HISTOPATHOLOGY OF THE BOVINE PLACENTA IN LEPTOSPIRA POMONA INFECTION

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Ray L. Morter
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The research reported herein is an extension of investigations conducted by the candidate and the major advisor at the University of Wisconsin. Materials collected in connection with this work have been processed and evaluated by the candidate at Michigan State University. The appendix contains two reprints of published articles dealing with the experimental design, bacteriological and serological studies pertaining to the histopathological results delineated in this thesis.

HISTOPATHOLOGY OF THE BOVINE PLACENTA IN *LEPTOSPIRA POMONA* INFECTION

by

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A THESIS

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R.L.M.

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INTRODUCTION

Abortions, usually occurring during the last trimester of pregnancy, have been reported as a manifestation of bovine leptospirosis.[2,3,4,5,8,9,10,13,14] In the United States bovine leptospiral abortions are due almost exclusively to Leptospira pomona infections and abortions may be the only manifestation of bovine leptospirosis. The pathogenesis of the abortions has not been defined. Te Punga and Bishop [15] have postulated three possible causes for leptospiral abortions: (a) pyrexia and systemic reaction resulting in abortion; (b) interrupted transfer of metabolites due to localized lesions in the maternal-fetal cotyledonary junction, with subsequent fetal death; (c) actual invasion of the fetus by the leptospiras with death and expulsion following active fetal infection. Ferguson et al.[4] propose the release of a toxic material from the leptospiras which is able to cross the placental barrier where it is presumed to destroy the red blood cells of the fetus. Alteration of the epithelium of the maternal crypts, the site of production of the hormones of pregnancy in some species, could result in hormonal imbalance and abortion.

Abortions occur two to three weeks after the acute systemic phase of leptospirosis and after the expelled fetus probably had been dead 24 to 48 hours. [2,4,9] Attempts to isolate leptospiras from fetal tissues, fetal fluids or the

placentas have generally been unsuccessful, indicating that L. pomona does not cross the placental barrier in pregnant cattle.[2,3,4,10,11] The report of Podgwaite et al.[12] of isolation of L. pomona from three aborted bovine fetuses has been reviewed by Ferguson.[4] The demonstration of leptospira-like bodies in fetal material by silver staining techniques has been reported but not substantiated by bacteriological evidence,[2,15] Leptospiras may be unable to survive in the environment of the autolyzing fetus. However, attempts to isolate the agent from living bovine fetuses, removed at necropsy, at various times following maternal infection, have been unsuccessful.[10,11]

Histopathological examination of the cotyledons of pregnant heifers, killed at selected intervals following infection, was undertaken to determine if alterations in the maternal-fetal cotyledonary relationships had occurred.

RESULTS

The clinical manifestations of the infection were mild. During the acute phase of the disease, the experimental heifers showed rectal temperatures of 103.2 to 104.4 F. Six of the animals evidenced anorexia, tachycardia and depression. Hemoglobinuria, icterus or diarrhea were not observed. The animals were killed 10 to 46 days following exposure.

Heifer 5 was sacrificed on the tenth post-infection day during leptospiremia. The fetus was approximately seven months old, viable and appeared normal at necropsy. Significant microscopic lesions were not observed in the fetal tissues. Grossly, the uterus and cotyledons appeared normal. Microscopically, the cotyledons showed large areas of hemorrhage with erythrocytic hemolysis and hemosiderosis at the junction of the allantochorion and the maternal tissue (Figure 3). The relationship of the chorionic villi to the maternal crypts was quite normal. Some vacuolation of the chorionic cells was observed. Similar changes occurred in the cotyledons of one heifer sacrificed twelve days after infection.

Heifer 2 was killed 25 days after infection. The fetus was viable and apparently normal, as were the uterus and cotyledons. Areas of hemorrhage at the junction of the

chorion and maternal tissue were observed microscopically. In the deeper portions of the cotyledons there were some hemorrhagic areas and degeneration of the fetal villi characterized by vacuolization (Figure 4). Mitotic figures were occasionally seen in the epithelium. The maternal crypts exhibited increased fibrous connective tissue.

A viable four-month fetus was obtained from heifer 6 at necropsy on post-exposure day 28, and the only gross lesion observed was one small grey-white area in the dam's renal cortex. The most pronounced histopathological findings involved the chorionic villi (Figure 5), some of which showed a vacuolar degeneration. Cellular detail was lacking in these villi and pyknosis was pronounced. The epithelial lining of the maternal crypts was disrupted in some areas and evidenced pyknosis (Figure 6). This would indicate that a degenerative process had resulted in a loss of fetal-maternal relationship in these areas. Increased amounts of connective tissue were present in the stroma of the maternal crypts.

Forty-six days after infection, heifer 7 was sacrificed. The fetus was viable and no gross lesions were observed in the fetus, placenta or uterus. Numerous grayish-white foci were evident in the maternal kidneys and extended through the cortex into the medulla. Microscopically the cotyledons were found to have many empty crypts, some nearly devoid of an epithelial lining. Adjacent crypts contained cellular

debris or degenerating villi (Figures 7 and 8). There was an increase of fibrous connective tissue in the maternal crypts.

Forty-seven days after infection the fetus of heifer 4 was alive. Gross lesions were limited to the maternal kidneys. The microscopic lesions of the cotyledons resembled those of heifer 7. These changes included increased amounts of connective tissue in the maternal cotyledon, degenerate chorionic villi and separation of the villi from the crypts.

Heifer 3 aborted 29 days after experimental infection or 22 days after showing a systemic reaction to the infection. The fetus appeared to have been dead 12 to 24 hours at the time of abortion and mild autolysis was evident. The fetal organs appeared edematous. The pleural and peritoneal cavities of the fetus contained increased amounts of a serosanguineous fluid as did the pericardial sac. The placenta was retained. The animal was sacrificed 24 hours following abortion. Microscopically, the uterus was edematous and the bases of the maternal crypts were congested. The normal architecture of the cotyledon was missing, the connective tissue proliferation obliterating most of the maternal crypts (Figure 9). The crypts were devoid of the cuboidal epithelial lining. The few remainingfetal villi were markedly necrotic (Figure 10). Leptospiras were isolated from the maternal kidneys but from none of the fetal nor placental tissues.

The gross and microscopic lesions in the maternal kidneys were similar to those reported previously.[5] Microscopically the capsules of the kidneys stripped with very little difficulty. The cortex had numerous grayish-white foci which measured up to five millimeters in diameter and in some instances the lesions involved the medulla and hilus. These lesions when observed microscopically revealed a marked infiltration of lymphocytes, a few plasma cells and macrophages. In the same areas some tubules showed atrophy and necrosis. About 47 days postinfection, fibrous connective tissue proliferation caused a thickening of some of the Bowman's capsules of the renal corpuscles. Increased fibrous connective tissue in the intertubular areas led to some tubular atrophy and necrosis.

Terminal serums from the heifers gave agglutination-lysis reactions at dilutions of 1:1,000 to 1:1,000,000. Leptospiras were not isolated from any of the fetuses nor were they demonstrated by the silver staining technique applied to fetal and placental tissues.

DISCUSSION

One of seven heifers experimentally infected with L. pomona aborted 22 days after the acute phase of the disease. The aborted fetus appeared to have been dead 12 to 24 hours and only slight autolysis was observed. The other six animals were killed 10 to 47 days following infection. Their fetuses were viable at the time of necropsy and appeared normal macroscopically as did the uteruses and placentas.

Microscopic changes, progressing from the tenth to the forty-seventh day, were found in the cotyledons, and were most marked in the cotyledons of the heifer which aborted. Ten days after infection areas of hemmorrhage and hemolysis appeared at the chorionic-maternal junction (Figure 3). By the twenty-eighth day the chorionic villi had a vacuolar appearance, pyknosis and karyorrhexis of the epithelium lining the maternal crypts was evident, and proliferation of the interstitial connective tissue between the crypts was apparent (Figures 4, 5, and 6). Forty-six and forty-seven days after infection there was an increase in fibrous connective tissue in the maternal caruncle, loss of epithelium lining the crypts and an absence of fetal villi (Figures 7 and 8). The normal architecture of the placentomes of the heifer which aborted was almost completely

masked by the increased amounts of connective tissue (Figures 9 and 10). Very few necrotic villi were present and a morphological relationship between the fetal and maternal tissues which would permit normal function appeared to be almost completely lacking. The pathogenesis of bovine leptospiral abortions apparently can be explained, therefore, by the alterations in the intimate relationships between the fetal and maternal systems that are necessary to support fetal development. The microscopic lesions demonstrate a series of changes of increasing severity from the time of the active infection in the dam until abortion occurs. These alterations in the placentome can interfere with the transfer of essential materials across the placental barrier resulting in fetal inanition and death. The dead fetus becomes, in essence, a foreign body and is expelled.

