in contrast to the situation in swine where large numbers of leptospirae may depress the initial antibody response, making it possible to observe concurrent leptospiremia and low level agglutinin-lysin titer (27).

Six of the 12 dogs shed L. pomona in the urine. Leptospiruria was demonstrable for 47 days after infection and leptospirae were detected in kidney tissue 54 days after infection. Very few leptospirae were shed in the urine. The majority of the specimens proven positive for the agent by hamster or guinea pig inoculation were negative when examined by darkfield microscopy. Several samples contained non-motile bodies which morphologically resembled leptospirae. These may have been immobilized or recently killed organisms. Dog urine is usually acid and frequently has a pH of less than 6.0. This reaction tends to render the urine leptospiracidal (35). In addition, agglutinin-lysins found in urine have an immobilizing and lysing effect on leptospirae. These conditions may help explain why the agent was not readily demonstrable. Dogs appear to be much less likely than cattle or swine to transmit the disease because of the few organisms present in urine. This indicates that dogs probably play a minor role in the natural dissemination of L. pomona.

Serological reactions for L. icterohemorrhagiae AB and L. canicola developed during the course of the experimental infections. These cross reactions appeared as early as the

8th day at the 1:10 level. Heterologous responses did not exceed but paralleled those for L. pomona. In cattle, experimentally infected with L. pomona, L. icterohemorrhagiae AB titers were higher than homologous titers during the initial infection phase (24). Galton (12) indicated that in a case of human leptospirosis the heterologous titer surpassed the homologous for approximately 50 days. Two case histories (9) on record at this clinic depict the situation in which serotype identification on the basis of examination of the patients' sera proved difficult. Two dogs from the same home became sick and were admitted for diagnosis and treatment. Both dogs showed icterus of all visible mucous membranes, and anorexia and malaise were noted. The younger dog died after 1 day and a necropsy examination was performed. Several white lesions were present in the renal cortex, which on microscopic examination appeared to be foci of interstitial nephritis. Grossly, the liver was enlarged and mottled with focal areas of necrosis. Guinea pigs, inoculated with kidney homogenates, developed titers for L. icterohemorrhagiae, but did not develop titers for either L. pomona or L. canicola during the 3 week period following inoculation. Unfortunately, leptospirae were not isolated. The older dog was treated successfully with 300,000 units of aqueous penicillin, and 0.25 gram of streptomycin sulfate daily for 8 days, and then 600 mg. erythromycin® daily for 7 more days. Two blood samples were taken from this dog approximately 10 days and 5 weeks after symptoms of leptospirosis appeared. Serum

agglutinin-lysin titers for the 3 serotypes were as follows:

	L. icterohemorrhagiae AB	L. pomona	L. canicola
10 day sample	1:100	1:1000	1:10
5 week sample	1:100	1:1000	1:1000

Therefore, unequivocal identification of the leptospiral species involved was not possible as can be noted from the above serological data.

Antibiotic therapy has been found to depress antibody response in swine fed aureomycin® (23). It is conceivable that certain components of the antibody are retarded or masked and the cross reacting principles are more in evidence. The author feels that antibiotic therapy during the early phases of antibody formation confuses the definitive serotype identification.

Most L. pomona canine infections appear to be subclinical or occur with mild, transient symptoms which are disregarded or overlooked by owners. Perhaps these infections are more common than suspected. This premise is borne out in the serological survey of Murphy et al. (33) in which 2.2 per cent of 357 apparently normal rural dogs were positive for L. pomona.

SUMMARY

Dogs have been found to be susceptible to infection with L. pomona. Leptospirosis occurred following either subcutaneous or oral exposure. Leptospirae of both porcine and bovine origin established infection and the renal carrier state.

Symptoms of leptospirosis were absent. In this respect canine infections resembled the mildest or subclinical form of the disease observed in goats, swine or cattle.

Pathological alterations were limited to the kidney and were observed in all dogs which developed serum agglutinins. Microscopically, alterations appeared as interstitial infiltration of lymphocytes, plasma cells and macrophages and varying degrees of renal tubular degeneration. The lesions were minimal in all but one.

The pathogenesis of the disease was observed through hematological, bacteriological, serological and histopathological techniques. Leptospiremia followed infection, usually occurring from the third through seventh days.

