EXPERIMENTAL INFECTION OF PREGNANT EWES WITH LEPTOSPIRA POMONA, AND INTESTINAL ABSORPTION OF LEPTOSPIRAL ANTIBODIES IN LAMBS

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY

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1957

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by

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A THESIS

Submitted to the College of Veterinary Medicine
Michigan State University of Agriculture and
Applied Science in partial fulfillment of
the requirements for the degree of

MASTER OF SCIENCE

Department of Microbiology and Public Health

1957

TABLE OF CONTENTS

LIST OF TABLES

TABLE Page

ACKNOWLEDGMENTS

The author wishes to express his sincere thanks to the W. K. Kellogg Foundation whose generosity made his stay in this country possible.

Thanks are also due to Dr. E. V. Morse both for providing facilities for, and for his continued interest in this study.

The valuable assistance rendered by Mrs. Athalie Lundberg and Mr. Leonard Eames in the course of this work is greatly appreciated.

INTRODUCTION

This investigation is one aspect of a general research program concerning leptospirosis presently being conducted at Michigan State University.

Numerous reports indicate that leptospirosis caused by Leptospira pomona is one of the most prevalent diseases of domestic animals in the United States (70, 71, 95). Therefore, the disease is of considerable economic importance to the livestock industry (46, 56). The public health significance is indicated by the fact that the disease is transmissible from swine and cattle to man and that farm animals constitute the most important reservoir of the etiological agent (28, 42, 43, 85).

Leptospira pomona is frequently incriminated as the cause of abortion in cattle and swine (9, 58, 95). The course and effect of Leptospira pomona infection in pregnant sheep have not been subjected to extensive investigations. The purpose of this work has been to study the bacteriological, hematological and clinical aspects of the disease in pregnant ewes, and to ascertain the effect of infection upon the fetuses at various stages of gestation. The antibody content of milk from the ewes, as well as the development of antibody titers in the sera of lambs ingesting colostrum, was determined.

Upon completion of this work, some experiments were carried out on the intestinal absorption of leptospiral antibodies in normal lambs of different ages by feeding high titer serum.

REVIEW OF THE LITERATURE

1. The Recognition of Leptospira pomona

In 1936 a leptospira was isolated from the blood of a dairy farmer living near Pomona in south Queensland, Australia, who had suffered from a fever of eight days duration. This strain was referred to as the Pomona strain and was later shown to differ serologically from other known serotypes of leptospirae (15, 19, 20, 40, 47, 91, 92). Subsequent to the establishment of the specificity of the organism, the name Leptospira pomona was proposed by Derrick in 1942 (20).

In 1938 an epidemic of leptospirosis in swine caretakers caused by a strain which was designated Monakow (type II) occurred in Russia (88). Sera from convalescents agglutinated this strain in high titers as did those from the pigs with which they had been associated. In the same year a strain designated Mezzano was isolated from a case of "rice field fever" in a man in Italy. Both the Russian and Italian strains have been shown to be identical with Leptospira pomona (2, 3, 27, 74). As early as in 1940, Mochtar (53) isolated Leptospira pomona from swine in Java. Leptospira pomona has been incriminated in Switzerland and Germany to be the causative agent of certain cases of serous meningitis in man affecting those persons who have been in close contact with pigs. Since

swine appeared to be the reservoir of the infectious agent, the disease became known as "swineherds disease" (27, 29, 75).

2. Bovine Leptospirosis

The first etiologically defined case of bovine leptospirosis in the United States was reported by Jungherr (41) in 1944. He was able to demonstrate the presence of leptospirae in kidney sections from diseased animals. The agent was not isolated; therefore, the serotype is not known. It has been assumed that it was L. pomona.

Previously, clinical syndromes of icterus, hemoglobinuria and abortions had been reported in cattle in the United States which in retrospect may very well have been leptospirosis (48, 70, 84).

Mathews (49) in 1946 was able to transmit a febrile disease of cattle to calves and guinea pigs, by the inoculation of blood from affected animals. Leptospirae could be demonstrated in tissue sections from some cases.

Baker and Little (4') in 1946 isolated an agent from cattle which had acute mastitis. At that time the agent was considered to be a virus. Later, however, the condition was shown to be due to a leptospira (4''). The microorganism was found to be serologically indistinguishable from Leptospira pomona (26). The course of Leptospira pomona infection in cattle may be inapparent or may be characterized by severe

symptoms of anemia, icterus and hemoglobinuria, especially in calves. Abortions are not infrequent and usually occur in the latter half of pregnancy (3, 24, 30, 33, 52, 70, 81, 87). It is not known whether the abortion is a physiological reaction or whether it is directly mediated by the leptospirae (23, 57). Preliminary evidence indicates a hemolytic endotoxin or exotoxin may be involved (23).

Only one report has been found describing the isolation of leptospirae from aborted bovine fetuses (64). The almost consistent failure to demonstrate leptospirae in aborted bovine fetuses is in great contrast to what has been described in porcine leptospirosis. Leptospirae can be found rather easily in aborted pigs or piglets born dead or weak (23).

Ferguson et al. (23) stated that there was an apparent lack of erythrocytes in the heart, blood vessels and other tissues of aborted bovine fetuses. This phenomenon has also been observed in naturally-occurring leptospiral abortion in cattle.

Infected cattle may shed leptospirae in the urine for three months after the infection (86). This probably represents the extreme rather than the usual duration of the carrier state in cattle (55).

3. Porcine Leptospirosis

Monlux, et al. (54) in 1948 found leptospirae in sections of kidneys from a diseased hog and this is thought to be the first case of porcine leptospirosis noted in the United States.

In 1950, Bohl and Ferguson (22) discovered that antibodies against Leptospira pomona were present in the sera of Ohio swine. The first isolations of this organism from infected swine in the United States were performed in 1952 by Gouchenour, et al. (25). Bohl and Ferguson (8) cultured leptospirae from an "apparently healthy," but serologically positive, pig. Recently a number of isolations have been made from aborted fetuses (12, 13, 73) and from stillborn and weak pigs (9). The serotype involved in these instances was L. pomona.

Although the organisms may be found in "apparently healthy" pigs, the disease in pregnant sows may be extremely severe. Abortions frequently occur as well as the birth of dead or weak piglets. These may be the only manifestations commonly found (4, 11, 73, 80). Icterus and hemoglobinuria commonly associated with the disease in cattle, sheep and horses have not been reported in swine in the United States. Infected pigs may harbor leptospirae in their kidneys for long periods of time. The microorganisms may be eliminated in the urine in large numbers (4). Thus, swine represent important carriers of these organisms and a potent source of infection for other animals and man.

The duration of the carrier state in swine has been investigated by Baker (4) who found it to last longer than five months. Schmid and Giovanella (78) reported that the elimination of leptospirae in the urine of experimentally-infected pigs commenced approximately three weeks after the infection and that the organisms were still present in large numbers for six months. The number of organisms decreased gradually and one year after the infection only very few, if any, leptospirae were observed in the urine.

Morse (55) found that in an experimentally-infected group of twenty pigs only one pig was shedding leptospirae on the 122nd day after inoculation. This was determined by intraperitoneal inoculation of urine into groups of hamsters. Two different strains, one of bovine origin and one of porcine origin, served to infect the hogs.

Extensive and detailed information concerning human, porcine, bovine and equine leptospiroses is given in the reviews by Walch-Sorgdrager (91), Broom (10), Reinhard (70), Bohl and Ferguson (8), and the monographs by Gsell (27), Van Thiel (90) and Rimpau (74).

4. Ovine Leptospirosis

Only a few reports have been published concerning leptospirosis in sheep. Therefore, it was deemed necessary to review these findings in more detail.

The Annual Report (1950-1951) of the New Zealand Department of Agriculture reported for the first time the occurrence of leptospirosis in sheep (31). Hartley (31) in 1952 described the findings on which this first diagnosis was based and also presented data concerning a more recent outbreak. In the first outbreak in 1950 the diagnosis of leptospirosis was made on the clinical finding of "redwater" and pathological changes similar to those observed in bovine leptospirosis. Moreover, leptospirae were observed in sections of liver and kidney stained according to the method of Levaditi. In the second outbreak, occurring in 1951, the clinical observations and pathological changes were essentially similar to those noted in the first outbreak. Darkfield examination of urine from a living male lamb revealed numerous viable leptospirae and the organisms were found to infect guinea pigs. Sera from recovered animals agglutinated a strain of Leptospira pomona to a titer of 1:2000. Webster (31) in a personal communication to Hartley, states that he had also been able to demonstrate live leptospirae in the urine from infected sheep. Ovine leptospirosis, as described by Hartley (31) occurred mainly in lambs although a few ewes contracted leptospirosis and died. Icterus and hemoglobinuria were the only prominent signs observed.

In a personal communication to R. L. Morter in 1956, Hartley stated that leptospirosis is quite prevalent as a clinical entity in sheep in certain areas in New Zealand.

Furthermore, he indicated that ovine leptospirosis usually occurred as explosive outbreaks among lambs on lush pastures which were heavily stocked. Affected animals showed a high temperature, the urine was invariably red in color and the animals often died within two or three days. He noted that survivors and animals which developed subclinical infections remained renal carriers for many months. Usually evidences of interstitial nephritis were lacking. Experiments were performed on several occasions to infect lambs, "hoggets" and pregnant ewes with known virulent calf leptospiral strains and with strains isolated from sheep, but no clinical signs except a mild rise in temperature and inconsistently leptospiruria were produced. The strains of leptospirae isolated from cattle and sheep in New Zealand appeared to be serologically identical and indistinguishable from Leptospira pomona (32).

Since Hartley's first descriptions of leptospirosis in sheep, further outbreaks have been recorded in New Zealand. Salisbury in 1953 investigated an outbreak of the disease in a small flock in which eight lambs died out of a total of seventy. After the last death occurred, urine samples were collected from the 145 animals which remained, and of these 13 were shown "to be positive" (76).

Seddon (79) in his paper on diseases of domestic animals in Australia, mentioned the occurrence of leptospirosis in three pet lambs on a farm where five weeks

previously there had been a severe outbreak of the disease among calves. All three lambs excreted leptospirae in their urine and one lamb was still doing so eight weeks after the onset of symptoms.

Salisbury and McDonald (77) reported that death is the first indication of leptospiral infection in sheep. Losses are greatest among young lambs and yearlings. Icterus and hemoglobinuria are usually present.

Webster and Reynolds (93) in New Zealand had the opportunity to study a severe outbreak of ovine leptospirosis in a large flock of 400 ewes and their lambs. Morbidity approached 100 percent, and there was a mortality of 18 percent. The symptoms were milder in the older sheep; most of which gave no clinical evidence of infection. A slight transient hemoglobinuria was observed in some of the older ewes. A strain isolated from that outbreak was identified serologically as L. pomona. The same authors also described another outbreak of ovine leptospirosis in a flock of 162 unweaned lambs. Thirty of the lambs died and there was clinical evidence of leptospirosis in 38 of the survivors, as follows: 19 had elevated temperatures up to 106° F; 5 had hemoglobinuria; while others were icteric and anemic. A strain of L. pomona was obtained by inoculation of blood from febrile lambs into guinea pigs. None of the ewes gave evidence of clinical infection, although some had a leptospiruria. The authors stress the fact that in both of these natural outbreaks the

lambs were unthrifty owing to prolonged wet weather producing rank unpalatable pastures and waterlogged conditions. The lambs in the first flock were also heavily burdened with intestinal parasites. Webster and Reynolds (93) further reported vaccination experiments in sheep with heat-killed whole culture. Twenty lambs were vaccinated in one trial and resisted challenge (three weeks after vaccination and again 11 months later) with 10 ml. of a 7-day old culture of L. pomona (96.7×10^6 organisms per ml.). Twenty control sheep became infected by the same challenge dose; 16 subsequently developed leptospiruria which persisted for up to 3 months. The shedding of leptospirae was followed by microscopic examination of sediments of urine samples stained by Fontana's method. Only one of a total of 28 control sheep developed the acute clinical syndrome which was considered as being as typical of leptospirosis in the young. The authors attributed this to the fact that the experimental animals had reached adolescence and had passed their most susceptible age. They also observed in the natural outbreaks that the majority of naturally infected sheep ceased to excrete leptospirae in the urine after 2 or 3 months. In some, however, leptospiruria persisted much longer; one sheep was shedding leptospirae consistently in the urine for 9 months.