Direct infection of the fetus by L. pomona lacks unequivocal bacteriological proof at this writing. The inability to demonstrate leptospiras in any of the seven fetuses is in agreement with most reports.[2,3,4,11] Six of the fetuses were viable at the time materials were taken for bacteriological examination. Therefore, destruction of leptospiras in the environment of an autolyzing fetus could not account for the negative bacteriological results. Isolations were accomplished from the cotyledons of one heifer during the leptospiremic phase of the disease but not from its fetus. This indicates that the organisms

approached but failed to cross the placental barrier during the leptospiremia of the dam. Similar results were obtained by Lindquist, Morse and Lundberg[6] with experimentally infected pregnant ewes. Leptospiras were isolated from the cotyledons of one ewe during leptospiremia. The fetal organs, blood and fluids from this ewe and all ewes sacrificed during the experiment were bacteriologically negative. Positive results must be obtained by accepted bacteriological methods to confirm the presence of L. pomona in fetal tissues. An inoculum composed of a 10 per cent emulsion of 200 mgs. of tissue in 0.85 per cent sterile saline, containing ten or less leptospiras will infect guinea pigs or hamsters.[1] If only a few leptospiras are present in tissues, they may not be recognized by darkfield examination or staining technique but can be recovered by guinea pig inoculation.[16] Silver impregnation techniques can be valuable in determining the presence of leptospiras in tissues but can not be considered a definitive diagnostic means for L. pomona infections, unless the tissues are found to be bacteriologically positive for leptospiras. L. sejroe, L. icterohemorrhagiae and L. canicola infections may result in the invasion of the bovine fetus. The leptospiras observed by some in fetal tissues stained by silver impregnation techniques may actually be one of the above three serotypes and not L. pomona. Clarification of the entire matter awaits experimental proof and should not be based upon conclusions drawn from naturally

occurring infections. Various argyrophilic tissue components can interfere with proper interpretation of silver stained histological preparations by untrained personnel.

Bovine leptospiral abortions occur three to four weeks following infection of the dam. The fetuses apparently die shortly prior to abortion. If fetal death were associated with the systemic reaction of the dam it is questionable if abortion would be delayed up to four weeks.

Fetal hematological determinations were not undertaken but intact erythrocytes were present in the histological preparations of the fetal tissues.

CONCLUSIONS

One of seven pregnant heifers infected with L. pomona aborted 29 days following infection.

Leptospiras were not isolated from the aborted fetus or any of the six fetuses which were viable at the time the dams were killed. Direct infection of the bovine fetus did not appear to be responsible for the abortions under these conditions. A series of histopathological changes was found in the cotyledons which could interfere with the development of the fetus and result in fetal death and abortion.

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APPENDIX

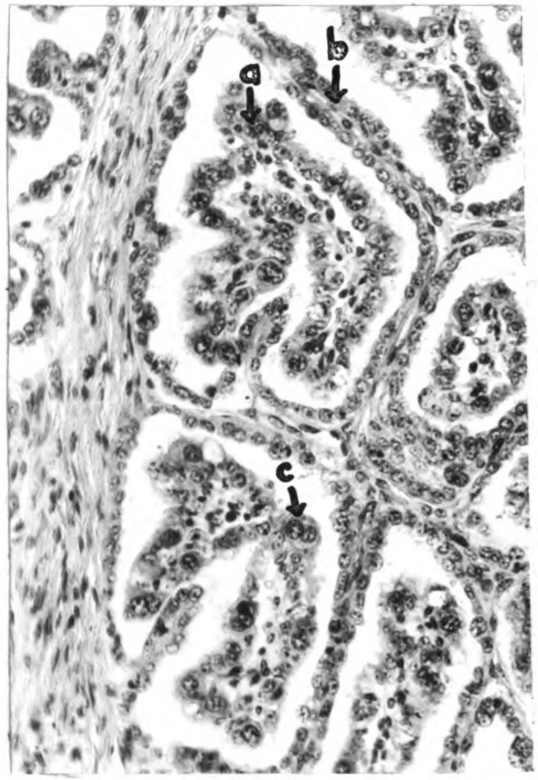

Fig. 1. X 175 Apparently normal bovine cotyledon. (A) Fetal villus, (B) Maternal epithelium with little connective tissue stroma, (C) Diplokaryocyte. (Separation of fetal and maternal tissues in an artifact.)

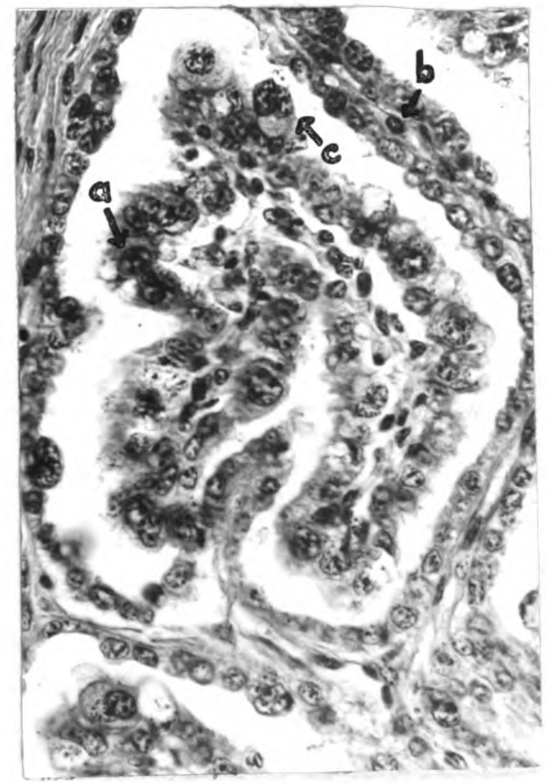

Fig. 2. X 325 Apparently normal bovine cotyledon. (A) Fetal villus, (B) Maternal epithelium, (C) Diplokaryocyte.

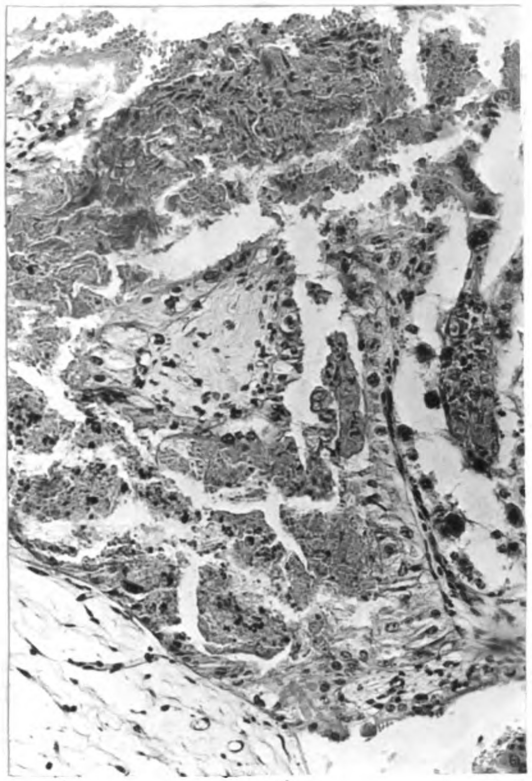

Fig. 3. X 300 Maternal-chorionic junction; area of hemorrhage and hemolysis the tenth day after infection.