The serum antibody response was marked in all infected dogs. Reactions for L. icterohemorrhagiae AB and L. canicola were constant findings, but the magnitude of the heterologous reactions never approached those for L. pomona.

Urinary shedding of the leptospirae commenced as early

as the fifteenth day, and persisted for as long as 47 days. The microorganisms were detected in the kidneys of one dog 54 days after oral exposure. Since the renal carrier state can be established following oral exposure, the dog might be responsible, in some rare instances, for the transmission of L. pomona infection to other lower animals and possibly man. However, the dog's role as a significant reservoir host for L. pomona is probably slight.

TABLE 1

Histories and Exposure Data for *L. pomona* Infected Dogs

Dog	Sex	Age	Source	Group	Inoculum*		
					Strain	No.Cells	Route
L23	Male	4 yrs.	City Pound	I	Bovine, Wickard	5×10^5	Subcut.
L24	"	1 yr.	" "	"	" "	"	"
L22	"	1 yr.	" "	"	Porcine, Ohio	5×10^2	"
L25	Female	3 yrs.	" "	"	" "	"	"
L26	"	6 mo.	Lab. Kennel	II	Bovine, Wickard	**	Oral
L27	"	" "	" "	"	" "	**	"
L28	"	" "	" "	"	" "	**	"
L29	"	" "	" "	"	" "	**	"
L30	"	8 mo.	" "	"	" "	5×10^4	Subcut.
L31	Male	" "	" "	"	" "	"	"
L32	Female	" "	" "	"	" "	"	"
L33	Male	" "	" "	"	Uninoculated Control		
L26	Female	7 mo.	" "	III	Bovine,Wickard Variant	5×10^5	Subcut.
L28	"	" "	" "	"	" " "	"	"

* Administered as 5 cc. of infected guinea pig blood as indicated.

** Fed infected guinea pig carcasses and blood. Guinea pigs were in leptospiremic phase of infection.

TABLE 2

Bacteriological Findings for Dogs Infected with L. pomona

Dog (Route)	Days Following Inoculation[a]						Antibody Response	
	Blood[b]		Urine[c]		Kidney			
	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Day	Highest Titer
L23 (Subcutaneous)	1,2,7-9	3-6	6,20	15,23,25	31		20	8[d]
L24 (Subcutaneous)	1,2,7-9	3-6	6,10		10		10	5[e]
L22 (Subcutaneous)	1-3,7,8	4-6			13		13	5[e]
L25 (Subcutaneous)	1,2,4,7,8	3,5,6		7		26	20	8
L26 (Oral)	3-8							
L27 (Oral)	3-7				7		7	3[e]
L28 (Oral)	3-8							
L29 (Oral)	3-8		46,47,50	21,25,32		54	14	8

TABLE 2, continued

Dog	Days Following Inoculation[a]							
	Blood[b]		Urine[c]		Kidney		Antibody Response	
(Route)	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Day	Highest Titer
L30 (Subcutaneous)	5,6	4		14	14		14	8
L31 (Subcutaneous)	4,6	5		21		21	15	8
L32 (Subcutaneous)	4,6	5	12,50	21,25,35, 40,46,47	54		14	8
L26 (Subcutaneous)		3-5,7	25,39,46	18,32	55		11	8
L28 (Subcutaneous)	3,5,7	4	18,25,32, 39,46		49		18	8

a Numbers indicate materials were examined.

b Each blood sample cultured in 5 tubes of Chang's medium.

c Inoculation of hamsters or guinea pigs. Development of serological titers within 18-21 days indicated leptospirae present in original inoculum.

d Maximal serological response of 25% agglutination-lysis or greater expressed as negative exponent to log base 10.

e Dog sacrificed during phase of ascending antibody response.

TABLE 3

Cross Reactions Between Leptospiral Serotypes Observed in Agglutination-lysis Test of *L. pomona* Infected Dogs

