EXPERIMENTAL LEPTOSPIROSIS: LEPTOSPIRA CANICOLA INFECTION IN CALVES

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ABSTRACT

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by Salah Eldin Imbabi

A study of experimental *L. canicola* infection was conducted on ten calves. Seven of these were infected by the subcutaneous route using inoculums of leptospiremic hamster blood. Clinical, bacteriologic, hemotologic, serologic and pathologic aspects of the disease were observed.

Clinical symptoms were manifested 14 to 24 hours following inoculation, and lasted up to postinoculation (P.I.) day five or six. Marked thermal response, temporary anorexia, general weakness, lethargy, stiffness of legs and in two calves, diarrhaea, were observed.

Leptospiremia was detected in all infected calves 12-24 hours after inoculation and it continued up to P. I. days four or five. Leptospiruria started in all infected calves between P. I. days 12 and 20 and was detectable up to day 37 in some calves. Leptospires were also in the brain of one calf on day six and in the kidney up to day 40 but the liver and spleen were negative as early as P. I. day six, as shown by guinea pig inoculation.

Specific serum antibodies against L. canicola were observed in significant titers consistently on the sixth day after inoculation in all infected calves. Maximum titers of up to 10^6 were recorded.

Moderate anemia with varying reductions in packed cell volume, hemoglobin and erythrocyte counts, was seen but there was no hemoglobinuria or jaundice. There was an initial leukocytosis with marked neutrophilia that lasted for two or three days followed by leukopenia which continued up to around day 10. A slight absolute lymphopenia and monocytosis were observed.

No impairment to renal function was noticed. This was evaluated by measurements of blood urea nitrogen and examination of urine for albumin, specific gravity, and pH, all of which gave normal results. Hepatic function was tested by the bromosulphalein clearance technique. Variable increases in the half time values, were observed in some instances, that did not correlate with other findings. The value of the data obtained on liver function in this experiment may be questionable.

At necropsy no significant lesions were found except in the kidney where small greyish foci were seen in the cortex. These foci extended into the medulla.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

INTRODUCTION

Outbreaks of leptospirosis in cattle, where L. canicola was shown to be the only cause of the disease, have been reported. The infection was confirmed both serologically and by actual isolation of organisms from sick calves.

Experimental evidence, however, was very scanty. Reports of only three calves infected on two different occasions were encountered. Nowhere in the literature was there any report of a controlled experiment to study the various clinical, clinical pathologic or immunological aspects of L. canicola infection in cattle.

The experimental work undertaken by the author is an attempt to meet this deficiency.

LITERATURE REVIEW

The leptospiroses are a group of diseases, caused by a variety of leptospiral serotypes.

The leptospiral etiology of Weil's disease was first recognized by Inada, et al. (1916), who classified the organism as Spirochaeta icterohaemorrhagiae. Since that time many serotypes of leptospira were recognized in man and other animals in many parts of the world. Mitchen and Azinov (1935) in Russia, were the first to associate leptospires with icterohemoglobinuria in a calf. Shortly after that, Clayton and Derrick (1937), reported the isolation of leptospira from a farmer near Pomona in Australia and designated this serotype as L. pomona. Jungherr (1944), was the first to recognize leptospires in stained kidney sections from three fatal bovine cases. Baker and Little (1948) isolated leptospires from cows suffering from a febrile disease characterized by thick blood-stained milk. This serotype was later identified by Gochenour, et al. (1950), as antigenically similar to L. pomona.

Leptospira are associated with many animal hosts, as revealed by Steele (1958). The concept that had prevailed in the past concerning host specificity is no longer generally accepted, Galton, et al. (1958). More and more evidence is accumulating, both of naturally occurring and

induced infections in host species by various leptospiral serotypes. Faine (1962) considered that the host of election in leptospirosis should be regarded as a result of a quantitative rather than a qualitative adaptation of host and parasite to one another.

Serologic evidence furnished by Alexander, et al. (1962), showed the extensive occurrence of L. sejroe agglutinins in bovine sera from ten states. Prior to that, Bryne and Chambers (1959) had shown the prevalence of this serotype in cattle of Maryland, a finding that had been reported from Florida by Galton, et al. (1956); from Georgia by Hale (1957); and Illinois by Ferris, et al. (1958). Leptospira hardjo was isolated from cattle in Louisiana by Roth and Galton (1960) and from a cow showing clinical symptoms of leptospirosis on a Pennsylvania farm, Clark, et al. (1961).

Mitchell, et al. (1960), described an outbreak of leptospirosis in cattle in Canada, associated with a serotype of the hebdomadis group, namely L. sejroe. Most of the infections, however, were caused by L. pomona but they have also encountered weak serologic reactions against L. canicola.

Leptospira pomona caused widespread epizootics in swine, Bohl and Ferguson (1952) and Yager, et al. (1952); and in cattle, York (1951), Gochenour (1950 and 1953), Reinhard (1952 and 1953), and Boulanger (1958). Leptospira pomona was also found to be the cause of an outbreak in

horses with manifestations of systemic disease, Roberts, et al. (1952). Some cases of leptospiral meningitis in man were caused by this serotype, Schaeffer (1951) and Schnurrenberger (1961).

Leptospira pomona was isolated from naturally occurring canine cases by Murphy (1957) and by Morter, et al. (1959), and successfully used to establish experimental infections in dogs by Cholvin, et al. (1959). Sheep and goats were also susceptible to experimental infection, Morse, et al. (1958).

While the foregoing clearly illustrates the point that a leptospiral serotype can infect more than one host species and that one host may be infected by more than one serotype; a further example is furnished by L. canicola. It was first isolated from a sick dog in the Netherlands by Klarenbeck (1933) and for many years was considered as confined to dogs and man. This concept, however, was later proved incorrect.

Olitzki (1951) produced serologic and cultural evidence of L. canicola in cattle, dogs, and pigs. Starr (1953) investigated a serious outbreak in pigs with parallel high incidence in dogs and human beings, and significant serum titers in two out of 21 cows examined. In the same year Wellington, et al. (1953) also encountered a serologically positive case of L. canicola in a cow with symptoms of "hematuria."

Van der Hoeden (1955a) reported that prior to 1952 the main etiology of leptospirosis in Israel was L. grippotyphosa, but that following 1952, L. canicola started to assume increasing importance in many parts of Israel where it accounted for several outbreaks among cattle in regions where its incidence in dogs was comparatively low. There were indications in one outbreak, that one dog which died from the disease, had contracted it by licking the boots of his master who was a herdsman, and of another dog contracting the disease by frequenting pens of infected calves. Infection in jackals was also common and many showed high serum agglutinins against L. canicola which was isolated from the kidneys of two. Again, van der Hoeden (1955) described four outbreaks that had occurred previously, namely in 1949, 1952, 1953, and 1954 respectively. In the first one, a three weeks old calf died after a short illness with symptoms of extreme weakness, lack of appetite, bumping pulsation of the heart and intense jaundice. From the kidneys of this calf a leptospira was isolated and later identified as L. canicola. The second outbreak involved mainly calves about five months old, some of which showed severe symptoms including hemoglobinuria and pyrexia and half of these were either killed or died from the infection. The third and fourth outbreaks were noticed among older cows. Symptoms included hemoglobinuria and premature birth with high serum agglutinins against L. canicola in both outbreaks.

The same author also established infection experimentally in a three and one-half month-old calf by ocular instillation of L. canicola culture obtained from a dog. The calf started to have a high temperature on the 9th day after infection and was found to be serologically positive against L. canicola on P. I. day 18. At necropsy, the kidneys had lesions of acute and subacute focal interstitial nephritis and many leptospires were seen in the bladder urine.

Once more van der Hoeden (1955c) described severe outbreaks in cattle in Israel due to L. canicola with high incidence in man and pigs and significant titers in apparently healthy horses, mules and donkeys in areas where cattle were infected. He postulated that cattle, swine, dogs and other animals were infected by water and vegetation contaminated by jackals and dogs and vice versa.

The incidence of L. canicola in dogs and pigs in Israel was also reported to be strikingly high, van der Hoeden (1956). In one case, 14 out of 33 symptomless pigs examined were serologically positive with titers of from 1:200 to 1:2000. Many pigs examined in slaughter houses were shown to have evidence of diffuse and focal interstitial nephritis with tubular degeneration and some glomerulonephritis. As for human infections in Israel, L. canicola was second to L. grippotyphosa and accounted for 31% of the cases reported by van der Hoeden (1958). He also reported L.

canicola infection in hedgehogs in which 27 out of 142 examined were serologically positive. The organism was isolated from 21 of these.

In Hungary, Kiszel, et al. (1957), found strong serologic evidence of L. canicola, among other serotypes, in dogs, pigs, cattle and horses. Bezdenezhnykh, et al. (1956), incriminated L. canicola, as the major cause of leptospirosis in swine in the Sakhalin islands.

In the United States, Williams, et al. (1956), while studying an outbreak of canicola fever in man, was also able to isolate L. canicola from dogs and swine in addition to finding significant serum agglutinins in cattle. In this instance human beings apparently were infected by using a small stream near which swine and cattle frequently grazed and which was also frequented by dogs.

A bacteriologically verified case of L. canicola in a new born calf was described by Turner, et al. (1958). The calf was presented for treatment with a history of blood in the urine and intense jaundice. The calf, on examination, had a very low red blood cell count, leukocytosis, and a high serum level of combined protein bilirubin with no free bilirubin. Inoculation of urine from the calf into mature chinchillas resulted in a disease similar to, but more pronounced than that caused by L. pomona. From the chinchillas L. canicola was isolated and used to experimentally infect two calves, readily establishing the infection with

localization in the kidney. The dam of the original calf and another cow in the herd were serologically positive for L. canicola. There was also a history of one abortion in the herd and the death of a dog that was in the same area, from liver disease.

In addition to farm animals and man, various surveys have shown the existence of various serotypes of leptospira among wild life Galton (1959). In many cases the exact role of these animals in the epizootiology and epidemiology of leptospirosis is not quite clear. Abdulla, et al. (1962), found that 26 out of 75 deer killed in various parts of Ontario were serologically positive for L. pomona with kidney lesions suggestive of leptospirosis in most of them. Leptospira pomona was isolated in four instances by inoculating kidney tissue suspensions in liquid media. Trainer, et al. (1963), also encountered significant serum titers against L. pomona and L. autumnalis in a ratio of four to one in white tailed deer in Wisconsin. Jackals and hedgehogs were considered as reservoirs for L. canicola in the case of the former and L. canicola and L. grippotyphosa for the latter in Israel and their important role in the epizootiology of the respective diseases was described by van der Hoeden (1955c) and 1958).

Lavrova (1962) found serologic and cultural evidence of leptospiral infections in rodents, namely Microtus arvalis, Pitymys majori, Apodemus agrarius and Rattus norvegicus and

considered them important in the epizootiology of leptospirosis. Parnas, et al. (1961), after a four year survey of swamp fever in Poland, reported that aquatic, field and swamp mammals, principally Mus musculus, Microtus arvalis, Arvicola terrestia and Microtus arviceps were the chief vectors of L. grippotyphosa, L. icterohaemorrhagiae, L. sejroe and L. anatum.

Forty-four out of 820 mammals (or 5.4%) examined by McKeever, et al. (1958), were found positive by isolation from kidney tissue, for various leptospiral serotypes. Opossum were positive for L. ballum and members of the L. mitishyos serogroup, grey fox for L. ballum, striped skunk for L. ballum and L. pomona, wildcat for L. ballum and L. pomona and raccoon for L. ballum, L. pomona, L. australis, L. grippotyphosa and L. hebdomadis serogroup. Reilly (1954) had also furnished serological evidence that the raccoon may be a reservoir for L. canicola.

Some workers have suggested arthropod vectors as having a role in the transmission of leptospirosis. Burgdorfer (1956) was able to infect the Argasid tick Ornithodoros turicata by letting it feed on guinea pigs and hamsters infected with L. pomona. He showed that the organisms persisted in the tissues of the tick for some time. Krepkogorskaia, et al. (1957), isolated L. grippotyphosa from the European tick Dermacentor marginatus S. found on cattle in Russia, where isolated cases of leptospirosis were seen.

Infection in nature takes place through the mucous membranes of the mouth, nose, eyes, and abraded skin, Te Punga and Bishop (1953), Stoenner (1957), and Steele (1958). Donatien, et al. (1951), however, reported that they could infect guinea pigs by feeding emulsified infected liver and kidney in addition to infection by the intranasal route.

Transmission by coitus or by infected bulls' semen was reported by Sleight, et al. (1961). They suggested that the semen became contaminated through contact with urine.

In nature, infection usually takes place either by direct contact with infected urine, voided by infected animals during the period of leptospiruria, Moore, et al. (1956), Te Punga, et al. (1953), Steele (1958), Galton, et al. (1958), Stuart (1956), or by contact with water that has been contaminated by urine from shedding animals, Te Punga, et al. (1953), van der Hoeden (1955), and Williams, et al. (1956). Gillespie, et al. (1957), succeeded in isolating L. pomona from water known to have been contaminated by cattle shedding leptospires in their urine.

The seasonal occurrence of outbreaks of leptospirosis was mentioned in several places in the literature. Mitchell (1959) stated that the majority of outbreaks in Canada occurred during the period of August to December. According to van der Hoeden (1958), outbreaks in Israel were reported mainly during the wet season. Stoenner, et al. (1956), postulated that the confinement of cattle during fall and winter

favored the spread of the disease.

In the individual animal, the manifestations of leptospirosis may be very severe, amounting to a fulminating syndrome with high mortality in young subjects, van der Hoeden (1955), Stoenner, et al. (1956), and Mitchell (1959). The disease, on the other hand, may be mild or even inapparent, and can only be detected by serologic methods, Gochenour (1953), Boulanger, et al. (1958), and Seibold, et al. (1961). Factors like type of host, age and resistance of the individual animal, Faine (1962), and the pathogenicity and virulence of the infecting strain, Alexander, et al. (1956), are thought to influence the outcome of the infection.

Body temperatures of 104-107 F., extreme weakness, depression, fast pulse, diarrhea and stiffness of legs were among the symptoms reported by various workers during the acute febrile phase which usually lasted for one to eight days, Gochenour (1953), Reinhard (1953), Moore et al. (1956), and Steele (1958).

Hemoglobinuria and jaundice, usually seen in young animals, were reported to occur in various outbreaks of leptospirosis, Reinhard (1951), Moore, et al. (1956), van der Hoeden (1955), Turner, et al. (1958), and Boulanger, et al. (1958). These were also produced experimentally in young lambs and pregnant ewes by inoculation of leptospira free filtrates containing hemolysins, Bauer, et al. (1961), and Sleight, et al. (1962), respectively. Alexander, et al.

(1956), discussed differences in hemolysin production among various serotypes and among various strains within serotypes. He thought this to be a function of the virulence of various serotypes and strains which might explain the differences in the severity of diseases produced by homologous serotype strains.

Initial leukocytosis which may be followed by leukopenia was reported, York, et al. (1951); Roberts, et al. (1952); Turner, et al. (1958); Lindqvist, et al. (1958); Sleight, et al. (1961); and Bertók, et al. (1961).

In cattle, abortion as a sequel to the acute clinical leptospirosis was reported by Fennestad, et al. (1956), who could produce it experimentally in two out of 21 pregnant heifers which aborted 23 and 28 days postinoculation, respectively. In many outbreaks of the disease, however, abortion may be the only symptom and according to many workers, it was noticed to take place in the last trimester of pregnancy, Fennestad, et al. (1956); Boulanger, et al. (1957); and Mitchell (1959). Retention of the placenta was reported by Fennestad (1958) and van der Hoeden (1955); mummified fetuses by Te Punga and Bishop (1953).

An atypical flaccid mastitis with a marked drop in milk which becomes thick, yellowish and sometimes blood-stained is being recognized as a manifestation of leptospirosis in lactating cows and by some workers it is considered pathognomonic, Gochenour (1953), Mitchell and

Boulanger (1959). Schnurrenberger, et al. (1961), investigated outbreaks of leptospirosis among 47 herds and reported that 30 herds had abortions as the most predominant sign; 11 herds had flaccid mastitis and six herds had breeding difficulties.

It was repeatedly reported that leptospiral meningitis was a common sequel in man, Saint Martin (1955); van der Hoeden (1955); Bertók, et al. (1961). It was observed in cattle by Hoag, et al. (1954).

Conjunctivitis in dogs and cattle and iridocyclitis in horses as sequels to leptospirosis was described by Cholvin, et al. (1958); Hoag, et al. (1957); and Hensser (1952).

Following infection, leptospires can be demonstrated in the peripheral blood during the acute phase of the disease, Gochenour, et al. (1953), and Moore, et al. (1956). In experimental infections, leptospiremia was demonstrable up to postinoculation (P. I.) day 10, York (1951); Cholvin, et al.(1959); Lindqvist, et al. (1958); and Sleight, et al. (1961). During leptospiremia, isolation can usually be made from many organs, including kidney, liver, spleen and brain. Leptospires, however, tend to disappear from these organs, except the kidney, by the end of leptospiremia, Gochenour, et al. (1953). Sleight, et al. (1961), could isolate L. pomona from the brain up to P. I. day 18. Such localization in the kidney, with tubular degeneration, interstitial nephritis, glomerular degenerative changes and

lymphocytic infiltration were the most consistent pathologic findings in the literature (Baker and Little, 1948; Hadlow and Stoenner, 1955; van der Hoeden, 1955; and Galton, et al. 1958).

Following localization, infected animals shed leptospires in the urine for variable periods and other animals and human beings may be infected by direct contact or indirectly through contaminated water and vegetation, Te Punga (1953); van der Hoeden (1955a); and Gillespie, et al. (1957). Morse, et al. (1957), stated that cattle and swine may shed leptospires for over 90 days.

Impairment of renal function and hepatic function was described by Low, et al. (1956), Gleiser (1957) in experimental dogs and by Arean (1962) in guinea pigs infected with L. icterohemorrhagiae. They were of the opinion that the renal insufficiency was due primarily to a reduction in the tubular maximal excretory capacity, a depression of the glomerular filtration rate and renal plasma flow leading to nitrogen retention.

The same authors also found that the jaundice that was seen in the acute phase of the disease correlated nicely with histopathological findings of hepatocellular injury resulting in functional impairment of the liver. Similar lesions were noticed in sheep, Bauer, et al. (1961), and in cattle and sheep, Sleight, et al. (1962), by inoculating leptospiral hemolysins. The latter, however, did not find

any indications of inflammatory reaction and postulated that the centrilobular necrosis and disruption of hepatic cords were attributable to anoxia as a result of hemolytic anemia.

Infection with a leptospira serotype induces production of antibodies which are detectable in the serum within a few days after the onset of the infection Te Punga, et al.(1953), Boulanger (1958), Mitchell (1959), and Seibold, et al. (1961). In experimentally infected animals, serum antibodies were detected as early as postinoculation day four in ewes with L. pomona, Smith, et al. (1960). In cattle with L. pomona, agglutinins were detectable between seven to ten days after the start of the febrile response, Moore, et al. (1956). Steele (1958) found them generally six to twelve days after onset, reaching maximum titers by the third to the fourth week. Van der Hoeden (1955) reported a titer of 1:20,000 against L. canicola in a sick calf on the sixth day of infection. A horse experimentally infected with L. pomona was serologically positive on the ninth day after the febrile response.

Antibodies can also be demonstrated in the urine, Rudge (1958), which in many instances may interfere with isolation of leptospires from the urine, Stuart (1956). It was reported that infection with one serotype did not protect against another serotype, Menges et al. (1960).