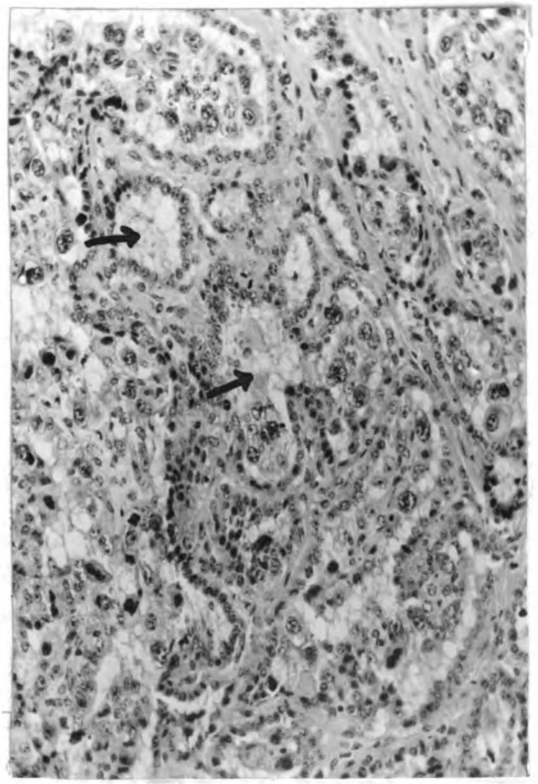

Fig. 4. X 300 Cotyledon of heifer 2, 25 days after infection showing vacuolization of fetal villus.

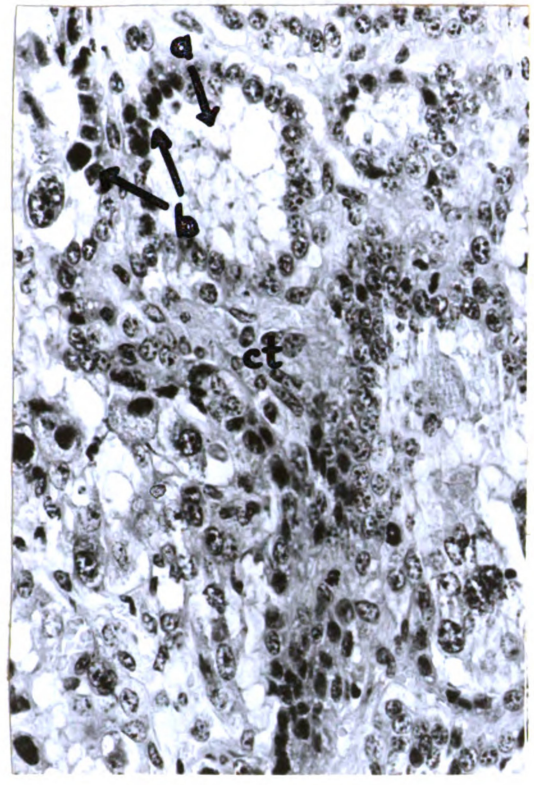

Fig. 5. X 135 Twenty-eight days after infection showing increased fibrous connective tissue and vacuolization of the fetal villi. (A) Vacuolar fetal villus, (B) Pykнotic nuclei in the maternal epithelium, (CT) Maternal connective tissue.

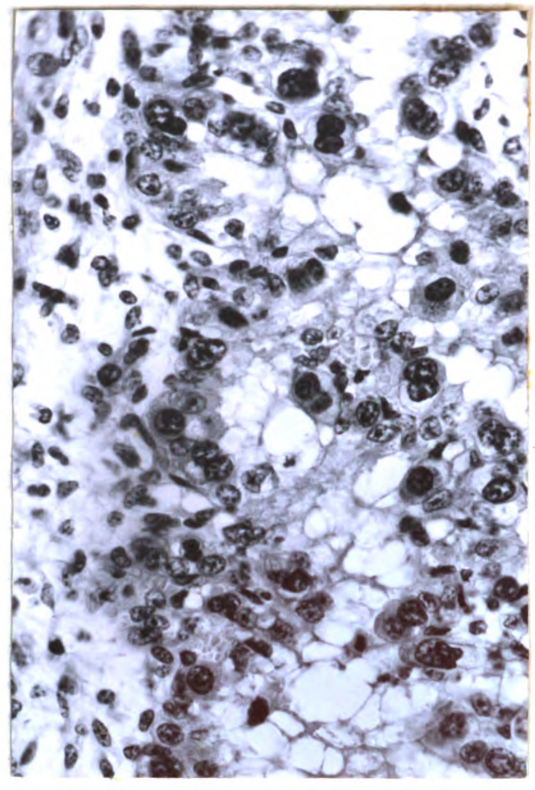

Fig. 6. X 300 Higher power of Figure 4.

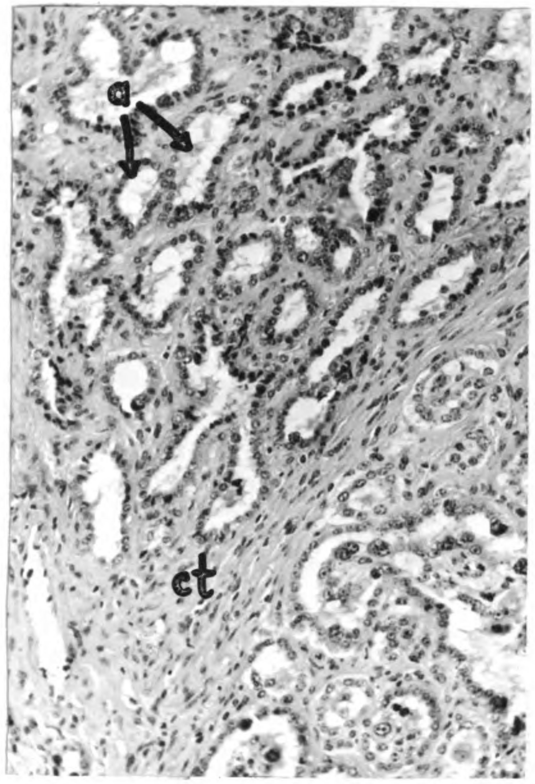

Fig. 7. X 135 Heifer 7, forty-six days after infection showing marked proliferation of connective tissue stroma of the cotyledon (CT), and empty crypts (A).

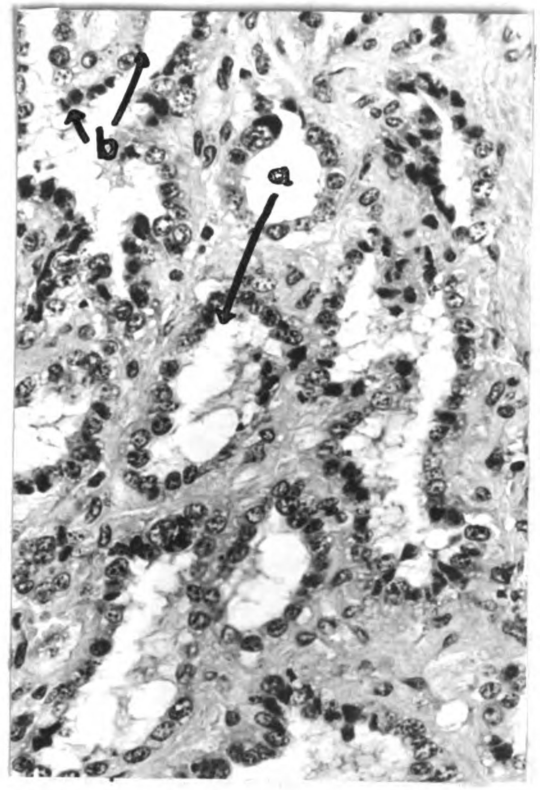

Fig. 8. X 300 Cotyledon of heifer 7, empty crypts with pyknotic nuclei and lack of continuity in the maternal epithelium.

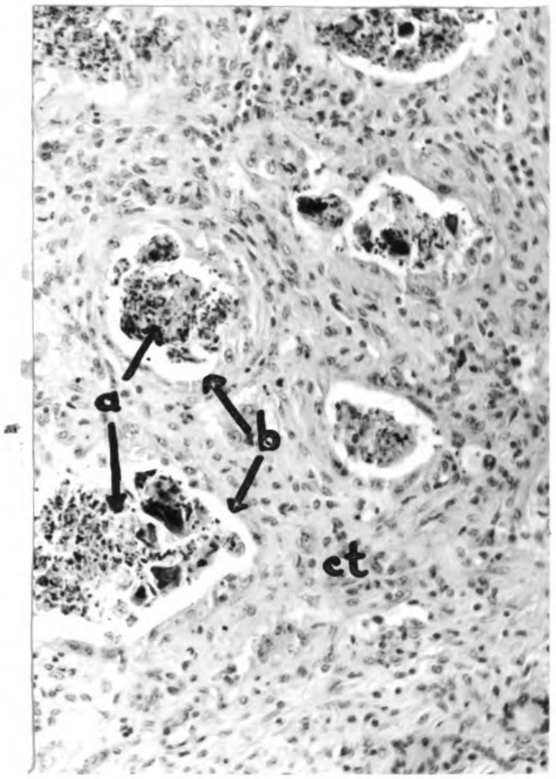

Fig. 9. X 135 Heifer 3 which aborted on the twenty-ninth day after infection demonstrating the loss of the normal cotyledonary architecture. (A) Necrotic remnants of fetal villi, (B) Absence of material epithelium, (CT) Maternal interstitial fibrous connective tissue.

