EXPERIMENTAL *LEPTOSPIRA POMONA* INFECTION OF BOARS, INCLUDING STUDIES ON TRANSMISSION OF INFECTION BY COITUS

By L. TAMMEMAGI, B.V.Sc., Dr.med.vet., G. C. SIMMONS, B.Sc., M. D. MCGAVIN, B.V.Sc., and C. G. LUDFORD, B.Sc.*

SUMMARY

Four boars inoculated intramuscularly with *L. pomona* showed agglutinins and excreted leptospirae in the urine.

No clinical evidence of infection was seen.

Leptospiruria first occurred at 25 days in one boar, at 28 days in two and at 31 days in one. It was intermittent in all four animals, extending over periods of 16 to 21 days.

At slaughter, nine weeks after infection, the kidneys of all four boars showed greyish-white lesions of variable size, shape and distribution. The histological picture of the lesions was that of a focal interstitial nephritis. No abnormality was seen in the liver or testes.

Two of the boars were each mated during leptospiruria with three non-infected sows. None of the sows developed leptospiruria or showed antibodies against *L. pomona*.

I. INTRODUCTION

Experiments done at this Institute have shown that *Leptospira pomona* is a cause of abortion and neonatal mortality in swine (Ryley and Simmons 1954*a*, 1954*b*) while *Leptospira hyos*, another leptospiral serotype often present in pigs in Queensland, has failed to show such properties (Tammemagi and Simmons 1956, 1958). To our knowledge, no experimental work has been done specifically on *L. pomona* infection in boars.

This paper describes observations made on four boars experimentally infected with *L. pomona*, and data on the subsequent mating of two of these boars during leptospiruria, each with three uninfected sows.

II. METHODS

The four Large White and Wessex Saddleback cross boars (Nos. 461, 462, 463 and 466) used in the experiment were all nine months of age and were members of a litter from one sow in the Institute herd. Of the six sows mated

* Senior Veterinary Pathologist, Senior Bacteriologist, Senior Histopathologist and Senior Serologist, respectively, Animal Research Institute, Yeerongpilly. (Queensland Department of Agriculture and Stock.)

to the infected boars, five (Nos. 425, 426, 427, 429 and 437) were gilts between 15 and 16 months of age, and one (sow 403), 24 months old, had had a litter some 8 months earlier. Four other sows, (Nos. 371, 400, 405 and 412), 20 to 24 months old, were mated to an uninfected boar and kept as controls. Of these, No. 412 was a gilt; sows 400 and 405 had farrowed once, and sow 371 twice, previously.

All animals were housed in separate concrete pens. Blood samples taken on several occasions prior to the commencement of the experiment did not contain antibodies for *Brucella suis, L. pomona* or *L. hyos.*

The four boars were inoculated into the gluteal muscles with 1·0 ml of a culture of *L. pomona* strain E.1377 previously used by Ryley and Simmons (1954) in their experimental work on leptospirosis in swine. The culture was obtained by sowing heart blood from a 21st guinea pig passage into Schuffner's medium and incubating for seven days.

Boars 462 and 463, while showing leptospiruria, were selected for serving three sows each. Boar 462 was mated to sows 425, 426 and 429, and boar 463 to sows 403, 427 and 437. When the infected boar was taken to the sow's pen, precautions were taken to minimize contact and so prevent the infection being transmitted by any route other than coitus. The sow was restrained with a rope around the upper jaw.

Immediately after service was completed, the boar was removed from the pen and the sow's skin and external genitalia washed and scrubbed with 2 per cent. lysol. Semen which dropped from the vulva immediately after service was removed from the floor, which was then liberally scrubbed with 2 per cent. lysol before the sow was released. Some sows continued to pass lumps of coagulated semen intermittently up to six hours after service. This material was not removed.

An attempt was made to arrange matings so that they occurred on days when the boars showed leptospiruria. Table 3 shows that this arrangement was successful in the mating of five of the six sows. Sow 429 was mated on the day when the boar's urine was negative for leptospirae, although the organisms were seen on the day prior to and on the day after mating. Each sow except 403 was mated only once to the selected boar; sow 403 returned to heat three weeks after the first mating and was mated again. During the first mating the boar's urine was positive for leptospirae, but at the second mating, which took place on two consecutive days, 15 days had elapsed since the boar had shown leptospiruria.