MATERIALS AND METHODS

Ten healthy calves, six males and four females ranging in age from five to eight months and in weight from approximately 250 to 450 pounds were used. The calves were examined for internal parasites and received appropriate anthelminthic treatment. They were serologically tested and found negative for antibodies against L. canicola, L. pomona and L. icterohemorrhagiae. The calves were divided into three groups by random selection. In the first group were calves 915, 924, and 925; in the second, calves 916, 918, and 923; and in the third, calves 919, 920, 921, and 922. Calves 915 from the first, 918 from the second, and 919 from the third group served as controls.

Pre-infection values for erythrocytes, leukocytes, differential leukocyte counts, hemoglobin and packed cell volumes (PCV), were determined at least twice before, and once on the day of infection prior to inoculation. Renal and hepatic functions were similarly evaluated.

Leptospira canicola, strain MOULTON which was propagated in Stuart's medium (Difco) enriched with 10% rabbit serum, was transferred to young hamsters, by intraperitoneal inoculation, where it was maintained by regular passage until the experiment was concluded.

Calves were infected by subcutaneous inoculation of 1.5 ml. of hamsters' heart blood, obtained approximately 48 hours after inoculation and collected under general anesthesia in heparinized syringes. The first group of calves received infected blood from the fourth; the second group from the 16th, and the third group from the 28th hamster passage respectively. Control calves received 1.5 ml. each of noninfected, normal hamster's blood.

Following inoculation, infected and control calves which were properly segregated were closely observed for any manifestations of disease or any deviation from the normal. Body temperatures were taken daily for the first 14 days and after that on various days until the animal was killed, Appendix I.

Blood was collected aseptically from the jugular vein and cultured daily for the first 14 days by placing two to three drops in ten ml. of Stuart's medium (Difco) enriched with 10% rabbit serum. This was then incubated at 30 C. for at least eight weeks and examined weekly for leptospires by dark field microscopy. Simultaneously, blood from each infected animal was inoculated into one hamster on postinoculation days two to five. The heart blood of these hamsters was collected approximately 72 hours later, diluted with saline, centrifuged and the supernatant fluid examined for leptospires by dark field microscopy.

Smears for daily differential leukocyte counts were made from fresh blood, promptly dried and stained with Wright's stain. One hundred cells from each of two slides from each animal were differentially counted, using a battlement system, MacGregor et al. (1940), and the average taken.

Blood samples for hemoglobin (HB) and packed cell volume (PCV) determinations and for erythrocyte and leukocyte counts, were collected in vacuum tubes containing ethylenediaminetetraacetate (EDTA) as anticoagulant.

Hemoglobin values in gm/100 ml. of blood were determined by the cyanmethemoglobin method and the packed cell volume by the capillary tube method.

Both erythrocytes and leukocytes were counted, using an electronic cell counter (Coulter), Mattern, et al. (1957), and Grant, et al. (1960) adjusted to thresholds 10 and 35 respectively and an aperture current setting of seven for both.

Sera were used for blood urea nitrogen (BUN) determination by means of an autoanalyzer, Skeggs, et al. (1957) and the results calculated as mg/100 ml.

Urine samples were collected, and portions from each were diluted in physiological saline and two ml. inoculated intraperitoneally into young guinea pigs weighing approximately 250 gm. The guinea pigs were killed three to four weeks later and their sera used for detection of specific antibodies against L. canicola. Agglutination and/or lysis

of 50% of leptospires in the antigen in a serum dilution of 1:100, was considered positive.

Furthermore, the urines were examined directly by dark field microscopy for leptospires and examined for occult blood, albumins, specific gravity and pH.

All above determinations, along with serological tests of the calves' sera by the microscopic agglutination-lysis test, Morse, et al. (1955), were conducted daily for the first 14 days postinoculation and thereafter at variable intervals until the animal was euthanized.

To assess liver function, ten ml. Bromosulphalein* solution containing 500 mg. phenoltetrabromphthalein-disodiumsulfonate was injected aseptically into the left jugular vein while recording the exact time. Approximately ten ml. of blood from the right jugular vein was collected after about five minutes and a second sample approximately five minutes later, accurately recording the time in minutes and seconds. The sera from the first and second samples were separated and the amount of dye contained in each was determined spectrophotometrically and the values used to determine the half time clearance.

The calves were killed and subjected to a thorough post mortem examination. This was done according to the following schedule:

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*Hynson, Westcott and Dunning, Inc., Baltimore, Md.

		Days Postinoculation
Group 1	Control calf 915	75
	Infected calf 924	75
	Infected calf 925	50
Group 2	Control calf 918	40
	Infected calf 923	40
	Infected calf 916	30
Group 3	Control calf 919	21
	Infected calf 921	21
	Infected calf 920	14
	Infected calf 922	6

At necropsy, samples of bladder urine and portions of liver, spleen, kidney and brain were aseptically collected. These tissues were separately emulsified as 10% by volume in physiological saline and two ml. of each injected intraperitoneally into young guinea pigs which were examined three to four weeks later for specific serum antibody reactions. Samples of lung, brain, liver, spleen, kidney, skeletal and heart muscles, rib, thyroid and adrenal glands, lymph node, testicle and seminal vesicle from males, and ovaries and uterus from females were collected in appropriate fixatives for histopathological examination.

EXPERIMENTAL RESULTS

Following subcutaneous inoculation with infected hamster blood, all infected calves showed a marked thermal response starting as early as 14 hours after inoculation in calves 920 and 921 and within 24 hours in the rest of the calves. The pyrexia continued up to the fifth or sixth day; the highest being on postinoculation (P. I.) days two and three. Temperatures of the three control calves remained normal throughout. Figure 1 shows the temperature curves for the seven infected and three control calves plotted from the group averages on P. I. days one to 13. Appendix 1 shows actual temperatures throughout the experiment.

During the febrile stage, all infected calves were visibly sick; with a rough coat, dry muzzle, anorexia, suppressed rumination, grinding of teeth, sluggish movements, and general lethargy. Respiration was shallow and accelerated and the pulse was pounding and fast. Calves 920, 921, 922, and 924 had diarrhea and calves 916, 920, 923, and 925 showed marked stiffness of all four legs on postinoculation days three, four, and five.

After this acute phase had passed, all infected calves gradually recovered and except for a visible loss in condition they all behaved normally after the seventh day.

Leptospiremia, starting as early as 12 hours (calves 920 and 921) and 24 hours after inoculation for the other five infected calves, was confirmed both by dark field microscopy of blood cultures and by hamster inoculations. Leptospiremia continued up to and including P. I. day four and in one case (922) on P. I. day five, after which no leptospires could be demonstrated, Table 1.

Hematologic Findings

As early as the second day (calves 916, 920, 921), or the third to fourth day after inoculation for the rest of the calves, the hemoglobin, packed cell volume and erythrocyte count started to fall steadily, reaching a maximum reduction around days six to eight. Maximum per cent reductions in hemoglobin, packed cell volume, and erythrocytes for infected and control calves, is shown on Table 2, and is illustrated graphically, Figures 2, 3, and 4. The actual values are shown in Appendices 2, 3, and 4, and the mean and standard deviations in Tables 4 and 5.

No hemoglobinuria and no icterus were noticed on any day throughout the experiment, but occult blood was detected in a few days in the urine of infected calves as summarized on Table 3.

Leukocytes

Five infected calves showed a noticeable increase in the total leukocyte counts, starting on P. I. day two and

amounting to a 60% increase in the case of calf 921, Appendix 5. This leukocytosis continued for about two to three days and was gradually diminished to a leukopenia starting on P.I. day five with lowest values around P. I. day seven, Figure 5, and Tables 4 and 5.

It can be seen from Appendix 6 and Figure 6 that the initial leukocytosis was due to an absolute neutrophilia, which was accompanied by a slight left shift in calves 916 and 921, Appendix 7 and Tables 4 and 5.

All calves had a decrease in the number of circulating lymphocytes starting P. I. days two to four and, except in one calf (924), the lymphopenia continued up to around P. I. day 14. In some of the calves the lymphocytes were reduced to about 30% of their pre-incoulation values, but the overall maximum reduction as calculated from mean values for all calves was about 33%, Figure 7 and Tables 4 and 5. Appendix 8 shows the absolute values of lymphocytes and it can be seen that while there was an absolute lymphopenia, some calves had a relative lymphocytosis after P.I. day five and throughout the period of leukopenia and neutropenia.

Monocytes, on the other hand, Appendix 9, were shown to have a definite rise starting around P. I. day four or five for most of the calves up to around P. I. day six. The control calves, however, showed a similar but less pronounced monocytosis, Figure 8 and Tables 4 and 5. All infected calves experienced an eosinopenia starting P. I.

day two up to P. I. day 13 when the eosinophils started to rise, Appendix 10 and Figure 9, and Tables 4 and 5.

Examination of the Urine

Except for occult blood that was detected on certain days, Table 3, the urine pH and specific gravity, did not show any more fluctuations than those seen in the same animals before inoculation or in the control calves. It may be of interest, however, to note that the lowest values for specific gravity in all infected calves occurred during P.I. days nine to eleven, Appendix 11. No albumin was detected in the urine throughout the experiment.

Renal Function Test

Renal function, measured by the blood urea nitrogen level, before and after the infection did not reveal any impairment. For all intents and purposes, both infected and control calves fluctuated similarly and remained well within the normal levels, Appendix 12. Table 6 shows mean values for blood urea nitrogen (BUN) before and after inoculation.

Hepatic Function Test

Infected calves had variable increases in the half time values for bromosulphalein clearance ranging from 0.54 to slightly over 4.00 minutes. Two of three control calves had increases of 1.32 and 6.8 minutes respectively. With regard to individual calves, the more noticeable increase occurred after P. I. day 13; while in the first 12 days

after infection, the delay was slight, Appendix 13. Table 7 shows mean values before and after inoculation.

All ten calves excreted varying amounts of bromosulphalein in the urine following intravenous injection of the dye. Urine was collected during the interval between the first and second samplings of blood or immediately following the second. The highest amount measured was 7.6 mg. in 62 ml. of urine which comprised about 1.5% of the total amount injected. This was easily noticed because of the purple color it gave to the alkaline urine. Its concentration was measured spectrophotometrically.

Leptospiruria

No leptospires were detected by dark field microscopy in any of the urine samples collected from the calves. Twenty-one of these samples, however, induced serum antibody response in guinea pigs following intraperitoneal inoculation. It was found that leptospiruria occurred in all infected calves between P. I. days 13 and 20. The last leptospiruria detected was on P. I. day 37.

Serum Antibodies

Specific serum antibodies against L. canicola were first detected on day six and reached a maximum between P.I. days 17 and 21. The test was considered positive if agglutination and/or lysis took place in the 1:100 serum dilution, provided it increased on subsequent tests. In

this experiment positive agglutination-lysis was encountered in dilutions of up to 1:1,000,000. Results are summarized in Table 9.

Post Mortem Findings

Carcass condition of four calves, 916, 920, 922, and 923, which were originally in excellent condition, were still quite good, while the three others, 921, 924, and 925, were in a rather poor condition and did not seem to have gained weight after recovery.

Calf 922, which was killed on P. I. day six showed a slight congestion of the liver and kidney, and a slight increase in the pericardial serous fluid. The suprascapular, femoral, popliteal and mesenteric lymph nodes were slightly enlarged and appeared edematous. One calf (921) had a slight endocarditis involving the mitral valve and calf 925 had some fibrinous pleural adhesions. Apart from these, no gross lesions were evident except in the kidneys, which showed greyish lesions varying from barely visible to about two to three mm. in diameter.

Persistence of Leptospires in Tissues

Kidney tissues from five of seven infected calves were shown by guinea pig inoculations to contain L. canicola. These five calves were necropsied within 40 days following inoculation; while guinea pigs inoculated from kidney tissues of

calves necropsied at 50 and 75 days and of control calves were all negative.

Only one guinea pig inoculated with brain tissue from calf 922, killed on P. I. day six was positive in a 1:100 dilution of serum.

All guinea pigs inoculated with homogenized livers and spleens from all calves were serologically negative, Table 10.

Histopathologic Examination

The most extensive lesions were present in the kidneys of calf 921 killed on P. I. day. 21. The renal lesions were characteristically well demarcated and primarily in the cortical region. The lesions were typified by intertubular and periglomerular infiltration of predominantly lymphocytes. In the affected areas the tubules were compressed and to a degree replaced by the inflammatory cells. The glomeruli were shrunken in some instances and Bowman's capsules were thickened. Many of the tubules near the periphery of the lesions were distended, Figures 10 and 11. All of the remaining infected calves, including 922 killed on P. I. day 6, had minimal renal lesions of a type similar to but much less extensive than calf 921, Figures 13 and 17. The control calves had no renal lesions of consequence. There was one small area of lymphocytes located perivascularly in the cortical region of calf 918.

There were a few focal accumulations of lymphocytes and neutrophils in the liver of 922 killed on P. I. day 6, Figures 14 and 15. In the regions of the portal triads of 920 and 922 there were greater numbers of lymphocytes than in the control calves or in the remaining infected calves.

The medulla of one adrenal gland of 922, killed on P. I. day 6, had an area of lymphocytic infiltration, Figure 16.

There were intertubular accumulations of lymphocytes in testicular sections of 921 killed on P. I. day 21. These lesions were not extensive or numerous, Figure 12.

No lesions were observed in the remaining tissues examined in the infected or in the control animals.

DISCUSSION

A marked thermal response with temperatures ranging between 104-107 F. and occurring during the acute phase of the disease was reported in cattle with Leptospira pomona, York, (1951), Gochenour (1953), Moore, et al. (1956), and Mitchell, et al. (1960). Van der Hoeden (1955) had reported high temperatures of up to 106 F. in one calf experimentally infected with L. canicola. Similar reactions were seen in sheep, Morse, et al. (1957), Lindqvist, et al. (1958), and in swine, Sleight, et al. (1960), with L. pomona infections.

In this experiment, the temperature of the infected calves started to rise as early as 14 hours after inoculation and at the same time, leptospires were detected in the circulating blood. This early leptospiremia with marked thermal response a few hours after inoculation is not common with other leptospiral infections in cattle, York (1951) or with L. canicola infections in man and dogs, Bertók, et al. (1961). It seems reasonable to speculate that this outcome may be due to one or more of the following factors. The comparatively large inoculum of hamsters' blood taken at the height of their infection may have had something to do with this. It will be noticed that the experimental group that were infected from the 16th and 28th hamster passage experienced the high

temperature peaks 24 hours before the first group, which was infected with blood from the fourth passage. One may expect enhancement of virulence to hamsters by repeated passage, and hence more leptospires per unit volume of blood, Bertók, et al. (1961).

On the other hand, this unusual response might have been induced because of incomplete adaptability of host and parasite to one another. It was seen in this experiment that L. canicola, killed hamsters in the first few passages around the 4th day following their inoculation. This period however, became gradually shorter and hamsters after the 10th passage were killed in approximately 48 hours. It was also noticed that calves inoculated with hamster's blood from the later passages had more heightened response and an earlier leptospiremia than the first group. These findings may indicate an enhancement of virulence to cattle also, with evident shortening of the lag phase after inoculation. It is postulated that this unusual response may have been responsible for the calves' ability to limit the progress of the infection.

All other symptoms of anorexia, accelerated pulse, lethargy and stiffness of legs were reported in the literature to occur during the acute phase of leptospirosis in calves, York (1951), van der Hoeden (1955), and Moore, et al. (1956). In this experiment such symptoms could have been caused by the fever alone.

The hemoglobinuria and jaundice that had been described to occur in calves during the acute phase of natural infection with L. canicola, van der Hoeden (1955) and (1956) and Turner, et al. (1958), and also with L. pomona, Reinhard (1951), Gochenour (1953),and Mitchel, et al. (1960), were not encountered in any of the calves in this experiment. Anemia, on the other hand, as a result of erythrocyte loss as indicated by successive counts and reduction in hemoglobin and PCV, apparently through hemolysis, was a common finding in all infected calves, but the reductions were not as great as those reported by Turner, et al. (1958).

The fact that no isolations were made from liver and spleen as early as P. I. day six, indicated that leptospires disappeared from these organs as early as they did from the circulating blood. Leptospira canicola was shown to be present in the brain on day six but no lesions either gross or microscopic were seen in brain or meninges. It may, therefore be speculated that localization in the brain, which is known to occur in humans, Dvoskin, et al. (1956), does not commonly occur in L. canicola infection in cattle.

Localization of L. canicola in the kidneys of cattle and its subsequent shedding in the urine for sometime after recovery, confirms van der Hoeden's findings (1955) and (1956) and those of Turner, et al. (1958). The shortness of the period of shedding being up to P. I. day 37 as compared to periods quoted for L. pomona, Morse, et al. (1957), was

evidently a result of the comparatively less extensive localization in the kidney seen in this experiment. This was evidenced by negative renal function tests and the freedom of urine from signs of nephritis, as can be seen from Table 6 and Appendix 11. Apparently serious damage to the kidney tubules, that was reported by Low, et al. (1956), Gleiser (1956), and Arean (1962) with L. icterohaemorrhagiae, did not occur in this experiment. This again could be attributed to the exaggerated response of the host marked by a high fever, early cellular response, early antibody production, and probably a parallel tissue immunity that may have limited the development of lesions.

Results obtained from the liver function tests in the seven infected and three control calves were not consistent and were therefore difficult to interpret. All calves had pre-inoculation clearance times comparable to the normals described by Cornelius and Kaneko (1963). Apart from a slight delay seen between days three and six, most of the calves had an increase in the 1/2 clearance time ranging from .54 to 4.00 minutes and two of three control calves showed a delay of 1.32 and 6.8 minutes respectively, Table 7 and Appendix 13. Except for this slight increase between days three and six, most of the calves had a relatively higher increase between P. I. days 13 and 20, and for the controls on days 30 and 40 respectively.

It is surmized that the delay in clearance shown after P. I. day 13 would not be accounted for by an inflammatory

hepatocellular injury that was described to occur in dogs infected with L. icterohaemorrhagiae, Low, et al. (1956), Gleiser, et al. (1957), or in guinea pigs, Arean (1962). This is based on the minimal gross and microscopic hepatic lesions, Figures 14 and 15, and on the finding that no leptospires could be demonstrated in the liver on P. I. day six, and were therefore assumed to have disappeared by the end of leptospiremia, and the simultaneous appearance of serum antibodies. Similar findings were reported with L. pomona by Gochenour, et al. (1953), and Sleight, et al. (1961).

It also appears that the relatively low hemolytic qualities exhibited by L. canicola in this experiment, as indicated by the comparatively less severe reductions in blood values and the absence of hemoglobinuria and jaundice, would not be expected to result in the severe hepatic degeneration and centrilobular necrosis that were reported to occur with L. pomona hemolysin in cattle and sheep, Sleight, et al. (1962).

Figures 14 and 15 are photomicrographs of sections taken from the liver of calf 922 killed on P. I. day six. The only lesions seen consisted of focal accumulations of neutrophils and lymphocytes. In the remaining experimental calves these accumulations were either negligible or absent. No signs of necrosis or disruption of hepatic cords were seen in any of the calves.

Variable amounts of bromosulphalein (B.S.P.) were recovered in the urine of all calves. Some workers have shown that the dye, following a single intravenous injection is practically exclusively taken up by the liver and excreted in the bile. Rosenthal and White (1925) found that extirpation of the liver left the B.S.P. almost *in toto* in the blood during the early period following injection. They also reported that if any, only traces are excreted in the urine. Cantarow, *et al.* (1941), found that practically 100% of the dye was removed from the blood by the liver in the first 30 minutes. The same was reported by Pratt, *et al.* (1952), and Cornelius and Wheat (1957), who described extrahepatic excretion as insignificant.

