LEPTOSPIRA POMONA INFECTION IN CATTLE AND
ITS ETIOLOGICAL ROLE IN ABORTION

DISSERTATION

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A graduate training program usually involves the help of several people. I would like to acknowledge in this small way my appreciation for the help I have received.

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INTRODUCTION

It is accepted today that the recognition of leptospirosal disease entity was first brought to our attention by Wiel in 1886. Leptospirosis has been considered to be a disease problem of consequence since that time in many parts of the world. In other areas such as the United States, leptospirosis has not been frequently diagnosed until recent years. Even today human infections are not common in this country, and it remains to be seen if this is due to a lack of recognition or whether our living conditions and habits truly decrease the incidence of infection.

In this paper, human leptospirosal infections are not our primary concern. Voluminous information on this subject is available for anyone's information. We are primarily interested in bovine leptospirosis and especially the phenomenon of abortion which is believed to occur as a sequela to acute infection.

The 1954 report of the Agricultural Research Service lists leptospirosis as the infectious disease causing the third greatest economic loss to the cattle industry. Realizing that ten years ago the disease was practically unrecognized as affecting cattle in the United States,
one must be impressed by the rapid change in viewpoint of our disease control authorities. It is not known whether leptospirosis has increased greatly in incidence in the last several years, or if our awareness of the disease and our ability to recognize it has increased. However, leptospirosis is definitely recognized as a problem today regardless of its past history.

The present study was undertaken to induce abortion with *L. pomona*, if possible, and to determine its cause. Other aspects of leptospirosis were investigated while using pregnant cattle.
Weil's Disease - Early History

Jaundice was known in ancient times, but more recently, it has been recognized as a symptom and not a specific disease. This enabled the recognition of several disease entities which have jaundice as one of several symptoms.

Stimson (1) reports seeing leptospiras in a kidney section from a patient believed to have died of yellow fever. Sellard (2) reexamined some of the original sections in 1940 and confirmed that the organisms were leptospiras.

Later Noguchi (3) repeated the error that leptospiras were the cause of yellow fever. He isolated the organism by guinea pig inoculation and designated it *Leptospira icteroides* after yellow fever (Typhus icteroides). He realized that it might have been *L. icterohemorrhagiae*.

Several years before, Inada et al. (4) identified leptospiasa as being associated with Weil's disease. They named the organism *Spirocheta icterohemorrhagiae* and were able to grow it in culture medium. They suggested that the organism had not been seen before because upon autopsy the liver is nearly devoid of organisms. Once the technique of isolation of the organism was established, it was found to be world-wide in distribution.
The Morphology of Leptospira

According to Bergey's Manual (5), Leptospira are finely coiled organisms that are 6 to 20 microns long. Both ends are bent into a semicircular hook when in a liquid medium. They maintain a spinning movement in liquid media and a vermiform type motion when in semisolid agar media.

Kelser (6) describes Leptospira as being 6 to 9 microns long and 0.25 to 0.3 microns in width. They are closely spiralled cylindrical organisms with pointed ends.

Morton and Anderson (7) reported their electron micrograph studies of L. icterohemorrhagiae and L. canicola. The length of these organisms were from 4 to more than 10 microns. Measurements greater than 10 microns could not be made for technical reasons. The width as measured by their method was considerably smaller than those previously mentioned, being only 0.07 to 0.14 microns. They also report the spirals to be 0.25 microns wide with a pitch of 0.3 to 0.6 microns. No granules were seen within the cells, nor were any flagella revealed. Differences in technique can readily explain the variations in measurements that have been obtained.

Jacob (8), also using the electron microscope, found abnormal forms in old cultures of a strain of L. canicola.
In addition to regular leptospiras, he found fine, thread-like forms with terminal egg shaped bodies. They did not occur when frequent subcultures were made. Contaminants could not be incriminated. The author considered the possibility of the forms being the result of growth in unfavorable medium and mentioned possible epidemiological significance.

Perhaps Woratz (9) had seen the same phenomenon when he recently reported spherical forms in older cultures grown on solid agar medium. After several weeks on this medium, the organisms either dissolved completely or changed into spherical shapes. He theorizes that these are resting forms which make permanent exogenous existence possible.

Nicholaev (10) examined leptospiras using dark-ground illumination and direct sunlight. He describes disc-like extremities that rotate with a corkscrew action similar to a ship's propeller. No one has confirmed this observation up to the present time.

Cultural Characteristics and Requirements

The diagnosis of leptospirosis has been hindered by the absence of a readily available culture medium. Few diagnostic laboratories keep culture media available for the isolation of leptospiras.
Inada (4) first isolated leptospira organisms using Noguchi's spirochetal medium. Many media have been developed since that time.

Of the semisolid type media, Chang's (11) is probably the most used today. He concludes that leptospiras live on protein and amino acids and cannot utilize simple sugars. Temperatures of 23° to 28°C. are optimum.

Many liquid media are used; but Schuffner's modification of Vervoort's medium, as given by Kelser (6), is a good representative type.

Although work has been done on the growth requirements of Leptospira, a completely synthetic medium has not yet been produced.

Stuart (13) has devised a medium that uses amino acids and salts for the basic solution. It can readily be made up and kept in stock. However, rabbit serum must still be added before use to get a complete medium. The final medium is placed in a water bath at 60°C. for 1 hour to inactivate the rabbit serum. In this article, Stuart points out that even traces of soap are lethal to leptospiras; and the inoculum should be heavy when transferring cultures.

Schneiderman et al. (14) have carried the problem further in their work with L. canicola. By fractionating the
serum, they found that salt-precipitated albumin fractions were about equal to dialized rabbit serum. Crystalline and alcohol-precipitated albumins had low activity.

In another article (15) they showed that in a chemically defined medium, lacking amino acids, but containing rabbit albumin, growth was stimulated by the addition of certain levels of arginine, aspartic acid, glutamic acid and proline. Growth was inhibited by 16 other amino acids. Thiamine was the only necessary vitamin. They were unable to replace the rabbit albumin with a mixture of amino acids simulating the amino acid composition of the albumin.

Serum, or a fraction thereof, is still necessary for growth; a small amount of hemoglobin enhances growth (11). Babudieri (16) used guinea pig blood to replace rabbit serum but found no increase in the growth rate. Serum from a wide variety of animals may be used if care is taken that no antibodies are present in the serum.

Zironi and Carlinfanti (17) recognized growth regulating factors influencing the development of cultures. After a culture reached its maximum growth, they would remove most of the organisms and again get heavy growth. This could be repeated 5 times with maximum results; but by the sixth repetition, the culture was less luxuriant. This
indicates a factor limiting growth which is not the exhaustion of the medium.

Later workers (18,19) with their investigation of requirements, help to explain the growth regulation to a large extent. The need for gaseous O$_2$ on the part of the leptospiras also explains the ring or disc of dense growth just below the surface of solid or semisolid media. Earlier, Chang (11) had also discussed the advantage of having the optimum O$_2$ present.

Rosenfeld and Greene (20) investigated the role of vitamins and enzymes in the metabolism of leptospiras. Some of the vitamins, they discovered, were beneficial in extremely low concentrations, but inhibitory in greater amounts. They theorized that an enzyme system producing molecular oxygen instead of nascent oxygen is responsible for better or continued growth. In their experiment, riboflavin, in a concentration that would prevent growth in several transfers, had no inhibitory effect when catalase was added. On the other hand, the addition of peroxidase would cause the cessation of growth more quickly. They suggested that H$_2$O$_2$ is probably formed and that catalase might produce molecular oxygen from it, while peroxidase might result in nascent oxygen.
The Leptospira media are excellent for supporting the growth of many bacteria. With the slow growth of leptospiras, bacterial contamination is often bothersome. It is not always true that these contaminants are harmful. Frander (21) found that Sarcina enhanced growth. In the medium she was using, a pH of 7.4 was optimum for development of leptospira. Growth was very poor at 6.4; but at 8.2 moderate growth still occurred.

Morrow et al. (22) gave us the first report of the growth of leptospiros on chorio-allantoic membranes of chick embryos. Eggs incubated for ten days were used and transfers made at 4 or 5 days. The embryos died at 6 to 8 days. Guinea pig virulence was still maintained after 20 passages through egg embryos.

Only a few of the media have been mentioned in this paper, but it is hoped that a sufficient background has been supplied to lead to a general understanding of the requirements of Leptospira.

The Serological Manifestations of Leptospirosis

In leptospirosis, antibodies generally appear the second week of the disease and reach a peak by the third or fourth week. The titer then diminishes gradually, but some titer may remain for many years (23).
Several serological tests are available, but the agglutination-lysis test is the one most used at this time. Schuffner's (24) discussion of the test gives the basic information as we know it today. This test is also explained by Wolff (23) in the World Health Organization's monograph series. A well grown culture of leptospires in liquid medium is added to serial dilutions of serum. After incubation at 32°C for 4 hours, a drop of the material is examined under a darkfield illuminated microscope. In negative serum samples, the organisms will be separate, fully motile and in maximum numbers depending upon the original concentration. In positive samples, there will be large numbers of the organisms clumped together; or the total amount of organisms will be drastically decreased. Usually both conditions occur in the same sample to a varying extent.

Schuffner (24) further reported on the use of formalin killed cultures in his laboratory. He cited the following advantages over live cultures: freedom from danger, results more distinct, no lysis in cases of early illness not due to leptospirosis and the ability to apply absorption tests if desired.

The technique was shortened to 15 minutes by Brown (25) who centrifuged the formalin treated cultures to increase antigen density. The test is performed on a glass plate and read with the aid of a hand lens.
Brown and Broom (12) discuss variable agglutinability of formalinized cultures and report that macroscopic tests are not as sensitive as the microscopic test.

Gardner (26) believed the formalized suspensions are difficult to prepare and used live organisms.