In the vaccination trials all vaccinated and control animals were thriving and fattening well when challenged. The one lamb which did manifest typical acute symptoms was the smallest and least thrifty. Hemoglobinuria was not

detected in any of the experimentally infected control lambs. The pathogenicity of the strain used was not questioned, since guinea pigs were readily infected, and severe illness developed in both authors due to accidental infection which occurred at the time the sheep were challenged.

Ovine leptospiral abortion was first observed in the United States by Beamer (6) in 1952. Many of the ewes on an Illinois farm aborted and several showed a positive agglutination-lysis test. The agent involved was not reported.

The first etiologically defined cases of ovine leptospirosis in the United States were described by Beamer et al. (7) in 1953. These authors reported an outbreak of the disease in a flock of 108 ewes shipped from New Mexico to a farm in Illinois. Five months later, six or seven deaths occurred, and shortly afterward, 19 of the remaining ewes aborted. Subsequently, depression and loss of condition were evident. The placental membranes were usually expelled within a few hours after the abortion. Of these 19 ewes, 15 died. At necropsy some ewes revealed icterus, swollen kidneys, mild fatty changes in the liver and marked hematuria. Although no leptospirae could be demonstrated histologically in the kidneys, darkfield examination of the stomach contents of an aborted fetus revealed motile organisms morphologically resembling leptospirae. A guinea pig inoculated with the stomach contents of this fetus died three days later. Histological examination of three other aborted fetuses revealed

forms which were suggestive of leptospirae. Unfortunately, the microorganisms could not be identified with certainty since cultures were not made. Serum samples from some of the ewes were examined for "leptospiral antibodies" by the agglutination-lysis test and some titers were found to be 1:1000. Although leptospirae were not demonstrated by cultural methods, the authors believe that the clinical and pathological findings, the results of the agglutination-lysis test and the demonstration of leptospirae from an aborted fetus warrant a diagnosis of leptospirosis. The investigattors did not indicate which serotype might be incriminated. A disease in calves which were on the same farm had been tentatively diagnosed as leptospirosis a few months previous to the outbreak in the sheep. A serum sample from one calf showed a positive agglutination-lysis titer of 1:1000. Again, it was not stated which species of Leptospira was involved.

Andres (1) reporting an outbreak of leptospirosis in cattle in 1954, mentioned that some yearling ewes on a farm aborted at about the time that the disease also appeared in the cattle. Evidence of Leptospira pomona infection was substantiated by positive serum titers.

Morter and Morse (59) in 1956, using experimentally infected calves as carriers, reported that four 3-4 year old ewes placed in contact with the infected calves on the seventh day following inoculation, did not show any evidence of infection. Heifers, pigs and one goat which were also in contact with these calves became infected.

In a more recent publication Morse et al. (61) stated that sheep are quite susceptible to experimental Leptospira pomona infection. Eight 4-5 months old lambs were used. Two strains of Leptospira pomona which had been maintained in continual hamster or guinea pig passage since their recovery from field cases were employed. One was obtained from an infected dairy cow and the other from the urine of an infected hog. Both strains proved to be pathogenic. The clinical manifestations of leptospirosis in sheep were characterized by pyrexia, anorexia, depression and polypnea. In some cases hemoglobinuria and icterus were observed, and in two lambs lameness was also noted. Five to eight days following inoculation, the lambs were found to pass through a leptospiremic phase of one to three days duration, during which the animals showed a high temperature. Sometimes leptospirae were detected in the blood on the day prior to the rise in temperature. Leptospiruria was demonstrable as early as the seventh day following exposure in one lamb, but generally leptospirae were not shed in the urine prior to the twelfth or fourteenth day. Leptospiruria was maximal and most consistent after 20 to 30 days. The authors further stated that darkfield microscopy consistently failed to reveal the presence of leptospirae in urine samples which were later proved to be positive by animal inoculation. The maximal duration of the renal carrier state for sheep was found to be 62 days. The majority of the sheep, however, did not excrete organisms in

the urine for longer than 44 days. Transmission from infected sheep to normal sheep, swine and goats did not occur. A marked decrease in the number of erythrocytes and low hemoglobin levels were noted. The differential counts remained essentially unaltered, although a mild leucocytosis could be observed in some of the sheep.

Due to the close relationship between the ovine and caprine species, the report by Van Der Hoeden (89) on Leptospira grippotyphosa infection in goats and the experimental caprine Leptospira pomona infections by Morse and Langham (60) are worth noting.

Van Der Hoeden in 1953 (89) described an outbreak of caprine leptospirosis in Israel. In this enzootic, which was found to be caused by Leptospira grippotyphosa, mainly goats were involved, although serological evidence of infection was found in several other species including sheep. Considerable losses were sustained only in goats. The main clinical findings were icterus, hemoglobinuria and abortion in the majority of the pregnant goats. The disease in sheep showed an atypical and benign course which was of short duration. The pathogenicity of a strain of Leptospira grippotyphosa isolated from a dead goat was tested in several species of animals. Two Arab sheep used in this experiment remained overtly healthy, but developed high agglutinin titers for the infecting organism. One sheep slaughtered forty-six days after the inoculation

showed interstitial nephritis but no leptospirae were observed microscopically, and cultures from this animal remained sterile.

Morse and Langham (60) observed that in experimentally-infected goats the course of Leptospira pomona infection appeared to be occult; the characteristic clinical manifestations of depression, anorexia, polypnea, hemoglobinuria and icterus were absent. Transmission of the disease from infected hogs to one goat was observed. Leptospiral antibodies were present in high titers in the sera of all animals and leptospirae were found in urine samples and in the kidneys of three goats at the time of necropsy. The pathological changes due to the disease in goats were also described.

5. Passive Antibody Transfer in the Newborn

The early reports concerning antibody transfer in the newborn appear to be quite contradictory, since results obtained from experiments with one species were thought to be valid for other species. Frequently, it was not definitely ascertained whether antibody in the newborn was derived from placental transfer or obtained through ingestion of colostrum containing antibodies. Furthermore, due consideration was not always given to the time factor involved in the intestinal absorption of colostral antibodies. No attempt will be made to cover in full the numerous publications that have

appeared on this subject. Attention will be mainly focused on some major facts and differences among various species of animals and some of the more recent observations in lambs.

Bauer (5) pointed out that colostrum plays an important role in the development of immunity in the newborn.

Famulener (21) systematically evaluated the function of early mammary secretions in the transmission of antibodies from the mother to offspring. Working with goats, he found that colostrum plays a greater role in the transmission of hemolysin-type antibody to the offspring than that which occurs due to placental transfer.

Howe (37) showed that globulins were lacking in the serum of the newborn calf. After the ingestion of colostrum the serum globulin content rose rapidly as compared to the sera of calves which did not receive colostrum.

Little and Orcutt (45) reported that calves born to "Brucella abortus immune cows" possessed no agglutinins to this organism in their sera at birth, but soon after ingestion of colostrum the agglutinins appeared. The same conclusions may be drawn from the work of Huddleson (39) and Connaway (18).

Smith and Little (82) investigated the significance of colostrum to the newborn calf. They showed that 75 to 80 percent of the calves that failed to get colostrum died of septicemia which they thought was due to Escherichia coli. Calves which received colostrum, only for a very short time,

survived. In a subsequent paper (83) the same authors confirmed their previous findings on the importance of colostrum to the newborn calf. They fed newborn calves, which were deprived of colostrum, a cow's serum containing high agglutinin titers for Brucella abortus. The serum was given during the first hours of life and the agglutinin content of sera from the calves was shown to contain high titers when tested a few hours later. Serum titers had not been evident at birth. The feeding of high antibody content serum to a calf slightly older than three days of age failed to raise the existing titer above that obtained by a previous feeding. The authors stated that the bovine placenta is impermeable to brucella agglutinins and that colostrum seemed to be the most efficient transporting agent for antibodies.

Ratner et al., in a series of publications (65, 66, 67, 68, 69), reviewed the literature on the subject and presented additional evidence for the importance of colostrum in antibody transport for a number of species. The authors also considered the mechanism of transplacental passage of antibodies which occurred. Transplacental passage of antibodies may well be due to anatomical differences of the placentae of different species of animals. These differences offer a possible explanation for the occurrence of in utero transfer in man, guinea pigs and rabbits, and for the absence of such transfer in the mouse and ruminants. They further concluded that in cattle, goats and sheep, colostrum is essential for the transmission of antibodies to the young.

Several reports (35, 36, 62, 63, 69) substantiate the importance of colostrum in producing antibody titers and probably engendering passive immunity in newborn pigs. It is generally agreed that the porcine placenta is impermeable to any appreciable amount of protective substances and that the function of antibody transfer is accomplished by ingestion of the colostrum. It is important to consider that this passive transfer of antibodies may interfere with active immunization in early days of life (36, 38).

Although there seems to be a general agreement that antibodies are absorbed *via* the gastrointestinal tract during the first hours of life, at least in certain species, comparatively little work has been published on the duration of this absorption phenomenon in the newborn.

Hill and Hardy (34) stated that the epithelial cells of the villi of kids and lambs up to 36 hours old contained large numbers of eosinophilic globules which they believed to be colostral proteins. This work was based upon histological and histochemical studies of intestinal epithelium of lambs and kids which were killed after receiving colostrum.

Mason *et al.* (50), working with lamb dysentery, i.e., *C. perfringens*, type B, found that the antitoxin in the colostrum of ewes was absorbed by newly-born lambs. Adsorption was shown to take place for as long as eighteen hours, but did not occur in a four day old lamb.

Comline et al. (17) demonstrated histologically droplets which were considered to be normal colostral globulins in the intestinal mucosa of 9 to 36 hour old calves. Only a very few droplets were observed within the cells when the colostrum was given to calves 63 to 65 hours after birth. The same authors (16), working with Brucella abortus agglutinins showed that the absorption took place in the small intestine of calves.

A review is given by McGirr (51), concerning the transmission of antibody substances from mother to offspring. This author presented the following table:

TABLE 1

COLOSTRAL TRANSMISSION OF ANTIBODY SUBSTANCES

Animal Species	Placental Type	Antibody Transmission		Period from Birth During which Intestinal Absorption of Antibodies Occur
		Placental	Colostral	
Man	haemochorial	+++	±	first few days
Mouse	haemochorial	+	+++	probably at least 20 da.
Rat	haemochorial	+	+++	" " " " "
Guinea pig	haemochorial	+++	-	" " " 10 "
Rabbit	haemochorial haemoendothelial	+++	-	" " " " "
Dog	endotheliochorial	±	+++	probably 10-12 days
Sheep	syndesmochorial	-	+++	less than 4 days
Goat	syndesmochorial	-	+++	" " " "
Cow	syndesmochorial	-	+++	24 hours
Pig	epitheliochorial	-	+++	no data available
Horse	epitheliochorial	-	+++	" " "

MATERIAL AND METHODS

Thirteen yearling western range ewes were purchased from the University flock in September, 1956. These animals were selected from a group of twenty-one ewes, the sera of which showed no antibody titer when tested with L. pomona and L. icterohaemorrhagiae AB antigens using a modified microscopic agglutination-lysis test (61). The ewes were kept on pasture and were fed hay and oats until the experiments were started. While still on pasture, the animals received two treatments with phenothiazine (25 grams per sheep per treatment) at one-month intervals to remove intestinal parasites. In addition, they were fed ad lib. commercial phenothiazine salt for one week after the last treatment. Two purebred Hampshire rams were allowed to stay for one month each with the ewes on pasture. To detect when the ewes had been bred, the rams carried a harness to which a piece of colored chalk was secured. The ewes were examined daily for colored spots on their loins, indicating that they had been bred. The ewes were again bled in December for agglutination-lysis tests. Normal values of hemoglobin, erythrocytes and leucocytes as well as differential leucocyte counts and nonprotein nitrogen (NPN) values were obtained. The agglutination-lysis tests with L. pomona antigen were all negative even at the lowest dilution (1:10). Standard clinical hematological techniques

were employed to ascertain levels for various blood cells and blood hemoglobin values (44). However, in the case of leucocyte counts of the newborn lambs, it was found necessary to increase the concentration of acetic acid in the diluting fluid to 4 percent to obtain complete hemolysis of red cells.

The hematological findings before commencement of the experiments and a history of the ewes, are presented in Tables 2 and 3.