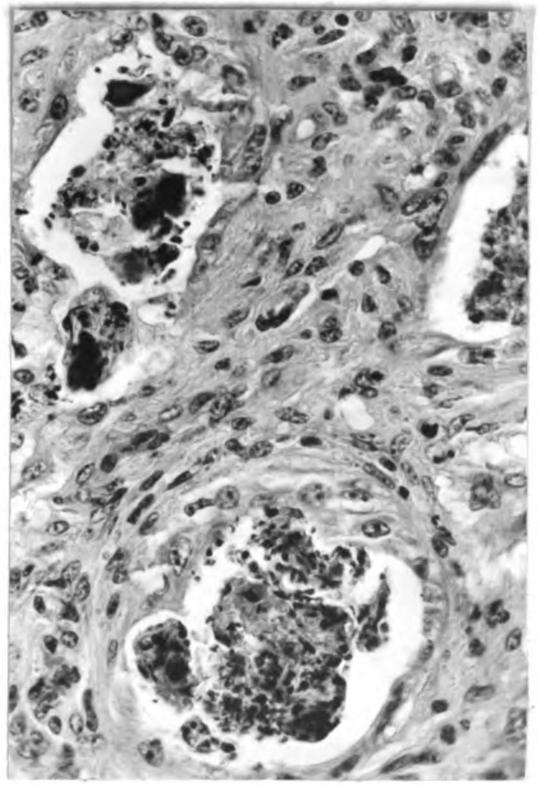

Fig. 10. X 300 Higher power of Figure 9.

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Experimental Leptospirosis. I. The Course of *Leptospira Pomona* Infection in Pregnant Heifers

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LEPTOSPIRA POMONA infection is a zoonosis and as such warrants the attention of both veterinarians and physicians. The disease is currently widespread in both cattle and swine in the United States. Leptospirosis is estimated as the third most important malady of cattle in the United States.[5]

Leptospiras are known to be present in the urine, milk, blood, and other tissues of infected cattle during various phases of the disease. Recent experimental work deals with the course of the infection in calves[11] and the efficacy of antibiotic therapy in cattle.[14] The disease in cattle, as indicated by surveys and field investigations, appears to be most acute and severe in the pregnant and lactating animal.[8] The study to be reported was directed toward elucidation of the course of *L. pomona* infection in the pregnant, nonlactating, dairy heifer.

MATERIALS AND METHODS

Seven, apparently normal, grade Holstein-Friesian heifers approximately 22 months of age served as the experimental animals. The serums of the animals at the outset were negative to the agglutination-lysis test for *L. Pomona* or *Leptospira icterohaemorrhagiae* at the 1:10 dilution,[7] and were negative, *i.e.*, reacted no higher than the 1:25 dilution, when examined by the rapid plate and tube agglutination tests for bovine brucellosis as recommended by the U. S. Department of Agriculture. Conception resulted from artificial insemination. During the course of the investigation, each heifer was maintained in an individual unit of an isolation building.

Heifers 1 through 4 were exposed to *L. pomona*, strain Wickard, during the sixth or seventh month of pregnancy, while heifers 5 through 7 were infected during the third to fourth month of gestation. The leptospiral strain was isolated by the senior author in 1953 from the urine of a cow in southern Wisconsin. The microorganism had been maintained by serial passage in guinea pigs for a number of months and was known to be virulent. The exposure inoculum consisted of heparinized blood (1 cc.. of heparin to 8 or 9 cc. of blood) obtained from 3 to 5 guinea pigs during the pyretic period of the infection. Each heifer was given 5 cc. of the blood by the subcutaneous route.

Heifers 1 through 4 were examined daily, *i.e.*, rectal temperature, heartbeat, and respiratory rates were determined for three days previous to exposure and for 15 days following exposure. Thereafter, examinations were made at five- to nine-day intervals until the experiment was terminated. Blood and urine samples were obtained for bacteriological or serological examination on alternate days during the 15-day postexposure period and subsequently at the time of each physical examination.

Heifers 5 through 7 were examined daily for two days previous to infection and for 14 days following infection; subsequent examinations were made on alternate days until the twenty-fourth day and at weekly intervals thereafter. Blood and urine were obtained for bacteriological or serological examination daily on days three through 14 following exposure and each day the heifers were examined thereafter.

At the time of necropsy, liver, spleen, kidney, lung, cotyledons and udder were obtained from the heifers and fetuses. Blood, urine, fetal stomach contents, and fluids from the uterine and fetal cavities were inoculated into five tubes of modified Chang's fluid medium.[2, 7]

The tissues were homogenized in sterile 0.85 per

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cent sodium chloride solution, and approximately 2 to 3 ml. of the 10 per cent tissue emulsion was inoculated by the intraperitoneal route into each of 2 to 4 guinea pigs weighing 200 to 250 Gm.; the guinea pigs were exsanguinated three to four weeks after inoculation and the serums examined[7] for the presence of *L. pomona* agglutinins. Urine and udder secretions of all heifers were inoculated intraperitoneally while the blood of heifers 1 through 4 was injected by the subcutaneous route into each of the 2 to 4 guinea pigs. The latter procedure was not followed for heifers 5 through 7 since it was found that *L. pomona* usually could be isolated directly, using modified Chang's medium.[2, 7]

Agglutination-lysis tests,[7] employing *L. pomona* strain Johnson as antigen, were conducted on each blood sample from the cattle and their fetuses. Udder secretion as well as fetal fluids, *i.e.*, amniotic, chorionic, or allantoic, when suitalble, were examined by the agglutination-lysis test using *L. pomona* as the antigen.

RESULTS

During the acute or early phase of the infection, *i.e.*, postexposure days one through 12, leptospiremia, fever, anorexia, depression, anemia, icterus, diarrhea, or hemoglobinuria sometimes occurred.[11-13] Heifers 3, 4, and 5 were mildly to moderately pyretic during this period. The observed highest rectal temperatures of these 3 animals were 103.6 F., 103.8 F., and 104.2 F., respectively. Malkmus and Oppermann[6] indicated that the normal bovine body temperature range is 100.4 to 103.1 F. The marked temperature rise of 105.0 F. to 106.0 F., commonly reported for bovine leptospirosis, was not observed.

During the period of leptospiremia, anorexia and depression occurred in 4 of 7 animals. Evidences of icterus were not detectable upon physical examination. However, the buccal mucous membranes of heifer 5 appeared to be paler than normal. None of the cattle were observed to void bloody urine during the course of the experiment. The urine of heifer 2 appeared to be darker than normal on the seventh day following *L. pomona* exposure.

During the febrile period, six to nine days following exposure, either the respiratory or heartbeat rate was slightly elevated[6] for 6 of the animals. Heifer 5 appeared to have an acute pneumonia, and a copious, clear nasal discharge was evident.