Microscopic agglutination tests are time consuming. Naturally, there was interest in the development of a test which could be read without the aid of a microscope. The macroscopic agglutination test, as applied to leptospirosis, has been established as a reliable procedure and discussed by several workers (27,28,29). Antigens for this test have been prepared that are supposed to be sensitive, specific and stable.

Borg-Petersen and Fagroeus (30) reported upon the influence of antigen density when conducting the agglutination-lysis test. The influence of antigen density upon the results of the test has created some uncertainty. They demonstrated that the titer increases as the antigen density decreases when living organisms are used. Also, the sensitivity of living antigen decreases with age; but the increase in density with age may counterbalance or overcompensate the decrease in sensitivity. More work is needed to elucidate various factors of variability in the agglutination-lysis test.
Stoerrer (40) has adapted the agglutination test to the capillary-tube technique and Van Hoeden (41) has used milk (whey) in place of serum for the agglutination test.

The complement-fixation test is also used for the diagnosis of leptospirosis (31,32,34,35). Carlinfanti (33) isolated an alcohol extractable substance common to many Leptospira species. Other workers (36) report a soluble specific complement-fixing antigen in the organism free culture liquor. This substance is heat stable, non-protein in nature and strain specific. Schneider (37) showed that the complement-fixing principles are in polysaccharide-containing cell fractions and are relatively free from protein. Schneider (38) further identified it in an aqueous cell free extract. He was not able to separate genus-reactive from serotype-reactive fractions. An antigen suitable for the complement-fixation test also has been obtained from sonic-vibrated leptospiras (39).

Serological diagnostic tests were compared by York and Johnston (42). They suggested that the complement-fixation test has advantages over the agglutination tests due to its ease of reading, use of contaminated samples and lack of serotype specificity in diagnostic work.
Laboratory Animals Susceptible to Leptospira

Noguchi (3) isolated the organism he called *Leptospira icteroides* by guinea pig inoculation. Today, guinea pigs are still one of the favorite animals for experimental or diagnostic work with leptospiras.

These animals are used for more than just propagation of the organisms. Schuffner (43) and later Bianchi (44) used guinea pigs to obtain pure cultures from contaminated sources. The animals were inoculated intraperitoneally and then bled several times starting about 10 minutes later. Apparently the leptospiras traverse the serosa more readily than other organisms and are first to enter the blood stream.

Van Riel (45) exposed the shaved abdomen of guinea pigs to water being tested for the presence of leptospiira. This gave the organism an opportunity to infect the animal with less danger of other contaminating organisms gaining entrance.

The Golden or Syrian hamster (*Cricetus auratus*) is now used to a large extent. They reproduce rapidly and are cheaper than guinea pigs for experimental or diagnostic work.

Randall and Cooper (46) preferred the hamster for work with *L. canicola*. Larson (47) states that in his work, *L. canicola* killed hamsters, but only produced a febrile reaction with guinea pigs and failed to infect mice.
Conversely, Morton (47) reports that the strain of *L. canicola* he used did not kill hamsters although *L. icterohemorrhagiae* was lethal in 5 to 8 days. Blood cultures were positive for both serotypes in 48 hours.

It has long been known that rats are the chief carriers of leptospirosis by acquiring persistent kidney infections. Already in 1917, Noguchi (49) was studying the serological relationship of leptospiiras isolated from rats originating from different parts of the world. In this article he suggested the genus name, *Leptospira*.

Hiroki (50) had little success in passing leptospiiras through mice, but Packohanian (51) had better results using American deer mice. Of 32 species or sub-species, 26 were susceptible.

Bernkopf (126) infected chickens and found that the organisms persisted in the blood stream for as long as 9 days, while Hoag, Gochenhour and Yager (52) produced leptospiremia in baby chicks. No illness resulted from intraperitoneal injection of the chicks, but organisms could be isolated from the blood for at least 5 days.

**Leptospirosis in Cattle**

The first report of leptospirosis in cattle came from Russia. Michin and Azinow (53) reported on the occurrence
of spirochetal jaundice in the North Caucasus in 1935. For some years there was confusion over whether or not the jaundice seen was caused by leptospiiras, but by 1940 the picture was clarified. Teskikh (54) and Zemskov (55) noticed similarity between leptospirosis in man and cattle. Semskov and fellow workers (56) named the organism *Leptospira icterohemoglobinurica*. The importance rats played as carriers of leptospirosis was recognized by Efimov (57).

In the last 10 years leptospirosis has been recognized for its important place among the diseases of cattle throughout the world. This is not surprising since human leptospiroses are so cosmopolitan.

There are reports of leptospirosis in cattle from Turkey (58), Tunisia (59), Algeria (60,61) and Italy (62). Wikerhauser (63) reported antibodies in over 60 of 300 apparently healthy cattle tested. Only 2 of this group reacted with *L. pomona* antigen while *L. sejroe* was responsible for nearly all the positive reactions. Maria and Quevedo (64) noted the presence of leptospirosis in Argentina. Among the reports from Australia are those of Peterson (65) and Simmons, Lawrence and Forbes (67) who studied leptospirosis in calves.
Jungherr (66) found leptospiiras in Levaditi's stained sections of kidney and liver from a cow. This animal was part of a herd in which cattle showed anorexia and bloody milk.

In the midwest, leptospirosis is known to be present in Ohio (68,69,70), Illinois (71,72) and Wisconsin (75).

Smith and Perry (73) presented a case history of a bull which was fatally infected in Canada. The young bull was found down and unable to rise. Positive diagnosis was made by examination of liver and kidney sections after staining by Levaditi's method.

Field and Sellers (74) described a fatal case history of *L. icterohemorrhagiae* in a calf in England.

These are but a few of the reported cases in cattle from various parts of the world. Other reports will be given while discussing various aspects presented by leptospirosis.

Signs of Leptospirosis in Cattle

Two years after Jungherr (66) gave the first report of leptospirosis in the United States, Baker and Little (76) reported a disease causing abnormal milk in New Jersey. Later papers by these authors (77,78) showed the condition
to be caused by leptospirosis. In the cattle they observed, the milk was blood stained in the severe cases; but in the more mild cases, it usually appeared thickened and yellow. There was a severe decline in milk production, and the udder became soft and flaccid. They were able to transmit the infectious agent to experimental animals, including other cattle. There was no abortion or icterus, and hemoglobinuria was rarely seen.

Sutherland and Morrill (79) stressed the presence of icterus, anemia and hemoglobinuria in the outbreak they saw in Illinois.

The disease as Mathews (80) saw it, ranged from a fatal to a mild and transitory form. The disease attracted attention primarily because abortions were prevalent. Experimental inoculations produced a mild form of the disease in calves.

Reinhard (81) inoculated calves with organisms from natural bovine cases. Fever started from the fourth to the ninth day and lasted 2 to 4 days. Neutropenia and lymphopenia occurred during the febrile period or shortly after, and a transitory anemia was usually observed.

In Australia, outbreaks of leptospirosis are more common in calves than in older cattle. Perhaps the higher
susceptibility of calves to severe, acute infection causes this to only appear to be true. Sutherland, Simmons and Kenny (82) reported on leptospirosis in calves in Queensland. They found a high incidence in the herd with sudden appearance, high mortality and hemoglobinuria. Darkfield examination of centrifuged urine was a useful diagnostic aid. On the basis of agglutination findings, *L. pomona* was the only serotype involved.

Wellington, Ferris and Stevenson (83) reported icterus and hemoglobinuria in most of the cases they have seen. *L. pomona* and *L. mitis* were causes of the disease in Victoria among cows, calves, horses and swine. They noticed that swine are nearly always on the premises where an outbreak has occurred. Clinical manifestations of the disease are usually limited to cows and calves, but swine are thought to be important carrier animals.

Ensor and McClure (84) reported that "red water" or hemoglobinuria is caused by *L. pomona*. Again, the mortality was much higher for calves. Swine were with the calves in 13 of the 15 infected herds. They may be only a reflection of the husbandry methods of New Zealand.

In Israel, the mortality is higher than in many areas according to the figures listed by Van Hoeden et al (85).
In an outbreak of *L. grippo-typhosa*, 10 of 12 animals showing acute signs died. They noticed abortions in heifers, and many cows had high titers before clinical signs were seen in the herd. This suggested the possibility of increasing virulence from serial passage within the herd.

Freund (86) indicated that leptospirosis is increasing in importance in Palestine. He described peracute, acute and chronic forms. The peracute occurs in pregnant animals; the onset is sudden with fever, black urine, dark brown mucous membranes and stasis of the digestive system. The acute form is present in milking cows, the onset being slow, the milk becoming pink and sometimes having blood clots. Jaundice appears along with stasis of the digestive tract, but these signs disappear in about 2 weeks. The chronic cases are similar to the acute except that relapses are common. Some cases show only digestive disturbances.

Little, Beck and McCahon (87) recorded an outbreak in Pennsylvania involving 33 cattle on 7 farms. The signs were fever, anorexia, depression, abortion and a thick yellow secretion from the udder. Organisms were isolated by guinea pig inoculation.

A report of leptospirosis in adult cattle in New York by Reinhard, Tierney and Roberts (88) lists similar signs;
but no abortions were noticed. Hemoglobinuria was prevalent in infected calves.

Winn (89) discussed other diseases causing icterus and anemia. He mentioned bracken fern poisoning, trichlorethylene extracted soy bean oil meal, bacillary hemoglobinuria and anaplasmosis.

Reinhard (90) gave a fine summarization of the signs of leptospirosis in cattle and also explained the physiological and pathological basis for some of the signs commonly seen. He further stated that stunted animals often resulted from leptospira infection in calves.

Serological and Immunological Factors in Cattle

Positive diagnosis of leptospirosis depends upon isolation of the organism. However, serological testing is much easier and more practical to accomplish in routine diagnosis. The sample is readily taken and the test may be performed whenever the sample arrives at the laboratory. Field cases are usually diagnosed serologically after the symptoms have disappeared.