Dog (Route)	Antigen	Days Following Exposure 6	8	10	11	12	14	15	16	19	20	21	22	24	27	32	39	46
L23	*L. pomona*:	0[a]	3[b]	5		5	5		5		8		8	7				
	L. ictero:	0	0	1		2	4		3		3		2	2				
(Subcut.)	*L. canicola*:	0	1	0		1	0		0		0		2	0				
L25	*L. pomona*:	0	2	3		5	6		7		8		8	8	7			
	L. ictero:	0	0	1		2	2		2		2		2	0	4			
(Subcut.)	*L. canicola*:	0	0	0		0	0		0		0		0	1	0			
L29	*L. pomona*:				3	5	8			6		6				7		
	L. ictero:				0	2	3			0		0				2		
(Oral)	*L. canicola*:				0	3	4			2		1				2		
L31	*L. pomona*:				2	3	7	8					6					
	L. ictero:				2	2	3	3					0					
(Subcut.)	*L. canicola*:				0	2	2	3					4					
L32	*L. pomona*:				6	6	8	7								8		
	L. ictero:				3	4	4	3								3		
(Subcut.)	*L. canicola*:				0	2	4	3								1		
L26	*L. pomona*:			5	8									7		6	5	6
	L. ictero:			0	3									0		1	1	0
(Subcut.)	*L. canicola*:			1	2									0		0	0	0

a Agglutination or lysis absent at 10^{-1} serum dilution. 25% agglutination-lysis considered positive.

b Maximal serological response expressed as negative exponent to log base 10.

TABLE 4

Hematological Data for Exposed Dogs and Uninoculated Control

Dog		Preinfection	Days Post-infection								
			5	6	7	12	13	14	21	22	26
L22	Hb	17.3 gm	17.6			14.5					
	rbc	7200000/mm^3	6470000			5360000					
	wbc	13600/mm^3	13500			13200					
L23	Hb	17.1		18.1				16.2		16.6	
	rbc	7230000		7090000				5650000		5650000	
	wbc	13250		17850				22500		17500	
L24	Hb	15.0		15.3							
	rbc	6420000		6050000							
	wbc	21700		23500							
L25	Hb	14.1	14.1				13.3		12.7		10.5
	rbc	5170000	5170000				5390000		4760000		4760000
	wbc	12750	12750				12850		13550		9150
L26	Hb	11.7			12.3			13.0			
	rbc	4870000			4850000			5070000			
	wbc	19550			18350			15750			
L27	Hb	12.7			12.7						
	rbc	4950000			5250000						
	wbc	17150			13650						

TABLE 4, continued

Dog		Preinfection	Days Post-infection								
			5	6	7	12	13	14	21	22	26
L28	Hb	10.7			13.3			12.7			
	rbc	3970000			5340000			5300000			
	wbc	18450			13250			19750			
L29	Hb	11.3			11.9			11.7			
	rbc	4860000			4430000			5110000			
	wbc	10050			8050			11950			
L30	Hb	14.0			13.0			13.0			
	rbc	6160000			5560000			5250000			
	wbc	16700			18000			11800			
L31	Hb	14.5			13.0			11.3			
	rbc	5660000			5220000			4370000			
	wbc	17650			27400[a]			26000			
L32	Hb	13.7			13.0			12.3			
	rbc	5350000			4940000			5350000			
	wbc	12050			11800			16300			
L33[b]	Hb	14.1			17.1			15.0			
	rbc	5570000			6000000			6460000			
	wbc	14600			13100			9200			

a Leucocytosis apparently due to abscess at site of inoculation of infective material.

b Uninoculated control dog.

Figure 1. Kidney of dog L32 necropsied 54 days after exposure to L. pomona, showing numerous whitish foci in the cortex. x1.7.

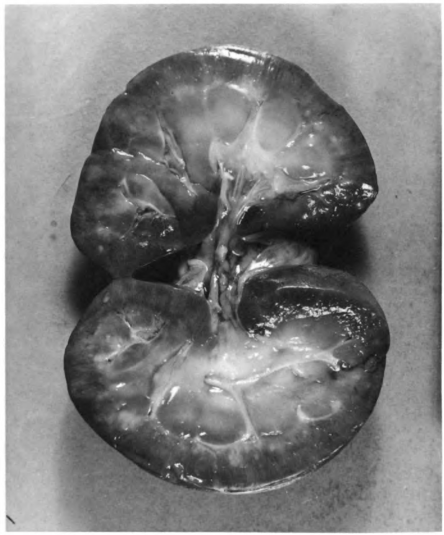

Figure 2. Cut surfaces of kidney (dog L32) showing grayish-white foci in the cortices and extending into the medulla. x1.7.