Necropsies were performed ten to 46 days following exposure. Multiple small white

TABLE I—Summary of Symptomatological, Pathological, and Bacteriological Observations of 7 Pregnant Heifers with Experimental Leptospira Pomona Infection

Heifer	Clinical features, normal unless indicated*	Necropsy findings	Isolation of L. pomona from:
1	Temperature 103 F. and heartbeat 88 on day 6; anoretic, urine slightly turbid.	Performed on day 12—focal, interstitial nephritis.	Blood on days 6 and 8+; urine on day 12#.
2	Highest temperature 102.4 F., heartbeat 100 on day 7; urine darker than normal on day 7.	Performed on day 25—focal, interstitial nephritis.	Blood on day 8+; kidneys#.
3	Temperature 102.6 F. on day 8, 103.6 F. on day 9; heartbeat 84 and 80, respectively; anoretic day 9; urine dilute on day 8; aborted at 29 days.	Performed on day 30—diffuse peritonitis, septic metritis, retained fetal membrane; focal, interstitial nephritis.	Urine on days 22 and 26#; kidneys#.
4	Temperature 103.8 F.; heartbeat 96; anoretic on day 7.	Performed on day 47—focal, interstitial nephritis.	Blood on days 6+ and 8+#; urine on days 13 and 25; kidneys#.
5	Temperature 103 F., respirations 38 on day 7; temperature 104.2 F., respirations 30; anoretic, clear mucous nasal discharge, loose fetid feces on day 8; temperature 103.1 F., respirations 45, decreased nasal discharge, pale buccal mucous membranes, and some appetite on day 9, heartbeat—slightly elevated	Performed on day 10—large areas of subcapsular cortical renal hemorrhage; abnormal amount synovial fluid in major joints; liver enlarged.	Blood on days 4 through 10+; kidneys, lungs, udder, liver-spleen and cotyledons#.
6	Temperature 103 F., respirations 38 on day 7, heartbeat slightly elevated	Performed on day 28—one small area of focal, interstitial nephritis.	Urine on day 19#.
7	Highest temperature, 102 F. on day 6, heartbeat slightly elevated	Performed on day 46—numerous areas of focal, interstitial nephritis in both cortex and medulla.	Blood on day 5+; urine on days 13, 19, 21 and 29#.

*All days are following exposure. + Chang's medium isolation. +# Leptospiras observed but not in pure culture. # Inoculated guinea pigs' serums positive on agglutination-lysis test.

foci of interstitial nephritis[11] were present in the cortex and extended several centimeters into the medullary portions of the kidneys of all the animals except heifer 6 which had only one such area in one kidney. The other organs of heifers 1, 2, 4, 6, and 7 as well as those of their fetuses appeared to be normal.

Heifer 3 aborted 29 days following exposure. The fetus, it was concluded, had been dead 12 to 24 hours at the time of abortion. The fetal membrane was edematous and friable; manual removal was not possible. The uterus contained a flocculent, mucoid, nonodorous fluid. The fetal organs and muscles of their abdominal and thoracic walls were edematous and moderate autolysis was evident. Approximately 200 cc. of bloody transudate was present in both the thoracic and abdominal cavities. The pericardial sac contained approximately 75 cc. of a similar fluid.

The day following abortion, the body temperature of heifer 3 was 101.8 F. and the heart rate was 140. The animal was killed and necropsy revealed an extensive edema of the posterior portion of the pelvic cavity, uterus, and bladder and a mild peritonitis and concurrent septic metritis. The omentum contained small areas of petechiation, and hemorrhagic areas, 0.5 to 1 cm. in diameter, were present on the cortical surfaces of the kidneys. At the time of abortion, the serum agglutination-lysis titer was 1:1 million, and the titer of the milk whey was the same.

Heifer 5 was killed ten days following exposure. With the exception of the kidneys, all organs appeared to be normal. The kidneys contained numerous areas of subcapsular and cortical hemorrhage. The animal had been reluctant to move during the three days prior to necropsy, and it was concluded that an acute arthritis or synovitis was present. Approximately 20 cc. of synovial fluid was contained in each of the scapulohumeral and hip joints. Leptospiras were not isolated from the synovia.

Leptospira pomona was obtained from the blood of 5 of the animals by direct culture in Chang's fluid medium. Leptospiras were present in the renal tissue of 4 cows as proved by the guinea pig-inoculation technique. Leptospiruria or renal residence of leptospiras or both were demonstrable by guinea pig inoculation procedures for all 7 heifers.

The presence of leptospiras in lungs, udder, liver, spleen, and cotyledons of heifer 5 was indicated by the inoculation of guinea pigs and subsequent development of agglutination-lysis titers.

Leptospiras were not isolated from any fetal materials obtained. Except as specified, leptospiral isolations were not made from the genital tract, liver, spleen, udder, or lungs of the heifers.

The clinical features, gross pathological and bacteriological observations made of these animals are recorded (table 1).

Leptospira pomona agglutinin-lysin titers of 1:10 appeared in the serums of the cattle seven to nine days following exposure. By the thirteenth postexposure day the titers were 1:100 to 1:10,000. The highest agglutinin titer observed was 1:1 million; it occurred 30 days following exposure. A milk whey titer of the same magnitude as the serum titer was observed. Agglutinin-lysins were not present in the urine, fetal fluids, or extrafetal fluids from any of the cattle examined. The results are summarized (table 2).

DISCUSSION

The major problem in the control of leptospirosis appears to be the detection of the animal which sheds leptospiras in the

TABLE 2—Serological Results Obtained with the 7 Heifers Infected with Leptospira Pomona

Heifer	Day**	Positive serum dilution*											
		2-7	9	11	13	20	25	30	47				
1		0	10^{-1}	10^{-2}	....	....	....	....	....				
2		0	10^{-1}	10^{-1}	10^{-2}	10^{-4}	10^{-4}	....	....				
3		0	10^{-1}	10^{-2}	10^{-4}	10^{-4}	10^{-6}	10^{-6}†	....				
4		0	10^{-1}	10^{-2}	10^{-2}	10^{-4}	10^{-4}	10^{-6}	10^{-6}				
	Day**	3-6	7-9	11	13	15	17	22	24	26	28-34	35-46	
5		0	10^{-1}	10^{-1}	....	....	....	....	....	....	....	....	....
6		0	10^{-1}	10^{-1}	10^{-2}	10^{-4}	10^{-4}	10^{-4}	10^{-3}	10^{-4}	10^{-3}	....	....
7		0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-4}	10^{-3}	10^{-4}	10^{-3}	10^{-3}	10^{-3} to	10^{-4}

*Agglutination-lysis test using L. pomona, strain Johnson, as antigen. Titer = highest dilution showing evidence of agglutination or lysis or both. **Day following exposure to L. pomona, strain Wickard. †Milk whey agglutinins present at 10^{-6} dilution level.

urine. Therapeutic efforts are generally directed toward alleviation of the acute and severe manifestations of the disease. The obviously ill animal is naturally considered as a prime source of contagium, but the occult case, as a urinary shedder of leptospiras, is equally as dangerous. The control of this disease in cattle must, therefore, be based not upon individual animal considerations but upon detection of the disease in all carrier animals within the herd. The asymptomatic carrier may shed *L. pomona* in the urine for at least 45 days and probably longer. Slaughtering of acutely ill animals will not eradicate the infection from the herd. Probably the majority of cattle which have agglutinin-lysin titers have been carriers and shedders at some period. In addition, a control program for leptospirosis must be based upon the broadest epizootiological concepts because of the wide host range of *L. pomona*, e.g., swine,[4] horses,[15] sheep,[1] goats,[9] and man.[3,16]

Leptospira pomona serum agglutinin-lysin titers may appear as early as seven to nine days following exposure. By the thirteenth day, the titer may be 1:100 to 1:10,000. After 25 days, a peak of 1:10,000 to 1:1 million may be anticipated.

The Leptospira antibody titers ascend rapidly and remain at 1:1,000 to 1:10,000 for 29 to 52 months.[8] Whey titers in colostral milk are comparable to those obtained for the serum of an individual animal.

There is little doubt that *L. pomona* is responsible for bovine abortions. The agent was isolated by the authors from the cotyledons of 1 experimental heifer. Leptospiras were also found, by Podgwaite *et al.*,[10] to be present in 3 aborted bovine fetuses. The exact mechanism of abortion is unknown. It appears that interference with the placental and fetal circulation, as evidenced by the extensive edema, may have been the cause or at least a contributing factor. The fetus examined in this investigation had been dead 12 to 24 hours prior to the abortion, which occurred 29 days following exposure. Evidence obtained from investigations[7,8] of a number of natural occurrences of bovine leptospirosis indicates that abortions take place three to four weeks following exposure to *L. pomona*. The abortion is not a manifestation of the acute phase of the disease. The serum and milk whey agglutinin levels at the time of abortion were 1:1,000 to 1:1 million.[7,8]

Gross renal lesions are usually observed even in the milder and asymptomatic cases of bovine leptospirosis. Considerable scrutiny at necropsy may be required to locate the characteristic yellow or white foci on the renal cortex[11] which descend 2 to 3 cm. into the medullary portions of the kidneys. Attempts to discover these lesions are well warranted, especially in the recently infected animal.