All boars and sows were examined daily and their rectal temperatures recorded twice daily. All dead piglets were necropsied and examined bacteriologically by the technique described by Ryley and Simmons (1954*b*).

Urine samples were collected daily from all boars for 63 days following inoculation and from the exposed sows for 42 days after service. The urine samples from sows were obtained by disturbing the sows about one hour after the morning feed and collecting the required sample into a sterile jar as they urinated. Collection from boars was done by restraining the animals in specially designed individual crates until they passed urine on to a tray, from which it drained through a funnel into a jar placed underneath. Urine samples were examined as described by Ryley and Simmons (1954*b*). No urine samples were collected from the control sows.

Blood samples were collected from the boars weekly for the first three weeks after exposure to *L. pomona* and later at intervals of two weeks. Samples from the sows of both exposed and control groups were collected every two weeks. The sera were kept at —25°C until the end of the experiment. All sera were then tested for agglutinating antibodies to *L. pomona,* on the same day and using the same batch of antigen. After incubation of the serum-antigen mixtures for 1·5 hr in a moist atmosphere at 37°C, the degree of agglutination was read under darkground illumination, using a magnification of 80.

Sixty-three days after exposure to infection, the boars were killed and the livers, kidneys and genitalia, including the accessory glands, were examined macroscopically. The testes and epididymides were weighed.

For histopathological examination, liver and kidney blocks were fixed in 10 per cent. neutral buffered formalin (Lillie 1948), and blocks of testis and epididymis in Bouin's solution. Tissue blocks were embedded in paraffin, cut at 7 microns and stained with haematoxylin and eosin. For identifying leptospirae, liver and kidney sections were stained by the technique of Warthin and Starry (Lillie 1948).

III. RESULTS

(a) Boars

(1) Clinical Observations

No ill-health was observed throughout the experiment in boars 462 and 463. Boar 461 had a watery diarrhoea and slight inappetence on the 14th and 15th days post-inoculation, and boar 466 showed slight inappetence on the 35th and 36th days after inoculation, i.e. on the 4th and 5th day after the commencement of leptospiruria.

Rectal temperatures throughout the experiment were within the normal range, the highest temperatures recorded being 103·2°F in boar 461, 103·1°F in boar 462 and 102·9°F in boars 463 and 466. These figures were comparable with those recorded during the 10 to 15 days' pre-exposure period.

(2) Urine Examination

Results of urine examination are shown in Table 1. Leptospiruria in all four boars was intermittent (Figure 1). On one day, morning and evening samples were collected from three boars. In boars 461 and 463, both the samples were positive for leptospirae, but in 461 the afternoon sample was negative although leptospirae were seen in the morning sample.

TABLE 1

DETAILS OF LEPTOSPIRURIA

Boar No.	Leptospiruria (days after inoculation)		Duration (days)	Number of Positive Daily Samples
	Commenced	Ceased		
461	28	45	18	12
462	28	48	21	13
463	25	42	18	13
466	31	46	16	6