Negligible amounts of B.S.P. in the urine of human beings following single intravenous injection was reported by Wheeler, *et al.* (1960). Carbone, *et al.* (1959), produced experimental data showing that small amounts of up to 2.7 $\pm$ 1.1 mg/100 ml. are excreted in the urine of normal animals while in others with hepatitis and obstructive conditions of the liver, up to 26.2 $\pm$ 5.1 mg/100 ml. were excreted in the urine. Klein, *et al.* (1933), on the other hand, claimed that a portion of the dye is taken up by the reticuloendothelial system, especially in the spleen, a finding which was also reported by Moses, *et al.* (1948). Dogs with experimental obstructive jaundice were shown to excrete in the urine from 30 to 50% of the amounts of B.S.P. originally injected, Giges, *et al.* (1952).

Brauer, et al. (1955), found that substantial amounts of B.S.P. were stored in various tissues following continuous infusion, and that the kidney had 5.3% and the urine from .2 to 1.7% of the amounts infused. He thought that excretion in the urine was sporadic and its occurrence unpredictable.

In this experiment the B.S.P. recovered in one instance was approximately 7.6 mg. in a 62 ml. urine sample. This might not have been the total amount in the bladder at the time of collection. There was no evidence of renal insufficiency or of serious hepatic dysfunction at the time the samples were taken as indicated by the various tests. In view of this, it is speculated that cattle may normally excrete in the urine, amounts of B.S.P. that may affect the sensitivity of the test in this species. More controlled experimental work is definitely needed to determine the role of the kidney in the excretion of B.S.P. in cattle.

Slight initial leukocytosis, which was in some instances followed by a slight leukopenia, had been reported in various animal hosts and man, York, et al. (1951); Roberts, et al. (1952); Lindqvist, et al. (1958); Sleight, et al. (1960); and Bertók, et al. (1961).

In this experiment, there was a noticeable leukocytosis amounting to about 160% of the pre-inoculation values in some calves, followed by a total leukopenia with up to 55% of the leukocytes disappearing, Figure 5. Of interest,

however, was the marked neutrophilia which reached about 400% in one case and averaged 230% of the pre-inoculation values for the seven infected calves during the first two to three days, Figure 6, Appendix 6. Once more the neutropenia was quite marked after postinoculation day five. It will also be noticed that the extremes of neutrophilia and neutropenia were encountered in the calves receiving the higher passage inoculum. This might be interpreted as either a true defense response against the increasing number of leptospires, or as a result of the febrile condition in calves.

Another interesting feature of the animal response was the monocytosis that was seen in all the infected calves and, to a certain extent, in the control calves. In some infected calves the monocytes reached over 20% on certain days during the leukopenia; but since a slight monocytosis was evidenced in control calves, it might be suggestive that at least in part, this could be a response to some factor in the hamsters' blood, Figure 8, Appendix 9.

Absolute lymphopenia was seen both during the initial leukocytosis and the subsequent leukopenia. Lymphocytes have been reported as part of the reaction in various tissues, i. e., kidney, in leptospiral infection. The only correlation between a similar lymphocytic infiltration of some tissues in this experiment Figures 10 through 17, and the number of circulating lymphocytes was a relative lymphocytosis, Appendix 8.

One calf, 921, killed on P. I. day 21 had the most extensive kidney lesions. These consisted of intertubular and periglomerular infiltrations, predominantly by lymphocytes. Tubular and, to a lesser extent, glomerular degenerative changes were seen in the affected areas primarily in the cortex, Figures 10 and 11. Renal lesions of the remaining calves were similarly typified but much less extensive, Figures 13 and 17. These findings seem to be in conformity with the short period of localization of L. canicola in the kidney and the short period of leptospiruria, seen in this experiment.

Intertubular accumulations of lymphocytes were seen in testicular sections of calf 921, killed on P. I. day 21, Figure 12. These were not extensive but they may, however, suggest a tendency of L. canicola to localize in testicular tissue. The extent and significance of this in connection with transmission by coitus may only be verified by further experimental work with adult bulls. Similar findings were reported to occur in male cattle experimentally infected with L. pomona, Atallah (1963).

All calves had a marked antibody response against L. canicola and their sera were positive in dilutions ranging from 10^1 to 10^6. Weak positives in dilutions of 1:10 were first noticed on P. I. day five. These were not considered significant until day six when positive agglutination-lysis reactions were obtained in serum dilutions of 1:100 and more,

reaching a maximum around the second to third week after infection. Similar findings were reported before with L. pomona infections, Moore, et al. (1956); Boulanger (1958); and Steele (1958).

The immune response in all infected calves is also comparable with the early thermal and cellular responses; antibodies having been detected as early as P. I. day five and reaching a maximum also quite early. This may further explain the termination of leptospiremia by P. I. day five and the short duration of renal localization.

SUMMARY AND CONCLUSIONS

An experiment was conducted on ten calves, seven of which were infected with L. canicola, to obtain valid experimental data on infection in cattle caused by this serotype. The following were considered to be the most pertinent aspects of the experimentally produced disease.

1. An initial acute febrile stage of short duration, characterized by a high temperature and other accompanying symptoms.
2. Anemia with moderate reduction in blood values occurred, but there was neither hemoglobinuria nor jaundice.
3. Leptospiremia was evidenced between P. I. days one to five followed by clinical recovery.
4. Cellular response was evidenced by initial leukocytosis and neutrophilia followed by a total leukopenia with relative lymphocytosis.
5. Humoral antibody response starting day six and reaching a maximum between days 12 to 21 with titers up to 10^6.
6. Evidence of localization in the kidney with subsequent shedding in the urine for 37 days as determined by guinea pig inoculations.

7. There was no impairment of renal function and only a slight impairment to liver function.
8. Gross and microscopic lesions were practically confined to the kidney.

The experiment confirms earlier findings by van der Hoeden, Turner, et al. and others, that L. canicola can establish itself in cattle; and, although clinical recovery took place promptly in all infected calves in this experiment, yet the apparent retardation to growth was quite substantial. Valuable experimental data on the course of infection, fate of L. canicola in cattle and the behavior of the latter as host species, were gained. This was definitely needed to remove, in part, the deficiency in the literature concerning this serotype in cattle.

Moreover, localization in the kidneys with subsequent shedding in the urine, increases the hazard to man, cattle, and other animal species, if proper precautions are not taken.

TABLE 1.--Summary of Leptospiremia, Leptospiruria, and Serum Antibody in Infected Calves

Calf No.	Postinoculation Days					
	First Lepto-spiremia	End Lepto-spiremia	First Lepto-spiruria	End Lepto-spiruria	First Serum Antibody	Maximum Serum Antibody
924	2	4	17	37	6	20
925	2	4	20	28	5	20
923	2	4	20	32	6	17
916	2	4	14	up to 30	6	21
921	1	4	14	up to 21	6	21
920	1	4	13	up to 14	6	11
922	2	5	...	...	...	...

TABLE 2.--Summary of Reductions in Hematologic Values of Experimental Calves

Calf No.	Values Tested	Average Values A.I.**	Lowest Values P.I.***	Amount Reduction	Percentage Reduction
915*	Hb.°	12.00	11.60	.40	3.3
	P.C.V.	32.80	32.00	.80	2.4
	R.B.C.x10^3	10056	9340	716	7.1
924	Hb.	11.80	8.40	3.40	28.8
	P.C.V.	33.60	22.00	11.60	34.5
	R.B.C.x10^3	9685	6940	2745	28.2
925	Hb.	12.20	9.20	3.00	24.6
	P.C.V.	32.50	25.00	7.50	23.0
	R.B.C.x10^3	8860	6815	2045	23.0
918*	Hb.	10.40	9.60	.80	7.7
	P.C.V.	30.50	28.50	2.00	6.5
	R.B.C.x10^3	7815	7220	595	7.6
923	Hb.	11.60	10.10	1.50	13.0
	P.C.V.	33.00	27.00	6.00	18.1
	R.B.C.x10^3	8745	7000	1745	20
916	Hb.	12.5	9.00	3.50	28.0
	P.C.V.	35.0	25.00	10.00	28.5
	R.B.Cx10^3	8510	6100	2410	28.3
919*	Hb.	10.30	10.20	.10	1.0
	P.C.V.	30.00	28.50	1.50	5.0
	R.B.C.x10^3	8180	7640	540	6.6
921	Hb.	10.30	8.10	2.20	21.3
	P.C.V.	30.00	24.50	5.50	18.3
	R.B.C.x10^3	8570	6 80	2490	29.0
920	Hb.	13.50	9.70	2.80	20.7
	P.C.V.	36.50	27.00	9.50	26.0
	R.B.C.x10^3	8720	6250	2470	28.0
922	Hb.	11.30	10.00	1.30	11.5
	P.C.V.	33.00	26.00	7.00	21.2
	R.B.C.x10^3	7840	6870	970	12.3

* Control calves
** A.I. = Ante-inoculation
*** P.I. = Post-inoculation

°Hb. gm. hemoglobin/100 ml. blood

TABLE 3.--Postinoculation Days on Which Occult Blood was Demonstrated in Urine of Infected Calves

Calf No.	Postinoculation Days															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	17	20
924	...	...	...	...	...	...	...	+	+	+	+	...	...	...	...	...
925	...	...	...	...	...	...	...	+	+	+	...	...	+	+	+	...
923	...	...	...	...	...	...	+	...	...	...	+	...	...	...	...	+
916	...	...	...	...	...	+	+	+	...	...	+	+	...	...	+	...
920	...	...	...	+	+	+	+	...	...	...	...	...	...	...	...	...
921	...	...	+	+	+	+	...	+	...	...	...	...	...	...	...	+
922	...	...	...	...	+	+	...	...	...	...	...	...	...	...	...	...
915*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
918*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
919*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

*Control calves

TABLE 4.--Mean ($\bar{X}$) and Standard Deviation ($\hat{O}$) of the Absolute Leukocyte Counts and Hematologic Values of Control Calves

Factors	Pre-Inocul.	Days Postinoculation 1	2	3	5	7	9	11	13
Total Leukocytes /mm^3	11352 ±525	11989 ±616	11938 ±945	12349 ±908	12886 ±882	11086 ±1436	11160 ±1126	11489 ±562	11361 ±900
Neutrophils/mm^3.	4033 ±285	3962 ±571	3570 ±788	4766 ±1600	4329 ±1393	2774 ±547	3003 ±556	3030 ±1884	3282 ±1692
Lymphocytes/mm^3.	6371 ±758	6867 ±486	6904 ±454	5810 ±904	7145 ±1435	7023 ±398	6956 ±560	7230 ±1902	7053 ±2262
Monocytes/mm^3.	601 ±106	762 ±172	832 ±321	817 ±325	905 ±186	846 ±211	939 ±568	1034 ±319	708 ±22
Eosinophils/mm^3.	304 ±181	395 ±161	590 ±484	665 ±492	428 ±333	440 ±400	300 ±243	193 ±73	216 ±172
Hemoglobin, gm./100ml.	10.90 ±.95	10.66 ±.85	11.60 ±.72	10.95 ±.56	11.08 ±.97	10.76 ±.97	11.03 ±.75	10.60 ±1.00	10.70 ±.90
Packed Cell Volume	31.10 ±1.50	31.33 ±1.25	32.82 ±2.00	31.66 ±1.50	31.33 ±2.40	30.66 ±2.00	31.66 ±1.40	31.50 ±2.20	32.00 ±3.60
Red Blood Cells millions/mm^3.	8.851 ±1.49	8.348 ±1.26	8.588 ±1.12	8.416 ±1.11	8.215 ±1.08	8.251 ±1.24	8.316 ±1.21	8.142 ±1.43	8.105 ±1.51

TABLE 5.--Mean ($\bar{X}$) and Standard Deviation ($\hat{O}$) of the Absolute Differential Leukocyte Counts and Hematologic Values of Infected Calves

Factors	Pre Inocul.	Days Postinoculation 1	2	3	5	7	9	11	13
Leukocytes/ mm^3.	10123 ±1940	10130 ±2555	11595 ±2657	11498 ±2328	8757 ±2666	7191 ±2570	7897 ±1518	8010 ±1213	5356 ±1410
Neutrophils/ mm^3.	2551 ±577	2663 ±1222	5223 ±2987	5851 ±2425	2482 ±1406	1392 ±654	1996 ±515	1936 ±548	2256 ±955
Lymphocytes/ mm^3.	6588 ±965	6115 ±1898	4744 ±2087	4453 ±1540	4683 ±1490	5191 ±1701	5087 ±923	5481 ±914	5037 ±1312
Monocytes mm^3.	660 ±316	546 ±228	572 ±241	628 ±336	1504 ±695	705 ±216	785 ±170	647 ±94	515 ±138
Eosinophils mm^3.	379 ±207	574 ±427	353 ±286	284 ±410	178 ±245	124 ±56	156 ±62	167 ±107	416 ±220
Hemoglobin gm./100ml.	11.88 ±1.00	11.22 ±.80	10.90 ±.80	10.43 ±.65	9.86 ±.90	9.67 ±.85	9.80 ±1.00	10.00 ±.70	9.78 ±.66
Packed Cell Volume	33.37 ±2.48	32.43 ±2.62	32.20 ±1.00	29.60 ±2.00	27.79 ±1.77	26.66 ±2.60	28.00 ±3.50	27.66 ±2.00	28.50 ±2.50
Red Blood Cells millions/mm^3.	8.704 ±.55	8.396 ±.507	7.891 ±1.00	7.525 ±.78	6.948 ±.50	6.996 ±.54	7.017 ±.19	7.172 ±.40	7.045 ±.39

TABLE 6.--Summary of Renal Function Test; Mean Values of B. U. N**Before and After Infection, in mg/100 ml. of Blood

Number	Mean for Pre-Inoc.	Mean for Postinoc.	Maximum Level Postinoc.	Minimum Post-inoc.	Day of Maximum Level
924	20.50	17.00	22.50	10.00	2
925	17.00	14.10	18.00	8.00	37
923	18.75	15.30	17.50	11.00	1,4,5,20
916	14.00	14.60	16.50	11.50	20
920	12.50	14.60	17.50	12.50	3
921	12.50	17.30	21.00	12.50	3
922	13.20	16.00	20.00	13.00	4
915*	11.75	13.30	16.50	8.00	9
918*	16.50	17.70	23.00	10.00	8,9
919*	14.00	12.80	17.00	9.00	1

*Control calves

**B.U.N. = Blood Urea Nitrogen

TABLE 7.--Summary of Liver Function Test; Mean, Pre- and Post-inoculation Half Times for Clearance of Bromsulphalein in Minutes

Calf No.	Mean Pre-Inoc. Half Time (m)	Mean Postinoc. Half Time (m)	Maximum Increase in Minutes	P.I. Day of Maximum Increase
924	3.68	4.05	2.64	20
925	4.32	4.83	2.00	20
923	2.13	3.01	1.63	17
916	3.99	4.38	2.71	14
921	4.58	4.21	.63	3
920	3.45	4.73	4.07	14
922	3.75	4.36	.54	5
915*	3.94	4.94	1.32	13
918*	5.18	5.68	6.80	40
919*	4.08	3.40	.27	3

*Control calves
m = minutes

TABLE 8.--Duration of Leptospiruria in Experimental Calves Confirmed by Guinea Pig Inoculations

Post-inoc. Days	Days Postinoculation									
	915*	924	925	918*	923	916	919*	921	920	922
1-12	...	...	...	...	...	...	...	...	...	...
13	...			...	...	...	...	...	+	
14				...	...	+	...	+	+	
17	...	+	...	...	...	+	...	+		
20	...	+	+	...	+	...				
21							...	+		
24	...	+	+	...	+	+				
27				...	...	+				
28	...	...	+	...	+					
30	...	+	...	...	...	+				
32	...	...	...		+					
37	...	+	...	...	...					
40	...	...	...	...	...					
43	...	...	...							
50	...	...	...							
63	...	...								
75	...	...								

+ = Positive agglutination-lysis in serum dilutions of 1:100 or more.
* Control animals

TABLE 9.--Antibody Titers** for L. Canicola in Sera of Infected Calves

Post-inoc. Day	Calves									
	915*	924	925	918*	923	916	919*	921	920	922
1-5	...	...	...	...	...	...	...	...	...	...
6	...	...	+2	...	+2	+2	...	+2	+2	+
7	...	+2	+2	...	+2	+1	...	+3	+3	
8	...	+2	+3	...	+4	+3	...	+4	+5	
9	...	+2	+3	...	+4	+3	...	+4	+4	
10	...	+2	+3	...	+5	+3	...	+4	+5	
11	...	+3	+3	...	+5	+4	...	+4	+4	
12	...	+3	+3	...	+5	+4	...	+4	+4	
13	...	+3	+3	...	+5	+4	...	+4	+4	
14	...	+3	+4	...	+5	+4	...	+4	+4	
17	...	+3	+4	...	+6	+4	...	+4		
20	...	+4	+5	...	+5	+4				
21	...			...		+6	...	+4		
24	...	+4	+5							
27	...	+4	+5	...	+4	+4				
28	...	+4	...							
30	...		+4	...	+4	+4				
32	...	+4	+4							
37	...	+4	+4	...						
40	...	+4			+3					

TABLE 9.--Continued

Post-inoc. Day	Calves									
	915*	924	925	918*	923	916	919*	921	920	922
43	...	+3	+4							
50	...	...	+3							
63	...	+3								
75	...	+3								
Killed	75	75	50	40	40	30	21	21	14	6

*Control calves
**The titers are expressed as the exponents of the highest ten-fold serial serum dilution where agglutination-lysis occurred.

TABLE 10.--Summary of Persistence of L. Canicola in Tissues of Infected Calves

Case No.	Kidney	Liver	Spleen	Brain	Days Post-inoculation
915*	...	...	...	...	75
924	...	...	...	...	75
925	...	...	...	...	50
918*	...	...	...	...	40
923	+	...	...	...	40
916	+	...	...	...	30
919*	...	...	...	...	21
921	+	...	...	...	21
920	+	...	...	...	14
922	+	...	...	+	6

*Control calves

+Positive by guinea pig inoculations. Agglutination and/or lysis of L. canicola antigen by the guinea pigs sera in dilutions of 1:100 or more was considered positive.