The ewes were divided at random into two groups, one of which consisted of numbers 737, 786, 832, 836, and number 794 as control. This group was infected at about midpregnancy (72-87 days of pregnancy). Each animal was inoculated subcutaneously with 5 cc. of heparinized blood obtained from infected guinea pigs. This blood was obtained when the maximal pyretic response (104.5-106.0) was manifested. Ewe 794 received 5 cc. of normal guinea pig blood. Prior to inoculation, each animal received 1 cc. of adrenalin solution (0.1 percent) subcutaneously.

The other group consisted of numbers 608, 761, 763, 764, 770, 777, 781 and number 758 as control. The latter group was infected in late pregnancy (131-136 days of pregnancy).

After serving as control in the first group, Ewe 794 was infected and was killed for autopsy at the height of the temperature response. Blood from Ewe 794 was used as inoculum for the second group, but failed to infect the animals, which

were then inoculated subcutaneously with 10 cc. of a 9-day-old blood culture from sheep in the leptospiremic phase. Each sheep, except Ewe 770 and Ewe 608, received approximately 3×10^8 leptospirae. The number of organisms was determined by counting in a Petroff-Hauser counting chamber. Ewes 770 and 608, which only received blood from Ewe 794, showed no signs of infection either clinically or serologically. Ewe 770 was kept with the group to determine if transmission would take place.

A portion of the culture used as inoculum in the second group of ewes was Seitz-filtered and 10 cc. of the filtrate was inoculated subcutaneously into Ewe 608. The filtrate had been frozen for 24 hours and thawed before inoculation. When tested for sterility in Chang's medium, however, it was shown to contain living leptospirae although none was observed on darkfield examination of the filtrate.

Ewe 758 was exposed 20 days after lambing with 10 cc. of heparinized blood from infected guinea pigs at the height of pyretic response. Samples of milk were inoculated in groups of hamsters (2-3 hamsters) from the first to the 13th day after inoculation.

During the experimental period, each group of ewes was kept in a room 10 x 18 feet. The controls were kept in a separate room approximately 5 x 10 feet.

As soon as possible after lambing, blood and colostrum samples from the ewes, as well as fetal membranes were obtained.

The blood and colostrum from the ewes were examined for the presence of L. pomona antibody titers. Samples of fetal membranes were homogenized in a tissue grinder and injected intraperitoneally into guinea pigs as described elsewhere. The blood from the lambs was cultured immediately in Chang's fluid medium, and hematological examinations as well as agglutination-lysis tests were performed.

The strain of L. pomona selected for exposure was originally isolated by Morse (57) from bovine urine obtained during active infection in a Wisconsin dairy herd. It has been maintained since isolation by continual passage through guinea pigs and has been designated strain Wickard.

The agglutination-lysis test, using living L. pomona, strain Johnson, cells as antigen, was conducted with sera at tenfold dilutions starting at 1:10. One-tenth ml. of the serum dilution was mixed with 0.1 ml. of the antigen culture. The tubes were either incubated in a thermostatically-controlled waterbath at 37° C for two hours or overnight at room temperature. The tubes were then examined microscopically at 100x using a microscope fitted with an Abbe condensor into which was fitted a star diaphragm. This produced a modified dark-field type illumination which was found to be satisfactory for reading the tests.

Detection of agglutinins in the urine was conducted in a similar manner, except that the urine was diluted 1:5,

1:10, 1:100 and 1:1000. Urine samples were obtained by temporary occlusion of the sheep's nostrils.

Milk whey was produced for the agglutination-lysis test by adding two drops of concentrated rennin solution to approximately five ml. of milk. The mixture was allowed to stand at room temperature for a few hours or overnight at 4° C. The whey was then separated by centrifugation. Tenfold dilutions were employed in the agglutination-lysis tests. All agglutination-lysis titers were based on the 50 percent endpoint unless otherwise indicated.

Animal inoculation techniques were employed to determine the presence of leptospirae in urine, milk and tissues. Two to four guinea pigs (250 g. average) or hamsters (four to five weeks old) were inoculated intraperitoneally. Approximately 10 percent tissue emulsion, suspended in 0.85 percent sterile sodium chloride solution, served as the inoculum. In the case of contaminated fetal membranes, thorough washing and rinsing in sterile saline, previous to homogenization, was performed. Hamsters received 0.5 to 1.0 cc. of urine; guinea pigs received 1 to 3 cc. of urine or 2 to 3 cc. of the tissue suspension. After 21 to 30 days the animals were sacrificed. If their sera contained L. pomona agglutinins, the inocula were considered to have contained L. pomona. The strain, L. pomona, Wickard did not kill guinea pigs or hamsters.

Leptospirae were cultured from blood in Chang's medium (14), containing 10 percent sterile filtered rabbit serum and 0.01 percent hemoglobin (Difco). The same amount of blood was drawn from the jugular vein of each sheep. Each of five screw-cap tubes, containing ten ml. of medium, was inoculated with 0.1 to 1.0 cc. of the blood sample. In a few instances, dilutions were made of the blood sample before inoc inoculation to ascertain the maximal number of organisms at different stages of infection. Cultures were incubated at 30° C. for approximately 31 days. Darkfield microscopy examination (600x) were made at 14 to 16 days and again at 28 to 31 days to detect leptospirae.

Normal lambs for studies on intestinal absorption of leptospiral antibodies were obtained from the University flock. The sera and colostrum from the mother ewes were tested for the presence of leptospiral antibodies and were found to be negative. The lambs were allowed to stay with the ewes during the experiments. Serum containing high antibody titers ($1:10^{-6}$ or $1:10^{-9}$) against L. pomona, was obtained from the infected sheep. The serum was Seitz-filtered and was administered to the lambs of different ages through a stomach tube. The sera of the lambs were tested at various intervals for the presence of leptospiral antibodies.

EXPERIMENTAL RESULTS

1. Group I

This group consisted of ewes numbered 737, 786, 832, 836 and 794 which served as the control.

The group was infected at about midpregnancy. The exact stages of pregnancy are given in Table 3. Blood cultures were made daily from the third to the twelfth day after exposure. The time and duration of leptospiremia are presented in Table 4. Agglutination-lysis tests were performed with serum samples obtained during the experimental period. The titers obtained are tabulated in Tables 5 and 6. The hematological data are presented in Tables 7 and 8. No reticulocytes were observed in blood smears with one exception, namely, Ewe 786. A count of 0.8 percent reticulocytes was obtained on the 11th day after inoculation in that animal.

The duration of urinary excretion of leptospirae was investigated from the 12th to the 73rd day after inoculation. At the same time the urine was examined for the presence of antibodies against L. pomona. The results obtained from these investigations are shown in Tables 9 and 10. The urine samples were all negative for hemoglobin when examined with the benzidine test (123). Hemoglobinuria or icterus were not observed. Darkfield microscopy (600x) consistently

failed to reveal the presence of leptospirae in urine samples which were later proved positive by animal inoculation.

All ewes except 836 and 794, which were killed for necropsy before lambing, lambed normally. The gestation periods are presented in Table 2. As soon as possible after lambing, samples of colostrum and sera were obtained from the ewes, as well as placental membranes. Colostrum and placental membranes did not contain viable leptospirae as determined by negative agglutination-lysis tests of sera from guinea pigs inoculated with these materials. Colostral whey and sera were examined for the presence of antibodies against L. pomona by the agglutination-lysis test. Serum samples were obtained from the lambs soon after birth and at different intervals until the end of the experimental period. The titers of sera from the ewes and the lambs as well as the titers of colostral or milk whey at various times after lambing, are shown in Table 11. Since the ewes in this group all lambed during the night, samples could only be obtained several hours after birth. The lambs had in all cases obtained colostrum by sucking the ewes. This accounted for the high serum titers obtained in the lambs at the time of the first bleeding.

Blood cultures were made from the newborn lambs. No leptospirae were observed in these cultures. Hemoglobin, red and white cell determinations as well as differential counts and reticulocyte counts were made of the blood

samples obtained from the lambs at the time of the first bleeding. The hematological results are found in Table 12.

The characteristic course of infection in Group I is shown in Figure 1.

Ewe 836 was killed for necropsy 23 days after infection, at 110 days of pregnancy. The serum titer of the ewe was 10^{-7} with L. pomona antigen. The ewe was killed by intravenous injection of "Halatol" and subsequent exsanguination. At necropsy the macroscopic lesions were as follows:

> There were a few scattered grayish-white foci measuring about 1 mm. in diameter in the cortex of the kidneys. The right kidney showed a scar measuring about 2 cm. in diameter at the end of this structure.
>
> There were a few calcified areas in the lungs probably due to parasites.

The uterus contained one live fetus. Cultures were made in Chang's medium from amnionic fluid, fetal stomach contents and fetal blood. The cultures remained sterile. Agglutination-lysis tests performed with L. pomona antigen on amnionic fluid, fetal serum and stomach contents were all negative. Emulsions of fetal organs (kidney, liver, spleen, lung) stomach contents and amnionic fluid were injected intraperitoneally into groups of guinea pigs. When these animals were sacrificed one month later, their sera were all negative in the agglutination-lysis test with L. pomona antigen.

Emulsions of kidneys from Ewe 836 were also injected into 5 hamsters and 4 guinea pigs. Two of the guinea pigs and 4 of the hamsters showed positive titers when sacrificed.

An emulsion of placental membranes from Ewe 836 was injected into 3 hamsters and 3 guinea pigs. These animals failed to develop titers against L. pomona.

Ewe 794 served as control in Group I. The ewe received 5 cc. of normal guinea pig blood subcutaneously at the same time as the rest of the group received blood containing leptospirae. The ewe was bled according to the schedule for the group. The hematological observations are presented in Tables 7 and 8. Ewe 794 was originally intended to become the source of inoculum for the next group of sheep. She was therefore inoculated subcutaneously with 33-day-old culture of L. pomona obtained from sheep in the leptospiremic phase. The inoculum contained 1.2×10^8 organisms, determined by counting in the Petroff-Hauser counting chamber. The ewe was then 123 days pregnant. Blood cultures were made 5 hours after inoculation. Two cc. of blood were cultured in each of 5 tubes containing 10 ml. medium. In addition, 5 tubes were inoculated with 0.2 cc. blood. After 13 days of incubation, 4 out of 5 tubes with 2 cc. inocula were positive, whereas only one was positive of the others. Blood cultures were then made daily in 10 tubes, 5 of which received 1 cc. inoculum and 5 received 0.2 cc., each. The blood cultures with 1 cc. inocula contained innumerable leptospirae at

darkfield examination 7 days later. The tubes with 0.2 cc. inocula only contained 8-12 organisms per field. On the third day following infection, tenfold dilutions of blood were inoculated into series of 5 tubes containing 10 ml. medium. After 22 days of incubation at 30° C., growth of leptospirae was observed in all 5 tubes inoculated with 10^{-1} dilution of blood, in 4 out of 5 tubes receiving the 10^{-2} dilution, and in 2 out of 5 receiving the 10^{-3} dilution. Only one out of 10 tubes inoculated on the fourth day after infection with 1 cc. blood, was positive, and all tubes inoculated on the fifth day, were negative for leptospirae. Blood was drawn on the fourth and fifth day from this animal and inoculated in 10 cc. amounts subcutaneously into ewes in Group II. Since the inocula obviously contained very few organisms, if any, the sheep in Group II did not show any evidence of infections during the 6 days which were allowed to pass before reinoculation with leptospira culture. The course of the infection in Ewe 794 is shown in Table 13.

Ewe 794 was killed for necropsy by intravenous "Halatol" injection and exsanguination 5 days following infection. The macroscopic lesions consisted of edematous lymphnodes and a few small greyish-white lesions in the kidneys. The uterus contained one live, male fetus, 38.5 cm long. Edema was observed in the neck region and scrotum of the fetus. The cotyledons were very congested. Cultures were made for leptospirae from fetal blood, stomach content and amnionic

fluid. These cultures remained sterile. No leptospiral antibodies were detected in fetal serum, stomach content or amnionic fluid. Samples of liver, spleen, lung, kidneys and stomach content from the fetus, as well as amnionic fluid, were pooled and injected into 5 guinea pigs. The sera of these guinea pigs did not contain antibodies against L. pomona when the animals were sacrificed one month later.

On the day of necropsy, the urine from Ewe 794 contained hemoglobin as shown by a positive benzidine test and lack of red cells upon microscopic examination of sediment. Cultures of urine remained sterile.