Icterus, anemia, and hemoglobinuria or hematuria are not constant findings in experimental or naturally occurring leptospirosis in pregnant heifers. Respiratory distress in the severely ill bovine animal is a striking feature of the malady. Gross pulmonary lesions are apparently absent.

Stiffness and reluctance to move have been reported in naturally infected cattle.[8] One of the experimental heifers experienced a severe, acute synovitis. This clinical feature of leptospirosis, though not constant, may be observed during the early phases of the disease.

SUMMARY

Seven pregnant heifers were infected with *Leptospira pomona* by subcutaneous inoculation. One became severely ill, 1 aborted, and 5 remained essentailly asymptomatic. Leptospiremia endured for one to five days in 5 of the animals while all 7 developed at least a transitory leptospiruria as evidenced by isolation of the agent from urine or kidneys. *Leptospira pomona* was recovered from the udder, cotyledon, liver, spleen, lungs, and kidneys of 1 acutely and severely ill animal killed ten days after the onset of infection. Leptospiras were not isolated from the tissues of any of the fetuses from these heifers. Agglutinin-lysin serum antibodies were demonstrable within ten to 14 days following exposure to *L. pomona*. The experimental evidence indicates that infected cattle, even in the absence of visible manifestations of the disease, constitute a potent reservoir of animal infection and human exposure.

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Experimental Leptospirosis. II. The Role of Calves in the Transmission of *Leptospira Pomona* Among Cattle, Swine, Sheep, and Goats

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LEPTOSPIROSIS (*Leptospira pomona* infection) has become recognized during the past ten years as a threat to the livestock population of this country. The epizootiology has been defined but requires further elucidation. Cattle may be renal carriers of leptospiras for periods of three months.[10] *Leptospira pomona* infections can be transmitted experimentally from infected calves to susceptible calves.[1] Burnstein and Baker[3] showed that infection may be transmitted from diseased pigs to other pigs and calves but they were unable to show the reverse, that leptospirosis can be transmitted to pigs by contact with *L. pomona*-infected calves. The role of the infected calf in transmission of leptospirosis to other cattle, pigs, sheep, and goats has not been thoroughly delineated. This work was undertaken to further the epizootiological knowledge and to make possible a study of the course of *L. pomona* infection in several domestic animal species.

MATERIALS AND METHODS

The strain of *L. pomona* selected for exposure was originally isolated from bovine urine obtained during active infection in a Wisconsin dairy herd.[7] It had been maintained since isolation by continual passage through guinea pigs and had been designated as strain Wickard.[8]

Six calves, 4 pregnant heifers, 4 weanling pigs, 4 sheep, and 2 goats which were negative to the serum agglutination-lysis test[8] at the initiation of the experiment served as experimental animals. The calves, which were from 1 week to 4 months of age, were infected with *L. pomona* by subcutaneous injection. They then served as carriers to which the other animals were exposed. The pregnant, grade Holstein-Friesian heifers were in the fifth to seventh month of gestation at the time of contact exposure. The pigs, which were approximately 8 weeks old, were held in isolation for five days before being placed in isolation units with the infected calves. The sheep, which were 3 and 4 years old, were procured from a flock which had no clinical history to indicate the presence of leptospirosis. Two, 4-month-old goats were obtained; they had been confined in a small pen since birth.

The animals were kept in four pens of a modern isolation unit in which principles of strict isolation were exercised and in a cattle shed where contact with other livestock was impossible and where human and vehicular traffic to the premises was restricted to authorized personnel. This reduced the potential of accidental infection to a minimum. The isolation pens provided 70 sq. ft. of floor space and were cleaned daily with warm (180.0 F.) water under pressure. The shed unit was 16 ft. by 20 ft. with approximately one half of the floor area paved with concrete and the remainder being a dirt floor. No drainage was provided. This unit was not cleaned during the course of the experiment.

The animals in the isolation pens were provided with a mixed ground grain ration with dried beet pulp incorporated as bulk for the ruminants. No litter was provided. The grain ration was fed in metal baskets or metal troughs, and the water was provided in suitable garbage cans. The uneaten hay provided litter for the animals in the shed unit.

The animals were initially dispersed (table 1) in the various units as follows: Each of three isolation pens was stocked with 1 infected calf, 1 heifer, and 1 pig; the fourth isolation unit contained 1 infected calf and 1 heifer; and the shed unit contained 2 infected calves, 1 pig, 4 sheep, and 2 goats. At the termination of the experiments involving 14 of the animals, the 6 remaining animals were placed in two pens of the isolation unit. The first pen contained 2 infected calves and 2 goats, while the second contained 1 infected calf and 1 ewe. The two groups were maintained for an additional 14 days and the experiment was then concluded.

The 6 calves were inoculated subcutaneously with 5.0 cc. of heparinized blood obtained from infected guinea pigs. This blood was obtained when the maximal pyretic response (105.0 to 106.0 F.) was manifested.[9] The normal animals were placed in contact with the infected calves seven days following inoculation. Rectal temperatures were taken

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TABLE 1—Distribution of Experimental Animals for Transmission of Leptospirosis

	Initial distribution†						Secondary distribution‡	
	Isolation unit (pen No.)						Isolation unit (pen No.)	
Animals	1	2	3	4	Shed	Animals	1	2
Calves*	1	1	1	1	2	Calves*	2	1
Heifers	1	1	1	1	..	.....	..	..
Swine	1	1	1	..	1	.....	..	..
Sheep	..	..	..	..	4	Sheep	..	1
Goats	..	..	..	..	2	Goats	2	..

* All calves experimentally infected and used as sources of infection for other animals.
† Exposure for 30 hours to 34 days.
‡ Exposure for an additional 14 days.

daily and the animals were observed for clinical signs of leptospirosis. Whenever a temperature rise, anorexia, polypnea, depression, or lethargy were manifested by any of the animals, blood samples were secured on that day and on two successive days. A minimum of five tubes of modified Chang's fluid medium[4, 7] were inoculated with approximately 0.05 cc. of the blood. Cultures were placed in an incubator at 28 C. and held for a maximum of 30 days. All tubes were examined at approximately weekly intervals by dark field microscopy for evidence of leptospiras. Tubes of the medium which proved to be contaminated were discarded. Serum was collected from the animals at three- to five-day intervals following the observation of symptoms and at weekly intervals thereafter until destroyed. The agglutination-lysis test was employed throughout for serological examinations.[8]

Approximately 3.0 cc. of urine, milk, and 10.0 per cent tissue emulsion of kidney in 0.85 per cent sodium chloride solution were inoculated intraperitoneally into each of 3 to 5 guinea pigs and each of 5 hamsters. Lactation resulted from 1 of the heifers from the nursing efforts of the infected calf. Chorionic or amniotic fluids, stomach contents, and saline emulsions of fetal bovine kidney, liver, and spleen were also inoculated into guinea pigs. Guinea pigs and hamsters were held for 18 to 30 days prior to exsanguination. Their serums were examined by the agglutination-lysis test.[8] Titers of 1:100 or higher were considered to be indicative of infection and proof of the presence of *L. pomona* in the original inoculum.

Animals were destroyed at various intervals during the course of the experiment to obtain gross pathological, serological, and bacteriological data.

EXPERIMENTAL RESULTS

1) Experimentally Infected Carrier Calves.—Rectal temperatures of the experimentally infected calves, taken daily, showed a febrile reaction with maximal temperatures of 103.8 to 106.5 F. at three to seven days postinoculation. Other symptoms observed for periods of two to three days were lethargy, constipation, mild catarrhal to purulent rhinitis, excessive lacrimation, polypnea, a slight nonproductive cough, and excessive micturition. Hemoglobinuria was manifested by all 3 Hereford calves but not by the 3 infected Holstein-Friesian calves. Hereford calf 67 died of acute leptospirosis eight days after inoculation; gross lesions were not found on necropsy. Hereford calf 69 had extreme hemoglobinuria on postinoculation days 12 to 15 and died on the twenty-fifth day; the course of the infection was complicated by a persistent diarrhea. Leptospiras were isolated from blood which was collected during pyrexia. Leptospiremia was proved for all 6 calves and 4 of them were found, by means of the guinea pig inoculation technique, to be shedding leptospiras in their urine. Calf 67, which died during acute leptospirosis, and calf 68 were never proved to be renal shedders.