Bernkopf et al. (91) used agglutination titers in conjunction with isolation of the organism to diagnose the disease in cattle. They also noted that several human
cases reacted with high titers to this bovine strain. A later report (92) showed that 9.5% of all cattle slaughtered during a survey gave a positive reaction to the bovine strain.

Bernkopf and Little (93) compared strains of *Leptospira* isolated in New Jersey with a strain from Palestine. The New Jersey strains all reacted alike, but the Palestine strain belonged to another serological group. Guinea pigs inoculated with the Palestine strain showed no reaction; but when inoculated with the New Jersey strain 3 weeks later, they developed fever. The fever rose 2 or 3 days later than in the controls which did not receive the first inoculation.

Later Gochenour, Yager and Wetmore (94) reported results of agglutination-lysis, absorption and animal protection tests which indicated a similarity of the American strains and *L. pomona*. One of the New Jersey strains was included in this report. Today, *L. pomona* is known to be the principal, in fact almost the entire, cause of bovine leptospirosis in the United States.

The distinct antigenic response received from *leptospira* organisms suggests methods of prophylaxis and therapy as well as diagnosis. The Russian workers became aware of
leptospirosis in their domestic animals before other coun-
tries and were in the lead in the use of serum and vaccines
for cattle. Lyubashenko (95) reported that by 1949 over
300,000 animals had been inoculated with their vaccine.
Nefed'ew (96) testified to the efficiency of this vaccine.
We are not aware of any critical method used to determine
the value of the product.

There are reports of the use of inactivated organisms
to produce immunity in hamsters (97,98). The products
gave good protection only against the identical serotype.
The authors pointed out the necessity of immunizing against
all serotypes that may be in the environment of the animal.

Bacterins are available and are being used for the
control of bovine leptospirosis in the United States
(99,100,101). The value of these products is still under
some question. Reinhard (90) pointed out the difficulty
of proving adequate protection to field type exposure.
He declared that 4 conditions must be met before a vaccine
for leptospirosis can be properly evaluated. The conditions
are as follows: uniform opportunity for field exposure of
vaccinates and controls, equal number of controls and
vaccinated animals, all animals tested before start of the
experiment and both groups should be followed serologically,
clinically and by culture. The field exposure conditions
are especially difficult to arrange, and some of the other conditions would require large amounts of work to achieve. Reinhard believes this will preclude the early availability of such information.

The bacterin referred to by Brown et al. (100,101) has been tested to some extent. However, all challenges were by injection. The vaccinates were deemed immune, primarily, by lack of clinical signs and antibody response. Although the development of a titer in the controls was constant and marked, the isolation of organisms and clinical response were not conclusive.

Olitzki et al (102) found high titers or even recovery from prior infection offered no guarantee of immunity against a severe challenge. They also reported that repeated injections with live organisms is the best method of boosting antibody titer.

It has been brought out that, frequently many cattle in individual herds show high titers to leptospirosis by the time the first signs are noticed in the herd. To vaccinate animals already carrying a titer with killed organisms would be of doubtful value (90).
The Pathology of Leptospirosis in Cattle

Awrorow (103) collected information from 40 natural cases and 4 experimentally infected calves. Spirochetes were demonstrated in histological sections of liver, kidney, lung, heart and various lymph nodes. These are presumably acute cases. He reported intense icterus and necrotic areas in the mucosa of the tongue, gums, muzzle and some in the skin, especially around the eyes. The subcutaneous and subserous connective tissues were very edematous. Histological sections of the liver showed foci of necrobiosis, mainly in the central parts of the lobules.

Wylie (111) took a different attitude from the results of his work with guinea pigs. Necrosis of the liver was an inconstant lesion in his experimental infections, and an addition of methionine significantly reduced the incidence. The degree of liver damage did not influence the intensity of jaundice or survival time of the guinea pigs. Renal damage was a constant factor; and in Wylie's opinion, renal failure was the cause of death. Whether or not this information would apply to cattle is a matter of conjecture.

Ungar and Bernkopf (104) observed lesions in the periportal liver tissue with round cell infiltration, but only occasionally saw the central necrotic foci generally reported. In the kidneys, there were some renal tubular
degeneration and interstitial infiltration. The experimental animals were 10 to 14 day old calves of which 8 of 12 died.

Cordy and Jasper (105) reported centrolobular necrosis of the liver and degeneration to the point of necrosis of the convoluted tubules of the kidneys. The kidneys also presented small gray foci on their surface and reddish brown spots which were masses of blood pigment. Ungar and Bernkopf (104) had also mentioned reddish brown kidneys, usually with hemorrhages on the surface.

Hadlow and Stoenner (106) studied the histopathology of a group of Hereford cattle that were naturally infected with L. pomona. Abortion had been the most marked sign. Three months after the acute phase of the infection, they found a widespread chronic, focal, interstitial nephritis. The renal tubule cells proliferated with focal defects in the basement membrane. There was portal and interlobular mononuclear cell infiltration in the liver. Although retained placenta was the aftermath of abortion, no significant abnormalities were found in the uterus.

Winn (89) reported icterus, thin watery blood, hemoglobinuria and whitish foci on the surface of the kidney. In his experience, the lesions upon post mortem were constant; but he stressed the need to know more about the mode
of spread, viability of the organism and other factors before we can control the disease.

Hoag and Bell (107) recently noticed a bilateral, transient uveitis in a calf artificially infected with *L. pomona*. They were successful in isolating the organism from one of the eyes.

A report of *L. icterohemorrhagiae* infection was made in England (108). Jaundice of all the tissues was the principal post mortem finding.

There are 2 reports (109,110) concerned primarily with abortion that mention lesions in the fetus. Icterus and subcutaneous hemorrhage were found. Sippel et al. (110) found the navel cord to be large and edematous.

Mathews (80) was one of the early reporters of leptospirosis in the United States. He recorded most of the lesions we now associate with leptospirosis. He mentioned hemoglobinuria and focal necrosis of the liver as well as chronic nephritis.

**Leptospira pomona in Swine**

Although leptospirosis in swine is world-wide, it is confined mainly to 2 serotypes, *L. pomona* and *L. icterohemorrhagiae* (112). This review will be limited to
L. pomona and, mostly, to some of the articles stressing abortion and its significance in swine leptospirosis. In North America (113), at least, L. pomona is the only Leptospira known to be harbored by swine as the natural host.

There is evidence that cattle and swine might serve as reservoirs of infection for each other. Bohl and Ferguson (70) found both species on the same farm to have antibodies for L. pomona. Burnstein and Baker (114) were able to infect calves and pigs by contact with infected swine. However, the transmission from cattle to swine was not accomplished. They were able to infect pigs at will by subcutaneous and intranasal inoculation but not by oral administration. The importance of swine in the spread of L. pomona was brought out.

Gochenour et al. (115) described an involved outbreak of disease in swine in which hog cholera virus, Salmonella choleraesuis and L. pomona were all recovered from an infected animal. Obvious difficulties in clinical diagnosis could be expected in such situations.

Bryan, Rhoades and Willigan (116) reported the isolation of L. pomona from 5 aborted pig fetuses by guinea pig inoculation.
Bohl, Powers and Ferguson (117) reported a herd in which only 7 normal litters were farrowed from 29 pregnant sows. Abortions, dead and weak pigs were observed in sows that were clinically healthy. *L. pomona* was isolated from a weak pig killed 6 hours after birth. Of the 19 sows tested, all were serologically positive for *L. pomona* and negative for brucellosis.

Artificial infection of sows with *L. pomona* have produced abortions or dead and weak pigs. Riley and Simmons (118) inoculated a pregnant sow that farrowed 32 days later. Four pigs were born dead, 3 died within 3 days and 1 remained healthy to weaning time. *L. pomona* was isolated from 3 of the pigs.

Powers (119) produced abortion in an artificially infected sow. He stated that abortions usually occur 2 to 3 weeks following the first clinical signs of infection in swine.

Sippel (120) pointed out that we do not see reports of abortion in swine from leptospirosis in Europe. He also discussed the differential diagnosis. Eperythrozoonosis, pitch poisoning, ascarids in the bile duct, moldy corn poisoning, crotalara, lupine and other plant poisonings all may produce icterus or anemia. Brucellosis should be considered if abortion is a sign.
Although the incidence of infection was not discussed in this review, it is accepted from available reports that it is considerable in the United States. Abortion is the principal sign seen in this country.

Leptospira Pomona in Horses

In 1948, Heusser (121) reported the results of the agglutination-lysis test which was conducted on a large number of horses. The majority of these horses having periodic ophthalmia were positive while the healthy animals were mostly negative to the test. The author did not transmit the disease nor recover organisms from the eye or blood of the affected horses. \textit{L. pomona} was one of the serotypes found.

A case was cited by Krapf and Brunner (122) involving \textit{L. pomona} infection in a farm worker. A horse on the farm developed ophthalmia soon afterward. The blood titer of the horse was 1/10,000 when taken 18 days later.

Yager, Gochenour and Wetmore (123) also examined a group of horses serologically. Of the horses affected with periodic ophthalmia, 96% were positive to \textit{L. bovis} and 86% positive to \textit{L. pomona}. Only 12% of the normal horses reacted to either test.
*L. pomona* was isolated from horses with an acute septicemic disease by Roberts, York and Robinson (124). This is reported as the first known instance of the actual isolation of *L. pomona* from horses. A mare, involved in the outbreak, foaled 2 weeks early. The colt was weak and died within 24 hours despite attempts to save its life. Icterus was present upon necropsy, but the colt was bacteriologically negative. The mare had aborted twice previously; however, isoerythrolysis was ruled out by checking the mare's and foal's blood.

Wellington et al. (83) reported that a mare aborted and showed a rising titer for *L. mitis*. Later they inoculated a pregnant mare with *L. pomona*; she foaled normally 140 days later.

Bryans (125) studied experimentally produced equine leptospirosis. Leptospiremia and fever were induced although no organisms could be isolated from the aqueous humor. No signs of periodic ophthalmia were produced.