Samples of cotyledons were injected into 3 guinea pigs. They all showed a positive serum reaction when killed one month later. Two guinea pigs were inoculated with emulsion of brain from Ewe 794. One died after 4 days. The other was heartbled for blood cultures when sick 9 days following inoculation. A positive culture was obtained from this guinea pig and one month later its serum showed a positive L. pomona titer. An emulsion of kidneys from Ewe 794 was inoculated into 4 guinea pigs, which upon sacrifice showed titers against L. pomona. The serum antibody titers of Ewe 794 on the fourth and fifth day of infection were 10^{-1} and 10^{-3}, respectively.

2. Group II

Group II consisted of ewes numbered 761, 763, 764, 770, 777, 781, 608 and 758 as control. This group was infected

during late pregnancy as shown in Table 3. Hematological observations made 3 months before infection are presented in Table 2.

As previously mentioned, Group II was inoculated with blood obtained from Ewe 794 during the febrile response 4 and 5 days after exposure. As serum samples obtained from Ewe 794 at the same time showed positive leptospiral agglutinin titers, it was felt that the chances of infecting group II with this material were minimal. Clinical and hematological observations were made daily for 6 days after the inoculation of blood from Ewe 794. These data are presented in Table 14. Blood cultures obtained daily during this period remained sterile and no serum antibody titers developed. The data obtained during this period will thus represent "normal" hematological findings in this group. No reticulocytes were observed.

A 9-day-old culture, obtained from the blood of an infected sheep was then used as inoculum. Ten ml. of culture, containing 3×10^7 organisms per ml. were injected subcutaneously into 761, 763, 764, 777 and 781. Ewe 770, which had only received blood from 794, was not inoculated and was allowed to stay with the infected ewes. Ewe 770 remained uninfected throughout the entire experimental period as determined by the absence of serum antibody titers. Urine from this animal did not contain antibodies or live leptospirae.

The ewe had one lamb, the serum titer of which remained negative for leptospiral antibodies. At lambing, neither serum or colostral whey from the ewe contained detectable antibodies. Thus, no transmission took place from the infected sheep to Ewe 770 or its lamb.

Ewe 608 received 10 ml. of a frozen and thawed filtrate of the leptospira culture. The culture was filtered through a Seitz filter. When tested for sterility in Chang's medium, the filtrate was, however, shown to contain viable leptospirae. Ewe 608 subsequently developed antibody titers both in the blood serum in the milk and in the urine. Blood cultures obtained from Ewe 608 from the first day following inoculation until the occurrence of antibodies in the serum on the seventh day after infection revealed no growth of leptospirae. The temperature also remained normal during this period. No leptospirae could be demonstrated by animal inoculation of urine from Ewe 608, as shown in Table 18. Temperatures and hematological data obtained after inoculation of Group II with L. pomona culture are found in Table 15. The duration of leptospiremia in this group is presented in Table 16. The serum antibody titers obtained at various intervals are recorded in Table 17. The duration of urinary excretion of live leptospirae is presented in Table 18, and the urine antibody titers are found in Table 19. Darkfield microscopy did not reveal the presence of leptospirae in Group II urine samples which were later proved to be positive by animal

inoculation. Only Ewe 777 showed hemoglobinuria. This occurred on the sixth day following infection and was determined by the benzidine test. Microscopic examination of the urine sediment did not reveal the presence of erythrocytes.

Ewe 763 was killed by intravenous injection of "Halatol" 4 days after infection, i.e., at 131 days of pregnancy. Except for pseudotuberculosis-like lesions in the submandibular lymph-nodes, no macroscopic pathological changes were observed. Blood cultures obtained about 6 hours before necropsy showed one positive tube out of 5. Blood cultures taken at the time of necropsy were all negative. Samples of colostrum, brain, kidneys and placental membranes from the ewe were injected into groups of guinea pigs. Only the kidneys were shown to contain viable leptospirae. The uterus contained one live fetus. No macroscopic lesions were observed in the fetus or placental membranes. Cultures from fetal blood, stomach contents and amnionic fluid did not reveal leptospirae. Agglutination-lysis tests with L. pomona antigen or fetal serum, stomach content and amnionic fluid were negative. Emulsions of fetal liver, spleen, kidneys and stomach contents as well as fetal blood and amnionic fluid did not contain leptospirae.

The ewes numbered 758, 761, 764, 770, 777, 781, and 608 lambed after normal gestation periods as shown in Table 2. Samples of serum, colostrum, amnionic fluid and fetal membranes were obtained immediately after birth from all of these

ewes. At the same time blood cultures were made from the newborn lambs. Sera obtained from the newborn lambs showed no antibody titers. All blood cultures were negative for leptospirae. Colostrum and samples from fetal membranes did not contain live leptospirae, with one exception. Two guinea pigs were inoculated with an emulsion of fetal membranes from Ewe 781. One of these guinea pigs died 4 days afterwards. The remaining one had a positive serum titer for L. pomona.

Ewe 781 did not allow its two lambs to nurse. One lamb died 3 days after birth. Emulsion of liver, kidney, spleen and brain did not contain leptospirae. The serum of this lamb had a 10^{-2} antibody titer, which was due to the fact that the lamb had managed to obtain some milk containing antibodies from other ewes. The other lamb of Ewe 781 was killed in extremis when 32 days old. This lamb had managed to survive by obtaining milk from other ewes. The lamb had for the past two weeks suffered from pneumonia with intermittent fever with temperatures of 106.3° F. Blood cultures obtained during the febrile periods were all negative for leptospirae. Although the serum from this lamb had shown a 10^{-2} titer on the fourth day after birth, it remained negative from the seventh day of age till death. At necropsy large areas of pneumonia were observed, which involved the apical and cardiac lobes as well as the anterior portions of the diaphragmatic lobes. Some caseous areas were observed

within the pneumonic consolidations. The liver had a very bright yellow color. Emulsions of kidney, liver and spleen, brain and lungs were injected into groups of guinea pigs. None of the guinea pigs showed any macroscopic lesions when sacrificed one month later. Only one out of 3 guinea pigs injected with lung emulsion showed positive leptospiral antibody titers, the sera of the other guinea pigs were negative. Cultures on blood agar plates from lungs, liver, spleen and brain revealed a gram negative rod in pure culture. The organism is difficult to classify, but has most characteristics in common with Brucella bronchiseptica. It does, however, liquefy gelatin and is urease negative, which may indicate that it may belong to the genus Alkaligenes.

Ewe 781 was killed for necropsy 34 days after lambing, i.e., 44 days after infection. The macroscopic pathological observations consisted of numerous greyish-white lesions extending through the depth of the cortex of the kidneys. The mediastinal and bronchial lymph nodes were greatly enlarged and showed caseous areas. A culture made from the bronchial lymph nodes revealed a pure culture of Corynebacterium pseudotuberculosis. Several large caseous areas were found in the diaphragmatic and cardiac lobes of the lungs. The kidneys, liver and uterine mucosa were shown not to contain vialble leptospirae by guinea pig inoculations.

Serum samples were obtained immediately after birth from the lambs in Group II. At the same time samples of

colostrum were obtained. Samples of lambs' sera and milk from the ewes were taken at various intervals till about 3 weeks after birth. The results of agglutination-lysis tests performed on these samples with L. pomona antigen are presented in Table 21.

Ewe 758, which had served as control in Group II and had been kept in a separate room, had two lambs. Serum samples from this ewe and its lambs, as well as milk whey, did not contain antibodies at the time of lambing. As a preliminary experiment concerning the absorption of antibodies through the intestinal tract, the lambs received milk or serum containing leptospiral antibodies through a stomach tube. At the age of 52 hours one lamb received 20 ml. sheep serum which had a titer of 10^{-4}; the other lamb received 75 ml. of milk collected from the infected ewes, and which showed a titer of 10^{-3}. The lambs were bled 14 hours later. The lambs did not show any reaction even in the 10^{-1} dilution in agglutination-lysis tests. At the age of 75 hours the lambs received additional amounts of serum (27 ml.) and milk (60 ml.) respectively. They were again bled after 18, 24, and 44 hours. No antibody titers were found in these samples.

Ewe 758 was inoculated subcutaneously with 10 cc. of blood obtained from infected guinea pigs during the pyretic response. This was done to follow the shedding of leptospirae in the milk, and to ascertain if the nursing lambs would contract the disease. The ewe was infected 30

days after lambing. Blood cultures were taken from the ewe from the day of inoculation until the ninth day. Milk samples were obtained from the first day through the thirteenth day following inoculation. Samples of milk were inoculated into groups of hamsters and examined for the presence of leptospiral antibodies. The temperatures of the ewe and lambs were recorded daily. No rise in temperature was observed in the lambs and no antibody titers were found in their sera when examined one month after the inoculation of the ewe. The results are summarized in Table 22. The urinary excretion of leptospirae in Ewe 758 was followed by animal inoculation. One out of 3 hamsters inoculated with urine obtained 25 days after infection showed antibody titer. Hamsters inoculated with urine obtained 17, 27 and 46 days following infection were negative. The antibody titers of the urine were negative on the 17th day and were 10^{-1} and 10^{-3} on the 27th and 46th day, respectively.

3. Absorption of Leptospiral Antibodies in Lambs

On the basis of the experiments carried out with the lambs of Ewe 758 it seemed likely that, either the lambs were too old (52 hours) and absorption did not take place, or the amount of antibody given was too small to be absorbed in detectable serum concentrations. An arbitrarily-chosen dose of 50 cc. of serum with a titer of 10^{-6} was given to a

10-hour old lamb weighing 6 pounds. The lamb was bled after 6, 12, 24, 30 and 36 hours, and showed the following titers, respectively: 10^{-3}, 10^{-3}, 10^{-4} and 10^{-4}. The lamb was bled again 48 hours after the administration of serum. The serum titer of the lamb had dropped to a $\pm$ reaction, i.e., 25 percent agglutination-lysis, in the 10^{-1}, 10^{-2} and 10^{-3} dilutions. At the same time, i.e., 48 hours after the first serum administration and 58 hours after birth, this lamb again received about 50 cc. immune serum. Subsequent bleedings and agglutination-lysis tests failed to reveal an increase in the serum titer.

Another lamb, 24 hours old and weighing 8 pounds, received through stomach tube, 75 cc. of a serum with a titer of 10^{-6}. Subsequent bleedings and agglutination-lysis tests did not reveal any titers in the serum of this lamb.

These preliminary experiments indicated, then, that serum antibodies, when administered *per os*, were absorbed from the alimentary tract of a 10-hour old lamb, and that no detectable antibodies were demonstrated in the serum of a lamb receiving serum at the age of 24 hours. Furthermore, the absorption seemed to occur quite rapidly as a titer of 10^{-3} was found 6 hours after the administration of serum. The amount of immune serum used, namely about 8.5 cc. per pound body weight, appeared to be sufficient to enable detection of serum antibody titer in the lamb. The lambs suffered no ill effects from the administration of serum *per os*.

Several workers (34, 50, 17, 51) had indicated that absorption of globulins or antibodies from colostrum might occur up to the age of 36 hours or even 4 days.

To obtain additional data a group of 9 lambs was given serum with an antibody titer of 10^{-9}. The ages of the lambs, their weights, amounts of serum administered as well as the results obtained, are presented in Table 23. It may be deducted from this table that absorption of antibodies took place from the alimentary tract of lambs which were approximately 15 hours old. The antibodies were not absorbed in detectable amounts in lambs older than about 25 hours. Since these experiments were conducted late in the lambing season, few lambs were available, therefore it was not possible to study lambs 15 to 24 hours old.

DISCUSSION AND CONCLUSIONS

1. The Disease in Sheep

Ovine leptospirosis does not seem to be prevalent in the United States. This may be due to the fact that the disease may run an inapparent course with a low mortality rate. The disease will then only be detectable by serological means. The author is not aware of any reported serological surveys pertaining to ovine leptospirosis in the United States. Ovine leptospirosis may, however, appear as a severe disease with symptoms of icterus and hemoglobinuria as well as a high mortality rate, especially in lambs. The disease as such is a problem to farmers in New Zealand where it causes considerable losses (31, 76, 77). The factors responsible for the difference in prevalence may be many and varied. American breeds of sheep have been found to be susceptible to experimental L. pomona infection. Differences in resistance of breeds of sheep, then, apparently do not play any part. The virulence of L. pomona may differ from one strain to another. Information pertaining to the comparative virulence of L. pomona strains is not known to the author. The difference in virulence, then, may account for the reported prevalence of ovine leptospirosis in some areas, e.g., New Zealand, and the scant reports of the disease in other areas, e.g., the United States.