Serum agglutination-lysis titers were first detected on six to 15 days after inoculation. When the 4 surviving calves were destroyed for necropsy, at 44 days postinoculation, their titers were 1:10,000,000 and leptospiras were not isolated from their kidneys or urine. Their kidneys contained numerous gray-white foci over the entire cortical surface. These foci extended several centimeters into the cortex and, in some instances, into the medulla. There was considerable resistance to the knife blade, indicating extensive fibrosis, when longitudinal slices were made of the kidneys (fig. 1). The livers of 2 calves were firm and mottled. Accumulation of peritoneal and pericardial fluids was observed in some of the calves. Calf 71 was extremely emaciated and unthrifty.

The clinical features and bacteriological findings for the 6 calves are shown in table 2.

2) Animals Infected by Contact Transmission.—Heifer 46 had a temperature of 104.0 F. six days after 30 hours of contact with calf 67 before the calf died. Pyrexia was followed by slight constipation. Other clinical signs were not observed. The heifer was slaughtered 33 days postexposure. On necropsy, the chief lesions were pronounced

gray-white foci of both kidneys; the other organs appeared to be normal. A living 4½-month-old fetus was present in the uterus. The chorionic, allantoic, and amniotic fluids were straw colored, and the capsules of both kidneys were extremely edematous. Attempts to isolate leptospiras from the maternal blood, urine, or kidneys, as well as from the fetal organs, were not successful. The terminal serum agglutination-lysis titer of heifer 46 was 1:1,000,000.

Heifer 73 was in contact with infected calf 70 for 19 days. A primary temperature rise to 104.0 F. occurred seven days following exposure and a serum titer of 1:10 appeared on the fifteenth day postexposure and, on the nineteenth day, she had a temperature of 104.4 F. and leptospiras were isolated from her blood and milk. On the twenty-fifth day following exposure, when she was destroyed, her temperature was 105.0 F., her respiratory rate 103 per minute, and she had a cloudy vaginal discharge. Agglutinins were demonstrable in a serum dilution of 1:10,000,000. Her 5-month-old fetus was alive, had strong heart action, but made no respiratory attempts. Amniotic fluids and fetal stomach contents were viscid, turbid, and yellow. The fetal viscera and mesenteries were slightly edematous. The cortices of the maternal kidneys contained discrete, grayish foci. All guinea pigs which were inoculated with fetal kidney and spleen material died 24 to 72 hours later. Leptospiras were not isolated from any of the fetal organs or fluids but were isolated from the maternal kidneys.

Heifer 76 showed clinical signs of leptospirosis during the twenty-fourth day of exposure to infected calf 72. Her temperature was 103.2 F. and her titer was 1:100,000; a titer of 1:10 had first appeared on the fifteenth day postexposure. At 27 days postexposure, when her temperature was 103.4 F. and her respirations 104 per minute, she was destroyed for necropsy. Her kidneys were friable and mottled with grayish white foci which extended deep into the cortex and medulla and there were numerous petechial hemorrhages in the renal cortex. The liver was firm in consistency and distinctly mottled. The gallbladder was slightly edematous. The fetus was alive but, on necropsy, showed edema of the kidney capsule, pericardium, and mesentery. Leptospiras were not demonstrable in the maternal kidneys or liver nor in the fetal kidneys, spleen, amniotic fluids, or stomach contents.

Heifer 84, which had been in contact with infected calf 68 which was not proved to be shedding leptospiras in the urine, developed no signs of leptospirosis except a temperature of 103.6 F. on the third day postexposure. Significant serum agglutina-

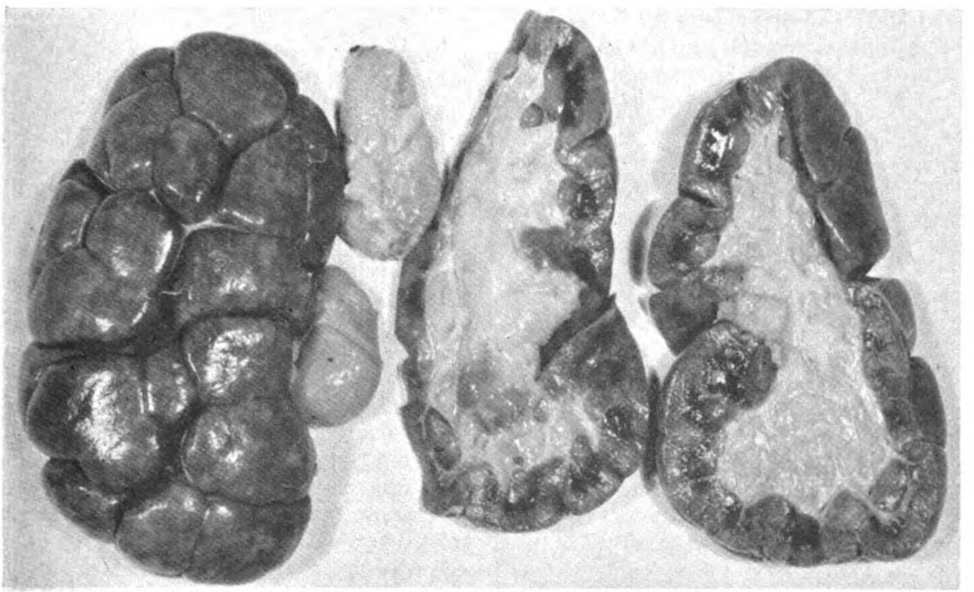

Fig. 1—Kidneys and renal lymph nodes of calf 71. Extensive cortical and medullary lesions can be observed, indicating chronic type of Leptospira pomona infection.

TABLE 2—Evidence of Leptospirosis Observed Among Experimentally Infected Caves*

Animal	Breed	Postexposure day	Clinical signs		Bacteriological results†		
			Temp. (F.)	Hemoglobinuria	Blood‡	Urine‡	Kidney
67	Hereford	6	103.8	+	+(6-7)	—	—
68	Holstein-Friesian	3	104.2	—	+(5)	—	—
69	Hereford	6	105.0	+	+(4-7)	+(10-20)	—
70	Holstein-Friesian	5	106.5	—	+(6,7)	+(16-26)	—
71	Holstein-Friesian	7	105.8	—	+(5-7)	+(10-28)	—
72	Hereford	5	104.8	+	+(5-10)	+(12-28)	—

* Subcutaneous exposure to Leptospira pomona, strain Wickard. These calves were used as infected animals for transmission experiments.

† Blood cultured directly in Chang's medium. Urine or kidney homogenates inoculated via intraperitoneal route in guinea pigs; development of serum-agglutininlysins indicated presence of Leptospira organisms in inoculum.

‡ Numbers indicate the days or period postexposure on which leptospiras were demonstrable.

tion-lysis titers failed to develop. She was destroyed for necropsy at 33 days postexposure. Her kidneys contained a few scattered, grayish foci which extended only a few millimeters into the cortex. Serums of guinea pigs and hamsters, which were inoculated with maternal kidney or fetal kidney, liver, spleen, amniotic fluid, or stomach contents, failed to give positive agglutination-lysis titers.

All 4 pigs developed leptospirosis following contact with the experimentally infected calves. Thermal responses were evidenced from the eighth to the eighteenth days postexposure, with peak temperature rises of 104.6 to 106.5 F. One pig appeared lethargic and developed a diarrhea concurrent with the pyrexia. The other 3 pigs remained asymptomatic except for the febrile responses. Leptospiras were isolated from the blood of 2 pigs. Serum agglutinin titers of $1:10^6$ to $1:10^8$ were present seven to twelve days following pyrexia in 3 of the pigs. The fourth, pig 99 which was in contact with calf 68, had a questionable agglutination-lysis serum reaction on the eighth day postpyrexia when it was destroyed. By guinea pig and hamster inoculations, leptospiras were proved to be in the kidneys or urine of all 4 pigs. Pronounced kidney lesions were not observed in any of the 4 pigs.

None of the 4 sheep manifested clinical signs of leptospirosis, none developed agglutination-lysis serum titers, and leptospiras were not isolated from their organs, blood, or urine.