Rossi and Kolochine-Erber (127) reviewed periodic ophthalmia. They found much support for the theory that leptospiras are the causative agents but considered the subject still to be confirmed.
Leptospira Pomona in Human Beings

Clayton and Derrick (128) first brought our attention to a new type of leptospirosis in Queensland in 1937. They reported the disease in a young dairy farmer near Pomona. Leptospiras were isolated by guinea pig inoculation. The disease was mild compared to other types of leptospirosis in Australia.

Derrick (129) reported that by 1942, 80 cases of leptospirosis had been diagnosed in Queensland Department of Health Laboratory and many additional ones by clinical diagnosis only. It was proposed that the organism be called L. pomona since it was serologically distinct from other Australian serotypes.

The previous articles did not incriminate any particular mode of infection. Bruere (130) reported an outbreak in which 3 men contracted the infection while working on a farm harboring infected calves. Swine were raised on the farm, and their pen drained into the calves' paddock.

Gsell (131,132) and Schmid and Giovanella (133) all agreed that swineherd's disease is caused by L. pomona. They pointed out the serological relationship between the human and swine populations. Nearly always the human cases were known to have been in contact with swine in some manner. Gsell also stated that the disease in human beings
is strictly limited to those areas of Switzerland where anti-pomona agglutinins can be found in pigs.

Kochochina-Gruber (134) reported that 2 laborers working with swine were infected with \textit{L. pomona}. The pigs carried agglutinin titers to the same organism.

Five cases of \textit{L. pomona} infection in man were reported by Sandler (135). These were the first recognized cases in Israel, and all cases had a direct contact with swine.

Schaeffer (136) described one of the largest single outbreaks seen in man. At Geneva, Alabama 50 out of 80 people became ill after swimming in a creek. Nearly all who were tested serologically showed a rising antibody titer to \textit{L. pomona}. Dead hogs had been seen floating in the creek. A serological survey of domestic animals in the area revealed some positive reactions when conducted 3 months later.

A case of acute iridocyclitis was reported by Beeson et al. (137). The patient, who was carrying a high titer of agglutinins to \textit{L. pomona}, had worked in an abattoir 7 months previously.
A case of acute iridocyclitis was reported by Beeson et al. (137). The patient, who was carrying a high titer of agglutinins to *L. pomona*, had worked in an abattoir 7 months previously.

Coffey, Dravin and Dine (138) discussed *L. pomona* infection in the United States. It was their opinion that more agglutination tests should be conducted in cases of iridocyclitis, aseptic meningitis and influenza-like diseases.

Spink (139) discussed a case of *L. pomona* infection in Minnesota. It closely resembled infectious mononucleosis and acute brucellosis.

It is clear that *L. pomona* has a definite public health significance, although the disease is mild compared to many other leptospiroses. In the United States it is probably responsible for the majority of the human leptospirosis. Since only domestic animals seem to be implicated in its spread, it is hoped that our increasing knowledge of animal leptospirosis will lead to its control.
The agglutination-lysis test was the sole serological method employed for the detection of leptospira antibody. The antigens were selected from young, vigorous cultures containing numerous organisms. Unless otherwise indicated, *L. pomona* (NIH) was used as the standard antigen.

The serum was diluted to one-half of the desired final concentration with saline before an equal amount of antigen was added in a Kahn tube. The tubes remained at room temperature for 1 to 4 hours before reading. Darkfield illumination was used with a magnification of 90 to 150 times to examine for agglutination or lysis. Approximately 50% of the organisms were lysed or agglutinated before a sample was declared positive. Control tubes were available to evaluate the normal concentration of any sample of antigen.

Several media were available for the growth of leptospira, but a modified form of Kelser's method of preparing Schuffner's medium was used in most instances. In addition, Chang's semi-solid medium was employed to a limited extent.

The modifications of Schuffner's were the use of Difco Neo-peptone, deionized water (Deeminizer), and pooled rabbit serum inactivated at 57°C for 30 minutes. Chang's
medium was also modified by the use of inactivated rabbit serum instead of horse serum.

In some instances, especially when contamination with other organisms was suspected, hamsters were inoculated intraperitoneally in addition to, or in place of, culture media. The hamsters were bled by cardiac puncture on the fourth day after injection. The blood obtained was then introduced into the media. The amount of blood used varied from 1 to 5 drops in each tube. It was customary to use 2 to 4 culture tubes per hamster, and 2 hamsters were inoculated with each type of material.

The prepared media were placed in test tubes using 5 to 7 ml. amounts in each tube. The inoculated tubes were incubated at room temperature except during the summer months, when a 30° C. incubator was used. Examinations for growth were made at 7 to 14 day intervals until 6 weeks had elapsed. Individual tubes were discarded before that time if gross contamination was found.

*Leptospira pomona* (H) was used for all inoculations. An account of the history of this strain is here indicated. The organism had been isolated 4 months previously from the urine of a cow which had aborted. Isolation was accomplished by the use of hamsters from which the leptospiros
were cultured and maintained in Schuffner's medium. This cow was in a herd of 47 adults, which had experienced 16 abortions during a period of 3 months. All the abortions were considered to be due to leptospirosis. Most of the cows, which had aborted, showed the following syndrome: anorexia for a few days, a marked drop in milk production, a flaccid udder, and thick yellow milk. Abortion occurred in most cases 2 to 4 weeks after these clinical signs. Hemoglobinuria was observed in 4 animals. Although only 1 cow was treated with a chemotherapeutic agent, none of the animals died. Seven weeks after the first cow was observed sick, 42 of the 47 animals were serologically positive with L. pomona. This organism was isolated from the urine of 3 cows in this herd.

Studies Using Pregnant Rabbits

Three does were injected intraperitoneally with 0.5 ml. of a Schuffner's culture of L. pomona (II). Two of the rabbits were inoculated on the seventeenth day after breeding and the third on the twenty-fifth day.

Blood was drawn for culturing on the fourth and sixth days after inoculation of the first 2 does. The third doe was bled on the second day when there was a rise of temperature.
At the termination of pregnancy, 2 young from each litter were sacrificed and liver and kidney emulsions were made. This material was used to inoculate culture media for the possible isolation of leptospira.

Studies Using Hamsters

Hamsters were used more extensively to determine the possible effects of leptospira upon pregnant females.

It was soon found that mating hamsters involved some difficulties. Pen type mating was not desirable since the exact date of mating was desired. After more than a week of attempting daytime matings unsuccessfully, further attempts were made in the evening. This concession to the nocturnal habits of hamsters was fortunate and resulted in copulation.

In the first trial 8 hamsters were bred. Usually 2 males were allowed to copulate with each female to obtain greater chances of fertilization. The females were 4 to 6 months old with their previous reproductive history unknown.

Inoculation of 2 hamsters took place on the fifth day after breeding, 3 were inoculated on the ninth day and 3 remained for controls. In this and succeeding trials, 0.1 ml. of a Schuffner's culture was used as
inoculum. All 8 females were bled and medium was inoculated for the identification of leptospira. Liver and kidneys from young of any resulting litters were cultured.

A more extensive trial involving 29 females was started sometime later. The apparently low conception rate was now recognized and all females were tried for rebreeding several times after mating had taken place. The return to estrus 4 to 7 days after service complicated the schedule of inoculations. It was hoped to have equal groups of females inoculated 1, 6 and 11 days after breeding and a fourth group of controls. However, this did not work out due to the uncertain breeding results.

Cultures were made of the mothers' blood and of tissues from the young by inoculating directly into media.

Studies Using Guinea Pigs

There were 2 separate groups of pregnant guinea pigs studied for the effect of *L. pomona* when inoculated during pregnancy. The first group contained 8 animals. By palpation, 3 were assumed to be about 25 days pregnant and 5 about 35 days pregnant. Two pigs from each stage of pregnancy were inoculated intraperitoneally with 0.2 ml. of a Schuffner's culture of *L. pomona* (H). This culture was the result of an inoculation with infected hamster
blood 10 days previously. The remaining 4 guinea pigs were left as controls. Temperatures were taken daily until a significant rise was demonstrated.

It was possible to obtain fetuses from 2 of the guinea pigs for culture. In one case a second fetus was removed by caesarian section after the first fetus had been aborted and partially devoured by the mother. Fetal kidney and liver suspensions were ground together and cultured via hamsters for the possible isolation of leptospirosas.

A second group of 9 guinea pigs was also utilized. Most of the animals were much advanced in their gestation period. The two in the earliest stage of pregnancy were about 35 days advanced. A Schuffner's medium culture that originated from infected hamster blood was inoculated intraperitoneally into 7 of the pregnant guinea pigs. Since the cultures did not contain large numbers of leptospirosas, 0.5 ml. was used for the inoculation.

Temperatures were taken at 12 hour intervals so that the initial temperature rise could be more closely estimated. Representative young, from aborted or normal litters, were sacrificed for examination. Blood and liver suspension was inoculated directly into media. The guinea
pigs were bled on the sixteenth day after inoculation for serological examination.

Studies Using Cattle

Two pregnant Holstein cows were made available for the first part of the work.

Prior to inoculation, Cow A was last bred 137 days, and Cow B, 196 days. Both cows were tested serologically for brucellosis, vibriosis and leptospirosis.

Although the route of inoculation and strain of *L. pomona* used in these 2 experimental cows was identical, the immediate source of the organism varied. Cow A was inoculated in the conjunctival sac with 2 ml. of a 4 day old Schuffner's culture, whereas, Cow B was inoculated with 2 ml. of a liver suspension from a young Syrian hamster which had been injected intraperitoneally with *L. pomona* (H) 4 days previously. The liver suspension revealed rather numerous leptospiras by direct darkfield examination.

In an attempt to recover leptospira organisms from the inoculated cows, various methods were used. Blood was inoculated directly into media and also intraperitoneally into hamsters. Milk and urine, in 1 to 2 ml. quantities,
were injected into hamsters. A few guinea pigs were also inoculated with urine, in which case they were bled for culture upon the demonstration of a febrile reaction.