The experimentally-produced disease in pregnant ewes does not exhibit the serious symptoms and high mortality rate sometimes reported in natural outbreaks. On the other hand, a disease is very often not reported unless it is the cause of serious loss of animals or decrease in yield. In certain areas, then, a higher incidence of the disease may be found, due to adequate diagnostic facilities and reporting services. The mild symptoms of disease commonly observed in experimental L. pomona infection of sheep, can only be attributed to leptospiral infection on the basis of bacteriological and serological investigations. This is in agreement with the statements made by Hartley (32) in New Zealand:

> We have, on several occasions, tried to infect lambs, hoggets and pregnant ewes with both known virulent calf strains and with sheep strains but have produced no clinical signs (except a mild rise in temperature and sometimes leptospiruria).

An important factor which will account for some of the differences observed between the naturally-occurring disease and the experimentally-induced disease, is the conditions under which the experiment is performed. Under natural husbandry sheep may be subjected to various factors predisposing for disease. These may be more or less heavy infestation with various parasites, e.g., lungworms or gastro-intestinal parasites. Sheep are sometimes kept on submarginal pastures which are incapable of supplying the animals with the necessary nutrients or essential minerals. Under such circumstances the animals may be rendered more

susceptible to infection. Animals used for experimental purposes are, on the other hand, commonly kept under good hygienic conditions, fed an adequate diet, and in general not subjected to undue stress. They are often routinely treated for parasites, as the case was in this experiment. Differences in climatic conditions and animal husbandry practices may be considered important factors influencing the prevalence and severity of ovine leptospirosis. Moderate temperatures and heavy rainfall will favor survival of L. pomona outside the host. Crowded pastures where several species may be feeding will enhance the possibilities of spread of the disease.

Several outbreaks of ovine leptospirosis (6, 7, 79) have been described following close contact with diseased cattle. Deer may serve as the source of infection since serological evidence indicates that leptospirosis has occurred in this game animal (94).

Although the inocula used in this experiment contained a much higher number of organisms than natural conditions would provide, the disease was very mild. The sheep passed through a pyretic stage during which symptoms of anorexia and polypnea were present. Hemoglobinuria occurred only in Ewes 794 and 777. In group I there was a marked and uniform decrease in hemoglobin levels and erythrocyte counts. But this drop was just as pronounced in the control sheep. The most marked drop in hemoglobin and red cell levels was observed

during the course of the disease in Ewe 794 (Table 13). At the time of necropsy, 5 days after inoculation, the urine contained hemoglobin. In group II marked depression in these levels was not detected. Hemoglobinuria may have been present in the experimental ewes for such short periods of time as to escape detection. On the other hand, an intravascular destruction of red cells with resulting hemoglobinemia may have occurred without hemoglobinuria. Hemoglobinuria only occurs when the amount of free hemoglobin in the circulating blood plasma exceeds the threshold of the kidneys for hemoglobin. An accurate means of following red cell destruction would be to record free hemoglobin in the blood plasma, or possibly erythrocyte fragility. This, however, has not been done in these experiments. Degrees of hemolysis judged by the color of the serum are subject to too many inaccuracies to be of much value.

Leptospiremia is readily demonstrated in experimental ovine leptospirosis. Leptospirae have been cultured from the blood as early as the first day after inoculation and as late as the seventh day. Tables 4 and 16 present the days after infection when positive blood cultures were obtained from the ewes. All 4 ewes in group I showed positive blood cultures on the fifth and sixth day after infection at the beginning of the febrile period. Two ewes (786 and 832) showed positive blood cultures as early as the third day after infection; the first blood cultures were made on the third day after

inoculation in this group. In group II (Table 16) 3 ewes showed positive blood cultures on the first day after inoculation. On the second, third and fourth day, all inoculated ewes (except Ewe 608) had positive cultures. The inoculum used in this group contained a very large number of leptospirae (3×10^7 per ml.). Ewe 794 which received 1.2×10^8 organisms yielded positive blood cultures 5 hours after subcutaneous inoculation. Blood cultures from Ewe 794 were positive for 4 days after inoculation. When the presence of leptospiremia (Tables 4 and 16) is correlated with the temperature of the animals (Tables 7 and 15), it is observed that positive blood cultures are more likely to be obtained during, or even before, the rise in temperature. In the case of Ewe 794, it was observed that the temperature was normal during the first two days after infection. Blood cultures obtained on these days were positive for leptospirae. The temperature rose to 104.3-105.4° F. on the third day after inoculation. Positive cultures were obtained on that day from blood diluted 10^{-3}. Two of 5 inoculated tubes were positive. On the fourth day after inoculation the temperature was 105.5° F. Only one out of 10 tubes inoculated with 1 cc. of blood was positive. Five tubes inoculated with 0.2 cc. blood were negative (Table 13). Positive blood cultures, then, were obtained from ewes 786, 832 and ewe 794, and from all infected ewes in group II (except Ewe 608) before any rise in temperature was observed. Leptospiremia thus occurs rapidly after

experimental infection of sheep with massive inocula of L. pomona. The term septicemia may well be used for the condition observed during the febrile period with symptoms of anorexia, polypnea and positive blood cultures. It is sometimes asserted that in septicemia organisms are multiplying in the blood, whereas in bacteremia they are derived from infected tissues. Such a distinction clearly cannot be drawn from the results of blood cultures. There are, however, indications that bacteria may multiply in the blood stream. The septicemic phase of any bacterial infection is probably the manifestation of a rapid and continuous invasion of the blood stream from foci which have been established earlier in the disease. The appearance of antibodies in the serum ends the septicemia as can be shown by comparing Tables 4 and 5 and Tables 16 and 17. In this connection the pathogenesis of relapsing fever (Borrelia recurrentis infection) is borne in mind. The antibody titers increase very rapidly from negative at the time of leptospiremia to 10^{-9} in a matter of 3 or 4 days (Tables 5 and 17). The titers may remain rather high (10^{-4} to 10^{-6}) for at least 3 months.

During, or soon after the rise in temperature a decrease in the number of leucocytes was observed in all infected ewes in group I (Table 7), in Ewes 761, 763 (Table 15) and in Ewe 794 (Table 13). A rise, however, in the number of leucocytes in Ewe 764 was observed. This may be accounted for on the basis of a concurrent suppurative

pododermatitis. Ewe 777 showed a slight decrease in the number of leucocytes at the rise in temperature, and then a rapid increase which occurred at the time of hemoglobinuria. Leukopenia, although of moderate degree, has been recorded in the course of leptospira infection in these sheep. This phenomenon is known to occur in certain infectious diseases. It has been observed in experimental leptospirosis in calves (70).

Some alterations were observed in the differential counts of the infected ewes (Tables 8 and 15). The percentage of eosinophils decreased during the infection in most of the animals. A slight relative increase in percentage of segmented neutrophils and a relative decrease in percentage of lymphocytes were also observed. Acute infections with neutrophilia are commonly associated with lymphopenia.

Antibodies were present in the urine of most of the infected animals (Tables 10 and 19). They did not appear in the urine until about 3 weeks after the infection. Tne possible sources of the urinary antibodies are either local antibody production in the kidneys or leakage of serum antibodies due to damage of renal tissue when the organisms became localized in the kidneys. Tests for protein (Heller's test, 44) performed on urine samples have all been negative, although antibody titers have been positive in 10^{-3} and 10^{-4} dilution of urine.

In the case of damage to renal tissue with subsequent leakage of serum into the urine, one would expect a positive reaction for albumen. The amount of serum protein leaking into the urine may, on the other hand, be very minute and still give a positive antibody titer. The test for albumen may then be too insensitive to detect traces of albumen. The presence of antibodies in the urine may interfere with the detection of leptospirae in the urine samples since the organisms are lysed. Inoculation of urine into animals should be performed as soon as possible after sampling to avoid inactivation of the leptospirae.

The maximal duration of leptospiruria has been found to be 35 days (Tables 9 and 18). Ewe 781, shedding leptospirae on the 35th day, was killed for necropsy on the 44th day after inoculation. Leptospirae were not harbored in the kidneys at the time of necropsy. The number of organisms excreted in sheep urine seems to be rather low. Organisms have not been observed in darkfield microscopical examination of fresh ovine urine. Only one or a few of a group of hamsters or guinea pigs inoculated with 1-3 cc. of the same urine sample may show serological evidence of infection. It thus appears that sheep experimentally infected with L. pomona are not very important as reservoirs and shedders of the organisms. This is in agreement with the results obtained by Morse et al. (61).

2. Necropsies

Ewes 763 and 794 were killed for necropsy 4 and 5 days after infection, respectively. Blood cultures obtained from Ewe 763 at the time of the necropsy were all negative. One out of 10 tubes inoculated with blood from Ewe 794 on the day before necropsy, was positive. All cultures obtained on the day of necropsy were negative. The serum antibody titer at the time of necropsy was 10^{-3} in Ewe 794 and negative in Ewe 763. Both ewes were pregnant (Table 3). Both fetuses were alive at necropsy. Cultures and animal inoculations failed to detect leptospirae in amnionic fluid, fetal blood and stomach contents and fetal organs. No antibodies were detected in amnionic fluid, stomach contents or fetal serum. In Ewe 763 only the kidneys were shown to contain viable leptospirae. The brain, samples of cotyledons and kidneys of Ewe 794 were shown to contain leptospirae. In ewes 763 and 794 leptospiral invasion of the fetuses or amnionic fluid had not taken place. Detectable transplacental antibody transfer to the fetuses had not occurred. These two ewes, 763 and 794, were killed at a time when the leptospirae were disappearing from the blood stream. In Ewe 794 the microorganisms obviously were still present in other tissues in addition to the kidneys, while in Ewe 763 only the kidneys harbored live leptospirae. Ewe 794 also exhibited the more marked gross pathological renal changes and hemoglobinuria.

Ewe 836 was necropsied 23 days after infection. The ewe was then 110 days pregnant. The serum titer at the time of necropsy was 10^{-7}. No antibodies were detected in amniotic fluid, fetal serum or stomach contents. The fetus was alive and did not harbor leptospirae, as determined on the basis of appropriate cultures or animal inoculations. Only the kidneys of Ewe 836 were shown to contain leptospirae. The absence of antibody titers in the fetus in the presence of high antibody titers in the ewe clearly indicates that transplacental transfer of leptospiral antibody does not occur; a fact which is further substantiated by the absence of leptospiral antibodies at the time of birth in lambs born to ewes with high serum antibody titers (Table 21).

Ewe 781 was killed for necropsy 34 days after lambing. Animal inoculation failed to reveal leptospirae in the tissues, including kidneys.

One lamb belonging to Ewe 764 died the day following birth. Another lamb which belonged to Ewe 781 died three days after lambing. None of these lambs showed any evidence of L. pomona infection proved by animal inoculations of tissue samples. The cause of death in these lambs remains undetermined. The second lamb of Ewe 781 was killed at the age of 32 days. The lamb had been ill for about 8 days. Blood cultures obtained during febrile attacks were negative for leptospirae. At necropsy extensive pneumonia was found. An organism resembling Brucella bronchiseptica was isolated

from the lungs, liver, spleen and brain. Guinea pigs injected with such material remained healthy and no lesions were observed when the animals were sacrificed one month after inoculation. At that time one out of three guinea pigs, inoculated with lung emulsion, had a titer against L. pomona. This phenomenon, for which there is no obvious explanation, may be attributed to a laboratory error.

3. Lambing and Lambs

The ewes lambed normally. The gestation periods are shown in Table 3. The gestation periods for the ewes lie all within the range of normal variation, which is 140 to 160 days. In group I samples of fetal membranes were obtained probably several hours after birth. In group II samples of fetal membranes were obtained immediately after birth. The fetal membranes, except those from Ewe 781, and all colostrum samples did not contain viable leptospirae. Injection of fetal membranes from Ewe 781 into 2 guinea pigs resulted in death in 4 days in one and an agglutination-lysis titer in the other guinea pig. A urine sample obtained 4 days after lambing was proved to contain leptospirae by animal inoculation. Whether the leptospirae were derived from the placental membranes themselves or from contamination with urine cannot be determined with certainty. Considering the presence of high serum antibody titer in the ewe and the absence of leptospirae in the blood of her lambs at birth, it would seem likely

that contamination with infective urine was the source of the leptospirae infecting the guinea pig.

