# EXPERIMENTAL INFECTION OF PIGS WITH LEPTOSPIRA HYOS SAVINO AND RENNELLA.

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#### SUMMARY.

Three of four sows inoculated intramuscularly with L. hyos showed agglutinins and excreted Leptospira in the urine. The only clinical evidence of infection was inappetence and slight rise of temperature in two of the sows.

Leptospiruria was evident at 15 days in one sow and at 21 days in the other two. It extended over periods of 20, 16 and 15 days respectively and was continuous with one sow and intermittent with the others.

No Leptospira were isolated from the sows when slaughtered up to four months after inoculation.

Of 29 piglets produced by the three infected sows, 4 were mummified and 6 were born dead. No Leptospira were isolated from the dead piglets.

The three infected sows and 11 piglets that were tested showed antibodies to L. hyos, the maximum titre being 1:3,000 in the sows and 1:1,000 in the piglets.

### I. INTRODUCTION.

In a review of leptospirosis, Ryley (1956) summarised the information on porcine leptospirosis due to *Leptospira hyos* Savino and Rennella (syn. *L. mitis* Johnson). He mentioned that field evidence, combined with serological results, suggested that infection with *L. hyos* may be associated with unsatisfactory breeding performance. A pregnant sow experimentally infected with *L. hyos* by Ryley and Simmons (1954a) produced a healthy litter and showed no clinical evidence of infection.

This paper describes observations made on four sows experimentally infected with L. *hyos* and three comparable uninfected sows as controls.

### II. METHODS.

The experimental sows were of the Large White breed reared at this Institute. They were the progeny of three different sows and were about the same age and conformation. Agglutination tests on sera collected at frequent intervals during the four months prior to infection were all negative for brucellosis and L. pomona and L. hyos. No Leptospira were seen in urine samples collected at the same time as the blood samples. The sows were mated with a Berkshire boar obtained from a piggery free from brucellosis and leptospirosis. The animals were fed and housed in the same way as reported by Ryley and Simmons (1954b).

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Four sows (Nos. 15, 24, 31 and 35) were inoculated intramuscularly with  $1 \cdot 0$  ml. of *L. hyos* culture obtained from sowing peritoneal fluid from the 20th guinea pig passage into Schuffner's medium and incubating for 15 days. Dark-ground examination at a magnification of 900X showed only two or three organisms per field. This strain was that used by Ryley and Simmons (1954a).

All sows were examined daily and rectal temperatures of the inoculated sows recorded twice daily throughout the experimental period. Urine samples were collected daily from the inoculated sows, and at weekly intervals from the controls. Blood samples were collected fortnightly from all sows and the sera kept at -25 deg. C. until the end of the experiment. Urine, sera and dead piglets were examined as described by Ryley and Simmons (1954b). All piglets were weighed during or immediately after farrowing except when farrowing occurred during the night. In this event, weighing was done on the following morning. Sera of surviving piglets were examined for *L. hyos* agglutinins at weaning when the piglets were 6–8 weeks old. The blood for this purpose was collected from the *vena cava cranialis*.

### III. RESULTS.

### Control Sows.

Details of gestation periods and litters of control and inoculated sows are given in Table 1. Sow 10 developed arthritis during mid-pregnancy, but sows 37 and 38 remained healthy. These three sows produced 30 live piglets and one mummified foetus. The duration of farrowing was two hours for sow 10 and 50 minutes for sow 37. Sow 38 farrowed during the night and was not observed.

	Stage of Pregnancy when Inoculated (days).	Gestation Period (days).	Number of Piglets.		
Sow No.			Born.	Born Dead.	Survived 2 Days.
15	69	116	13	0	12
24	35	109	12	7	4
31	84	110	9	3	3
35	69	114	8	0	8
10		115	11	1	10
37		117	9	0	9
38		114	11	0	10

Table 1.

GESTATION PERIODS AND LITTERS OF INOCULATED AND CONTROL SOWS.

Urine samples collected during the experiment were all negative for Leptospira; no agglutinins to L. *hyos* were detected in blood samples taken from the sows or piglets.

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## Inoculated sows.

### Sow 15.

This sow was inoculated 69 days after mating and showed neither leptospiruria (Fig. 1) nor serum agglutinins for the entire period of observation. After farrowing she became progressively emaciated and was finally destroyed *in extremis* 49 days later. At autopsy the liver was yellow with distinct marbling. Both kidneys were enlarged (18 x 8 x 5 cm.) and whitish in colour.



Fig. 1.

Examination of Urine for Leptospira.

Histologically, the liver sections showed an extensive centri-lobular fatty degeneration and fatty infiltration, leaving only a narrow rim of normal liver cells around the periphery of the individual liver lobules. In the kidney there was almost total destruction of the tubular epithelium with marked reduction in the number of active glomeruli. In places the glomerular tufts had largely or totally disappeared, leaving a distorted Bowman's capsule with an empty lumen. No cellular or interstitial tissue reaction was evident. No Leptospira were seen by histological examination of liver or kidney and none were detected by culture or guinea pig inoculation.

The litter of this sow was born 116 days after mating and consisted of 13 healthy piglets, of which one was killed by the sow three hours after birth. Sera obtained from the remaining piglets at weaning were negative for L. hyos agglutinins.