Temperatures were taken twice daily and the cows were bled at intervals for serological and hematological studies.

Upon abortion the fetal liver, kidneys, stomach contents, placental fluids and tissue from cotyledons were inoculated into hamsters. Blood obtained on the fourth day from these hamsters was inoculated into Schuffner's and Chang's media. These tissues were also inoculated on blood agar plates, part of which were incubated in 10% CO₂.

Several pregnant cattle were available for the final investigation. These animals were divided into 3 groups of 3 each. The identification and stage of pregnancy of each cow at the time of inoculation are available in Table 7.

*L. pomona* (H) was used for all 3 groups, but the immediate history of the organisms varied between groups. The organism, when inoculated into the first group, had been passed serially through 2 hamsters, a heifer, and then back through a hamster. A homogenate of hamster
blood, kidney, liver and spleen tissues ground in Schuffner's medium was used for the inoculum.

An 11 day old Schuffner's culture from blood of cow 1199 of the first group, was injected into hamsters. After 4 passages through hamsters, a blood and tissue homogenate was prepared and used for inoculating group 2.

The immediate history of the culture used for the third group was more complex. A young bull (107) had been injected with organisms from the seventh serial passage in hamsters. When the bull was demonstrating a febrile reaction, blood was withdrawn and inoculated into hamsters. Ground liver and blood from these hamsters was introduced into the cattle.

In all groups, the inoculum contained sufficient leptospiros to be readily visible upon darkfield examination. Intramuscular injections using 5 ml. of inoculum, were employed throughout.

There were 2 yearling Jersey bulls included in the experiment. Bull 97 was inoculated with the same inoculum used for the second group of cows. Bull 107 was mentioned previously.
In addition to the routine observations made upon bull 97, semen was collected by means of rectal massage of the ampulla and accessory organs and also by use of the electro-ejaculation technique. Attempts were made to culture leptospira by the intraperitoneal injection of 1 ml. of semen into hamsters.

Material for the following information was collected after the inoculation of the cows: rectal temperature, presence of leptospirosis, blood count, antibody titer and presence of leptospirosis. The leptospirosis, antibody titer and leptospirosis, were determined by methods already explained, excepting that direct blood culture was the only method used for the examination for leptospirosis. Red, white and differential blood counts were made and hemoglobin was estimated with a Dare hemoglobinometer. In some instances the urine was examined by darkfield after centrifugation at 10,000 R.P.M. for 5 minutes in place of or in addition to hamster inoculation.

When abortions occurred, the fetuses were examined and cultured to the extent warranted by their state of decomposition. Darkfield examination and hamster inoculations were made of the blood and tissues. Blood agar plates and other media were inoculated with material from
the fetus to isolate any other organisms that might be present.

Additional procedures were employed upon several of the pregnant cows. A laparotomy was performed in the left flank of cow 2290 on the ninety-eighth day after inoculation. Homogenized blood and liver in Schuffner's from the twenty-first serial passage through hamsters was injected through the uterine wall into the cervical region of the fetus. The fetus was active at this time.

Cow 603 was injected intramuscularly on the ninety-eighth day after inoculation with the same inoculum prepared for the injection of the fetus of cow 2290.

A caesarian section was performed upon cow 1181 on the thirteenth day after inoculation. A live heifer calf was removed and the following fetal tissues were cultured via hamster inoculation: stomach contents, heart blood, liver and placental fluid.
RESULTS

Pregnant Rabbits

Two of the does were inoculated on the seventeenth day after breeding. The body temperature of both does remained normal during the 10 days. One doe had a litter of 12 living and apparently normal young on the thirty-second day of pregnancy. The other had no young nor were there any signs in the cage of an abortion. If an abortion occurred, it must have been early in the gestation period because no physical signs of pregnancy became evident. The third rabbit, which was inoculated on the twenty-fifth day after breeding, had rectal temperatures of 104.0° F. and 105.0° F. respectively, 2 and 3 days later. These were the only instances of a temperature reading above normal for the 3 rabbits. The doe apparently suffered no ill effects and gave birth to a litter of 6 live young on the thirty-first day after breeding.

Leptospiiras were isolated from the bloodstream of only 1 doe. This was the doe that had no young. The rabbit that exhibited the temperature increase was not positive by blood culture at the time of the rise.

No leptospiiras were isolated from emulsions of fetal tissues. Some of the individual culture tubes were contaminated with other organisms and were discarded. The majority
of tubes had little or no bacterial growth of any kind.

Pregnant Hamsters

In the first trial with hamsters, 2 of the 3 controls showed no signs of pregnancy nor were any young found in the cage. The third one died as a result of the cardiac puncture technique but did prove to be pregnant upon necropsy.

One of the 2 hamsters inoculated on the fifth day after breeding also had no young, but the other delivered 8 live young on the sixteenth day of gestation. There was some mortality during the first day but this was apparently due to unsuitable environmental conditions. Two of the young were sacrificed, 4 died the first day and 3 developed normally past weaning age.

No young were saved from the 3 hamsters inoculated on the sixth day after breeding. One was found dead having been in dystocia. Another had only a partially eaten fetus left in her cage, while the third showed definite signs of parturition, but no young were found in the cage.

All of the inoculated hamsters yielded positive cultures when bled on the fourth post-inoculation day. The control group gave negative results from culture.
In the next trial, 9 hamsters were inoculated the day following breeding. One gave birth to a litter at the end of a normal gestation period, but 4 others apparently did not conceive. The remaining 4 hamsters died 8 to 9 days after inoculation. Upon necropsy, 2 were found to be pregnant. The fetuses were normally developed for that stage of the gestation period. Decomposition was too extensive to make attempts to isolate leptospira advisable.

There were 4 hamsters inoculated 6 days after breeding. One gave birth normally, and 3 showed no signs of pregnancy.

Only 3 hamsters were inoculated at 11 days, and none of them showed signs of parturition. Some hamsters scheduled for inoculation at this time had been rebred already, and it was now known that the conception rate was apparently low. Therefore, more were kept as controls for the purpose of arriving at a better estimate of the true breeding efficiency.

Two hamsters were inoculated and then rebred in the next few days when they were found to be in estrus. One of them gave birth normally while the other showed no signs of having conceived.
There were 11 control hamsters receiving no inoculations. Of this group 5 delivered young while 6 did not.

All but 3 of the hamsters receiving intraperitoneal injections of \textit{L. pomona} developed a leptospiremia as evidenced by blood culture. Of this group, 2 hamsters had no young; while the third, one of the group rebred after inoculation, farrowed healthy young.

The young of both inoculated and control groups survived equally well, and no leptospiras were cultured from any of the hamsters sacrificed at birth.

\textbf{Pregnant Guinea Pigs}

The significant temperature rises and time of abortion are given in Table 1. The first guinea pig was diagnosed pregnant by palpation. The time of abortion is not definite, but it occurred not more than 2 days prior to examination on the ninth post-inoculation day. The control group maintained normal temperatures, but one aborted shortly after arrival at the laboratory. The other 3 guinea pigs delivered live young at term that grew normally past weaning age.

The fetuses from the inoculated group were positive for leptospiras when cultured. There were no fetal tissues available from the control that suffered an abortion.
Table 2 shows the outcome of pregnancy for the 7 guinea pigs inoculated with *L. pomona* (H) in the second trial.

The remaining 2 served as uninoculated controls. One farrowed normal young, but the other aborted about 20 minutes after her rectal temperature was taken the first day of the experiment. Young from both controls and 4 of the inoculated animals were cultured without any leptospiras being recovered.

Many of the culture tubes became contaminated with other organisms. This was probably the result of not culturing through hamsters.

Both the Schuffner's and Chang's media available at this time did not support growth very well. This might have influenced the results.

Of the 7 inoculated females, 3 developed a temperature higher than 104.0°F the fourth day after inoculation. The temperatures of the other guinea pigs remained within normal limits for the 8 days they were recorded. One of the controls consistently maintained a temperature ranging from 103.0°F to 104.0°F. Blood cultures were made twice when the temperature was the highest, but no organisms were recovered. The animal was suckling 3 young during this time and was showing no other signs of illness.
**TABLE 1-Temperature Rise and Time of Abortion of Guinea Pigs Inoculated with 0.2 ml. *L. pomona* (H) for Group 1**

<table>
<thead>
<tr>
<th>G. pig No.</th>
<th>Time (days) after inoculation</th>
<th>Temperature (°F)</th>
<th>Outcome of Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>104.0</td>
<td>102.6</td>
<td>102.2</td>
</tr>
<tr>
<td>2</td>
<td>102.6</td>
<td>104.5</td>
<td>103.5</td>
</tr>
<tr>
<td>3</td>
<td>104.4</td>
<td>103.2</td>
<td>102.5</td>
</tr>
<tr>
<td>4</td>
<td>102.8</td>
<td>102.2</td>
<td>104.9</td>
</tr>
</tbody>
</table>
TABLE 2—Time of Temperature Rise and Parturition of Guinea Pigs After Inoculation with *L. pomona* (H) for Group 2

<table>
<thead>
<tr>
<th>G. pig No</th>
<th>Time of temp. rise</th>
<th>Time of parturition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>Aborted - 5th day</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>1 live - 1 dead fetus - 5th day</td>
</tr>
<tr>
<td>3</td>
<td>4th day</td>
<td>Aborted - 7th day</td>
</tr>
<tr>
<td>4</td>
<td>4th day</td>
<td>Aborted - 10th day</td>
</tr>
<tr>
<td>5</td>
<td>4th day</td>
<td>Not pregnant - 5th day</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>3 live - 1 dead fetus - 7th day</td>
</tr>
<tr>
<td>7</td>
<td>None</td>
<td>Aborted - 5th day</td>
</tr>
</tbody>
</table>
The guinea pigs' serological reaction to the leptospira agglutination-lysis test is given in Table 3. Animals 1 and 6 had died before the blood samples were taken. The controls were negative at the 1/20 dilution.