Colostrum and milk whey from infected ewes were shown to contain antibodies against L. pomona (Tables 11 and 21). On some occasions the titers in the whey were higher than in the blood serum of the ewe. The sera of the lambs did not contain leptospiral antibodies at birth. A rapid appearance of titers occurred after the lambs had obtained colostrum. In most lambs the antibody titers of the sera disappeared approximately 3 weeks after birth. Blood samples obtained from Ewe 770, which was not infected, and her lamb, remained negative. No antibody titer was found in colostral or milk when from that ewe. Transmission of the disease, then, from the ewes to their lambs did not take place. The presence of antibodies in the sera of these lambs during the most active shedding period, would appear to offer an explanation. Ewe 770 and her lamb, however, did not have serum antibody titers and should have been quite susceptible. Transmission did not occur even to these animals.

4. Leptospirae in Milk

At the time of lambing, i.e., from 7-81 days after infection (Table 3), none of the colostrum samples contained leptospirae. Ewe 758 was infected to determine the extent of excretion of leptospirae in the milk. Leptospirae were

shed in the milk from the fifth to the ninth day after inoculation (Table 22). Blood cultures were positive only on the sixth and seventh day following inoculation. The two lambs of Ewe 758, which nursed the ewe for one month during this experiment, did not show any fever and did not develop antibody titers. Transmission of the disease, then, did not take place, although the milk contained leptospirae for five days. At the time of inoculation of the ewe, the lambs were 30 days old. One lamb received high titer serum while the other received high titer milk at 52 and 75 hours of age. Antibodies were not demonstrated in the lamb's sera. The number of organisms shed in the milk was probably too low to cause infection through ingestion. No symptoms of mastitis occurred in Ewe 758. It is borne in mind at this point that Baker and Little (4') isolated L. pomona from acute mastitis in cattle.

5. Antibody Transfer to the Newborn

It has previously been pointed out that no detectable antibodies were found in the sera of ovine fetuses, although the serum titers of the ewes were very high. This has also been found to be true for lambs at the time of birth. Shortly after the lambs had nursed infected ewes, antibodies appeared in their sera. The serum titers of the lambs increased rapidly during the first two days after birth and often reached higher values than those of the sera or milk

wheys of their mothers (Tables 11 and 21). Transplacental transfer of leptospiral antibodies did not occur in detectable amounts in the lambs. The presence of antibodies in the sera of young lambs born to infected ewes is due wholly to intestinal absorption from high titer colostrum. The antibody titers of the whey decreased rapidly after the first few days of nursing. The titers of the lambs decreased slowly to nondetectable levels approximately 3-4 weeks after birth.

The sudden occurrence of serum antibodies in the lambs after the first nursings and the subsequent disappearance of titers in spite of continuous ingestion of milk containing antibodies stimulated the following studies on the influence of the age upon antibody absorption.

A 24-hour-old lamb did not show antibody titers after receiving 75 ml. of serum with a titer of 10^{-6}. Absorption was then shown to take place in a 10-hour-old lamb which received through stomach tube 50 ml. serum with a titer of 10^{-6}. The results of further experiments with lambs of various ages are shown in Table 23. On the basis of these experiments it may be concluded that the age factor plays an important role in the intestinal absorption of colostral antibodies in the newborn. Absorption of antibodies occurs in newborn lambs up to the age of 15-16 hours. At the age of 24 hours or older, detectable amounts of leptospiral antibodies have not been found in lambs. Due to

a lack of lambs for further studies on this phenomenon, the age period from 15-24 hours was not investigated.

SUMMARY

A study was made on experimental infection of pregnant ewes with Leptospira pomona. The ewes were inoculated subcutaneously either with blood from infected guinea pigs in the leptospiremic phase or with a culture of leptospirae. The ewes became ill after an incubation period of 3-6 days. They showed elevated temperatures and had a leptospiremia which lasted from 2-5 days. In some ewes, leptospirae were present in their blood before the rise in temperature. Hemoglobinuria was observed in only 2 of 12 infected ewes. The ewes subsequently developed serum antibody titers, and shed leptospirae in the urine. The maximal duration of leptospiruria was found to be 35 days. Leptospiral antibody titers were first found in the urine approximately the third week after inoculation.

A non-infected ewe and its lamb, which were allowed to stay with the infected sheep, did not contract the disease.

The fetuses of 2 ewes, killed at the end of the leptospiremic phase (4-5 days after inoculation) and the fetus of an ewe killed 23 days after inoculation, were alive at the time of necropsy. The fetal tissues, blood or amnionic fluids did not contain leptospirae or leptospiral antibodies.

The rest of the infected ewes and the control ewes lambed normally. Leptospirae were not detected in their

colostrum at the time of lambing. Placental membranes did not contain leptospirae, with one exception. It is questionable whether the microorganisms in the latter case were derived from the placental membranes themselves, or were due to contamination with infected urine.

High leptospiral antibody titers were found in colostral whey at birth. No titers were found in newborn lambs. A few hours after sucking, the sera of the lambs showed antibody titers and the whey titers of the ewes dropped rapidly.

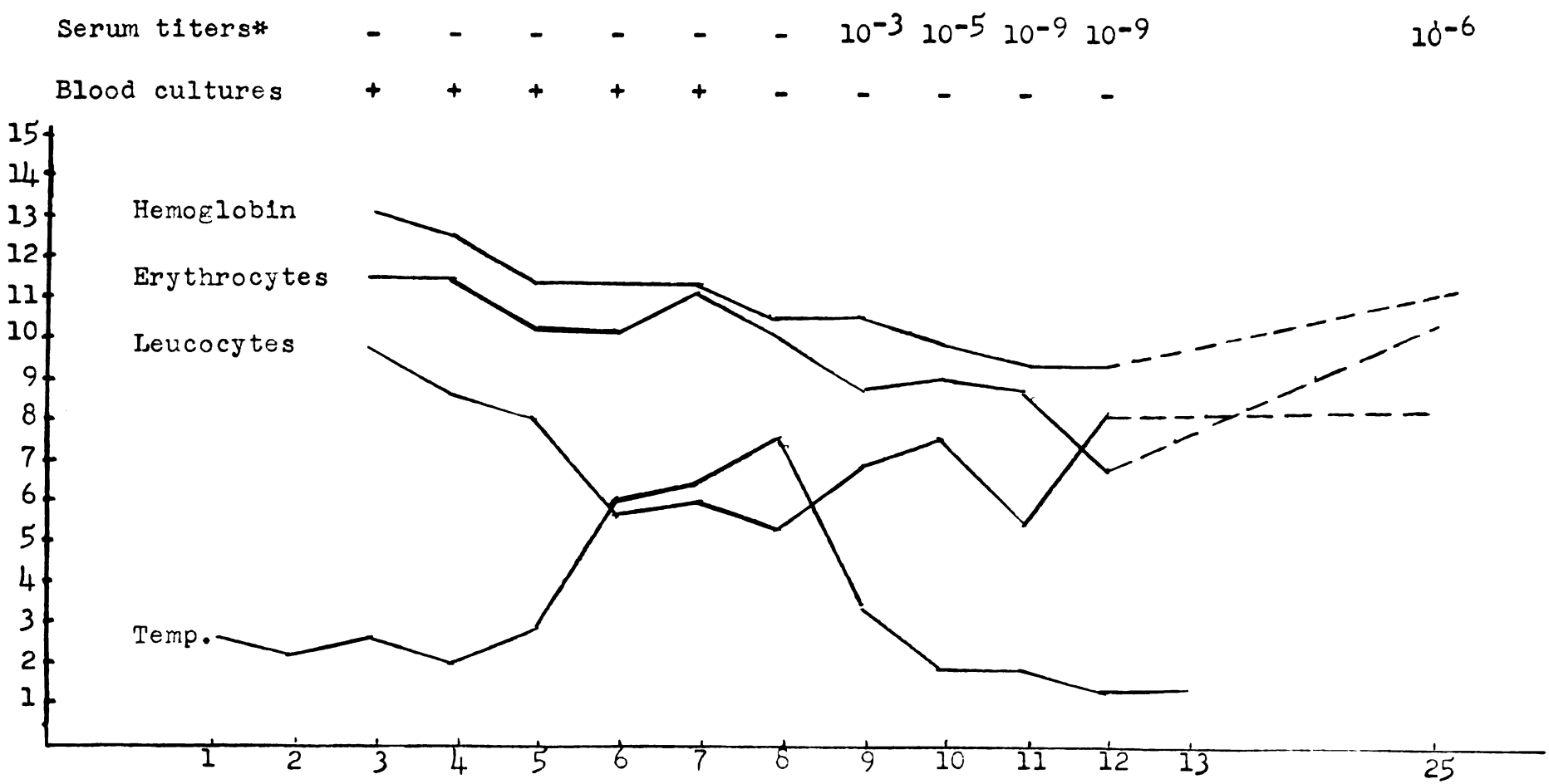
One ewe was inoculated 30 days after lambing. Leptospirae were shed in the milk from the fifth to the ninth day after inoculation. The duration of leptospiremia in that ewe was from the sixth through the seventh day following inoculation. The nursing lambs did not contract leptospirosis, although the ewe was shedding leptospirae in the milk.

Hematological observations were made during the course of the infection in the ewes. A slight decrease was noted in hemoglobin levels and erythrocyte counts in some of the ewes. The significance of this is doubtful, as a decrease in these figures was also noted in the control ewes. During the pyretic response, there was a decrease in the total number of leukocytes and a relative neutrophilia and lymphopenia. There was also a decrease in percentage of eosinophils during pyrexia.

A study was also made on the influence of the age of the lambs upon intestinal absorption of leptospiral antibodies. Milk or serum containing antibodies, was administered through a stomach tube to normal lambs of different ages. Absorption occurred in lambs younger than 15 1/2 hours old, but not in lambs older than 24 hours. Unfortunately, lambs were not available for studies on antibody absorption in the period from 15 1/2 to 24 hours of age.

FIG. 1

Characteristic course of infection obtained for Group I, represented by Ewe 832



Abscissa: days after infection
Ordinate: temperature in degrees F. + 100.
leucocytes x 10^3 per cmm.
erythrocytes x 10^6 per cmm.
hemoglobin in grams per 100 ml. blood

*Expressed as 50 percent agglutination-lysis

TABLES 2 through 23

TABLE 2

NORMAL HEMATOLOGICAL DATA (DECEMBER, 1956)

Ewe No.	Hemo-globin	Erythro	Leucocytes	Differential Counts						NPN
				Eosin	Band	Segm	Lympho	Mono	Retic	
608	13.7	11.70	8400	6	1	25	68	0	0	49.0
737	12.0	11.15	12450	2	0	34	63	1	0	41.5
758	13.4	10.16	9300	3	2	16	78	1	0	43.0
761	13.3	11.50	12850	3	0	18	78	1	0	50.0
763	14.1	9.18	7100	5	5	27	61	2	0	43.0
764	13.7	10.64	7250	4	2	36	58	0	0	41.5
770	13.9	10.99	9850	0	3	22	72	3	0	43.0
777	13.3	12.24	7900	7	2	25	65	1	0	43.7
781	11.3	9.22	9400	4	1	32	63	0	0	43.0
786	16.2	13.73	8300	6	0	19	74	1	0	43.0
794	12.7	13.00	7550	1	1	14	84	0	0	53.0
832	13.2	11.39	11550	6	0	27	66	1	0	59.0
836	12.7	10.30	8600	5	1	23	70	1	0	54.0

Hemoglobin in grams per 100 ml. blood

Erythro = erythrocytes in millions

Eosin = eosinophils

Band = juvenile neutrophils

Segm = polymorphnucleated neutrophils

Lympho = lymphocytes

Mono = monocytes

Retic = reticulocytes

NPN = mg percent nonprotein nitrogen in blood serum

TABLE 3

HISTORY OF THE EWES

Identification Number	Date of Breeding	Date of Lambing	Duration of Gestation (days)	Duration of Gestation before Inoculation (days)
608	10/20/56	3/15/57	146	136
737	10/21/56 or 11/6/56	4/5/57	165 or 149	85 or 68
758	10/19/56	3/19/57	151	control, infected after lambing
761	10/20/56	3/12/57	143	135
763	10/24/56	killed before lambing		131
764	10/21/56	3/12/57	142	134
770	10/20/56	3/20/57	151	not infected
777	10/19/56	3/11/57	143	136
781	10/24/56	3/14/57	141	131
786	10/19 + 11/2/56	3/29/57	147	72
794	10/21/56	killed before lambing		123
832	10/19/56	3/23/57	155	87
836	10/19/56	killed before lambing		87

TABLE 4

DURATION OF LEPTOSPIREMIA IN GROUP I,

DETERMINED BY BLOCD CULTURES

Ewe No.	Days after Inoculation									
	3	4	5	6	7	8	9	10	11	12
737	-	-	+	+	-	-	-	-	-	-
786	+	-	+	+	-	-	-	-	-	-
832	+	+	+	+	+	-	-	-	-	-
836	-	-	+	+	+	-	-	-	-	-

TABLES 5 and 6

SERUM ANTIBODY TITERS OF THE EWES IN GROUP I*

TABLE 5

L. pomona titers

Ewe No.	Days after Exposure																
	3	4	5	6	7	8	9	10	11	12	23	25	59	69	78	82	97
737	-	-	-	-	1	±	3	4	7	6		4	4			3	4
786	-	-	-	-	1	3	4	6	7	7		5	5		5		6
832	-	-	-	-	-	±	3	5	9	9				7			6
836	-	-	-	-	-	±	3	5	10	9	7	7	6				

TABLE 6

L. icterohaemorrhagiae, AB titers

Ewe No.	Days after Exposure												
	3	4	5	6	7	8	9	10	11	12	23	25	59
737	-	-	-	-	-	-	±	3	3	3		2	
786	-	-	-	-	-	-	±	3	1	3		3	3
832	-	-	-	-	-	-	±	2	2	3			
836	-	-	-	-	-	-	±	2	3	2	2		

*The titers are expressed as negative exponents of the highest serum dilutions showing 50 percent agglutination-lysis.