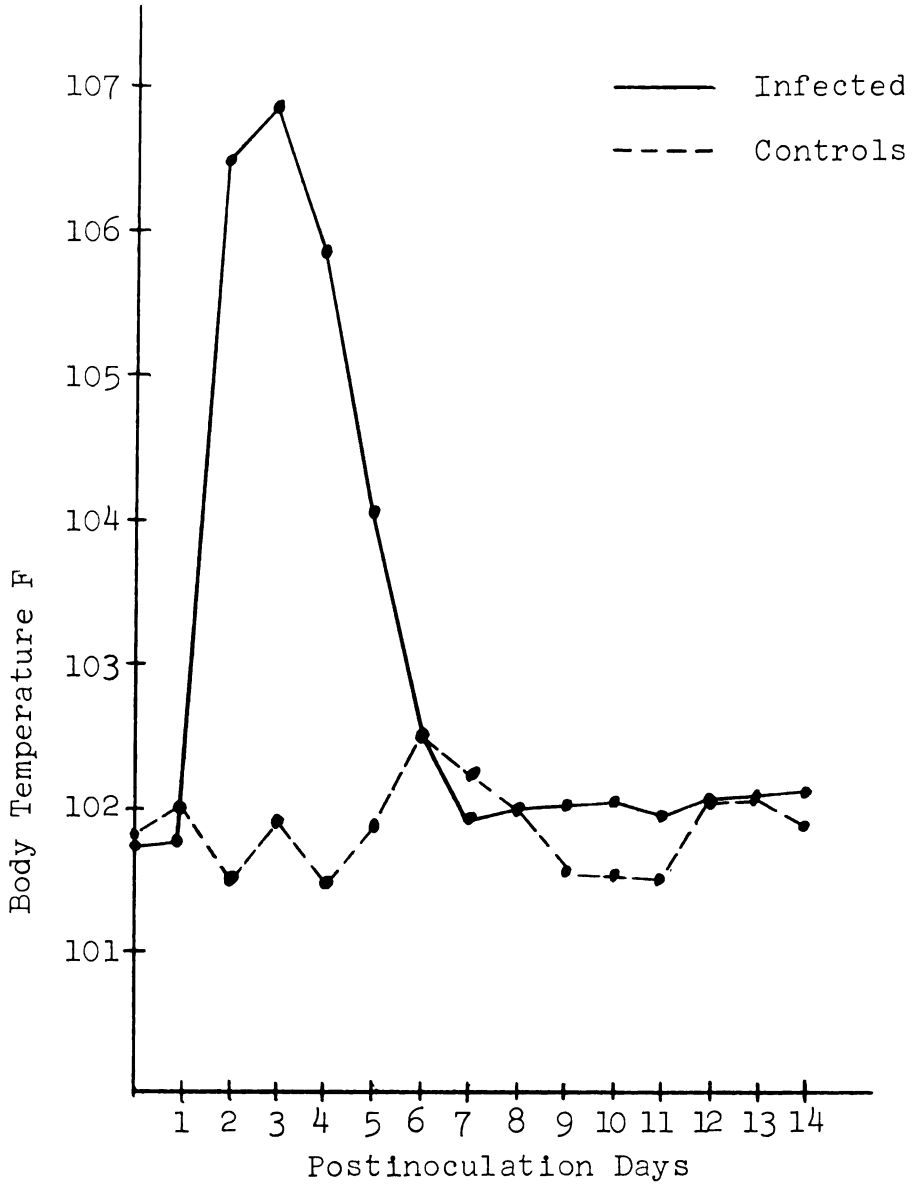

Figure 1.--Mean Values of Daily Temperatures of Experimental Calves

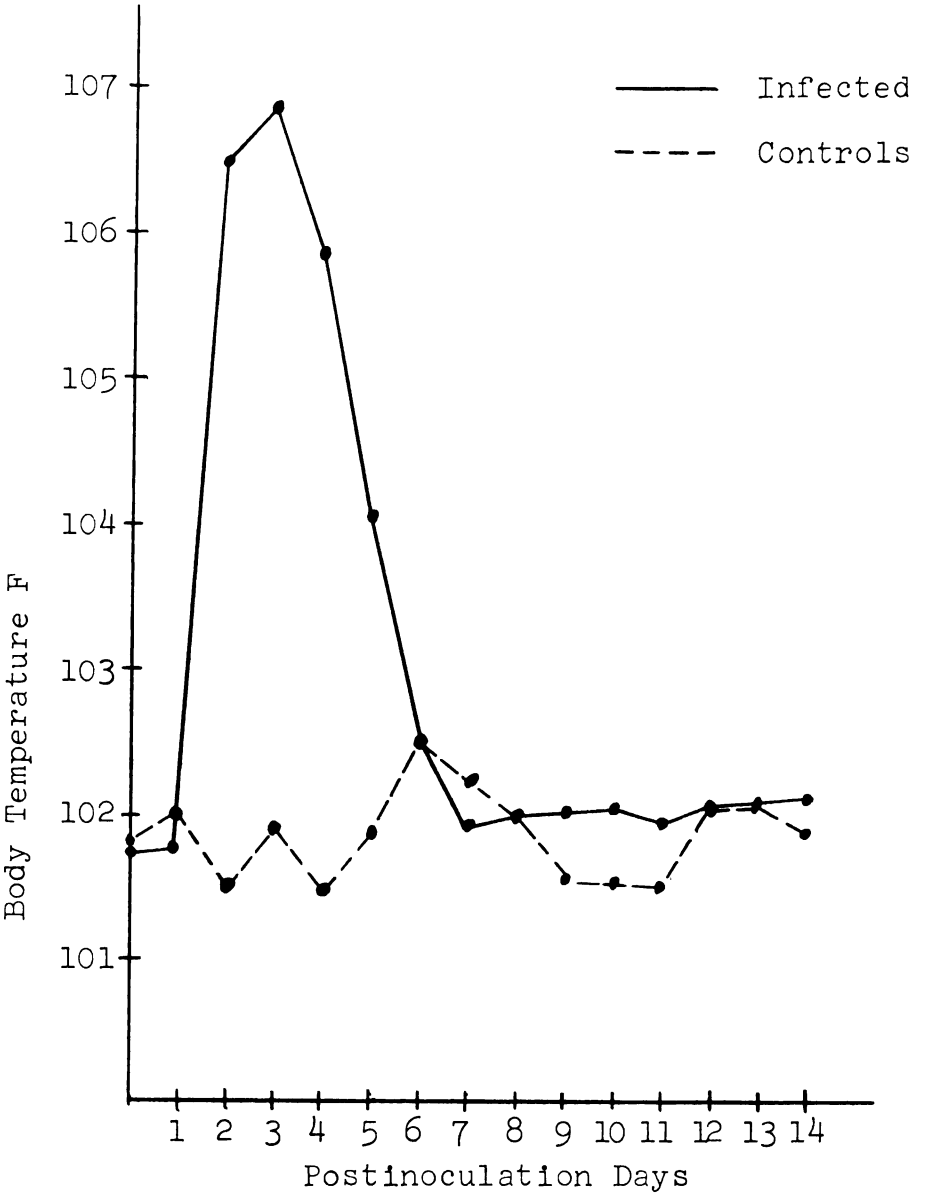

Figure 1.--Mean Values of Daily Temperatures of Experimental Calves

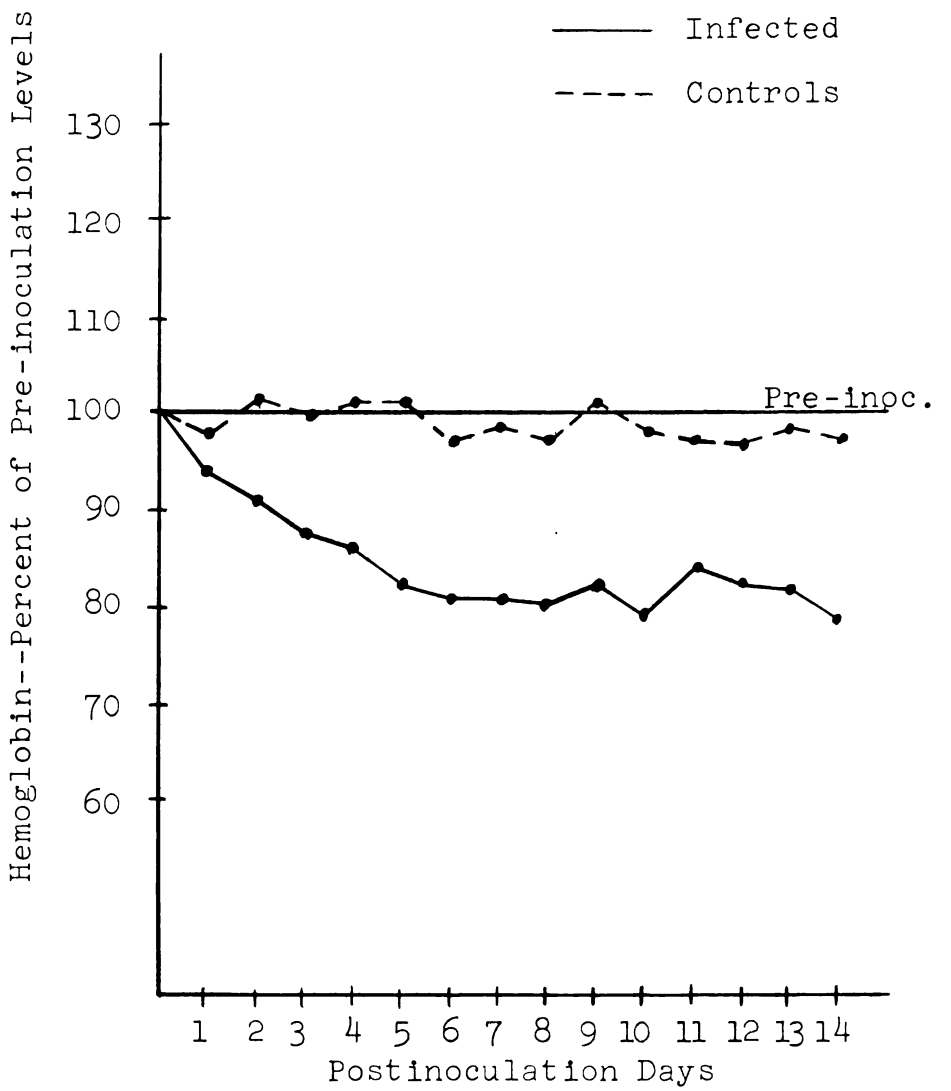

Figure 2.--Mean Hemoglobin Levels of Experimental Calves, Expressed as Percent of Pre-inoculation Values

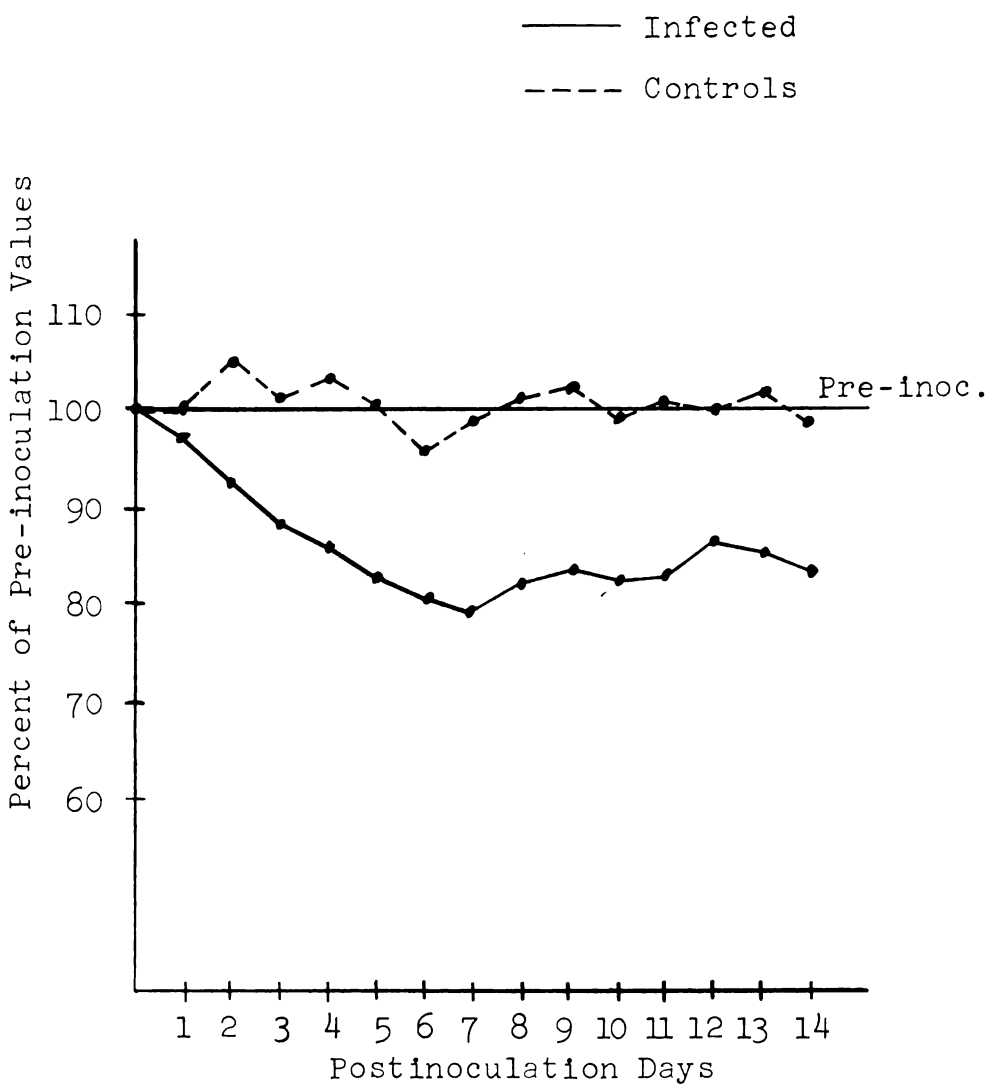

Figure 3.--Packed Cell Volume; Daily Mean Values, Expressed as Percent of Pre-inoculation Levels

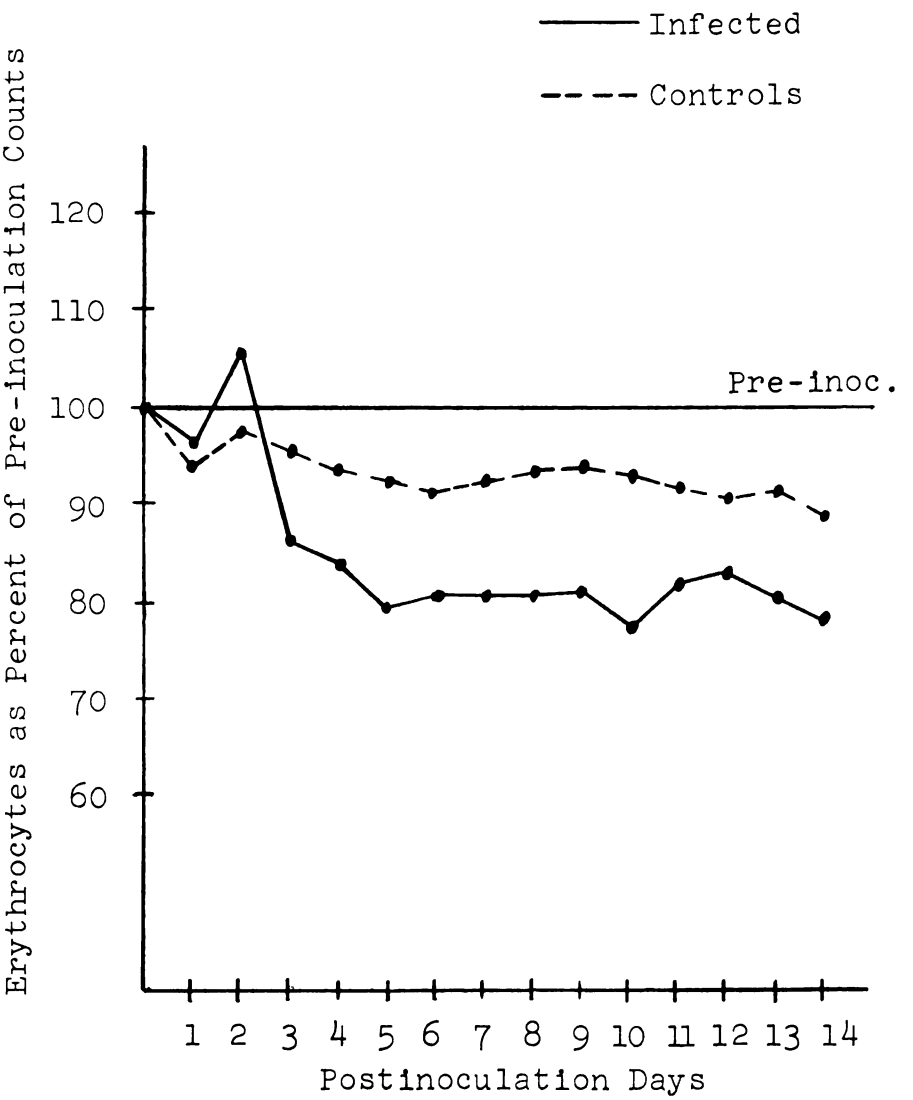

Figure 4.--Mean Values of Daily Erycthrocyte Counts Expressed as Percent of Pre-inoculation Levels

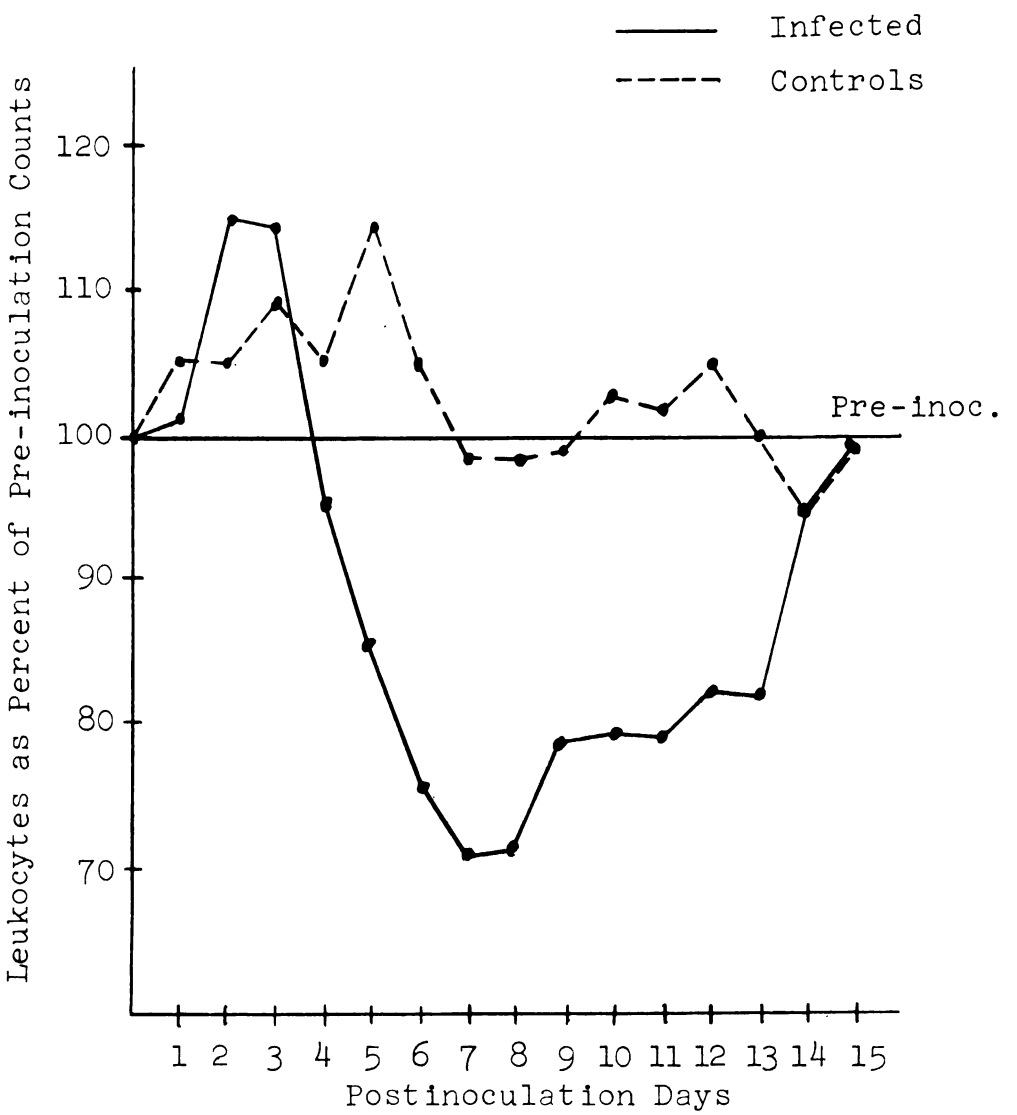

Figure 5.--Mean Values of Daily Leukocyte Counts of Experimental Calves, Expressed as Percentage of Pre-inoculation Levels