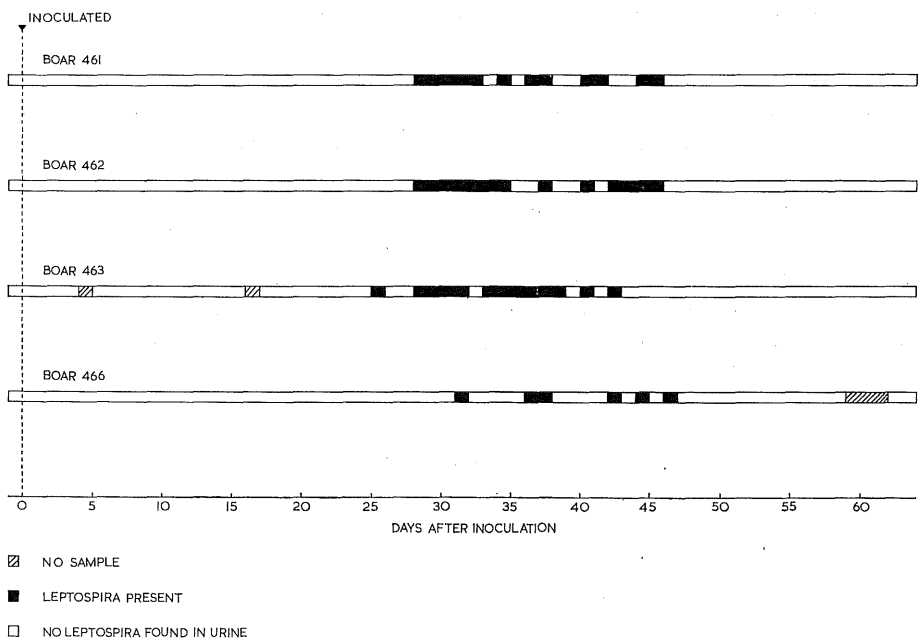

Fig. 1.—Examination of urine for leptospirae.

The number of leptospirae in the urine was never high and they were often dead and misshapen.

(3) Serology

The sera from the 10 sows showed no agglutinins to *L. pomona* throughout the whole trial.

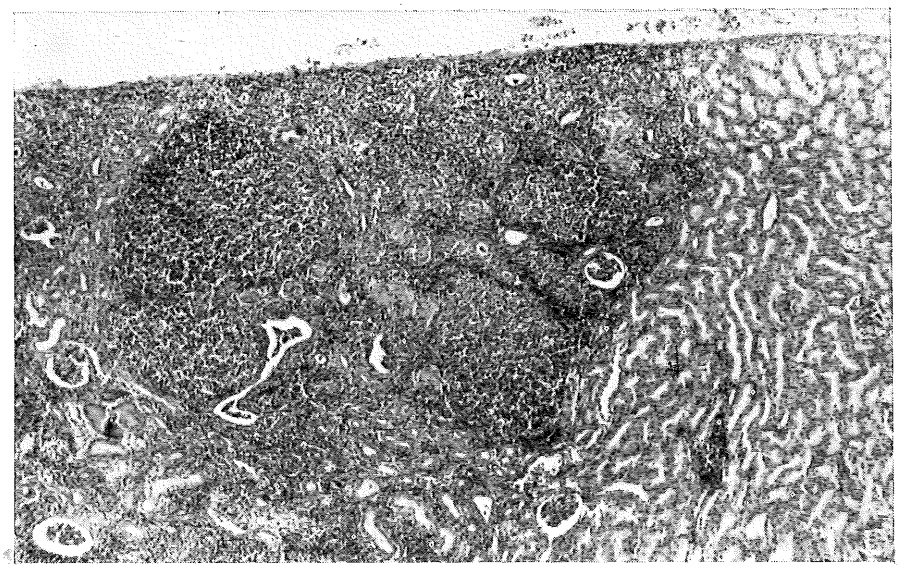

Fig. 2.—Kidney. X 50. Haematoxylin and eosin. Note the destruction of the subscapsular parenchyma. (X 16⅔, enlarged X 3. Wratten filters 15 and 58.)