Pregnant Cattle

Clinical signs of infection were seen only in Cow B. A rise in temperature was first noticed on the evening of the sixth day. It reached a peak of 105.0°F. on the eighth and subsided to normal on the tenth day. At the height of the temperature curve, the animal was definitely depressed and feed was refused. The milk flow, which had been scant, practically ceased at this time. The remaining secretion became thick and yellow in appearance. Blood was not detected in the urine. These clinical signs disappeared as the temperature receded, and the cow remained apparently normal until the twenty-eighth day when a fetus was aborted. Two days later the cow showed signs of acute metritis. In 24 hours the acute signs subsided, but the placenta was retained for 1 week.

Hematological studies also indicated changes solely in Cow B. After the initial temperature rise, there was a slight decrease in total leukocytes and an increase in immature neutrophiles. These differences were not marked and of doubtful significance. During the metritis, however,
<table>
<thead>
<tr>
<th></th>
<th>G.Pig 2</th>
<th>G.Pig 3</th>
<th>G.Pig 4</th>
<th>G.Pig 5</th>
<th>G.Pig 7</th>
<th>Controls (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titer</td>
<td>1:1280</td>
<td>1:1280</td>
<td>1:320</td>
<td>1:2560</td>
<td>1:1280</td>
<td>Neg at 1:20</td>
</tr>
</tbody>
</table>
a shift to immature neutrophiles and a decrease in total leukocytes persisted for 2 weeks. There were no noticeable changes in the hemoglobin or red blood cell picture.

Table 4 gives the results of attempts to isolate the organisms at different times and by different methods. Leptospiras were isolated from the urine of Cow B on the twenty-sixth day, but several attempts before and after were unsuccessful. No leptospiras were recovered from Cow A although identical procedures as were employed with positive results from Cow B were used.

There were also no leptospiras identified by cultural methods or Levaditi's staining technique from fetal or placental tissues. Examination by darkfield illumination revealed no motile organisms present in the stomach contents. Attempts to recover \textit{Vibrio fetus} and \textit{Brucella abortus} by cultural methods were also unsuccessful.

Table 5 shows the serological titers by the agglutination-lysis test using \textit{L. pomona} antigen. The later titers for Cow A are not given here. These serum samples were not tested until some time after the others. The serum, which was refrigerated but not frozen, apparently decomposed to the extent that the antibody content was affected. The titers ranged from 1/80 to 1/360 and were considered to be
an unreliable means of evaluating the true titer on the basis of the results obtained from the earlier tests.

No positive reactions to the agglutination-lysis test were found when the sera were tested with other species of leptospira except *L. autumnalis*. Here some incomplete reactions did occur in dilutions up to and including 1/160. However, in no dilution was there a typical reaction of at least 50% agglutination or lysis.

Agglutination titers for *Vibrio fetus* and *Brucella abortus* are given in Table 6. The cows originated from a herd with no recent history of brucellosis. These cattle were not vaccinated although calfhood vaccination has since been instituted in the herd from which they originated.

The fetus, delivered the twenty-eighth day after inoculation, showed no signs of degeneration and appeared to have been dead but a short time before delivery. Necropsy revealed no definite abnormalities. The fat deposits in the intercostal spaces might have been more yellow in appearance than normal, and the omentum was also yellowish in color. Whether this was beyond the normal coloring of a fetus at that stage of development could not be determined definitely.
<table>
<thead>
<tr>
<th>Days after inoculation</th>
<th>Blood via media</th>
<th>Blood via hamster</th>
<th>Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>*</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>7</td>
<td>*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Positive; - negative; 0 not attempted
TABLE 5-Titers of Agglutination-lysis Test for Cows A and B

<table>
<thead>
<tr>
<th>Days after inoculation</th>
<th>Cow B</th>
<th>Cow A</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>1/20</td>
<td>1/320</td>
</tr>
<tr>
<td>12</td>
<td>1/2,560</td>
<td>1/5,120</td>
</tr>
<tr>
<td>16</td>
<td>1/10,240</td>
<td>1/5,120</td>
</tr>
<tr>
<td>22</td>
<td>1/10,240</td>
<td>0*</td>
</tr>
<tr>
<td>26</td>
<td>1/5,120</td>
<td>0*</td>
</tr>
<tr>
<td>36</td>
<td>1/5,120</td>
<td>0*</td>
</tr>
<tr>
<td>54</td>
<td>1/5,120</td>
<td>0*</td>
</tr>
</tbody>
</table>

* Samples unsatisfactory for testing
## TABLE 6—Titers of Cows A and B for Brucella abortus and Vibrio fetus

<table>
<thead>
<tr>
<th>Time after inoculation (days)</th>
<th>Brucella abortus</th>
<th>Vibrio fetus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>-7</td>
<td>Inc/25</td>
<td>Inc/25</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>1/25</td>
</tr>
<tr>
<td>6</td>
<td>Inc/25</td>
<td>Inc/50</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>Inc/50</td>
</tr>
<tr>
<td>18</td>
<td>Inc/25</td>
<td>1/25</td>
</tr>
<tr>
<td>24</td>
<td>-</td>
<td>Inc/25</td>
</tr>
<tr>
<td>30</td>
<td>-</td>
<td>Inc/25</td>
</tr>
<tr>
<td>38</td>
<td>-</td>
<td>Inc/25</td>
</tr>
<tr>
<td>54</td>
<td>-</td>
<td>Inc/25</td>
</tr>
<tr>
<td>73</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 7 shows the days that *L. pomona* was cultured from the bloodstream of the infected cows and the days when elevated rectal temperatures were recorded. Isolation of organisms from the urine was attempted at various times, and more were made with some cows than others. Most of the successful attempts of isolating leptospira from the urine were made about 1 month after inoculation. One exception was cow 1088 whose urine was positive on the one hundred-second day. This animal was slaughtered 4 months after inoculation, but hamsters were not infected by kidney emulsion from the cow.

The outcome of pregnancy and length of the gestation period when inoculated are listed in Table 8 for the second trial of cows.

The aborted fetus from cow 1199 was considerably dehydrated. Negative cultures were obtained from 4 hamsters injected with ground liver tissue. Rectal palpation performed 2 weeks prior to the abortion revealed that the middle uterine artery had lost some of the characteristic surge that is noticeable in advanced pregnancy. Probably, the fetus was already dead at this time.

The day before cow 1124 aborted, it was noticed that udder development had advanced rapidly, and there was some
TABLE 7-Time of Increased Temperature and Positive Blood Culture of Cows Infected with *L. pomona* (H)

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Days of Temperature increase</th>
<th>Days blood culture positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1118</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>1088</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>1199</td>
<td>5</td>
<td>4, 5</td>
</tr>
<tr>
<td>2290</td>
<td>4</td>
<td>2-5</td>
</tr>
<tr>
<td>603</td>
<td>3,4</td>
<td>2, 3</td>
</tr>
<tr>
<td>986</td>
<td>5</td>
<td>2, 3, 5, 6</td>
</tr>
<tr>
<td>1124</td>
<td>3,4</td>
<td>2, 3</td>
</tr>
<tr>
<td>1151</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1181</td>
<td>3, 4, 5</td>
<td>1-5</td>
</tr>
<tr>
<td>Cow No.</td>
<td>Days of Gestation</td>
<td>Outcome</td>
</tr>
<tr>
<td>---------</td>
<td>------------------</td>
<td>----------------------------------------------</td>
</tr>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1118</td>
<td>263</td>
<td>Calved normally</td>
</tr>
<tr>
<td>1088</td>
<td>240</td>
<td>Calved normally</td>
</tr>
<tr>
<td>1199</td>
<td>124</td>
<td>Aborted 47 days after inoc.</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2290</td>
<td>29</td>
<td>Decomposed fetus*</td>
</tr>
<tr>
<td>603</td>
<td>101</td>
<td>Progressing normally</td>
</tr>
<tr>
<td>986</td>
<td>-</td>
<td>Not pregnant</td>
</tr>
<tr>
<td><strong>Group 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1124</td>
<td>187</td>
<td>Aborted 19 days after inoc.</td>
</tr>
<tr>
<td>1151</td>
<td>210</td>
<td>Live calf, 1 week early</td>
</tr>
<tr>
<td>1181</td>
<td>202</td>
<td>Caesarian section</td>
</tr>
</tbody>
</table>

* Found in vagina 7 days after 2nd inoculation
relaxation of the pelvic ligaments. Rectal palpation revealed no abnormalities, but the calf was carried forward in the abdominal cavity to such an extent that it could not be palpated completely enough to determine fetal movement. After delivery, the fetus appeared as though it had died very recently and was normally developed for the period of gestation. The thymus was enlarged, and both eyes showed corneal opacity. The liver possessed a slight yellowish color on cross section. The pleural cavity contained about a pint of fluid that was strongly stained with hemoglobin but having no red blood cells.

A fetal blood sample contained no antibodies for \textit{L. pomona}. Spleen, pleural fluid and blood were injected into hamsters, but no leptospiras were recovered from the hamsters. Direct cultures of the aqueous humor and pleural fluid into Schuffner's also revealed no leptospiras.

Exudate was obtained from the vagina of cow 2290 when the fetal debris was noticed a week after inoculation of the fetus. Several dead leptospira were noticed upon darkfield examination, but the 2 hamsters that were inoculated with this exudate died of purulent infection within 2 days.
Cultures of the exudate on blood agar plates produced a heavy mixed growth of predominately coliform and streptococcus organisms. The other 2 aborted fetuses gave no significant growth of organisms by this method of culture.

All cattle showed a marked serological response even though organisms may not have been isolated from the bloodstream. The first 2 groups gave a peak response of at least a trace of agglutination at the 1/102,400 dilution. The third group gave the somewhat lower titers of a trace at 1/6,400 for cow 1181 and a trace at 1/25,600 for the remaining 2 cows.

No significant erythrocyte or hemoglobin changes were found in the pregnant cow groups, although transient decreases of leucocytes were noticed in some of the cattle.