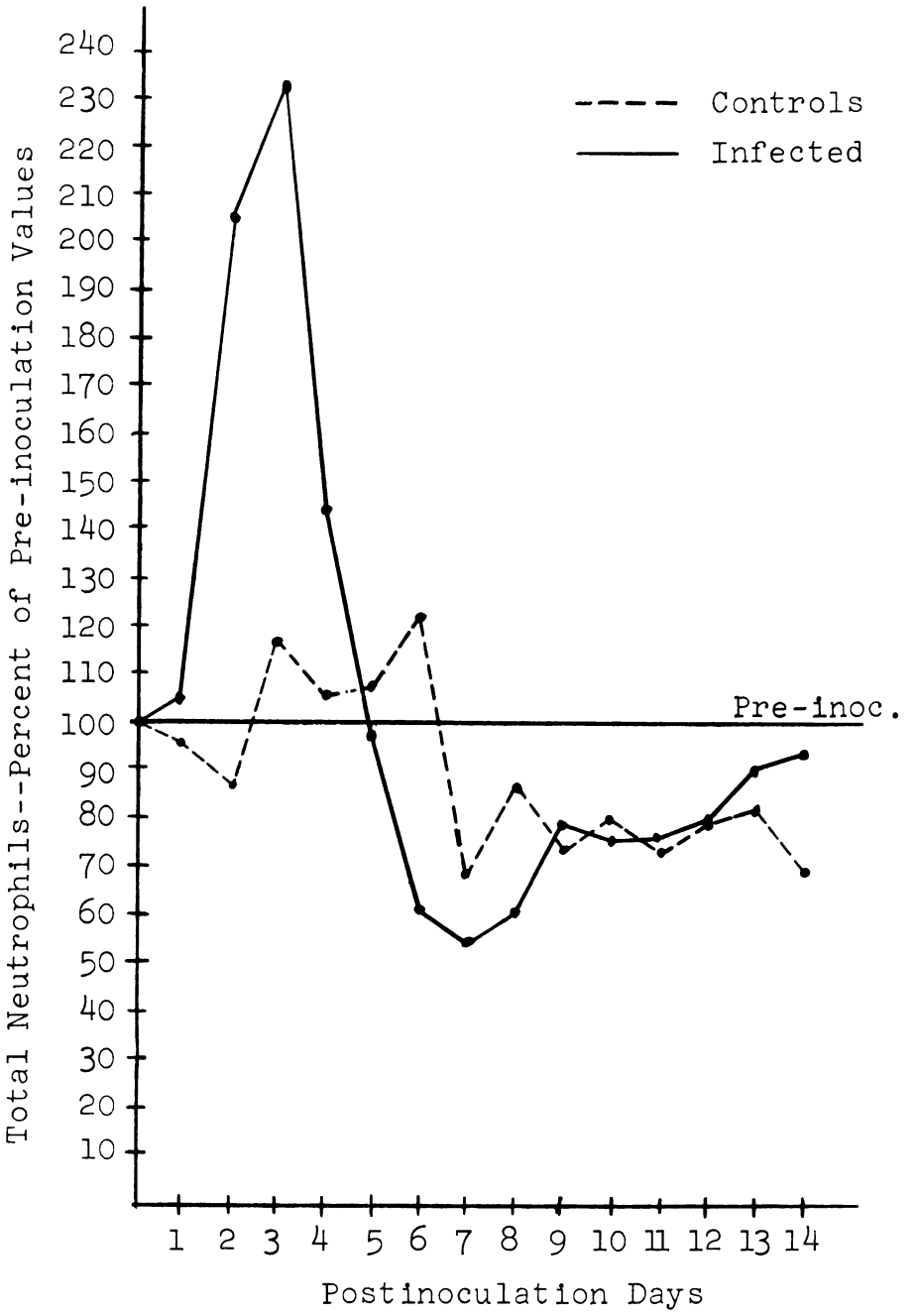

Figure 6.--Daily Mean Values for Total Neutrophils Expressed as Percentages of Pre-inoculation Values

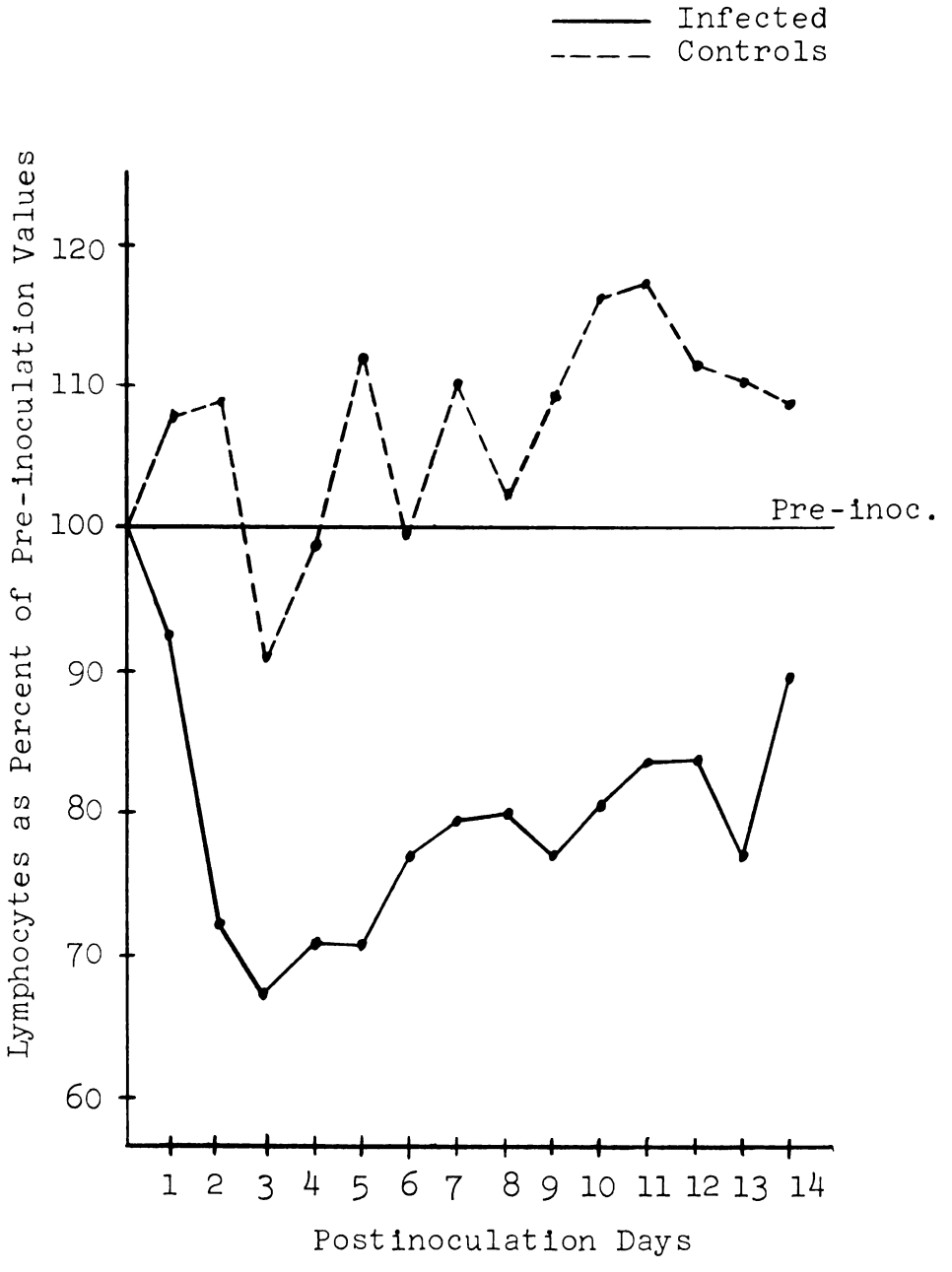

Figure 7.--Daily Mean Values of Lymphocyte Counts Expressed as Percentages of Pre-inoculation Values

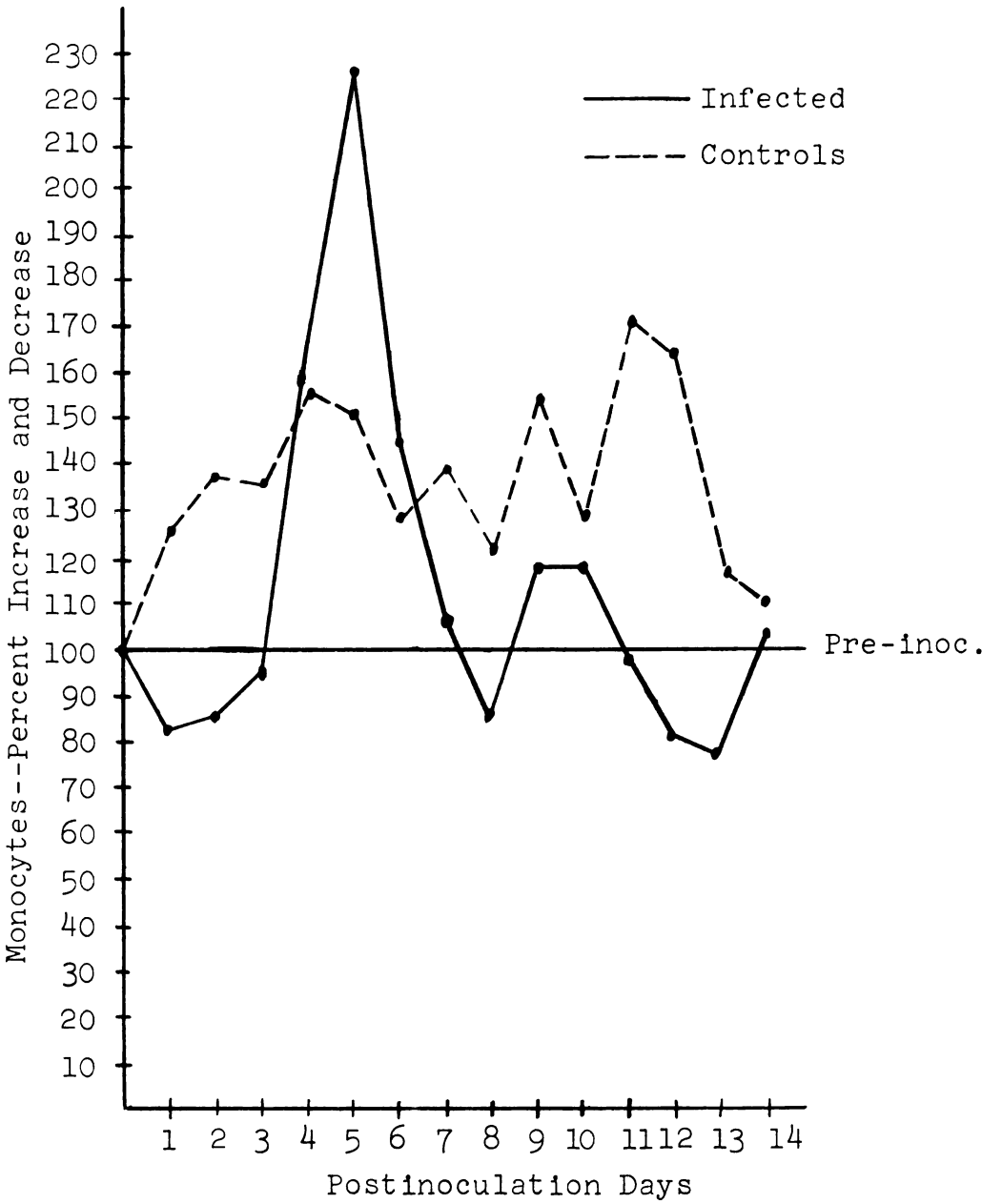

Figure 8.--Showing Daily Mean Values of Monocytes Expressed as Percent of Pre-inoculation Counts

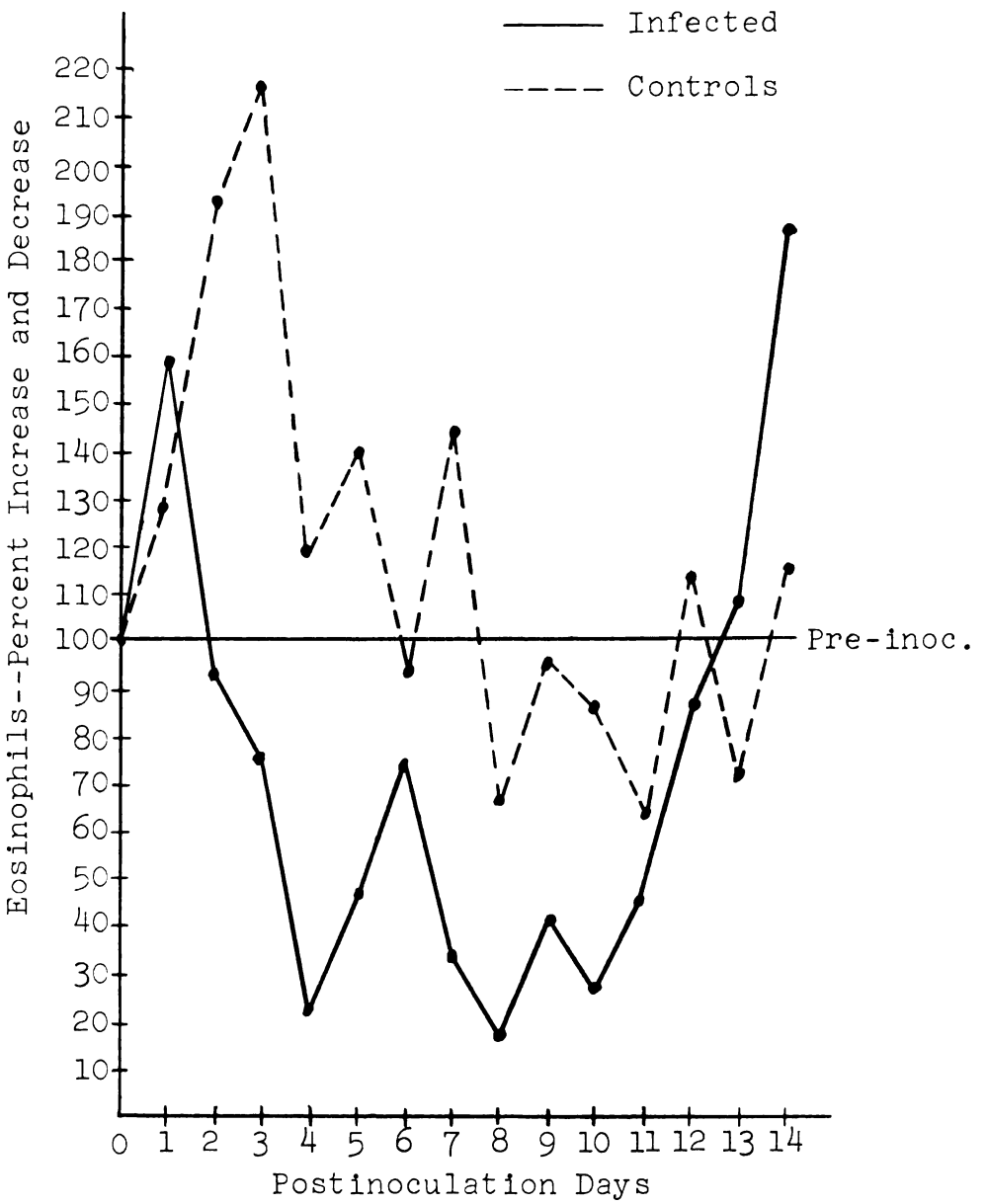

Figure 9.--Eosinophils Mean Values of Daily Counts, Expressed as Percentages of Pre-inoculation Levels

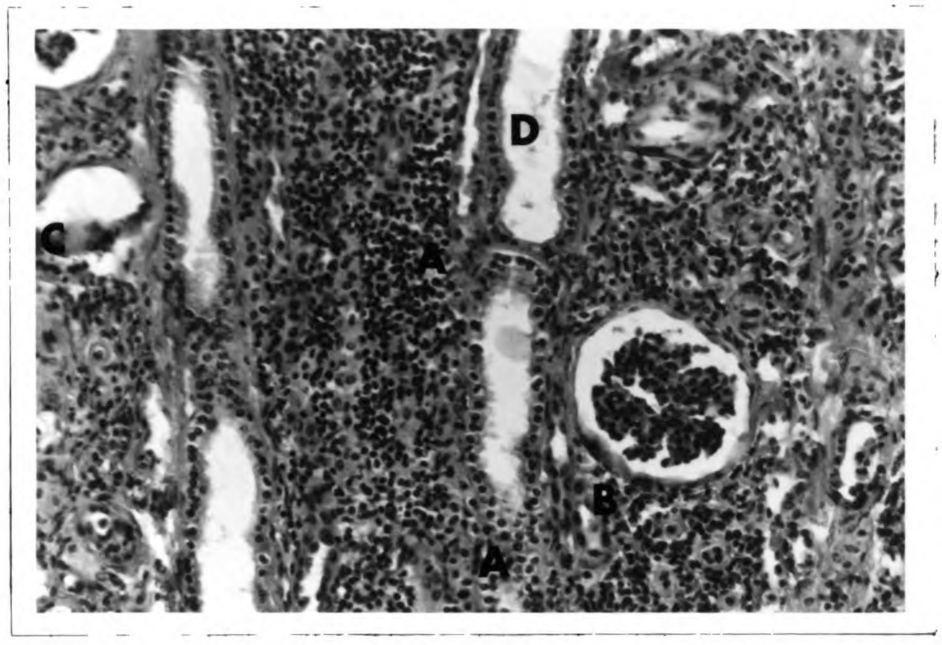

Figure 10.--Kidney Section, Calf 921. Killed on P.I. day 21 to show (A) intertubular infiltration, (B) periglomerular infiltration predominantly by lymphocytes, (C) shrunken glomerular tuft, (D) dilated tubule. x 187.

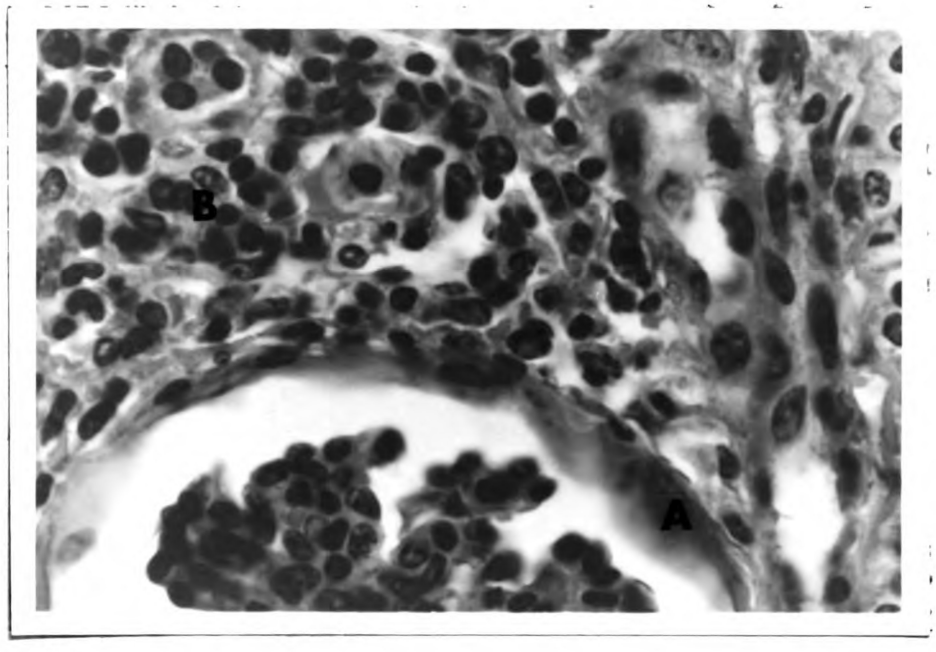

Figure 11.--Kidney Section, Calf 921. Note (A) thickened Bowmans capsule, (B) predominance of lymphocytes in periglomerular region. x 750.