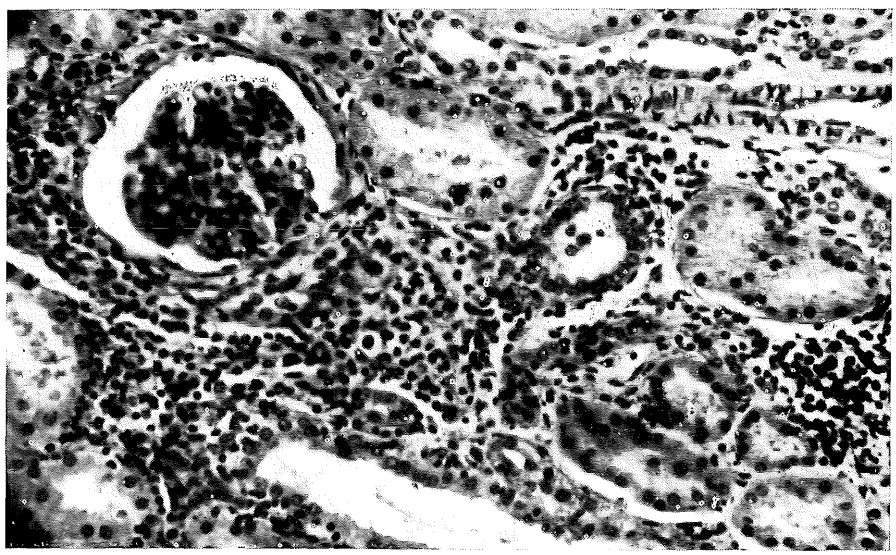

Fig. 3.—Kidney. X 225. Haematoxylin and eosin. An early focus of intertubular and periglomerular mononuclear infiltration. (X 75, enlarged X3. Wratten filters 15 and 58.)

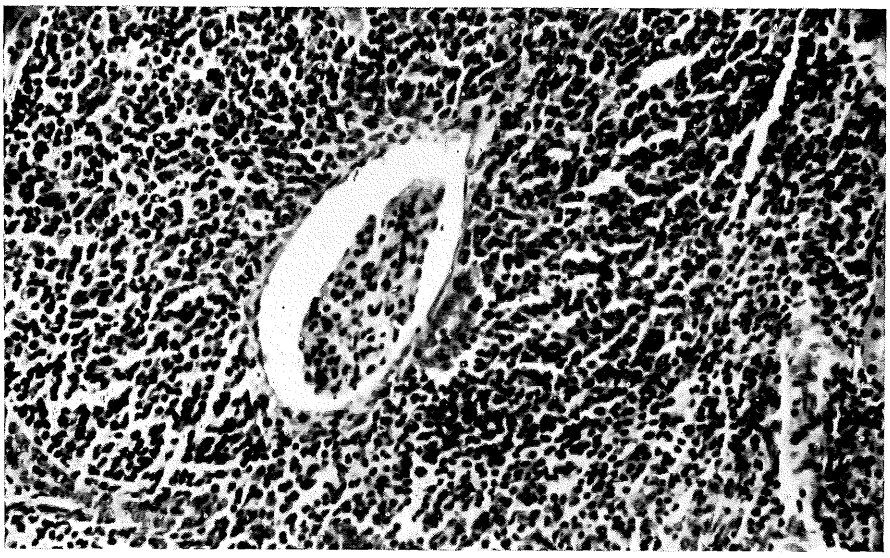

Fig. 4.—Kidney. X 225. Haematoxylin and eosin. Massive mononuclear infiltration causing complete destruction of the tubules. An atrophic glomerulus lies surrounded by infiltrating mononuclear cells which have completely destroyed the renal tubules. (X 75, enlarged X 3. Wratten filters 15 and 58.)