### Sow 24.

Inoculated 35 days after mating, the sow commenced excreting Leptospira in the urine on the 21st day after inoculation and remained positive until the 32nd day; the sample on the 33rd day was negative; no sample was collected on the 34th day but leptospiruria occurred again on the 35th and 36th days (Fig. 1). Thereafter, the urine remained free of Leptospira for the following 91 days, when the sow was slaughtered. Leptospira were never numerous in the urine. The first blood sample, taken 13 days after inoculation, had a titre of 1/100; by the 36th day the titre had risen to 1/1,000.

On the afternoon of the 28th day the sow's temperature was  $103 \cdot 7$  deg. F., compared with the usual readings of up to  $103 \cdot 0$  deg. F. Inappetence with constipation occurred during the 29th to 35th days. On the 60th day a slight lameness and stiffness developed; the condition lasted for 10 days. During this period the sow again showed inappetence and constipation. No abnormality was seen when the sow was slaughtered four months after inoculation, and Leptospira were not detected in liver or kidney by histological and cultural examination or by guinea pig inoculation.

Farrowing occurred after a gestation period of 109 days and lasted three hours. Twelve piglets were born, of which four were mummified and three dead. Two of the live piglets were overlain by the sow when two and six days of age. The three surviving piglets were weaned at four weeks of age because of cessation of milk supply. The size of the mummified foetuses suggested that death had occurred very early in pregnancy. Two of the piglets born dead showed blood-stained fluid throughout the subcutis and in the pleural and peritoneal cavities. The third had petechiae on the epicardium. Leptospira were not detected in any of the dead piglets. The three surviving piglets had a titre of 1/30 to L. hyos at weaning.

### Sow 31.

This sow was infected 84 days after mating. Leptospiruria commenced on the 15th day after inoculation and lasted for 20 days. Organisms were being excreted on the day of farrowing (Fig. 1). They were never numerous in the urine. The agglutination titre was 1/100 13 days after inoculation and increased to 1/1,000 by the 29th day. The titre was still 1/1,000 when the animal was slaughtered six weeks later.

Apart from a rise of temperature on the 34th day and a poor appetite on this and the following three days, no ill health was observed. When slaughtered three months after inoculation, no gross abnormality was present, but histological examination of kidney showed vacuolation of the glomerular tufts and occasional perivascular foci of lymphocytic infiltration in the renal cortex. No Leptospira were detected.

The gestation period in this sow was 110 days. Three dead and six live piglets were born. Three of the live piglets died on the day after farrowing. The duration of the farrowing is not known, as it occurred during the night. Nothing abnormal was noted in two of the dead piglets, but three showed blood-stained fluid throughout the subcutis and in the pleural and peritoneal cavities. One piglet that survived for 24 hours showed fibrinous peritonitis and reddening of the intestine, the lining of which was covered with yellow mucus.

No Leptospira were detected in any of the dead piglets by cultural and histological examination or by guinea pig inoculation. The sera of the three surviving piglets were not tested for L. *hyos* agglutinins.

### Sow 35.

Sow 35 was infected 69 days after mating. Leptospiruria commenced on the 21st day after inoculation and except for negative findings on the 32nd and 33rd days continued until the 37th day (Fig. 1). Leptospira were never numerous in the urine. Serum taken 13 days after inoculation had a titre of 1/100. The titre rose to 1/3,000 during the next two weeks, and six weeks later had fallen to 1/1,000. Except for a slight rise of temperature from 65th to 67th day no ill health was noticed. No abnormality was seen when the sow was slaughtered four months after inoculation and no Leptospira were detected.

Farrowing occurred after a gestation period of 114 days and lasted four hours. Eight strong piglets were born.

At weaning three piglets had a titre of 1/1,000 and five 1/300 to L. hyos.

### DISCUSSION.

These experimentally produced infections with *L. hyos* suggest that this organism produces little or no clinical evidence of infection in pigs. A transient inappetence and slight rise in temperature during or soon after leptospiruria were the only symptoms observed.

Lameness occurred in one control and in one inoculated pig. It may have been caused by the concrete floors in the pens.

Abortion was not a clear feature of infection, as of the three infected sows, one farrowed at full term and the other two farrowed only 4–5 days before the anticipated date. The duration of farrowing appeared to be more protracted than in the controls, but the numbers are insufficient to permit accurate conclusions to be drawn.

Also, no definite conclusions can be drawn regarding the effect on the piglets. One sow infected in mid-pregnancy gave birth to a healthy litter. The other two sows, one of which was infected in early pregnancy and one in late pregnancy, produced 21 piglets, of which four were mummified, six born dead and three more died within the next 24 hours. The mummified foetuses were from the sow infected in early pregnancy.

No liver lesions as described by Ryley and Simmons (1954b) were seen in the dead piglets. In no instance were Leptospira demonstrated in the piglets that were dead at birth or that died soon after, even though on one occasion the sow was excreting viable Leptospira.

Leptospiruria commenced later with L. hyos than with L. pomona infection as found by Ryley and Simmons (1954b). Further, the number of Leptospira in any one sample was not great, and the duration did not exceed three weeks, compared with up to 83 days in the case of L. pomona infection (Ryley and Simmons 1954b).

Serological results are of interest inasmuch as the maximum titre was 1/3,000, compared with 1/30,000 in sows infected with *L. pomona* (Ryley and Simmons 1954b).

The evidence obtained in these experiments is inconclusive, but warrants further study on the effect of L. *hyos* infection on the breeding performance of pregnant sows.

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