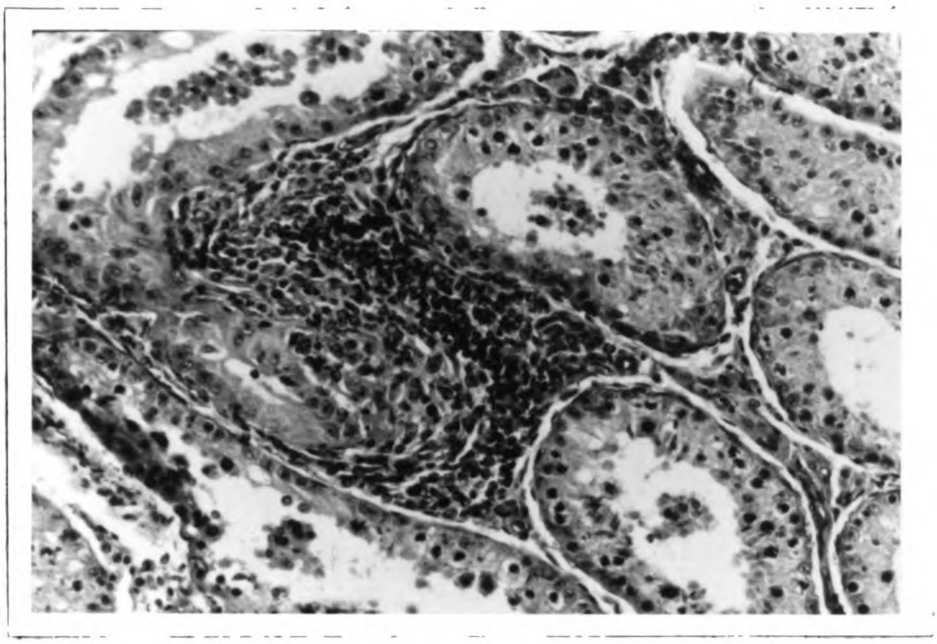

Figure 12.--Testicle, Calf 921. Note interstitial infiltration predominantly by lymphocytes. x 187.

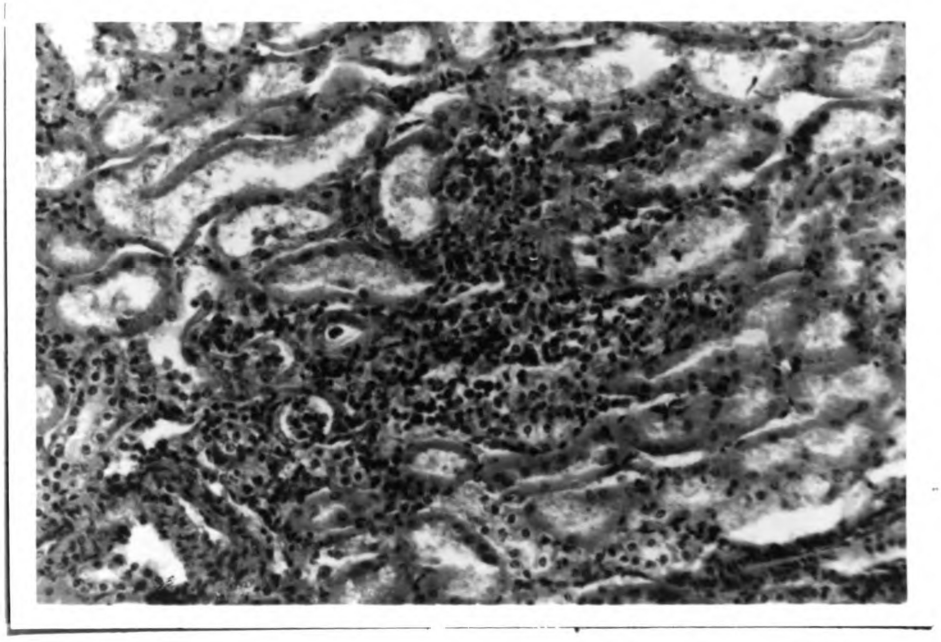

Figure 13.--Kidney Section, Calf 922. Killed on P.I. day 6 with intertubular infiltration by lymphocytes. x 187.

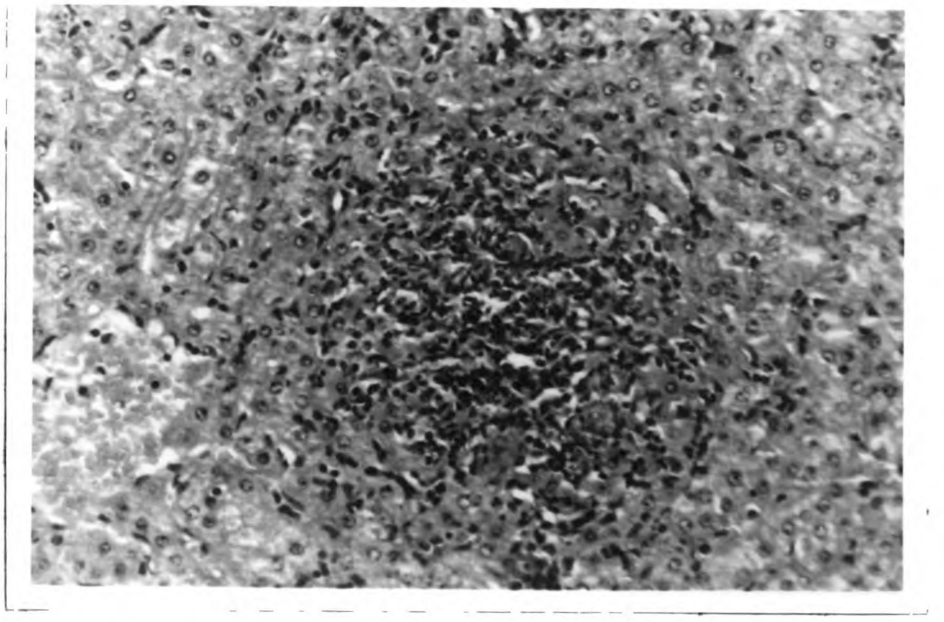

Figure 14.--Liver Section, Calf 922. To show focal areas of lymphocytic infiltration. x 187.

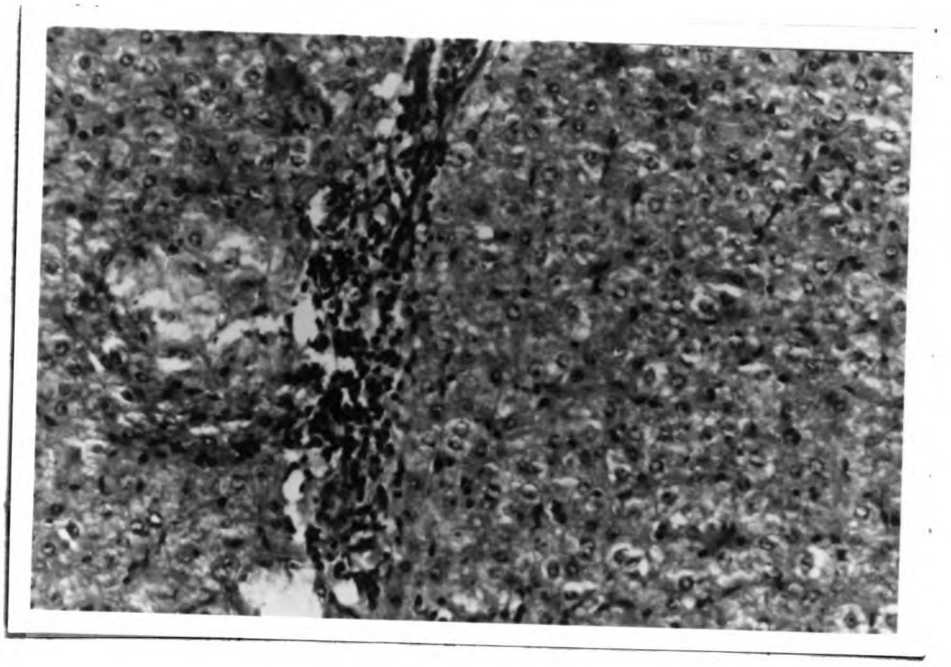

Figure 15.--Liver Section, Calf 922. Note lymphocytic infiltration in the region of portal triads. x 187.

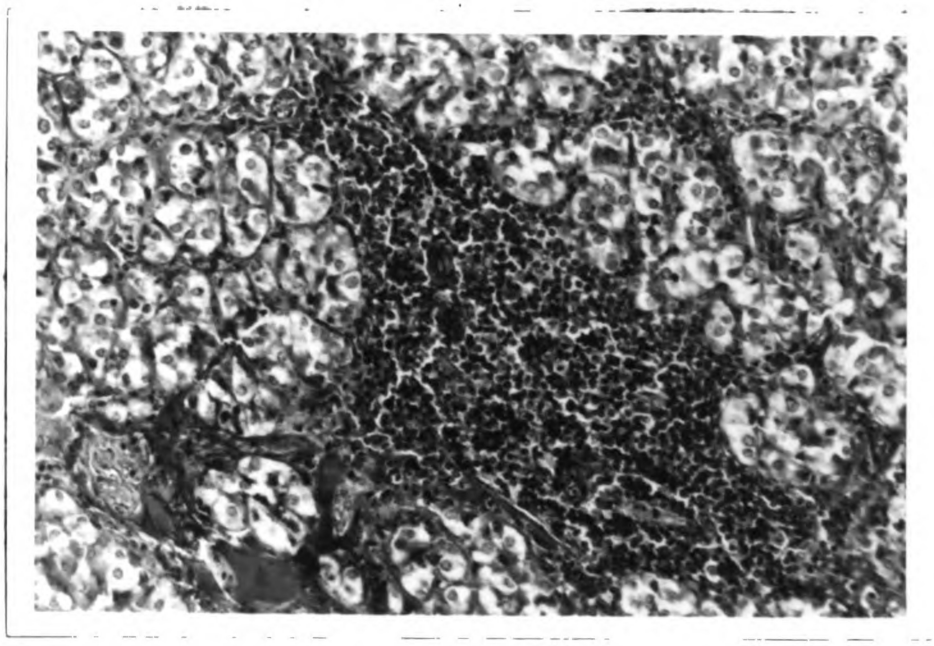

Figure 16.--Medulla of Adrenal Gland, Calf 922. With focal infiltration predominantly by lymphocytes. x 187.

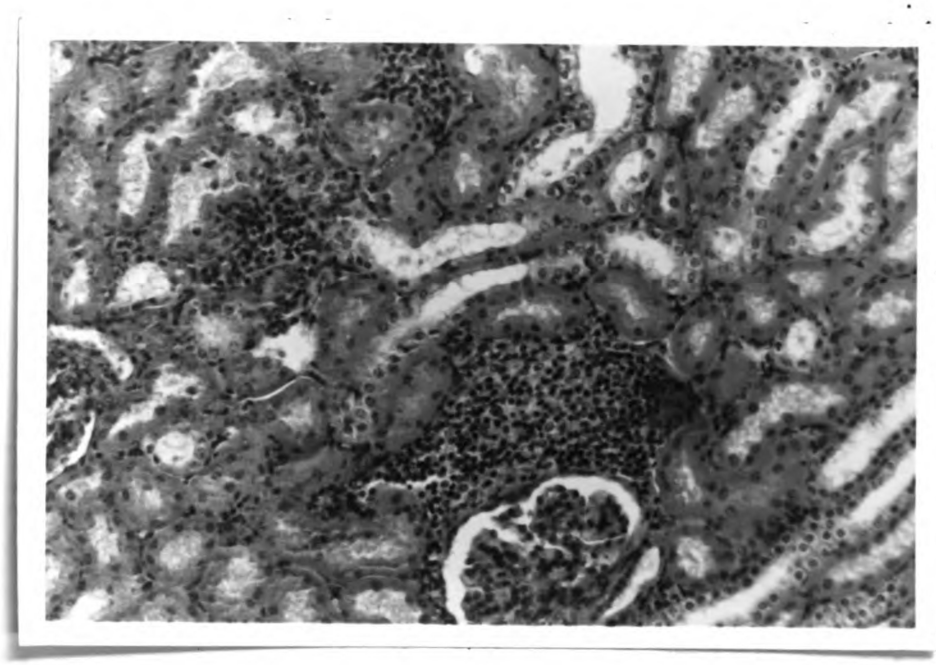

Figure 17.--Kidney Section, Calf 923. Killed on P.I. day 40. Typical intertubular lymphocytic infiltration. x 187.

BIBLIOGRAPHY

Abdulla, P. K., Karstad, L., and Fish, N. A. Cultural and and Serological Evidence of Leptospira in Deer in Ontario. Canad. Vet. J., 3 (March, 1962), 71-78.

Alexander, A. D., Smith, O. H., Hiatt, C. W., and Gleiser, C. A. Presence of Hemolysins in Cultures of Pathogenic Leptospires. Proc. Soc. Exp. Biol. and Med., 91 (1956), 205-211.

Alexander, A. D., and Evans, L. B. The Significance of Leptospira Sejroe Agglutinins in Bovine Serums. Amer. J. Vet. Res., 23 (March, 1962), 267-273.

Arean, V. M. Studies on thePathogenesis of Leptospirosis, II: A Clinicopathologic Evaluation of Hepatic and Renal Functions in Experimental Leptospiral Infections. Laboratory Investigations, 11 (1962), 273-288.

Atallah, O. A. Experimental Leptospirosis. Pathology of Leptospira pomona Infection in Male Cattle. M. S. Thesis, Michigan State University, 1963.

Baker, J. A., and Little, R. B. Leptospirosis in Cattle. J. Exp. Med., 88 (1948), 295-307.

Bauer, D. C., Eames, L. N., Sleight, S. D., and Ferguson, L. C. The Significance of Leptospiral Hemolysins in the Pathogenesis of Leptospiria pomona Infections. J. Infect. Dis., 108 (March-April, 1961), 229-236.

Bertók, L., Kemenes, F., and Szarka, G. A Case of Laboratory Infection in Man with Rodent Adapted Leptospira canicola. Acta. Vet. Hung., 11 (1961), 441-446.

Bezdenezhnykh, N. I. and Kashanova, N. I. Leptospirosis of Swine in Sakhalin Island. Zhur mikrobiol, Epidemiol and Immunobiol, 4 (1956), 101-104 (Abst.).

Bohl, E. H., and Ferguson, L. C. Leptospirosis in Domestic Animals. J.A.V.M.A., 121 (Dec., 1952), 421-428.

Boulanger, P., and Smith, A. N. Serological Investigations of Leptospirosis in Canada II: Preliminary Agglutination Studies of Cattle Sera with Leptospira pomona and Leptospira canicola antigens. Canad. J. Comp. Med., 21 (Jan., 1957), 4-11.

Boulanger, P. Agglutination Tests on Leptospirosis in Cattle. Canad. J. Pub. Health, 49 (April, 1958), 142-147.

Boulanger, P., Mitchell, D., Smith, A. N., and Rice, C. E. Leptospirosis in Canada III: A Study of the Importance of the Disease in Cattle as Shown in Combined Serological, Clinical, and Bacteriological Investigations. Canad. J. Comp. Med., 22 (April, 1958), 127-143.

Brauer, R. H., Pessotte, R. L., and Krebs, J. S. The Distribution and Excretion of S 35 Labled Sulfobromophthalein Administered to Dogs by Continuous Infusion. J. Clinic. Investig., 34 (1955), 35-43.

Burgdorfer, W. The Possible Role of Ticks as Vectors of Leptospirae :1: Transmission of Leptospira pomona by the Argasid Tick, Ornithodoros turicata and the Persistence of the Organism in its Tissues. Exp. Parasitol., 5 (1956), 571-579.

Byrne, R. J., and Chambers, C. F., Jr. A Serological Survey for Leptospiral Antibodies in Maryland Cattle. J.A.V.M.A., 134 (June, 1959), 498-502.

Cantarow, A., and Wirts, C. W. Excretion of Bromosulphalein in the Bile. Soc.Exp.Biol. and Med.,47 (1941),252-257.

Carbone, J. V., Grodsky, G. M., and Hjelte, V. Effect of Hepatic Dysfunction on Circulating Levels of Sulfobromophthalein and Its Metabolites." J. Clinical Investigations, 38 (1959), 1989-1996.

Cholvin, N. R., Morse, E. V., and Langham, R. F. Experimental Leptospira pomona Infection in Dogs. J. Infect. Dis., 104 (Feb., 1959), 92-100.

Clark, L. G., Kresse, J. I., Carbrey, E. A., Marshak,R.R. and Hollister,C.J. Leptospirosis in Cattle and Wild Life on a Pennsylvania Farm. J.A.V.M.A., 139 (1961), 889-891.

Clayton, G., and Derrick, E. The Presence of Leptospirosis of a Mild Type-Seven Day Fever in Queensland." Med. J. Aust., I (1937), 647-654.

Cornelius, C. E., and Wheat, J. D. Bromosulphalein Clearance in the Horse. A Quantitative Liver Function Test. Am. J. Vet. Res., 18 (1957), 369-374.

Cornelius, C. E., and Kaneko, J. J. Clinical Biochemistry of Domestic Animals. New York and London: Academic Press, 1963.

Donatien, A., and Gayot, G. Certain Modes of Infection in the Guinea Pig with Leptospira icterohaemorrhagiae. Arch. Inst. Pasteur Algerie, 29, 4 (1951), 289-297.

Dvoskin, S., and Hook, E. W. Canicola Fever with Meningitis. A Report of 3 Cases and an Epidemiological Study at Fort McClellan, Ala. Am. Med. Assoc. Arch. of Int. Med., 97 (June, 1956), 793-797.

Faine, S. Factors Affecting the Development of the Carrier State in Leptospirosis. J. Hyg., 60, (1962), 427-434.

Fennestad, K. L., and Petersen, C. Borg. Experimental Leptospirosis in Pregnant Heifers. Nord. Vet. Med., 8 (1956), 815-833.

_____. Fetal Leptospirosis and Abortion in Cattle. J. Inf. Dis., 102 (May-June, 1958), 227-236.

Ferris, D. H., Hanson, L. E., Alberts, J. O., Calhoun, J. C., and Marlowe, R. Epizootiological Studies on Leptospirosis in Illinois. Proc. CDC Conference for Teatures of Vet. Pub. Health Workers, (June, 1958), 354-358.

Galton, M. M., Acree, J. A., Lewis, A., and Pratter, E. C. Leptospirosis in Domestic Animals in Florida with Reference to Cattle. J.A.V.M.A., 128 (Jan., 1956), 87-91.

Galton, M. M., Menges, R. W., and Steele, J. H., Epidemiological Patterns of Leptospirosis. Annals of the N. Y. Acad. of Sc., 70 (June, 1958), 427-444.

Galton, M. M. Current Knowledge of Wild Animal Hosts of Leptospires in the United States. Southwestern Vet., 10 (Spring, 1959), 67-72.

Giges, B., Man, J. D., and Sharon, W. S. Bromosulphalein Retention in Obstructive Jaundice. Proc. Soc. Exp. Biol. and Med., 79 (1952), 375-376.

Gillespie, R. W. H., Kenzy, S. G., Ringen, M., and Bracken, F. K. Studies on Bovine Leptospirosis III. Isolation of Leptospira pomona from Surface Waters. Am. J. Vet. Res., 98 (Jan., 1957), 76-80.

Gleiser, C. A. Experimental Canine Leptospirosis III: Histopathologic Changes. J. Inf. Dis., 100 (May-June, 1957), 249-256.

Gochenour, W. S., Yager, R. H., and Wetmore, P. W. Antigenic Similarity of Bovine Strains of Leptospirae (United States) and Leptospira pomona. Proc. Soc. Exp. Biol., N. Y., 74 (1950), 199.

Gochenour, W. S., Jr. Manifestations of Bovine Leptospirosis. Vet. Med., 48 (June, 1953), 218-220, 243.

Grant, J. L., Metvin, C., Britton, A. B., and Kurtz, T. H. Measurement of Red Blood Cell Volume with the Electronic Cell Counter. Am. J. Clin. Path., 33 (Feb., 1960), 138-143.