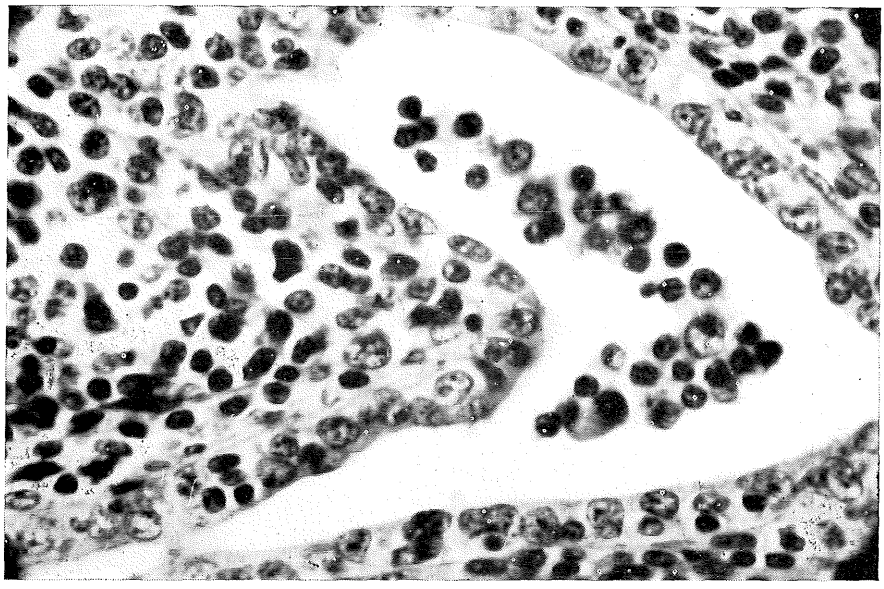

Fig. 5.—Kidney. X 750. Haematoxylin and eosin. Macrophages and lymphoid cells border an isolated tubule which contains a cellular cast. (X 250, enlarged X 3. Wratten filters 15 and 58.)

The results of the agglutination tests on the infected boars' sera are shown in Table 2. The maximum titres ranged from 1/1,000 to 1/3,000. The peak was reached within 14 days after infection in boars 461 and 462, but only after the 49th day in the other two boars. The secondary rise in the antibody titre in boars 461 and 462 between the 49th and 63rd days is not considered significant, since it represents an increase of only one dilution in the titration and is within the expected experimental error of this type of test.

TABLE 2

RESULTS OF AGGLUTINATION TESTS ON SERA FROM INFECTED BOARS

Boar No.	Days After Inoculation						
	0	7	14	21	35	49	63
461 ..	0	300+	3000	3000	1000	1000	3000
462 ..	0	30	1000	300	300	300	1000
463 ..	0	30	300	1000	1000	3000	1000
466 ..	0	30	300	300	300	1000	1000

Key : + Reciprocals of titres. Read at approximately 50% agglutination.
0 No agglutinins.

NOTE.—Leptospiruria commenced in boars 461 and 462 on the 28th, in boar 463 on the 25th, and in boar 466 on the 31st day after inoculation.

(4) Pathology

No gross lesions were seen in the livers or genitalia from the boars. The weights of the testes, including epididymides, were 325/320 g (left/right) in boar 461, 365/350 in boar 462, 325/325 in boar 463 and 410/395 in boar 466.

The kidneys in all four boars showed numerous greyish-white circumscribed lesions about 2 mm in diameter and a number of smaller, less well-defined foci scattered subcapsularly or situated more deeply in the cortex. The lesions were particularly numerous in boar 463.

(5) Histopathology

Histopathological examination showed significant changes only in the kidneys. These changes were those of focal interstitial nephritis. Non-purulent inflammatory foci of varying size were scattered through the renal cortices from the subcapsular area to the cortico-medullary junctions (Figure 2.) The medullae were free of lesions. The majority of the inflammatory cells were either lymphoid cells or macrophages, but eosinophils, neutrophils and plasma cells were seen occasionally. In early lesions, inflammatory cells were invariably found perivascularly about the interlobular arteries, but foci were also often seen intertubularly (Figure 3) and sometimes periglomerularly. As the lesions advanced in size, tubules were destroyed and glomeruli became atrophic (Figure 4). In the large foci, the normal kidney architecture was lost (Figure 2), but an occasional shrunken glomerulus or tubule could be detected in a mass of

mononuclear cells (Figure 4). Some of the surviving tubules contained syncytial masses with several tubule cell type nuclei. An occasional tubule in a mass of mononuclear cells contained cellular casts (Figure 5). No leptospirae were detected in any of the liver and kidney sections examined.

(b) Sows

None of the six sows exposed to the infection developed leptospiruria as judged by examination of urine samples. Serum samples from the exposed and control sows throughout the trial were negative for the presence of *L. pomona* antibodies.

The live piglets born to the exposed and control sows were bled periodically but none of them developed antibodies against *L. pomona.*

The gestation periods in the sows (Table 3) ranged from 114 to 117 days, compared with 113 to 116 days in the contrcl sows.