± : 25 percent agglutination-lysis in the 10^{-1} dilution of serum.

TABLE 7

TEMPERATURES, HEMOGLOBIN VALUES, ERYTHROCYTE AND TOTAL LEUCOCYTE COUNTS OF GROUP I

Days after Inoculation	Ewe 737				Ewe 786				Ewe 832			
	Temp.	Hgb.	Erythro.	Leuco.	Temp.	Hgb.	Erythro.	Leuco.	Temp.	Hgb.	Erythro.	Leuco.
3	100.4	13.0	12.25	9600	102.6	13.7	12.40	7600	102.6	13.1	11.49	9750
4	102.6	14.1	12.84	8900	104.0	14.5	11.88	5750	102.0	12.5	11.47	8600
5	105.0	13.3	12.48	9050	106.4	12.5	10.38	5500	102.8	11.3	10.22	8000
6	106.0	13.7	11.53	9000	106.0	13.3	12.18	6750	106.0	11.3	10.13	5700
7	102.4	14.1	9.77	9210	105.4	11.9	10.89	5800	106.4	11.3	11.12	6000
8	102.3	13.7	11.14	6000	103.8	11.0	11.70	4250	107.5	10.4	9.98	5300
9	101.8	12.3	11.17	6800	101.0	11.9	10.16	5800	103.4	10.5	8.66	6850
10	100.8	13.5	12.94	7750	101.3	11.9	10.53	6450	101.8	9.8	9.00	7500
11	101.5	12.9	12.35	8050	100.2	11.5	10.15	7100	101.8	9.3	8.62	5450
12	101.8	14.5	11.75	7500	101.0	12.1	11.52	7050	101.2	9.3	6.75	8050
25		13.9	12.34	7900		14.1	11.73	6550		11.0	10.33	8250

Temp. = temperature in °F.
Hgb. = hemoglobin in grams per 100 ml. blood
Erythro. = erythrocytes in millions per cmm.
Leuco. = leucocytes per cmm.

TABLE 7, continued

Days after Inoculation	Ewe 836				Ewe 794			
	Temp.	Hgb.	Erythro.	Leuco.	Temp.	Hgb.	Erythro.	Leuco.
3	102.8	11.7	10.67	7550	101.4	14.5	12.20	6200
4	102.0	11.3	10.35	6450	102.0	13.7	11.60	7450
5	104.0	12.0	10.30	6650	102.0	13.0	11.16	8150
6	106.9	12.7	11.47	5450	101.4	13.3	11.12	7500
7	105.6	12.0	10.58	5700	102.0	12.5	11.23	6700
8	106.6	10.0	9.56	3350	101.6	12.0	10.30	7400
9	103.4	10.5	9.10	4200	101.5	11.3	9.55	6000
10	101.6	10.1	8.53	6400	102.2	12.3	10.55	6000
11	102.0	9.8	9.91	6500	102.0	11.7	10.34	5850
12	102.2	10.1	9.04	6500	100.8	11.3	8.81	6200
25						11.7	9.81	6100

TABLE 8

DIFFERENTIAL LEUCOCYTE COUNTS OF GROUP I

Days after Inoculation	Ewe 737					Ewe 786					Ewe 832					Ewe 836					Ewe 794 (control)				
	E	B	S	L	M	E	B	S	L	M	E	B	S	L	M	E	B	S	L	M	E	B	S	L	M
3	4	0	30	65	2	9	0	30	61	0	6	0	22	70	2	6	0	23	68	3	7	0	24	68	1
4	6	0	23	71	1	9	0	33	57	1	4	0	24	70	2	9	2	27	60	2	7	0	16	75	2
5	3	1	39	56	1	1	15	32	52	0	6	0	20	73	1	9	0	28	62	1	5	4	15	74	2
6	0	0	23	77	0	0	8	20	72	0	1	2	47	50	0	0	0	61	39	0	4	1	16	78	1
7	1	5	24	69	1	1	14	30	55	0	1	21	27	51	0	1	22	42	35	0	6	3	28	62	1
8	3	0	24	73	0	1	0	36	63	0	0	0	52	47	0	0	1	53	45	1	3	0	20	77	0
9	4	0	13	82	1	2	0	20	78	0	1	0	24	75	0	0	0	35	63	2	5	0	19	76	0
10	2	0	17	80	1	1	0	20	78	1	0	0	20	77	3	2	0	31	66	1	4	0	24	72	0
11	2	0	36	62	0	1	0	25	73	1	1	0	22	75	2	1	0	24	73	2	5	0	27	68	0
12	2	0	17	81	0	1	0	28	70	1	0	0	16	84	0	6	0	21	72	1	2	0	15	82	1
25	6	0	17	77	0	8	0	21	71	0	8	0	21	71	0	*6	0	28	65	1	8	0	26	66	0

E = eosinophils
B = juvenile neutrophils
S = polymorphnucleated neutrophils
L = lymphocytes
M = monocytes

*At necropsy 23 days after infection.

TABLE 9

DURATION OF URINARY EXCRETION OF LEPTOSPIRAE IN GROUP I, DETERMINED BY ANIMAL INOCULATIONS

Ewe No.	Days after Inoculation									
	12	18	22	24	29	31	33	40	59	73
737	+	-		-	-		-	-	-	-
786	-	-	+		-	+	+	-	-	-
832	-	-	+		+		-	-	-	-
836*	+	-	-							

*Ewe 836 killed for necropsy on the 23rd day after inoculation.

TABLE 10

ANTIBODY TITERS FOR L. POMONA IN THE URINE OF GROUP I*

Ewe No.	Days after Inoculation								
	12	18	22	24	29	33	40	59	73
737	-	-		-	-	-	-	-	-
786	-	-	-		-	-	-	2	2
832	-	-	-		-	2	3	2	2
836	-	-	-						

*The titers are expressed as negative exponents of the highest urine dilutions showing 50 percent agglutination-lysis with L. pomona antigen.

TABLE 11

ANTIBODY TITERS FOR L. POMONA IN COLOSTRUM (OR MILK) AND SERA OF EWES, AND SERA OF LAMBS IN GROUP I*

Number of Ewe or Lamb	Days after Birth																					
	<1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	18	19	24	26	45	52	58
serum 737	4																				4		
milk 737	7	4	3				2					2											
lamb 737	3	5	4		5		4					3						±			-		
serum 786	6																					6	
milk 786	7	6	4	3	3	2	4		4	3				4			3					2	
lamb 786	7	6	7	7	8	5	7	7	6	6		6		7			6			4		-	
serum 832	7																						6
milk 832	6	4	4	3		3	3	3	3	2	2	2	2		2	2		3	2				2
lamb 832	7	6	9	8	7	7	7	5	8	5	6	4	5		5	5	6	5	4				-

*The titers are expressed as the negative exponents of the highest serum dilutions showing 50 percent agglutination-lysis.
± = 25 agglutination-lysis in 10^{-1} dilution.

TABLE 12

HEMATOLOGICAL OBSERVATIONS OF THE LAMBS IN GROUP I AT BIRTH

Lamb of Ewe No.	Hgb.	Erythrocytes	Leucocytes	Differential Counts					R
				E	B	S	L	M	
737	12.3	9,330,000	9350	0	2	42	55	1	0
786	15.3	11,400,000	7650	0	0	41	59	0	0
832	12.3	9,570,000	4400	0	0	55	45	0	0

Hgb. = hemoglobin in grams per 100 ml. blood
Erythrocytes per c.mm.
Leucocytes per c.mm.

E = eosinphils
B = juvenile neutrophils
S = polymorphnucleated neutrophils
L = lymphocytes
M = monocytes
R = reticulocytes

TABLE 13

THE COURSE OF L. POMONA INFECTION IN EWE 794*

Time after Inoculation	Temp.	Blood Culture	Hgb.	Erythrocytes	Leucocytes	Differential Counts					Serum Antibody Titers†
						E	B	S	L	M	
5 hours	102.8	+	12.7	11,930,000	6450	3	0	30	66	1	-
1 day	102.9	+	11.5	9,350,000	6750						
2 "	102.8	+									
3 "	104.3	+	9.8	9,330,000	7000	10	0	37	52	1	
4 "	105.8	+	8.5	7,790,000	5450	1	0	49	50	0	10^{-1}
5 "	105.2	-	9.8	8,050,000	5100	3	0	49	48	0	10^{-3}

*Killed for necropsy 5 days after inoculation.

Temp. = temperature in °F.
Hgb. = hemoglobin in grams per 100 ml. blood.
Erythrocytes per cmm.
Leucocytes per cmm.

E = eosinophils
B = juvenile neutrophils
S = polymorphnucleated neutrophils
L = lymphocytes
M = monocytes

†Antibody titers are expressed as the negative exponents of the highest serum dilutions showing 50 percent agglutination-lysis.

TABLE 14

TEMPERATURES AND HEMATOLOGICAL DATA OBTAINED IN GROUP II AFTER INOCULATION OF BLOOD FROM EWE 794*

Days after Inoculation	Temp.	Hgb.	Erythro.	Leuco.	Differential Counts E	B	S	L	M
			Ewe 758 (control)						
1	101.2	12.7	10.03	8650	4	0	37	58	1
2	101.3	12.3	9.33	7150	9	0	44	61	1
3	102.0	12.3	8.96	9600	6	0	49	44	1
4	101.0	11.3	8.38	8350	11	0	33	56	0
5	102.0	11.3	8.53	9900	7	0	39	54	0
6	102.9	10.4	9.57	7550	11	0	48	41	0
			Ewe 761						
1	101.8	11.3	9.91	9500	4	0	42	51	3
2	101.7	11.7	9.04	11250	2	0	34	64	0
3	101.8	10.8	10.98	11350	3	2	39	54	2†
4	101.3	10.7	9.48	11850	6	0	28	65	1
5	101.6	10.4	9.00	11150	7	0	32	61	0
6	102.1	10.7	10.19	10000	7	0	33	58	2
			Ewe 763						
1	101.9	13.2	10.51	10850	9	0	37	53	1
2	101.8	12.3	9.79	9200	9	0	33	58	0
3	102.4	11.7	9.44	7800	7	0	38	54	1†
4	102.5	12.0	9.52	9100	10	0	30	59	1
5	102.3	12.5	9.81	7700	9	0	27	64	0
6	102.5	11.2	9.23	8650	7	0	37	56	0
			Ewe 764						
1	103.1	11.7	10.61	9650	2	1	58	39	0
2	102.9	11.7	9.70	9700	2	0	44	54	0
3	102.3	11.2	10.10	10450	2	0	55	43	0
4	101.2	11.5	9.98	10500	2	0	60	38	0
5	102.7	11.0	10.37	11550	0	0	53	47	0
6	102.4	9.6	8.87	9650	5	0	50	42	3
			Ewe 770						
1	101.5	13.7	11.02	8250	4	0	31	65	0
2	101.6	13.0	9.83	10100	2	0	41	57	0
3	102.4	12.2	11.23	9350	6	0	26	68	0
4	101.1	12.0	8.83	8050	7	0	21	72	0
5	102.3	12.9	9.70	10950	7	0	34	59	0
6	102.0	11.3	9.04	7950	3	0	36	58	3