Hadlow, W. J., and Stoenner, H. G. Histopathological Findings in Cows Naturally Infected with Leptospira pomona. Am. J. Vet. Res., 16 (1955), 45-56.

Hale, M. W. Laboratory Report on Leptospirosis in Georgia. J.A.V.M.A., 133 (Aug., 1958), 196-197.

Hensser, H. The Etiology of Periodic Ophthalmia. Schweiz. Arch. Tierheilk, 94, (1952), 296-306.

Hoag, W. G., and Bell, W. B. Bovine Leptospiral Meningitis. J.A.V.M.A., 124 (1954), 379-380.

Hoag, W. G., and Wilson, B. B. Isolation of Leptospira pomona from a Bovine Eye. J.A.V.M.A., 125 (1957), 381-382.

Inada, R., Ido, Y., Hoki, R., Kaneko, R., and Ito, H. The Etiology, Mode of Infection and Specific Therapy of Weils Disease (Spirochaetosis icterohaemorrhagica). J. Exp. Med., 23 (1916), 377-402.

Jungherr, E. Bovine Leptospirosis. J.A.V.M.A., 105 (1944), 276-281.

Kiszel, J., and Fuzi, M. "Investigation of a Leptospiral Outbreak in Domestic Animals in a District of Southeastern Hungary. Act. Micro. Biol. Ac. Sci. Hungarica, 4, 4 (1957), 377-389, Abst.

Klarenbeck, A., and Schuffner, W. A. P. Het Voorkomen Van een Aftwijkend Leptospira Ras in Neederland." Nederl. Tijdschr. v. Geneesk, 77 (1933), 4271-4276, Abst.

Klein, R. I., and Levinson, S. A. Removal of Bromosulphalein from the Blood Stream by the Reticuloendothelial System. Soc. Exp. Biol. and Med., 31 (1933), 179-181.

Krepkogorskaia, T. A., and Remenestova, M. M. The Isolation of Strains of Leptospires from the Tick Dermacentor marginatus S. from Cattle. J. Microbio., Epidem and Immunobiol., 28 (1957), 251-252.

Lavrova, M. Ya. The Role Played by Rodents in the Nidi of Leptospirosis in the Lower Course of the Kuban River (On the Problem of Interrelationships between Rodents and Farm Animals as the Source of Leptospirosis Infection). Zool. Thur., 41, (1962), 1067-1074, Abst.

Lindqvist, K. J., Morse, E. V., and Lundberg, A. M. Experimental Leptospira pomona Infection in Pregnant Ewes. Cornell Veterinarian, 48 (July, 1958), 277-290.

Low, D. G., Hiatt, C. W., Gleiser, C. A., and Bergman, E. N. Experimental Canine Leptospirosis. I: Leptospira icterohemorrhagiae Infections in Immature Dogs. J. Infect. Dis., 98, (1956), 249-259.

Low, D. G., Bergman, E. N., Hiatt, C. W., and Gleiser, C. A. Experimental Canine Leptospirosis. II: Renal Function Studies. J. Inf. Dis., 98, (1956), 260-265.

MacGregor, R. G. S., Richards, W., and Loh, G. L. The Differential Leukocyte Count. J. Path. and Bact., 51 (1940), 337-368.

Mattern, C. F. T., Bracket, F. S., and Olson, B. J. Determination of Number and Size of Particles by Electrical Gating: Blood Cells. J. Appl. Phys., 10, (1957), 56-70.

McKeever, S., Gormon, G. W., Chapman, J. F., Galton, M. M., and Powers, D. K. Incidence of Leptospira in Wild Mammals from South Western Georgia with a Report of New Hosts for Six Serotypes of Leptospires. J. Trop. Med. and Hyg., 7 (Nov., 1958), 646-655.

Menges, R. W., Galton, M. M., and Habermann, R. T. Culture and Serologic Studies on Four Dogs Inoculated with Two Leptospiral Serotypes, Leptospira pomona and Leptospira canicola. Am. J. of Vet. Res., 21 (May, 1960), 371-376.

Michin, N. A., and Azinov, S. A. Spirochaetal Jaundice of Cattle in North Caucasus. Sovyet Vet., 10 (1935), 23; Abst. (1937), Vet. Bull. 7:419.

Mitchell, D. Bovine Leptospirosis in Canada. Allied Vet, 30 (April-May, 1959), 54-58.

Mitchell, D., and Boulanger, P. Leptospirosis in Canada; An Atypical Mastitis in Cattle Due to Leptospira pomona. Canad. J. of Comp. Med., 23 (Aug., 1959), 250-255.

Mitchell, D., Boulanger, P., Smith, A. N., and Bannister, G. L. Leptospirosis in Canada. V: Infections in Cattle with a Serotype of the Hebdomadis Group. Canad. J. Comp. Med. and Vet. Sci., 24 (Aug., 1960), 229-234.

Moore, T., and Rice, C. E. Serological Investigation of Leptospirosis in Canada. I: Introduction and Preliminary Compliment--Fixation Studies of Cattle Sera with Commertially Prepared Leptospira pomona Antigen. Canad. J. Comp. Med., 20 (Oct., 1956), 362-373.

Morse, E. V., Krohn, A. F., and Hall, R. Leptospirosis in Wisconsin. I: Epizootiology and Clinical Features. J.A.V.M.A., 127 (1955), 417-421.

Morse, E. V., Morter, R. L., Langham, R. F., Lundberge, A. M., and Ullrey, D. E. Experimental Ovine Leptospirosis. Leptospira pomona Infection. J. Inf. Dis., 101 (1957), 129-136.

Morse, E. V., and Langham, R. F. Experimental Leptospirosis III: Caprine Leptospira pomona. Infection. Am. J. Vet. Res., 19 (Jan., 1958), 139-144.

Morter, R. L., Ray, J. A., and Chapel, D. F. Leptospira pomona Isolation from Naturally Occurring Canine Infections. J.A.V.M.A., 135 (Dec., 1959), 570-571.

Moses, C., Critchfield, F. H., and Thomas, T. B. The Importance of Dye Removal in the Bromosulfalein Test of Liver Function. J. of Lab. and Clin. Med., 33 (1948), 448-452.

Murphy, L. C., Cardeilhac, P. T., Alexander, A. D., Evans, L. B., and Marchwicki, B. S. Prevalence of Agglutinins in Canine Sera to Serotypes Other than Leptospira canicola and Leptospira icterohaemorrhagiae. Report of Isolation of Leptospira pomona from a Dog. Am. J. Vet. Res., 19 (Jan, 1958), 145-151.

Olitzki, A. L. Research on Leptospirosis in Israel and the Near East. Acta Medica Orientalia, 10, 11/12 (1951), 255-259, Abst.

Parnas, J., Kazimierz, L., Tadeusz Daborwski, and Koslak, A. Strains of Leptospirae Evoking Swamp Fever in Southeastern Poland; A Four Year Survey. J. Inf. Dis., 108, (1961), 243-246.

Pratt, E. B., Burdick, F. D., and Holmes, J. H. Measurement of Liver Blood Flow, Using the Bromosulphalein Dye Method." Am. J. Physiology, 171 (1952), 471-478.

Reinhard, K. R. "A Clinical Pathological Study of Experimental Leptospirosis in Calves. Am. J. Vet. Res., 12 (1951), 282-291.

_____. Bovine Leptospirosis. Army Med. Survey Graduate Sch. Med. Public. No. 1, (1952), 126-139.

_____. "Newer Knowledge of Leptospirosis in the United States. J. Exp. Parasit., 2 (1953), 89-115.

Reilly, J. R. The Raccoon as a Wild Life Reservoir of Leptospira canicola. New York Fish and Game J., 1 (1954), 220.

Roberts, S. J., Charles, J. York, and Robinson, J. W. An Outbreak of Leptospirosis in Horses on a Small Farm. J.A.V.M.A., 121 (Oct., 1952), 237-242.

Rosenthal, S. M., and White, E. C. Clinical Application of the Bromosulphalein Test for Hepatic Function. J. Am. Med. Assoc., 84 (1925), 1112.

Roth, E. E., and Galton, M. M. Isolation and Identification of Leptospira hardjo from Cattle in Louisiana. Am. J. Vet. Res., 21 (May, 1960), 422-427.

Rudge, J. M. Observations on the Efficiency of Animal Inoculation for Isolating Leptospirae from Kidney Tissue. N. Zealand Vet. J., (Feb., 1958), 15-16.

Saint Martin, M., and Charborneau, J. H. Meningitis Due to Leptospira canicola. First Report of Occurrence in Canada. Canad. Med. Assoc. J., 73 (1955), 454-458.

Schaeffer, M. Leptospiral Meningitis: Investigation of a Water Born Epidemic Due to Leptospira pomona. J. Clin. Investig., 30 (1951), 670-671.

Schnurrenberger, P. R. Bovine Leptospirosis, A Hazard to Man. J.A.V.M.A., 139 (Oct., 1961), 884-888.

Seibold, H. R., Keech, H., and Bokelman, D. L. Subclinical Leptospirosis Among Cattle. J.A.V.M.A., 138 (April, 1961), 424-430.

Skeggs, L. T., Jr. An Automatic Method for Colorimetric Analysis. Am.J.Clin. Path., 28 (1957), 311-322.

Sleight, S. D., Langham, R. F., and Morter, R. L. Experimental Leptospirosis: The Early Pathogenesis of Leptospira pomona in Young Swine. J. Inf. Dis., 106 (May-June, 1960), 262-269.

Sleight, S. D., and Williams, J. A. Transmission of Bovine Leptospirosis by Coitionand Artificial Insemination. J.A.V.M.A., 138 (1961), 151-152.

Sleight, S. D., and Lundberg, A. M. Persistence of Leptospira pomona in Porcine Tissues. J.A.V.M.A., 139 (Aug., 1961), 455-456.

Sleight, S. D., and Langham, R. F. The Effects of Leptospira pomona Hemolysin on Pregnant Ewes, Cows, and Sows. J. Inf. Dis., 3 (July-Aug., 1962), 63-77.

Smith, R. E., Reynolds, I. M., and Sakai, T. Experimental Leptospirosis in Pregnant Ewes III: Pathological Features. Cornell Vet., 50 (1960), 115-122.

Starr, L. E. Leptospira canicola Outbreak. Proc. Pub. Health Vet. Meeting, Atlanta, Ga. (1953), 46-48.

Steele, J. H. Epidemiologic Aspects of Leptospirosis. Pediatrics, 22 (Aug., 1958), 387-394.

Stoenner, H. G., Crews, F. W., Crouse, A. E., Taschner, L. E., Johnson, L. E., and Wohleb, J. The Epizootiology of Bovine Leptospirosis in Washington. J.A.V.M.A., 129 (Sept., 1956), 251-259.

Stoenner, H. G. The Sylvatic and Ecological Aspects of Leptospirosis. Vet. Med., 52 (1957), 553-555.

Stuart, R. D. The Importance of Urinary Antibodies in the Diagnosis of Leptospirosis. Canad. J. Microbiol., 2 (1956), 288-297.

Te Punga, W. A., and Bishop, W. H. Bovine Abortion Caused by Infection with Leptospira pomona. N. Z. Vet. J., (Dec., 1953), 143-149.

Trainer, D. O., Hansen, R. P., Pope, E. P., and Carbrey, E. A. The Role of Deer in the Epizootiology of Leptospirosis in Wisconsin. Am. J. Vet. Res., 24 (Jan., 1963), 159-167.

Turner, L. W., Roberts, C. S., Wiggens, A. M., Alexander, A. D., and Murphy, L. C. Leptospira canicola Infection in a Newborn Calf. Am. J. Vet. Res., 19 (Oct., 1958), 780-784.

Van der Hoeden, J. The Epidemiology and Epizootiology of Leptospirosis in Israel. J. Trop. Med. and Hyg., 58 (Sept., 1955), 202-204.

_____. Epizootiology of Leptospirosis (canicola) in the Bovine and Other Species in Israel. J. Am. Med. Assoc., 126 (March, 1955), 207-210.

_____. Leptospira canicola in Cattle. J. Comp. Path. and Therap., 65 (1955), 278-283.

_____. Leptospirosis canicolaris in Pigs and Its Probable Transfer to Human Beings. J. Inf. Dis., 98 (Jan.-Feb., 1956), 33-38.

_____. Leptospirosis in a Mid-eastern Country. Proc. of VI International Congress on Tropical Medicine and Malaria., 4 (Sept., 1958), 5-13.

_____. Leptospira Infections in Hedgehogs. J. Inf. Dis., 103 (Nov.-Dec., 1958), 225-238.

Wellington, N. A. M., Ferris, A. A., and Stevenson, W. J. Leptospirosis Among Farm Animals in a Dairying District. Aust. Vet. J., 29 (1953), 212-217.

Wheeler, H. O., Epstein, R. M., Robinson, R. R., and Snell, E. S. Hepatic Storage and Excretion of Sulfobromophthalein Sodium in the Dog. J. Clin. Investigations, 39 (1960), 236.

Williams, H. R., Murphy, W. J., McCroan, J. E., Starr, L. E., and Ward, M. K. An Epidemic of Canicola Fever in Man With the Demonstration of Leptospira canicola Infection in Dogs, Swine, and Cattle. Clinical and Epidemiological Studies., Am. J. Hyg., 64 (1956), 46-69.

Yager, R. H. and Gochenour, W. S., Jr. Leptospirosis in North America. Am. J. Trop. Med., 1 (1952), 457-461.

York, Charles J. Aspects of Control in Bovine Leptospirosis. Proc. U. S. Livestock Sanitary Assoc., 55th Annual Meeting, (Nov., 1951), 295-300.

APPENDICES

APPENDIX 1.--Daily Temperatures of the Experimental Calves

	Days	Calves Numbers and Temperatures F									
		Control 915	924	925	Control 918	923	916	Control 919	921	920	922
Pre-inoc.		101.8	101.8	102.0	101.6	102.0	101.5	102.0	101.9	102.0	101.0
Postinoculation	1	101.8	101.8	102.0	102.1	101.6	101.8	102.6	102.2	102.4	100.9
	2	101.6	103.7	103.2	101.0	106.8	107.4	102.0	106.9	107.2	107.2
	3	101.8	105.6	106.5	101.4	105.6	106.8	102.4	105.2	106.0	106.9
	4	102.0	104.6	104.2	100.6	105.6	106.4	101.8	104.7	104.3	105.8
	5	102.2	105.0	102.9	101.6	103.0	103.8	101.9	104.9	104.3	105.2
	6	102.4	102.3	101.9	102.8	101.9	103.0	102.8	102.0	103.8	103.1
	7	101.6	101.3	102.3	102.9	101.8	102.0	102.8	101.8	102.1	
	8	101.6	102.3	101.8	102.0	101.8	101.8	102.4	102.4	102.2	
	9	101.9	102.6	102.0	101.4	101.0	101.4	101.7	102.6	102.8	
	10	101.0	102.4	102.0	101.6	101.6	102.0	102.0	102.3	102.2	
	11	100.8	102.0	101.0	101.2	101.4	102.0	102.2	102.2	102.2	
	12	102.0	102.2	101.6	102.5	102.0	102.5	102.2	102.2	102.1	
	13	101.8	103.0	101.6	102.0	102.5	102.1	102.6	102.9	102.0	
	14	102.0	103.3	102.6	101.8	102.2	101.8	101.8	102.0	102.0	
	17	100.6	102.0	101.6	102.2	101.7	102.1	102.0	101.9		
	21	101.0	101.6	102.5	102.0	101.8	101.9	102.0	102.0		
	24	102.6	101.8	102.0			102.4				
	30	102.0	102.4	102.0	102.2	102.0	102.0				
	37	102.2	102.0	101.8							
	40	103.0	103.6	102.8	103.0						
	50	102.2	102.2	102.4							
	63	103.4	102.8								
	75	102.0	101.5								

APPENDIX 2.--Daily Hemoglobin Values of Experimental Calves

		Calves Numbers and Hemoglobin Values in gm./100 ml. Blood									
	Days	Control 915	924	925	Control 918	923	916	Control 919	921	920	922
Pre-inoc.		12.00	11.80	12.20	10.40	11.60	12.50	10.30	10.30	13.50	11.60
Postinoculation	1	11.50	10.90	11.40	9.80	11.60	10.10	10.70	10.45	12.50	11.60
	2	11.90	11.50	11.50	10.60	11.90	9.80	10.70	10.20	10.40	11.00
	3	11.60	11.40	10.60	10.60	11.00	9.95	10.65	9.40	10.20	11.20
	4	12.40	11.25	10.90	10.30	11.70	10.10	10.50	8.10	10.65	10.70
	5	12.20	9.90	10.10	10.60	11.45	9.50	10.45	8.40	9.70	10.00
	6	11.90	9.30	10.30	9.80	10.60	9.20	10.20	8.40	9.85	10.00
	7	11.60	8.40	10.25	9.70	10.45	9.20	11.00	9.25	10.50	
	8	11.90	9.20	9.20	9.60	10.10	9.15	10.40	9.90	10.80	
	9	11.90	8.40	9.60	10.55	9.60	11.20	10.65	9.40	10.65	
	10	11.60	8.40	9.40	10.55	10.55	9.00	10.40	8.90	10.50	
	11	11.70	9.40	9.40	9.60	10.25	10.55	10.50	9.40	11.00	
	12	11.30	8.60	9.80	10.10	11.20	9.80	10.00	9.40	11.00	
	13	11.70	9.90	9.60	9.90	10.90	9.60	10.50	8.85	9.85	
	14	11.30	8.60	9.10	9.15	10.25	9.15	10.60	10.00		
	17	12.40	9.85	10.25	9.00	10.15	9.60				
	21	11.90	8.40	10.20	9.60	10.25	10.40	10.00	9.40		
	27	11.40	9.10	10.60	10.15	11.10	10.55				
	30	11.50	8.50	10.85	11.10		10.70				
	37	11.70	8.70	10.90	11.10	11.20					
	40	11.00	9.60	10.60	11.65	11.50					
	43	11.65	9.30								
	50	11.70		10.70							
	75	11.20	11.50								

APPENDIX 3.--Daily Packed Cell Volumes (P.C.V.) of Experimental Calves

	Calves Numbers and P.C.V./100 ml. of Blood									
	Days	Control 915	924	925	Control 918	923	916	Control 919	921	920	922
Pre-inoc.		32.80	33.60	32.50	30.50	33.00	35.00	30.00	30.00	36.50	33.00
Postinoculation	1	31.50	30.00	29.00	30.00	33.00	34.50	32.50	30.50	36.00	34.00
	2	35.00	32.00	30.00	32.50	32.00	31.50	31.00	30.00	30.50	32.50
	3	33.00	30.00	31.00	30.00	30.00	28.50	32.00	26.00	29.50	32.50
	4	35.00	28.00	28.50	30.50	31.00	29.00	31.00	24.50	30.50	31.00
	5	34.00	27.00	27.00	29.50	29.50	29.00	30.50	25.00	27.00	30.00
	6	32.00	27.00	26.50	28.50	30.00	27.50	28.50	24.50	27.50	26.00
	7	33.00	22.00	26.00	29.00	27.00	27.00	30.00	29.00	29.00	
	8	34.00	26.50	26.00	29.00	27.00	25.00	31.50	29.50	30.50	
	9	32.50	23.50	25.00	30.00	31.50	26.00	32.50	30.00	32.00	
	10	32.00	23.50	25.00	31.00	30.00	29.00	30.00	28.00	29.50	
	11	34.00	27.00	27.00	30.00	30.00	25.50	30.50	26.00	30.50	
	12	33.00	27.00	26.50	30.50	32.50	31.00	30.00	25.00	31.00	
	13	35.00	27.00	26.00	28.00	32.00	30.00	33.00	26.00	30.00	
	14	32.00	25.00	26.00	27.00	29.00	29.50	34.00	29.50		
	17	37.00	26.00	27.50	28.00	31.00	27.00				
	21	35.00	24.50	28.50	27.50	30.50	29.50		29.00		
	27	34.00	27.00	31.50	30.00	32.00	31.00				
	30	37.00	25.00	30.50	31.50		31.50				
	37	35.00	26.00	33.00	32.50	32.00					
	40	35.00	27.50	30.00	35.00	32.00					
	43	33.00	27.50	30.50							
	50	34.00	31.00								
	75	35.00	32.50								