A total of 44 piglets, eight of which were dead at birth, was born to the six sows. The size of the litters varied from 5 to 10 (Table 3). In the control group, 38 piglets (one dead) were born to the four sows, with litter sizes varying from 5 to 13.

(c) Piglets

Of the eight dead piglets in the exposed group, four belonged to a litter of five from sow 425. Their weights ranged from 2·0 to 3·0 lb. In all four, there was excess serosanguineous fluid in the pleural and peritoneal cavities. One of them showed a yellow fatty liver, but otherwise no significant abnormality was seen in these piglets.

The dead piglet in the litter from sow 403, weighing 1·9 lb, was covered with foetal membranes and was decomposed. Eight of the nine litter mates had body-weights from 3·0 to 4·5 lb. The other weighed only 2·2 lb, and it was, a few hours later, crushed by the sow.

The weight of the dead piglet, 4·0 lb, from sow 427 was comparable with that of the live litter mates (3·5 to 4·5 lb). Necropsy showed no gross abnormality in the piglet.

The remaining two dead piglets, 2·1 and 2·6 lb body-weight, were in litter from sow 437. No gross abnormality was seen in either piglet. The live piglets ranged in weight from 4·0 to 4·5 lb.

The only dead piglet in the control group was born to gilt 412. It was decomposed and the body weight, 1·9 lb, was the lowest for this litter, which ranged from 2·9 to 3·8 lb. No significant changes were revealed at the necropsy.

TABLE 3

BREEDING DATA OF EXPOSED AND CONTROL SOWS

Sow No.	Mated to Boar No.	Leptospira Present in the Boar's Urine on Day of Service	Date of Service	Date of Parturition	Gestation Period (days)	Number of Piglets Born	
						Total	Born Dead
Exposed Sows—							
425	462	+	8/ix/59	31/xii/59	114	5	4
426	462	+	31/viii/59	26/xii/59	117	5	0
429	462	−	7/ix/59	30/xii/59	114	10	0
427	463	+	3/ix/59	27/xii/59	115	8	1
437	463	+	31/viii/59	25/xii/59	116	7	2
403	463	+	4/ix/59				
			Remated		115	9	1
		−	23–24/ix/59	16/i/60			
Control sows—							
371	Herd boar ..	..	15/ix/59	7/i/60	113	9	0
400	Herd boar ..	..	14/v/59	4/ix/59	113	13	0
405	Herd boar ..	..	11/v/59	2/ix/59	114	11	0
412	Herd boar ..	..	11/v/59	25/ix/59	116	5	1

No Brucellae or Leptospirae were isolated from any of the dead piglets by culture and guinea pig inoculation, and darkground examination of the pleural and peritoneal fluids gave negative findings in all instances.

A wide range of contaminants, such as alpha haemolytic streptococci, micrococci, Proteus and a Bacillus, were cultured from all the dead piglets, but coliforms in addition were constantly recovered from the various tissues, including the brain and foetal membranes from each of the four dead piglets of sow 425.

IV. DISCUSSION

The experiment has shown that boars can readily be infected with *L. pomona* by the intramuscular route. The four boars subjected to infection developed leptospiruria and showed specific antibodies in their blood.

The leptospiruria in the boars in this experiment commenced somewhat later, but was more uniform in duration than that experienced at this Institute by Ryley and Simmons (1954*b*) in their experimental infection of sows with *L. pomona.* Although the same strain of *L. pomona* was used in both experiments, they recorded leptospiruria for periods of 7 to 83 days in sows after a comparatively short incubation period of 9 to 19 days, as against an incubation period of 25 to 31 days' and 16 to 21 days' duration in the present trial. Of interest was the intermittent nature of leptospiruria in the boars in the present trial as compared with a more continuous shedding of leptospirae in sows in the experiment of Ryley and Simmons (1954*b*). This phenomenon may be due to some difference in the virulence of the leptospiral culture rather than the sex of the animals, as a similar observation was made by Tammemagi and Simmons (1956, 1958) in two trials with *L. hyos.* However, the apparent intermittency could be a reflection of the limitation of the darkfield examination in detecting small numbers of leptospirae, or simply due to concentration differences on passage of urine in boars. That this may be so is supported by the result that a morning urine sample was positive in one instance when the evening collection from the same boar was negative.