TABLE 14, continued

Days after Inoculation	Temp.	Hgb.	Erythro.	Leuco.	Differential Counts E	B	S	L	M
			Ewe 777						
1	102.5	13.7	11.90	12100	9	0	56	35	0
2	102.3	13.3	11.19	9450	15	0	50	35	0
3	102.1	13.0	11.21	10650	8	0	56	36	0
4	102.5	13.0	12.28	12650	11	0	46	42	1
5	102.2	13.0	10.01	12150	8	0	45	45	2
6	101.8	11.0	9.22	10000	4	0	59	37	0
			Ewe 781						
1	102.4	11.0	8.84	9900	5	0	51	44	0
2	101.7	11.2	8.75	8900	7	0	33	60	0
3	102.6	10.4	8.96	8300	6	0	40	54	0
4	102.0	10.7	7.90	9150	10	0	32	58	0
5	102.3	10.4	9.00	8150	9	0	31	60	0
6	102.4	9.3	8.04	9600	8	0	33	57	2
			Ewe 603						
1	101.9	12.0	9.38	11650	9	0	31	60	0
2	101.5	12.0	8.50	9500	9	0	39	52	0
3	101.6	11.3	9.32	8400	6	0	34	59	1
4	102.0	11.0	8.94	7000	9	0	34	56	1
5	102.4	11.3	9.80	7850	6	0	43	49	2
6	102.4	11.3	9.27	9600	5	0	34	50	1

Temp. = temperature in °F.
Hgb. = hemoglobin in grams per 100 ml. blood
Erythro.= erythrocytes in millions per cmm.
Leuco.= leucocytes per cmm.
E. = eosinophils
B = juvenile neutrophils
S = polymorphnucleated neutrophils
L = lymphocytes
M = monocytes

*Blood contained too few leptospirae to infect ewes. Data serve as control information.

†Basophils

TABLE 15

TEMPERATURES AND HEMATOLOGICAL DATA OF GROUP II FOLLOWING INOCULATION WITH 3 x 10^8 LEPTOSPIRAE

Days after Inoculation	Temp.	Hgb.	Erythro.	Leuco.	Differential Counts				
					E	B	S	L	M
			Ewe 758 (control)						
2	102.2								
3	102.5								
4	102.5								
5	102.0	10.7	9.01	6400	9	0	49	42	0
6	102.3	11.1	8.85	7450	10	0	38	52	0
7	102.6	10.3	8.28	7000	15	0	45	39	1
8	102.2								
			Ewe 761						
2	102.4	10.3	8.88	10450	7	0	36	56	1
3	103.0	10.5	8.82	7250	9	0	44	47	0
4	104.0	11.3	9.23	6950	6	0	30	63	1
5	103.0	10.4	9.08	6200	3	0	28	69	0
6	101.8	10.4	8.25	7750	3	0	22	75	0
7	101.8	10.5	8.33	9300	7	0	30	63	0
8	102.6	10.1	8.34	7650	2	0	30	67	1
			Ewe 763						
2	102.6	10.7	8.63	9750	7	0	35	57	1
3	104.5	11.0	8.83	7450	7	0	43	54	1
4	104.7	10.7	8.83	5700	6	0	38	55	1
			Ewe 764						
2	103.8	11.3	9.89	8500	0	0	51	48	1
3	105.9	9.0	8.03	10000	0	0	78	22	0
4	105.6	10.7	9.42	11600	0	0	78	22	0
5	104.3	11.3	9.80	11950	0	0	64	35	1
6	104.5	11.3	8.95	9250	0	0	74	26	0
7	101.8	11.3	9.84	11700	2	0	72	25	1
8	103.8	11.0	9.32	12500	3	0	56	40	1
			Ewe 770*						
2	102.7	10.6	8.90	9100	4	0	36	60	0
3	102.2	11.7	9.60	9700	4	0	29	67	0
4	102.2	10.4	8.91	9450	3	0	34	63	0
5	102.2	11.0	8.87	9350	1	0	18	81	0
6	102.1	11.0	8.87	9950	1	0	38	60	1
7	101.6	10.7	9.69	11600	4	0	24	70	2
8	102.8	11.5	9.53	9500	1	0	29	70	0

TABLE 15, continued

Days after Inoculation	Temp.	Hgb.	Erythro.	Leuco.	Differential Counts E	B	S	L	M
				Ewe 777					
2	102.7	11.7	9.57	10950	8	0	54	38	0
3	104.3	12.7	10.31	12000	5	0	58	37	0
4	103.6	11.7	10.40	8750	0	0	65	34	1
5	104.1	12.0	9.36	9100	0	0	77	23	0
6	102.5	11.3	9.25	12050	0	0	73	27	0
7	100.3	10.7	8.95	15000	0	0	71	25	4
8	102.3	9.3	8.78	10900	3	0	45	51	1
				Ewe 781					
2	102.8	9.6	8.35	7650	2	0	45	52	1
3	105.5	8.8	8.35	9400	3	0	62	35	0
4	103.5	9.6	9.10	7550	0	0	56	43	1
5	103.8	9.3	8.35	8450	0	0	51	49	0
6	103.8	9.8	8.61	7800	1	0	51	48	0
7	100.8	9.0	8.00	8500	7	0	38	52	3
8	102.5	9.8	7.60	8750	2	0	29	67	2
				Ewe 608					
2	102.0	10.5	8.59	9000	8	0	43	47	2
3	102.0	10.3	8.77	6000	7	0	40	53	0
4	102.0	10.1	8.74	7150	6	0	39	55	0
5	102.2	9.8	7.50	9250	8	0	32	60	0
6	101.8	10.4	9.07	8500	7	0	41	52	0
7	102.4	10.1	8.77	9800	10	0	47	40	3
8	102.3	10.1	8.30	7150	5	0	13	82	0

Temp. = temperature in °F.
Hgb. = hemoglobin in grams per 100 ml. blood
Erythro.= erythrocytes in millions per cmm.
Leuco. = leucocytes per cmm.
E = eosinophils
B = juvenile neutrophils
S = polymorphnucleated neutrophils
L = lymphocytes
M = monocytes

*Not infected

TABLE 16

DURATION OF LEPTOSPIREMIA IN GROUP II, DETERMINED BY BLOOD CULTURES

Ewe No.	Days after Inoculation						
	1	2	3	4	5	6	7
761	-	+	+	+	-	-	-
763	-	+	+	+	-	-	-
764	+	+	+	+	-	-	-
777	+	+	+	+	-	-	-
781	+	+	+	+	-	-	-
608	-	-	-	-	-	-	-

TABLE 17

SERUM ANTIBODY TITERS IN GROUP II*

Ewe No.	Days after Infection										
	4	5	6	7	8	10	14	17	31	37	67
761	-	2	4	6	8		7		5		4
764	-	-	2	5	8		9		7	5	5
777	-	-	4	7	7		9	9	7	7	4
781	-	-	3	7	6		9		8	9	
608	-	-	-	1	2	4	5		2		2

*Titers are expressed as the negative exponents of the highest serum dilutions showing 50 percent agglutination-lysis with L. pomona antigen.

TABLE 18

DURATION OF URINARY EXCRETION OF LEPTOSPIRAE IN GROUP II, DETERMINED BY ANIMAL INOCULATION

Ewe No.	Days after Inoculation					
	14	23	35	52	65	81
761	+	-	-	-	-	-
764	-	-	-	-	-	-
777	-	+	+	-	-	-
781*	+	+	+			
608		-	-	-	-	-

*Ewe 781 killed for necropsy 44 days after infection, kidneys negative.

TABLE 19

ANTIBODY TITERS IN THE URINE OF GROUP II*

Ewe No.	Days after Inoculation					
	14	22	35	52	65	81
761	-	-	-	1	2	2
764	-	-	3	3	2	3
777	-	-	2	2	1	3
781	-	2	2			
608	-	-	-	1/2	-	-

*The titers are expressed as the negative exponents of the highest urine dilutions showing 50 percent agglutination-lysis with L. pomona antigen.

TABLE 20

HEMATOLOGICAL DATA OF LAMBS IN GROUP II AT BIRTH

Lamb	of Ewe	Hgb.	Erythro..	Leuco.	Differential Counts E	B	S	L	M	R
1	758	15.5	11.45	5750	0	0	42	58	0	0
2	758	14.1	10.06	3400	0	0	22	78	0	0
1	761	13.3	9.37	5350						
2	761	13.7	9.26	6850						
1	764	15.0	11.59	4050	0	0	20	80	0	0
2	764	14.7	11.17	4500	0	0	19	81	0	0
1	770	14.5	11.15	2450	0	0	42	58	0	0
1	777	14.1	10.70	4250	0	0	10	90	0	0
2	777	14.5	10.95	4750	0	0	30	70	0	0
1	781	17.1	11.44	3650	0	0	19	81	0	0
2	781	15.7	10.27	3450	0	0	18	82	0	0

Hgb. = hemoglobin in grams per 100 ml. blood.
Erythro. = erythrocytes in millions per cmm.
Leuco. = leucocytes per cmm.
E = eosinophils
B = juvenile neutrophils
S = polymorphnuclcated neutrophils
L = lymphocytes
M = monocytes
R = reticulocytes per thousand red cells

TABLE 21

ANTIBODY TITERS FOR *L. POMONA* IN COLOSTRUM AND MILK WHEY, AS WELL AS SERA OF EWES AND LAMBS IN GROUP II

Lamb or Ewe No.	Sample	Days after Birth																
		0	1	2	3	4	5	6	7	9	12	17	18	19	20	21	23
Lamb 1 761	serum	-		6		5		4		3						±	
Lamb 2 761	serum	-		4		5		4		3	3					±	
Ewe 761	whey	4		5		5		4		3						3	
	serum							7									5
Lamb 2 764	serum	-		2		2		±		-						-	
Ewe 764	whey	2		5		4		3		2						2	
	serum							9									
Lamb 770	serum	-									-						
Ewe 770	whey	-									-						
	serum	-										-					
Lamb 1 777	serum	-			3		2					2				-	
Lamb 2 777	serum	-			3		3					1		-		-	
Ewe 777	whey	3			7		4					2			3		
	serum		7									9			9		
Lamb 1 781	serum	-		3		2			-				-				
Lamb 2 781	serum	-		3	2												
Ewe 781	whey		7	4		7			5								
	serum					9											
Lamb 608	serum	-	3		2			3				-					
Ewe 608	whey	3	4		4			1									
	serum				5												

*The titers are expressed as the negative exponents of the highest serum or whey dilutions showing 50 percent agglutination-lysis. ± = 25 percent agglutination-lysis in the 10^{-1} serum dilution.

TABLE 22

THE COURSE OF L. POMONA INFECTION IN A LACTATING EWE (758) EXPOSED 30 DAYS POST PARTUM

Item	Days after Inoculation												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Temperature °F.	102.0	102.0	102.8	102.0	102.5	105.2	106.1	104.0	103.0	102.0	102.1	102.9	
Blood culture	-	-	-	-	-	+	+	-	-				
Serum titers*	-	-	-					1	1	3	6	6	6
Milk titers*	-			-	-	-		-	-	2	6	6	6
Leptospirae in milk**	-	-		-	+	+	+	+	+	-	-	-	-

*The titers are expressed as the negative exponents of the highest serum and whey dilutions showing 50 percent agglutination-lysis.

**Determined by intraperitoneal inoculations into groups of 3 hamsters.

TABLE 23

DEVELOPMENT OF SERUM TITERS IN LAMBS TO WHICH ANTISERUM WAS ADMINISTERED PER OS AT VARIOUS AGES*

Lamb No.	Weight in Pounds	Serum cc.	Age hours	Hours after Serum Administration						
				0	7	10	19	43	47	52
57	9	72	31	-	-		-	-		-
58	8.5	68	30 1/2	-	-		-	-		-
59	8.5	68	29 1/2	-	-		-	-		-
60	9	72	29	-	-		-	-		-
61	11	88	26	-	-		-	-		-
62	10	80	25 1/2	-	-		-	-		-
64	9	32	25 1/2	-	-		-	-		-
80	9	72	15 1/2	-		±			±	
81	10.5	84	15	-		1			2	

*The titers are expressed as the negative exponents of the highest serum dilutions showing 50 percent agglutination-lysis.

± = 25 percent agglutination-lysis in the 10^{-1} serum dilution.

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