APPENDIX 4.--Erythrocyte Counts of Experimental Calves

		Calves Numbers and Erythrocyte Counts x $10^6/mm^3$.									
	Days	Control 915	924	925	Control 918	923	916	Control 919	921	920	922
Pre-inoc.		10.56	9.68	8.86	7.81	8.74	8.51	8.18	8.57	8.72	7.84
Postinoculation	1	9.660	9.210	8.770	7.140	8.320	7.670	8.245	8.460	8.400	7.945
	2	9.880	9.690	8.700	7.930	8.145	6.970	7.955	7.100	7.230	7.405
	3	9.670	8.850	8.135	8.030	7.140	7.230	7.550	6.785	6.725	7.810
	4	9.650	7.200	7.075	7.241	6.600	7.130	8.050	7.200	7.550	8.370
	5	9.440	7.800	7.220	7.565	7.040	6.625	7.640	6.650	6.250	7.055
	6	9.340	7.240	7.120	7.220	7.400	6.880	7.840	6.600	7.300	6.870
	7	9.660	7.275	7.310	7.260	7.000	6.705	7.835	6.080	7.605	
	8	9.875	7.400	7.098	7.280	7.100	6.610	7.810	6.830	7.080	
	9	9.660	6.940	6.815	7.320	7.320	6.960	7.970	6.890	7.180	
	10	9.550	7.115	6.815	7.220	7.120	6.100	7.955	6.890	7.115	
	11	9.645	7.740	7.015	7.780	6.980	7.460	8.000	6.598	7.240	
	12	8.900	7.000	6.900	7.030	7.400	7.000	8.220	7.901	7.570	
	13	9.325	7.841	6.875	6.420	6.960	6.910	8.570	6.865	6.820	
	14	8.430	7.830	6.325	6.590	6.810	6.350	8.660	7.390		
	17	10.190	7.465	6.600	6.650	7.040	6.890	7.880			
	21	9.790	7.225	7.150	6.810	7.030	7.310	8.100	7.165		
	27	9.490	7.795	7.590	6.910	7.160	6.880				
	30	10.190	6.900	7.625			7.000				
	37	9.115	7.010	8.210	7.690	7.900					
	40	9.450	6.800	8.535	8.170	8.435					
	43	9.215	7.575								
	50	9.490	8.000	8.760							
	75	10.540	9.400								

APPENDIX 5.--Daily Total Leukocyte Counts of Experimental Calves

		Calves Numbers and Total Leukocyte Counts/mm^3.									
	Days	Control 915	924	925	Control 918	923	916	Control 919	921	920	922
Pre-inoc.		11150	8022	12210	10930	9722	11889	11978	7100	10914	11009
Postinoculation	1	12175	7289	9470	11302	12530	13732	12491	7020	11102	10960
	2	10610	7320	9526	11782	15147	14133	13422	11420	12288	11150
	3	11836	7763	8909	11904	14870	14468	13442	11170	12059	11246
	4	11289	8240	9733	11099	10332	12301	13330	7048	11974	7726
	5	13586	9270	7670	11895	10291	13432	13179	4841	8204	7590
	6	12576	8892	7713	11310	8038	9694	12895	3190	7391	9543
	7	11299	7609	8891	11152	9084	9256	10808	3208	5100	
	8	12730	9024	8895	10585	7815	8433	9855	4542	5472	
	9	11521	9153	8857	9903	7888	8560	12057	4965	7969	
	10	11378	8825	9312	12563	8149	8437	11050	5765	7878	
	11	11536	7438	8882	12027	8376	9431	10905	5980	7957	
	12	12376	7612	9199	12496	9550	9532	10732	6310	8041	
	13	11686	7322	9792	12058	9223	9375	10340	6114	8321	
	14	10069	12346	11095	11530	10866	10090	10516	5318	7901	
	17	11818	8761	9149	10596	9684	9845		4097		
	21	11460	8445	9195	11693	10183	10978	10059			
	27	13782	7875	7949			12767				
	30		8762	9357	12610	10326	12911				
	37	15064	8275	9423							
	40		8180	10126	11965	8013					
	43	13009	8571	10076							
	50	10915	8843	10320							
	75	14804	9900								

APPENDIX 6.--Daily Segmented Neutrophil Counts of Experimental Calves

		Calves Numbers and Segmented Neutrophil Counts/mm^3.									
	Days	Control 915	924	925	Control 918	923	916	Control 919	921	920	922
Pre-inoc.		3791	1764	2442	4262	2916	3091	3234	1491	2619	2642
Postinoculation	1	3650	1166	2556	3390	3132	4806	4496	1965	2039	2626
	2	2864	1024	3143	3181	7422	7207	4563	7194	2220	5313
	3	4024	1941	3564	3333	8327	6510	6183	5473	8079	4866
	4	3838	3305	4379	3440	2996	2706	5598	2372	6226	2527
	5	4075	2410	1073	3092	2264	3895	5798	988	2543	2467
	6	4401	711	539	4184	1205	1744	5657	738	2586	2576
	7	3276	913	2133	2341	2089	1204	2594	454	1326	
	8	5346	1443	2312	2540	1406	1434	2661	695	1149	
	9	3110	2196	1771	1980	2209	1626	3135	1210	1753	
	10	4551	1853	3352	1382	1711	1687	3647	1136	1418	
	11	4729	1190	2131	1082	2345	1886	3053	1704	875	
	12	4701	989	2575	1499	1910	2764	3112	1956	1287	
	13	4782	951	3133	1446	2305	3187	3412	2446	1248	
	14	3355	1975	3328	1614	2607	3632	3154	1106	1343	
	17	4254	1489	2798	2331	2227	2954				
	21	5042	1689	2942	3157	1527	2415	2817	2124		
	27	4548	1414	2225							
	30		1840	2058	1891	2168	3227				
	37	8285	2068	2355							
	40		1963	3240	2752	1102					
	43	4553	2399	2317							
	50	4038	1768	2683							
	75	6069	2277								

APPENDIX 7.--Non-segmented Neutrophil Counts

		Calves Numbers and Non-segmented Neutrophil Counts/mm^3.									
	Days	Control 915	924	925	Control 918	923	916	Control 919	921	920	922
Pre-inoc.		335	80	244	...	...	...	479	71	218	...
Postinoculation	1	...	...	95	226	...	...	125	70	113	77
	2	106	73	190	...	151	1271	...	914	111	228
	3	118	...	446	238	297	723	403	...	362	573
	4	...	...	...	111	103	369	...	145	239	94
	5	...	185	...	119	205	1343	132	...	...	...
	6	377	...	...	...	80	193	...	32	148	99
	7	113	...	...	...	...	185	...	...	51	...
	8	...	...	266	...	234	84	...	...	...	
	9	346	92	...	198	552	513	241	58	...	
	10	...	88	93	...	81	169		60	...	
	11	115	74	...	...	...	283	109	126	...	
	12	247	152	...	...	96	95	107	183	...	
	13	...	73	98	...	...	94	207	...	...	
	14	...	...	...	...	...	202	210	...	80	
	17	118	262	...	105	...	98	...	...		
	21	...	84	...	...	...	...	101	...		
	27	276	79	...		...					
	30	...	86	...	...	103	129				
	37	301	...	...							
	40		...	...	120	...					
	43	130	86	...							
	50	...	...	...							
	75	...									

APPENDIX 8.--Lymphocyte Counts of Experimental Calves

		Calves Numbers and Lymphocyte Counts/mm^3.									
	Days	Control 915	924	925	Control 918	923	916	Control 919	921	920	922
Pre-inoc.		6244	5294	8058	5683	6124	7965	7186	4757	6875	7045
Postinoculation	1	7426	5348	6250	6555	8520	7689	6620	4071	7476	3553
	2	6578	5124	5144	7423	6811	4663	6711	2626	7327	1518
	3	6983	5589	4187	6071	5799	6655	5376	4803	2652	2767
	4	5983	3718	4379	6548	5068	7503	6225	1839	4909	5522
	5	8287	5283	5599	7612	5865	5372	5535	1531	4185	4935
	6	7042	7024	5630	6107	5948	6688	5786	1764	3178	5252
	7	7118	5782	6312	7360	6267	6756	6592	3224	2805	
	8	7128	6768	5692	6986	5548	6324	5518	3624	3666	
	9	7603	5949	6199	6635	4388	5221	6631	3747	5020	
	10	6257	5912	5122	10050	5296	5568	5856	4006	5514	
	11	5768	5280	6039	9381	5276	6318	6543	3849	6126	
	12	6186	5632	5887	9496	6303	5909	6224	3362	5950	
	13	6034	5345	5385	9646	5902	5156	5480	2446	5991	
	14	6169	8765	6657	8532	6845	5145	5994	2499	4898	
	17	6854	5957	5885	6887	6294	5513				
	21	5730	5573	5700	7132	6415	6806	5934	3984		
	27	7993	4871	4928							
	30		5257	6175	9711	6402	7488				
	37	5121	4965	5748							
	40		5071	5873	7657	4968					
	43	7154	4971	6650							
	50	5675	6013	6708							
	75	7402	6543								

APPENDIX 9.--Daily Monocyte Counts of Experimental Calves

		Calves Numbers and Monocyte Counts/mm^3.									
	Days	Control 915	924	925	Control 918	923	916	Control 919	921	920	922
Pre-inoc.		669	481	1343	656	389	594	479	497	654	660
Postinoculation	1	852	364	473	565	626	830	874	491	453	586
	2	954	658	857	471	606	565	1073	685	555	76
	3	591	1086	712	1190	297	579	672	782	844	95
	4	1241	1156	973	666	2169	1599	933	387	579	936
	5	1086	1112	997	714	1955	2686	922	606	1395	1776
	6	754	889	1465	679	723	872	900	641	1035	1090
	7	790	684	355	669	636	1018	1080	772	765	
	8	254	631	533	846	547	590	1084	596	547	
	9	460	732	709	792	710	1027	1567	577	956	
	10	568	706	745	879	978	843	884	658	788	
	11	692	669	622	1322	503	660	1090	631	796	
	12	866	532	460	1124	573	667	966	427	563	
	13	455	439	588	844	368	656	827	372	666	
	14	362	617	666	691	652	605	946	492	711	
	17	472	262	482	847	484	591				
	21	458	337	275	1169	1425	1207	905	398		
	27	689	549	556							
	30		526	749	756	929	1618				
	37	1355	413	754							
	40		573	506	957	641					
	43	910	514	504							
	50	873	619	413							
	75	888	594								

APPENDIX 10.--Daily Eosinophil Counts of Experimental Calves

	Calves Numbers and Eosinophil Counts/mm^3.									
	Control			Control			Control			
Days	915	924	925	918	923	916	919	921	920	922
Pre-inoc.	112	401	122	330	292	238	471	284	655	660
1	244	510	95	565	251	412	375	421	1246	1082
2	106	439	190	589	151	424	1074	...	888	380
3	118	466	...	1071	149	...	806	111	121	1145
4	226	83	...	333	...	123	533	97	...	280
5	136	278	...	357	...	134	791	64	82	691
6	...	267	77	339	80	194	514	32	443	892
7	...	228	89	781	91	93	540	91	153	
8	...	180	89	212	...	...	591	50	109	
9	...	183	177	297	79	86	482	173	239	
10	...	265	...	251	81	84	553	120	79	
11	230	223	88	241	251	283	109	...	159	
12	371	304	276	375	669	95	322	183	241	
13	114	512	587	120	646	281	414	53	416	
14	181	987	443	692	761	505	210	...	869	
17	118	788	482	424	678	689				
21	229	760	276	234	815	549	302	133		
27	276	942	238							
30		1051	374	252	723	387				
37	...	825	565							
40		572	506	479	1202					
43	260	600	604							
50	327	442	516							
75	444	495								

Postinoculation

APPENDIX 11.--Results of Urine Examination Showing pH and Specific Gravities

	Days	Calves Numbers With pH* and S.G.**									
		Control 915	924	925	Control 918	923	916	Control 919	921	920	922
Pre-inoc.		*8.1 **1.020	8.4 1.030	8.0 1.030	8.75 1.010	8.0 1.026	8.1 1.016	8.0 1.035	8.1 1.035	8.0 1.030	8.0 1.026
Postinoculation	1	*8.3 **1.020	8.6 1.030	8.7 1.032	8.5 1.035	8.0 1.034	8.6 1.027	8.2 1.037	8.3 1.038	8.1 1.032	8.2 1.033
	2	*8.4 **1.015	8.4 1.032	8.3 1.030	8.5 1.030	7.7 1.040	8.4 1.041	8.2 1.035	8.3 1.036	8.3 1.033	8.4 1.032
	3	*8.6 **1.018	8.3 1.031	8.3 1.028	8.7 1.032	7.8 1.037	8.3 1.035	8.2 1.030	8.4 1.028	8.4 1.040	8.0 1.034
	4	*7.7 **1.018	8.0 1.030	8.1 1.028	8.7 1.032	7.9 1.035	8.7 1.036	8.2 1.034	8.4 1.034	8.2 1.030	8.2 1.035
	5	*7.9 **1.020	8.7 1.037	8.0 1.022	8.7 1.031	8.1 1.031	7.6 1.026	8.15 1.028	8.2 1.034	8.2 1.035	8.0 1.038
	6	*8.0 **1.020	8.2 1.031	8.0 1.030	8.6 1.030	7.8 1.035	8.0 1.027	8.4 1.035	8.5 1.030	8.1 1.028	8.0 1.035
	7	*7.8 **1.011	8.1 1.030	7.6 1.020	8.8 1.035	7.5 1.032	8.3 1.030	8.2 1.033	8.4 1.031	8.2 1.035	
	8	*7.7 **1.010	8.4 1.027	8.0 1.018	7.9 1.028	7.4 1.030	7.9 1.030	8.1 1.035	8.3 1.038	7.8 1.032	

Postinoculation										
9	*	7.7	8.7	8.0	8.8	7.9	7.4	8.3	8.1	8.1
	**	1.005	1.030	1.018	1.033	1.016	1.015	1.025	1.027	1.038
10	*	8.0	8.3	8.3	8.0	8.0	8.0	8.3	8.2	8.0
	**	1.010	1.028	1.029	1.035	1.026	1.028	1.030	1.015	1.026
11	*	7.5	8.2	7.7	8.2	7.8	8.1	8.5	8.4	7.8
	**	1.005	1.010	1.008	1.030	1.025	1.024	1.030	1.035	1.010
12	*	8.9	8.5	7.75	8.8	7.7	7.5	8.6	8.4	8.0
	**	1.015	1.031	1.030	1.034	1.026	1.025	1.034	1.035	1.037
13	*	8.1	8.6	8.2	8.5	7.6	8.1	8.5	8.6	8.2
	**	1.012	1.032	1.030	1.024	1.033	1.033	1.038	1.035	1.036
14	*	8.0	8.2	8.2	8.1	7.9	8.35	8.5	8.45	8.0
	**	1.013	1.030	1.030	1.020	1.033	1.029	1.034	1.035	1.034
21	*	8.7	8.65	8.2	8.1	8.1	8.0	8.45	8.6	
	**	1.008	1.035	1.035	1.035	1.030	1.032	1.032	1.035	
27	*	8.15	8.8	8.0	7.8	7.8	8.0			
	**	1.017	1.036	1.020	1.020	1.025	1.025			
30	*	8.1	8.1	8.7			8.2			
	**	1.020	1.025	1.030			1.024			
37	*	8.8	8.35	7.5		7.2				
	**	1.018	1.025	1.025		1.040				
40	*	7.8	8.65	7.6	8.5	8.0				
	**	1.025	1.026	1.024	1.030	1.028				
50	*			7.7						
	**			1.029						
64	*	8.4	8.5							
	**	1.025	1.028							
75	*	8.3	8.8							
	**	1.035	1.018							

APPENDIX 12.--Renal Function Test; Levels of Blood Urea Nitrogen (B.U.N.) of Experimental Calves

		Calves Numbers and Mg.B.U.N./100 ml. Blood									
	Days	Control 915	924	925	Control 918	923	916	Control 919	921	920	922
Pre-inoc.		10.30	19.00	17.00	15.00	16.50	12.75	16.30	16.00	15.00	16.00
Postinoculation	1	14.50	21.50	17.50	19.00	17.50	16.00	17.00	14.00	13.50	15.50
	2	14.00	22.50	17.00	17.00	15.50	15.00	9.00	14.50	14.00	13.00
	3	11.00	17.00	12.50	18.50	15.50	15.00	13.00	21.00	17.50	18.50
	4	8.00	14.00	13.50	19.00	17.50	16.00	15.00	19.00	16.00	20.00
	5	10.00	15.00	13.50	13.50	17.50	15.00	15.50	17.50	15.50	15.50
	6	11.00	15.50	13.00	13.00	14.50	16.00	12.00	16.00	13.50	13.00
	7	11.50	15.50	14.50	21.00	14.50	15.50	14.50	14.50	12.50	
	8	15.00	10.00	14.00	23.00	14.00	14.00	15.00	16.00	13.50	
	9	16.50	16.00	9.50	23.00	14.00	13.00	16.00	16.50	13.50	
	10	15.50	18.50	12.50	21.00	12.50	11.50	12.50	17.50	15.00	
	11	13.50	16.50	12.50	19.00	13.50	12.50	11.00	12.50	13.00	
	12	12.50	21.50	12.00	18.50	14.00	14.00	10.00	21.00	15.00	
	13	16.00	16.50	8.00	18.50	12.50	14.00	9.00	20.00	16.00	
	14	15.50	14.50	12.50	15.50	14.00	16.00	14.00	18.50	15.00	
	17	14.50	18.00	15.00	10.00	11.00	14.50	12.50	18.00		
	21	14.00	18.00	15.50	13.50	17.00	16.50				
	27	12.00	17.00	15.50	13.50	16.50	16.50				
	30			17.00			15.00				
	32	15.00	18.50								
	37	15.50	19.50	18.00							
	40	11.50	21.00	17.00	13.50	16.50					
	50	13.50	15.00	17.50							
	75	10.50	16.50								

APPENDIX 13.--Results of Liver Function Test Showing Half Time Values for Bromosulphalein Clearance

	Calves Numbers and Half Time Values in Minutes									
	Days	Control 915	924	925	Control 918	923	916	Control 919	921	920	922
Pre-inco.		3.94	3.68	4.32	5.18	2.13	3.99	4.08	4.58	3.45	3.75
Postinoculation	1	3.08	3.45	4.95				3.54	4.77	3.07	4.67
	2				5.17	3.44	3.12				
	3							4.35	5.21	4.29	
	5	3.93	3.69	5.28							4.29
	6							4.14	3.44	5.25	4.14
	7				4.75	3.62	4.25				
	8		3.43	5.08							
	9				5.82	3.65	4.75				
	10							2.84	4.13	3.53	
	13	5.26	5.55	4.95							
	14				4.14	2.56	6.70	2.66	3.93	7.52	
	17				3.72	3.76	4.78				
	20	4.88	6.32	6.30	5.33	2.82	2.88				
	21							2.77	3.78		
	28	4.56	2.82	.308							
	30				11.96	1.52	4.19				
	40	5.81	3.75	4.86	4.49	2.72					
	50			4.18							
	61	4.19	3.97								
	75	4.20	3.45								