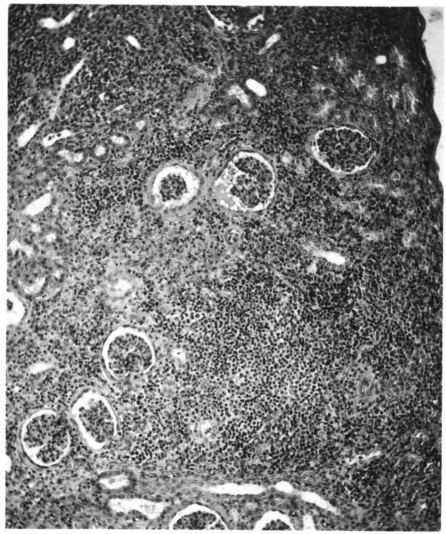

Figure 3. Section of kidney (dog L32) showing interstitial nephritis with infiltration of lymphocytes, plasma cells and macrophages. x115.

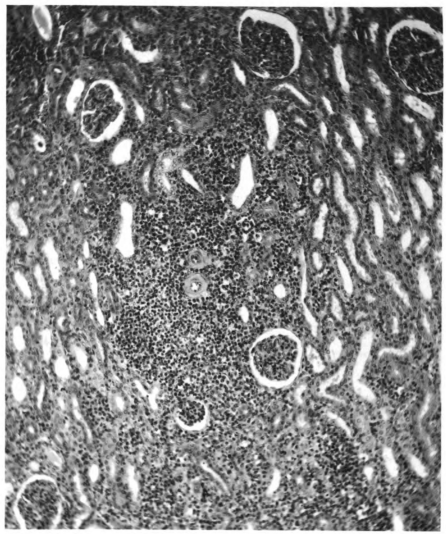

Figure 4. Section of kidney (dog L32) showing perivascular infiltration of lymphocytes and plasma cells. x115.

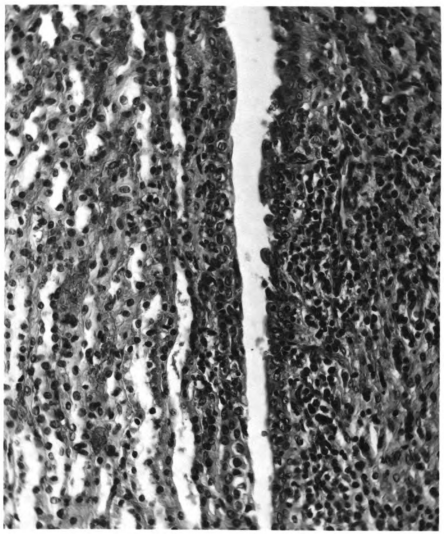

Figure 5. Medullary portion of kidney (dog L32) showing infiltration of lymphocytes and plasma cells. x250.

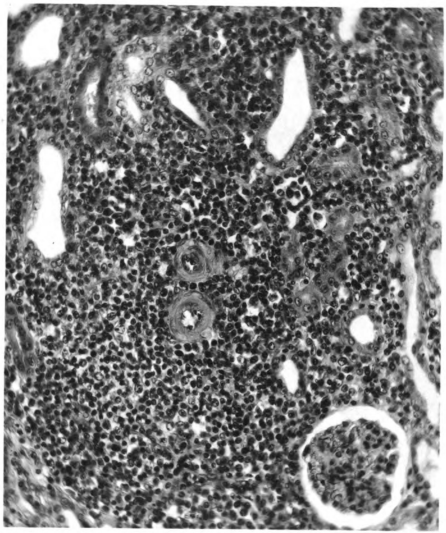

Figure 6. Section of kidney (dog L32) showing tubular degeneration and atrophy. x250.

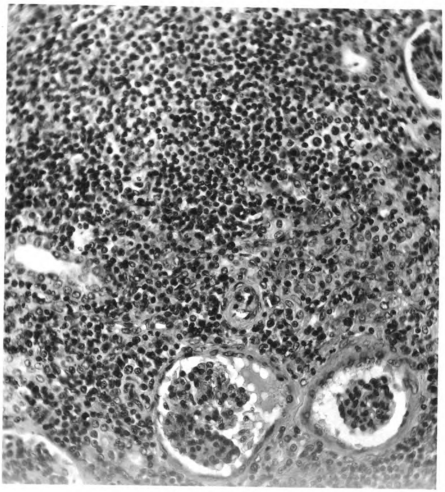

Figure 7. Section of kidney (dog L32) showing protein loss in the renal corpuscle. x250.

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