The 2 goats were in contact with calves 69 or 71 for 26 days without developing clinical leptospirosis and their serums did not contain leptospiral agglutinins. Both goats were then placed with infected calves 70 and 72. Nine days later, goat 87 had a temperature of 104.0 F., was lethargic, anorexic, evidenced general malaise, and leptospiras were isolated from the blood in Chang's medium.[4,7] Goat 87 was slaughtered five days after the pyrexial response. The terminal agglutination-lysis test titer was 1:100,000. Three of 5 guinea pigs which were inoculated with urine and 3 of 5 guinea pigs which received kidney emulsion from goat 87 developed significant temperatures of over 104.0 F. and leptospiras were isolated from their blood. Goat 88 remained asymptomatic as well as serologically and bacteriologically negative. Symptomatological and bacteriological findings for the animals infected by contact are summarized in table 3.

Agglutination-lysis tests of serums of all species were performed using *L. pomona*, strain Johnson, and *Leptospira icterohaemorrhagiae* AB, as antigens. The results indicated a cross serological reactivity. Some of the serums which had a titer of 1:100,000,000 for *L. pomona* evidenced an agglutination-lysis reaction in dilutions of 1:100,000 for *L. icterohaemorrhagiae*, AB. Most serums with a 1:1,000 *L. pomona* reaction produced agglutination or lysis of *L. icterohaemorrhagiae* AB, antigen at a dilution of at least 1:100. The serological data are summarized in table 4.

DISCUSSION

A possible inference that breed or type may affect susceptibility to leptospirosis is indicated by the death of 2 Hereford calves as well as by the acute hemoglobinuria which was observed only in the Herefords. Only mild transitory signs of leptospirosis were observed in the other calves. Marsh[6] reported leptospirosis in Hereford cattle that was characterized by a severe hemoglobinuria and a 90 per cent mortality among the calves. In Wisconsin, leptospiral infections have involved calves in only three of 41 dairy herds studied, with hemoglobinuria being uncommon or inconstant.[7]

However, a rapid, acute course, a rather constant finding of hemoglobinuria, and some deaths have been reported for beef cattle and dairy calves.[5]

Small numbers of leptospiras may be shed in the urine of calves.[3] Ringen *et al.*[11] suggested that approximately 750 leptospiras (*L. pomona*) per inoculum are required to produce 100 per cent infection in guinea pigs. Sixty-six guinea pigs were inoculated, in the present experiment, with 2.0 cc. of urine from known infected calves. The serums of 18 of these guinea pigs were negative for leptospiral agglutinins and 13 of the 18 negative serums were from guinea pigs which received the urine of calf 68, which apparently contained insufficient organisms to be infective for guinea pigs. Infection was not transmitted from calf 68 to the heifer but it was to a pig in the same pen, which suggests the possibility of a greater degree of susceptibility of swine than of cattle. Forty-four days after subcutaneous inoculation, leptospiras were not detected in the urine or kidneys of the 4 surviving calves by guinea pig or hamster inoculation. The renal-carrier and shedder state in these calves was of short duration but of a serious nature.

Evidence of transmission, by pen contact, from known infected calves to 3 of 4 pregnant heifers, all 4 pigs, and 1 of 2 goats indicates that calves may be important sources of infection. This seems to be the first authenticated occurrence of leptospirosis (*L. pomona*) in a caprine host in North America but *Leptospira grippotyphosa* infections in goats have been reported from Israel.[12]

Leptospirosis occurred in 1 heifer following 30 hours of contact with an infected calf. The ease of transmission from calves to other susceptible hosts is evidenced by the fact that these animals, with the exception of the group kept in the shed, were maintained in sanitary surroundings much better than those which would be maintained under ordinary farm conditions.

The fact that the sheep did not become infected, either in the shed or the isolation unit, may have been due to their age (3 to 4 years) or to a species characteristic of low susceptibility. *Leptospira pomona* infections in sheep have been described.[2] Further studies on the susceptibility of sheep to *L. pomona* and on the transmission among sheep and from sheep to other species are indicated.

Abortions did not occur and leptospiras were not isolated from the bovine fetuses, all of which were alive when the heifers were subjected to necropsy. Marked edema of the fetal kidney capsule was the most prominent gross pathological lesion. Serum agglutination-lysis titers were not evident in the fetuses of infected cattle.

TABLE 3—Bacteriological, Clinical, and Necropsy Observations of Animals Which Evidenced Leptospirosis* Following Contact Transmission

Animal	Clinical				Bacteriological‡			Necropsy	
	Day†	Temp. (F.)	Respirations	Other	Blood	Urine	Kidneys	Day†	Observations
Heifer 46	6	104.0	60/min.	Constipation.	−	−	−	33	Dam:gray-white, renal foci. Fetus: alive; fluids in body cavities, edematous kidney capsules.
Heifer 73	7	104.0	.....	.....	+(19)	+(25)	+	25	Dam: same as for heifer 46. Fetus: alive; edema of mesenteries and viscera; yellow, viscid allantoamniotic fluids.
	25	105.0	103/min.	Lochia.					
Heifer 76	24	103.2	.....	.....	−	−	−	27	Dam: petechiae, gray-white foci on kidneys; liver firm and mottled. Fetus: alive; edema of renal capsule and mesenteries.
	27	103.4	104/min.	.....					
Pig 77	8	105.4	.....	.....	−	+(21)	+	21	Kidneys: ecchymotic hemorrhages, few gray-white foci.
	18	104.8	.....	.....					
Pig 78	14	106.5	.....	.....	+(16)	−	+	17	Kidneys: few scattered gray foci.
Pig 99	13	104.6	.....	.....	−	+(21)	+	21	Kidneys: few hemorrhagic foci.
Pig 600	15	105.4	.....	Diarrhea, depression.	+(13)	+(21)	+	21	Kidneys: cortical petechiae and discrete gray-white foci.
Goat 87	9	104.0	.....	Depression, anorexia.	+(10)	+(14)	+	14	No significant lesions.

* All experimental animals with demonstrable serum-agglutinin titers of 1:100,000 (or higher) except pig 99.

† Days are postexposure. Goat 87 data represented for second exposure period.

‡ Blood cultured directly in Chang's medium. Urine and kidneys proved by guinea pig or hamster inoculation technique. Numerals in parenthesis indicate days postexposure when isolations were made.

TABLE 4—Agglutination-Lysis Titers of Serums of Animals Infected by Contact Transmission*

Animal	Days postexposure						
	0 to 5	6 to 10	11 to 15	16 to 20	21 to 25	26 to 30	31 to 40
Heifer 46	—	—	—	1:10	$1:10^{2}$	1.10^{2}	$1:10^{6}$
Heifer 73	—	—	1:10	1:10	$1:10^{7}$	.....	.....
Heifer 76	—	—	1:10	1:10	$1:10^{5}$	$1:10^{5}$	.....
Heifer 84	—	—	—	—	1:10	1:10	.....
Pig 77	—	—	—	$1:10^{3}$	$1:10^{7}$	.....	.....
Pig 78	—	—	$1:10^{3}$	$1:10^{3}$	$1:10^{6}$	.....	.....
Pig 99	—	—	—	—	1.10	.....	.....
Pig 600	—	—	$1:10^{3}$	$1:10^{4}$	$1:10^{8}$	.....	.....
Goat 87	—	—	1:10[5]†	.....	.....	.....	.....

* Titer expressed as dilution end point reading. Considered positive if 50 per cent agglutination or lysis or both occurred.

†Days after start of second exposure period.

A public health problem is suggested by the isolation of leptospiras from the milk of heifer 73. The concentration of leptospiras in 4.0 ml. of milk was sufficient to infect guinea pigs and might well be a threat to human health.

The serological results here recorded indicate a cross reaction between *L. pomona*, strain Wickard, and *L. icterohaemorrhagiae* AB.

SUMMARY

Six young calves were experimentally infected with *Leptospira pomona*. Only the 3 Herefords developed hemoglobinuria; 1 died on the eighth day postinoculation and another on the twenty-fifth day. All 6 developed leptospiremia and 4 shed leptospiras in their urine.

This infection was spread, by contact, to 3 of 4 pregnant heifers, to all of 4 pigs, to 1 of 2 goats, but to none of 4 sheep. Clinical signs of infection appeared six to 24 days after exposure. The course of leptospirosis in calves, heifers, pigs, and a goat, as well as the serological results obtained following infection, are discussed.

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