Cow 1199 of the group 1 cattle had a leucocyte count of 6,100 on the fourth day after inoculation. Counts performed before and after this time indicated levels around 9,000. The other 2 cows demonstrated no indication of a change in the leucocyte level.

Table 9 shows the leucocyte count for the cows in group 2. Differential counts gave no noticeable change in the comparative numbers of the different types of cells.
<table>
<thead>
<tr>
<th>Days after inoculation</th>
<th>2290</th>
<th>Cow No.</th>
<th>603</th>
<th>986</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8,500</td>
<td>7,450</td>
<td>8,250</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6,150</td>
<td>4,750</td>
<td>8,650</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6,350</td>
<td>3,550</td>
<td>5,650</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5,200</td>
<td>4,800</td>
<td>4,350</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>5,450</td>
<td>5,900</td>
<td>8,150</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>8,100</td>
<td>8,650</td>
<td>6,000</td>
<td></td>
</tr>
</tbody>
</table>
Complete blood counts were not conducted regularly upon the group 3 cows, but samples taken on the fourth and sixth post-inoculation day revealed no changes from the normal numbers.

The reaction of the young bulls to the injection of \textit{L. pomona} was much more marked than the reaction of the other cattle. Table 10 shows the temperature response and the isolation of organisms from the blood stream of these animals. Table 11 gives the changes in the blood picture that occurred during the course of the infection.

Bull 97 was off feed slightly during the febrile response but showed no other clinical signs. Bull 107 died on the seventh day after inoculation. No desire was indicated for feed or water the last 4 days. There was a definite depression and an incontinence of a red to wine colored urine. Death was probably hastened by exertion when photographs were taken about 1 hour before death occurred.

Many lesions were found upon necropsy.\textsuperscript{1} Icterus was generally present. There were subcutaneous petechial hemorrhages. A large blood clot (3 inches in length and 1 inch in diameter) was present on the thoracic end of the thymus. The kidneys were covered with dark petechial

\textsuperscript{1} We are indebted to Dr. Vance Sanger for performing the autopsy.
<table>
<thead>
<tr>
<th>Time after inoculation</th>
<th>Temperature</th>
<th>Blood culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>97</td>
<td>107</td>
</tr>
<tr>
<td>0</td>
<td>100.3</td>
<td>101.4</td>
</tr>
<tr>
<td>1</td>
<td>100.3</td>
<td>101.4</td>
</tr>
<tr>
<td>2</td>
<td>100.8</td>
<td>103.2</td>
</tr>
<tr>
<td>3</td>
<td>103.6</td>
<td>105.1</td>
</tr>
<tr>
<td>4</td>
<td>103.4</td>
<td>105.4</td>
</tr>
<tr>
<td>5</td>
<td>102.4</td>
<td>103.8</td>
</tr>
<tr>
<td>6</td>
<td>104.0</td>
<td>103.4</td>
</tr>
<tr>
<td>7</td>
<td>101.8</td>
<td>96.4</td>
</tr>
</tbody>
</table>

* Positive; - negative; 0 not attempted
* Positive via hamster inoculation only
### TABLE 11—Partial Hemograms of the Young Bulls after Inoculation with *L. pomona* (H)

<table>
<thead>
<tr>
<th>Days after inoculation</th>
<th>Hemoglobin Gm/100 ml.</th>
<th>r.b.c. millions/ml.</th>
<th>w.b.c. thousands/ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>97* 107</td>
<td>97* 107</td>
<td>97* 107</td>
</tr>
<tr>
<td>0</td>
<td>16.5 16.5</td>
<td>6.02 7.34</td>
<td>11.1 13.0</td>
</tr>
<tr>
<td>3</td>
<td>- 16.3</td>
<td>- 7.61</td>
<td>11.85</td>
</tr>
<tr>
<td>4</td>
<td>14.5</td>
<td>6.01</td>
<td>6.80</td>
</tr>
<tr>
<td>5</td>
<td>14.0 16.1</td>
<td>5.70 10.75</td>
<td>6.05 11.60</td>
</tr>
<tr>
<td>6</td>
<td>13.0</td>
<td>5.49</td>
<td>8.75</td>
</tr>
<tr>
<td>7</td>
<td>13.0 5.3</td>
<td>4.82 4.80</td>
<td>8.45 1.40</td>
</tr>
<tr>
<td>10</td>
<td>11.7</td>
<td>3.83</td>
<td>11.80</td>
</tr>
</tbody>
</table>

* Reached normal levels 21 days after inoculation
hemorrhages, and the left kidney contained a small infarct. The blood appeared thin and watery.

Hamsters were inoculated with secretions from the genito-urinary tract of bull 97 several times. The first attempt, 13 days after inoculation, involved rectal massage of the ampulla and accessory glands. A watery semen sample having about 40% to 50% motility was obtained. Leptospiroses were isolated from the inoculated hamsters.

An electro-ejaculator was used to secure the second semen sample 1 week later. The concentration was greater than the first sample, but it was still considered somewhat low. No organisms were isolated from the hamsters injected with this sample. A urine sample collected 7 days later did contain live organisms, although further attempts were unsuccessful due to the death of the hamsters shortly after inoculation.
DISCUSSION

In the past, rabbits have not been considered valuable experimental animals for the investigation of leptospira infections. Although the scope of this work was too limited to draw any definite conclusions, the results would agree with that observation.

It is impossible to decide if there is any significance connected with the fact that the rabbit from which leptospiras were isolated had no young. This doe was thought to be pregnant on the seventeenth day after breeding. The inoculation of pregnant rabbits was not pursued any further, for it was hoped that a more satisfactory subject could be found.

It was already known that, at that time, *L. pomona* (H) was not highly lethal to hamsters. Some young (4 weeks old) hamsters would die 8 to 10 days after inoculation, but adult hamsters could be expected to live. However, leptospiremia was generally present in hamsters after inoculation. It will be recalled that only 3 hamsters, of those inoculated, were not proved to have leptospiras in the bloodstream 4 days later. No information is available from the data that might explain the apparent lack of infection in these three hamsters. Of
course, a leptospiremia could have been missed due to the time of bleeding; or the cultural attempts might not have been sufficiently thorough. Regardless of the cause, the percent of successful inoculations was still satisfactory.

The greatest difficulties were the low conception rate and the various troubles at parturition time. The percent of pregnancies achieved was so low that it would be difficult to determine if any embryonic mortality occurred from leptospiroa infection. It is known that there is no high incidence of abortion and; indeed, there is no indication that any abortions took place.

Hamsters are quite nervous at the time of parturition. The conditions under which these hamsters gave birth contributed to their nervousness. Many other animals were in the room and several workers were going in and out. Many of the young were eaten by their dams immediately after birth, presumably due to these environmental conditions. The examination of newly born young for the presence of leptospires was hindered by this cannibalism.

This strain of *L. pomona* induced abortion readily in guinea pigs. The results in the second group are not as clear cut as in the first, but abortion was very evident. The second group were more advanced in pregnancy. This explains why some full term young were born. Van Thiel (141)
has mentioned that inoculation of guinea pigs near term is more apt to result in dead fetuses being delivered.

Only 2 guinea pigs delivered live fetuses. Both were near term when inoculated. The fetuses, both alive and dead, appeared fully developed when they were born. Of the 4 born alive, all but 1 lived and developed normally. No fever was demonstrated in these 2 dams, but it should be recalled that 2 other pregnant guinea pigs also did not have a fever but still abortion occurred.

The only other criterion of infection was the development of specific antibody. It can be seen in Table 3 that the absence of a rise of temperature was not detrimental to the development of the titer.

Placental transmission of leptospirosis in guinea pigs has been observed before. Takagi (140) reported that organisms of Weil's disease and Akiyami fever were present in the placentas of infected guinea pigs. No leptospiras were isolated from the fetuses. It was the opinion of Takagi that the ciliated cells and other placental tissues protected the fetus from invasion. Costa and Trovisier (142) were able to isolate leptospiras from the amniotic fluid of a guinea pig by inoculating another animal with a portion of the fluid. Van Thiel (141) cites other work in
the course of which leptospiras were isolated from the liver of the fetus.

This would be confirmed by the results of the group 1 guinea pig trial. Leptospiras were isolated via hamster inoculation from tissues of both aborted fetuses and those obtained by Caesarian section. Placental transmission is possible in guinea pigs.

In several instances, abortion took place a sufficient time after the occurrence of fever that antibodies were probably present in the maternal blood stream. Apparently there was no transmission of antibodies to the fetal circulation.

The primary interest of the investigation of this disease in cattle was abortion, but other aspects are also worthy of discussion.

The immediate previous history of the organism is apparently important for leptospiras. Of the first 2 cows, the one receiving organisms in culture medium did not show clinical signs although the antibody response was adequate. Comparisons between only 2 cows are of little value. However, the results from the remaining cows indicate an increasing virulence as judged by clinical response,
recovery of the organism from the blood stream and temporary leukopenia.

The organism was undergoing serial passage through hamsters while this apparent increase in virulence was taking place. Since this was the only change in handling the leptospiroses, this may be suspected as the cause of increased virulence for cattle. We would not necessarily expect passage of an organism through one host to increase its virulence for another host. Perhaps leptospiroses suffer a non-specific attenuation while in artificial media, and animal passage restores its normal virulence for all animals.

Accounts of outbreaks of leptospirosis often report a practically asymptomatic course for adult cattle, but calves show clinical signs and death is common. The 2 males used in this work point out this difference in response in relation to age. They were about 1 year old and could hardly be classed as calves, but their response to the infection was more marked in all respects except antibody titer.

2 Dr. Aziz Hamdy conducted these studies. A more detailed report will be made by him.
The possible presence of leptospiras in semen is a source of concern to artificial breeding associations. A bull with a renal localization of leptospiras could contaminate his semen. Organisms were isolated from a sample obtained by rectal massage. The sample resulting from the use of electrical stimulation gave negative results. The latter contained a higher concentration of spermatozoa. Perhaps the sample collected by massage was diluted with urine, and larger numbers of organisms were present for that reason. If the only contamination of semen is from residual urine in the urethra, few organisms would be expected in the semen sample.