None of the boars showed febrile reactions such as were observed by Burnstein and Baker (1954) and Ferguson and Powers (1956) in sows infected experimentally with *L. pomona.*

The macroscopical, as well as the microscopical, kidney lesions in the boars were similar to those observed in pigs by Burnstein and Baker (1954), Langham, Morse, and Morter (1958), and Sleight, Langham, and Morter (1960). Our description corresponds well with that given by Pallaske (1960) and Nieberle-Cohrs (1952) for focal interstitial nephritis. Pallaske (1960) makes the point that interstitial nephritis in the pig tends to be focal rather than diffuse as seen in man and occasionally in the calf.

Infection has been produced by pen contact from infected pen mates (Schmidt and Giovanella 1947; Morter and Morse 1956; Burnstein and Baker 1954), by subcutaneous infection (Burnstein and Baker 1954), by installation of culture into the conjunctival sac or nostrils (Schmidt and Giovanella 1947; Burnstein and Baker 1954; Ferguson and Powers 1956) and by deposition of leptospirae into the vagina (Ferguson and Powers 1956). Though the intravaginal route as reported by Ferguson and Powers was successful in setting up leptospirosis in the sows, our work produced no evidence that this will occur naturally even though the boars may be showing leptospiruria at time of service.

The failure to produce infection in the sows may have been due to the small number of leptospirae transferred at service, since the number of organisms shed by the boars in the urine was small and therefore the likely contamination of genitalia must be correspondingly so.

Our experiments did not indicate that the fertility of the boars had become impaired as a result of the *L. pomona* infection.

The incidence of still-born piglets, one or two per litter, is not of any great significance, as we have seen this occur in our breeding stock, mainly in litters consisting of 10 or more piglets per litter. The birth of four dead piglets to sow 425 is considered to be probably due to *Escherichia coli* infection. This organism was recovered from a wide range of piglets' organs, including brain and foetal membranes. Generally, coliforms are common post-mortem invaders, but as the sow subsequently showed a persistent bacteruria, and *E. coli* was repeatedly isolated from the urine, it is possible that the sow was affected with a chronic infection of the urinary tract which may have extended to the genital tract, so causing prenatal death of piglets.

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REFERENCES

BURNSTEIN, T., and BAKER, J. A. (1954).—*J. Inf. Dis.* 94:53.

FERGUSON, L. C., and POWERS, T. E. (1956).—*Amer. J. Vet. Res.* 17:47.

LANGHAM, R. F., MORSE, E. V., and MORTER, R. L. (1958).—*Amer. J. Vet. Res.* 19:395.

LILLIE, R. D. (1948).—"Histopathologic Technic". (Blakiston Co.: Philadelphia.)

MORTER, R. L., and MORSE, E. V. (1956).—*J. Amer. Vet. Med. Ass.* 128:408.

NIEBERLE AND COHRS. (1952).—"Lehrbuch der Spez. Path. Anatomie der Haustiere" 3rd Ed. (Gustav Fischer: Jena).

PALLASKE, G. (1960).—"Pathologische Histologie." 2nd Ed. (Gustav Fischer: Jena.)

RYLEY, J. W., and SIMMONS, G. C. (1954*a*).—*Aust. Vet. J.* 30:203.

RYLEY, J. W., and SIMMONS, G. C. (1954*b*).—*Qd J. Agric. Sci.* 11:61.

SCHMID, G., and GIOVANELLA, R. (1947).—*Schweiz. Arch. Tierheilk.* 89:1.

SLEIGHT, S. D., LANGHAM, R. D., and MORTER, R. L. (1960).—*J. Inf. Dis.* 106:262.

TAMMEMAGI, L., and SIMMONS, G. C. (1956).—*Qd J. Agric. Sci.* 13:169.

TAMMEMAGI, L., and SIMMONS, G. C. (1958).—*Qd J. Agric. Sci.* 15:137.

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