The possibility of leptospiras becoming established in the tubules of the testicle does exist. Brucella abortus localizes there, and Vibrio fetus has been isolated from hamster testicles (143). This aspect of the disease was not investigated in our work, but it is worthy of consideration.

Many reports in the literature emphasize abortion as one of the outstanding signs of the disease in cattle (77,85,106,109,110,145). The only report of an experimentally induced case of abortion in cattle was incidental to an experiment on other phases of leptospirosis research.
The animal aborted 22 days after the onset of fever, which agrees very well with the results of this experiment.

Only 3 of the cows used for this investigation aborted; however, abortion occurs primarily in cattle that are 5 to 8 months pregnant. If only cattle at this stage of pregnancy are considered, the number of abortions is much more impressive. Not counting the cow on which a Caesarian was performed, 5 cows were at this period of gestation. The cows which aborted showed clinical signs of infection and leptospirosis. The height of antibody titer gave no indication of impending abortion since all animals developed titers of 1/5,120 or more.

Abortion in cattle is not rare, and its causes are probably many in number. Such conditions as endocrine disturbances, trauma and nutritional deficiencies are mentioned in this connection; but infectious diseases cause a large number of premature births that occur in cattle.

For many years brucellosis was thought to be the principal bacterial cause of abortion. When abortions continued in the brucellosis-accredited herds it became evident that this was not the whole answer. Trichomoniasis and vibriosis then took their place in the growing list of causative agents of bovine abortion, and other organisms have been occasionally involved.
Leptospirosis is the latest addition to the list of those bacteria causing outbreaks of abortion. This probably accounts for many abortions we did not understand before; but, without a doubt, time will add more microorganisms to the list.

The exact manner in which bacteria produce abortion is not always completely clear. It is believed that the bacteria usually invade the conceptus and cause the death of the fetus directly or damage the placenta to the extent that it interferes with fetal nutrition.

In other bacterial diseases, the organisms are found in the fetal tissues. In this investigation, organisms were readily found in guinea pig fetuses from infected mothers, but cultural methods, staining techniques and dark-field examinations were all negative when used to attempt to demonstrate leptospiras in aborted bovine fetuses. This has been the experience of practically all workers.

Ringen et al. (144), in their previously mentioned report of an abortion in an experimental cow, could not culture organisms. They saw leptospira-like organisms in histopathological study. Fetal tissues contain large amounts of connective tissue fibers which stain similarly to leptospiras when silver impregnation stains are used.
For that reason it would be desirable to make actual isolations of leptospiras when working with fetal tissues.

A most interesting and also most vexing report was made by Podgwaite et al. (146). They recently reported success in isolating leptospiras from 3 of 11 aborted fetuses. Darkfield examination showed leptospira-like organisms in the thoracic and peritoneal fluids of 2 of the 3 positive fetuses. Organisms were not seen or cultured from the stomach fluid, liver, or kidneys of the 3 fetuses.

They used the baby chick inoculation method of Hoag et al. (52) for their isolation of leptospiras. This method is recognized as effective, but it is doubtful that it is superior to hamster inoculation. Although there are slight differences in technique, it is difficult to explain the conflicting results obtained by various investigators.

It may be theorized that the isolation of leptospiras has not been made from aborted fetuses because the material was not fresh when the isolation was attempted. This author and his co-workers made a special effort to examine the material as soon as possible. The Caesarian section was also a part of that attempt. More important was the fact that leptospira-like organisms were found in the
remains of the fetus inoculated "in utero", although this fetus had macerated to the extent that only bones and exudate remained. Some other factor must be held responsible for the lack of success since Podgwaite et al. did not infer any particular haste in their examination of the fetuses.

Two other striking discrepancies exist between the report of these workers and the existing literature. First, 2 of the 3 dams were negative serologically to L. pomona at the time of abortion. On the basis of a limited number of tests, the titer never rose to more than 1/100. It is doubtful if any worker in the field of bovine leptospirosis would consider these abortions due to leptospirosis if serological evidence alone was considered. Most reports state that abortion takes place after the antibody titer has risen to substantial or maximum levels.

The second difference involved the third cow. This animal had a 1/1,000 titer when slaughtered one month after abortion took place, and still organisms were isolated from the liver. Probably in no other place in the literature is there a report of the isolation of leptospiras from the liver of any animal at this stage of the disease.
Organisms have been isolated from the fetus when abortion due to leptospirosis occurred in certain species of animals. Leptospiras were isolated from swine fetuses (116,117,118) and also from sheep fetuses (148). The results of this investigation and those of other workers (141) prove that leptospiras can be isolated from guinea pig fetuses. Ward and Turner (147) isolated Leptospira icterohemorrhagiae from normal rat fetuses at the eighteenth day of gestation.

That leptospiras would be present in bovine fetuses would not be at all surprising. In fact, their apparent absence as reported by most workers is more surprising. However, the single report (146) of the recovery of leptospiras from bovine fetuses needs confirmation by other workers.

Leptospira-induced abortions in cattle occur from 2 to 6 weeks after acute signs of the disease are noticed. This holds true not only for the experimental animals discussed in this paper but also in field outbreaks of the disease. Many times, but not always, the fetus appears to have been dead but a few hours before delivery. If the leptospira gained entrance to the fetal tissues while the leptospiremia was present in the dam, they were relatively innocuous for a period of time.
Assuming that the organisms did multiply in the fetus, their absence at the time of examination must be explained. On the basis of one report (146), isolation is possible from some fetuses; but there remains many unexplained failures.

The lack of a sufficient supply of oxygen might be the reason why organisms are not found. The oxygen supply in a dead fetus would decrease quickly and Leptospira could not be expected to survive long under those conditions. This theory would be difficult to uphold in the face of the fact that leptospiras have been isolated from fetuses of other species. Also in our investigation with the artificially inoculated fetus, organisms were found in the completely macerated fetus.

The type of placenta might play an important role in the transmission of leptospirosis to the fetus. The guinea pig placenta represents the hemoendothelial type which is the most intimate connection between the fetal and maternal circulatory systems. The cow has many more layers of cells between the 2 circulatory systems. Although the bovine placenta is commonly listed among the syndesmochorial group along with sheep, it may actually be more nearly epitheliochorial in nature (149). It must be kept in mind that even if this difference in placental type is significant, abortion
does take place in both species and has to be caused in some manner by *Leptospira* or its metabolic products.

A review of the literature indicates that abortions are rare in early pregnancy. The results of this investigation would help to confirm this viewpoint although only 2 cows were in the early stages of pregnancy when inoculated. This would indicate that the conditions which produce abortion do not exist during infections in early pregnancy. It is possible that early leptospira infection might produce abortion at a later time, but if this is the case, it has not been recognized. There is also no evidence to point to unrecognized early embryonic mortality.

An explanation for the lack of early abortion is not evident. The placenta is less permeable to most agents in the early stages of pregnancy (149). Also, the oxygen carrying capacity of the fetal blood is lower at this time. Possibly one or both of these factors are responsible for the absence of abortion.

In this investigation, 2 cows were inoculated after the eighth month of pregnancy. They delivered apparently normal calves at term. The time between inoculation and normal parturition was too short to expect abortion, but no organisms were isolated from the blood stream of the calves. At least one calf had not nursed before the blood
sample was collected and no antibody titer for \textit{L. pomona} was found in this sample. If the organisms were present in the calves, this would have been an excellent opportunity to recover them. This is another instance where organisms were not recovered in spite of seemingly favorable circumstances.

It is clear that leptospiral infections do cause abortion, but the basic reason for the abortion in infected bovines remains unexplained.
SUMMARY

The primary objective of this investigation was to discover if abortion could be induced by infection with *L. pomona* and to gain an insight to the cause of abortion during leptospira infection. While utilizing pregnant cattle, other phases of leptospira infection were also investigated.

Several experiments were designed using different species of animals.

In the work with rabbits, 3 bred does were inoculated with a strain of *L. pomona*. Only 1 doe developed a leptospiremia. The resistance to infection and asymptomatic course of the disease discouraged further studies with rabbits.

More extensive experimentation with pregnant hamsters failed to produce evidence of abortion. A low conception rate and improper environment for parturient hamsters made the work more difficult. Several litters of hamsters were born to dams that were inoculated at various stages of pregnancy. The young developed normally and showed no signs of infection.

Abortion was produced consistently in guineas pigs. It occurred 5 to 10 days after inoculation, and leptospires
were isolated from several of the aborted fetuses. Of 11 animals inoculated, 2 were near term and farrowed fully developed litters; but several were born dead. There were 7 that definitely aborted fetuses. The remaining 2 were found to be not pregnant 5 and 9 days after inoculation although no signs of placenta or fetuses were seen. They had been diagnosed pregnant by palpation before inoculation.

There were 3 abortions of experimentally infected cows. Evidence points to these abortions as being produced by \textit{L. pomona} although no organisms were isolated from the fetuses. The abortions occurred approximately 2 to 4 weeks after signs of leptospirosis were seen in the dam. This has also been the experience of workers investigating field out-breaks.

A caesarian section was performed on a cow infected 13 days before. The calf was alive when removed, but no organisms could be found upon immediate examination of the calf by darkfield and cultural methods.

A laparotomy was performed upon another previously inoculated cow, and the live calf was inoculated in the cervical region with \textit{L. pomona}. The inoculation was made through the uterine wall, and the calf was not disturbed any further. One week later the macerated remains of the fetus were removed from the dam. Non-motile leptospira
were seen in the exudate, but cultural attempts were unsuccessful.

In the course of the experiment, it was possible to establish leptospiremia and leptospiruria in the majority of the cattle inoculated.

After several passages serially through hamsters, the organism produced more pronounced clinical signs in cattle. One attempt to inoculate a cow with organisms in a culture medium resulted only in an antibody response.


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