FURTHER OBSERVATIONS ON THE EXPERIMENTAL INFECTION OF PIGS WITH *LEPTOSPIRA HYOS* SAVINO AND RENELLA

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SUMMARY.

Five sows inoculated intramuscularly with L. hyos **showed agglutinins and excreted Leptospira in the urine. No clinical abnormality attributable to infection with** L. hyos **was observed.**

Of 58 piglets produced by the five infected sows, 5 were mummified and 6 were born dead. No Leptospira were isolated from the dead piglets.

I. INTRODUCTION.

In a previous paper, Tammemagi and Simmons (1956) reported observations made on four pregnant sows experimentally infected with a strain of *Leptospira hyos*. The aim of their experiments was to show whether infection with *L. hyos* during pregnancy had any effect on the litter. The results obtained were inconclusive but further experiments were considered to be warranted.

This paper reports observations made on another five pregnant sows experimentally infected with a different strain of *L. hyos*.

II. METHODS.

The methods were the same as used previously (Tammemagi and Simmons 1956), except that a different strain of *L. hyos* was used. This strain was isolated from porcine urine obtained from a pig in a commercial piggery. Ten millilitres of urine was centrifuged and the deposit injected intraperitoneally into a guinea pig. The guinea pig was killed six days later and the liver and kidney ground with sterile sand and saline. This tissue suspension was inoculated into a guinea pig, which had a temperature of 104·6 deg. F. six days after inoculation. Heart blood was taken on the same day on which pyrexia occurred and inoculated into several tubes of Schuffner's medium. These inoculated tubes were incubated at 36 deg. C. for 39 days, when one was distributed into several $\frac{1}{4}$ in. diameter Pyrex test tubes, which were sealed. The sealed cultures were held at room temperature for 73 days, when one was opened and the contents sown into 5 ml. Schuffner's medium. After 13 days' incubation at 30 deg. C., numerous Leptospira were present. About 2 ml. sterile Schuffner's

medium was added and this diluted culture was used to inject five pregnant sows intramuscularly with 1 ml. each. Agglutinin absorption tests showed that the strain isolated was *L. hyos*.

All sows were examined daily and rectal temperatures recorded twice daily. Urine samples were collected twice each week from the control and daily from the inoculated sows.

III. RESULTS.

(1) Control Sows.

Table 1 gives details of gestation periods and litters of control and inoculated sows. The four control sows produced 46 piglets, three of which were stillborn. One sow farrowed after a gestation period of 116, two after 117 and one after 119 days. Duration of farrowing was not observed, as all four farrowed during the night.

Table 1.

GESTATION PERIODS AND LITTERS OF INOCULATED AND CONTROL SOWS.

Sow No.	Stage of Pregnancy When Inoculated. (Days.)	Gestation Period. (Days).	No. of Piglets.		
			Born.	Born Dead.	Survived Two Days.
37	84	117	16	2	13
149	110	116	13	5 (3 mummified)	6
154	86	116	10	0	9
199	61	116	9	3 (2 mummified)	6
202	25	119	10	1	8
188	..	117	12	0	10
190	..	117	11	1	10
192	..	119	13	2	11
198	..	116	10	0	9

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

(2) Inoculated Sows.

(a) Sow No. 37.

This sow was inoculated 84 days after mating.

Leptospiruria commenced 20 days after infection and occurred on 12 subsequent days with negative samples interspersed (Fig. 1). The last positive sample was obtained on the 73rd day after infection. Leptospira were never numerous and were often dead and misshapen.

The first serum sample, taken seven days after inoculation, had a titre of 1/100 to *L. hyos*. This rose to 1/1000 on the 28th day, and then fell to 1/100 on the 145th day after inoculation (Table 2).

The temperature of the sow was between 99 and 102 deg. F. for 37 days after inoculation, after which an occasional rise of the afternoon rectal temperatures accompanied by slightly reduced appetite was noted on days coinciding with high air temperatures.

Table 2.

RESULTS OF AGGLUTINATION TESTS ON SERA FROM INFECTED SOWS.

Sow No.	Days After Inoculation.								
	7.	14.	28.	42.	56.	70.	84.	105.	145.
37 ..	100*	300	1,000	1,000	1,000	1,000	300	300	100
149 ..	30	300	1,000	1,000	300	300	300	300	†
154 ..	100	300	1,000	1,000	1,000	300	300	100	30
199 ..	30	100	1,000	1,000	1,000	1,000	1,000	300	100
202 ..	100	100	300	300	300	300	300	100	‡

KEY: * Reciprocals of titres. Read at approximately 50% agglutination.
† No agglutination.
‡ No sample.

After a gestation period of 117 days, 14 live and two dead piglets were born over a period of two hours. Leptospiruria occurred prior and subsequent to farrowing. No abnormality was seen in the two dead piglets.

No Leptospira were demonstrated by culture, guinea pig inoculation or histological examination of the liver and kidney of the stillborn piglets or of those that died later from overlaying or as a result of haemorrhage caused by blood collection from the anterior vena cava.

All live piglets were bled before suckling and no agglutinins to *L. hyos* were present.

The sow, when slaughtered five months after inoculation, showed several subcapsular, pinhead-sized, greyish foci in both kidneys. On histological examination, these foci were seen to consist of accumulations of lymphocytes. Occasional foci of a similar type were also present in the deeper cortex and in the medulla. No Leptospira were seen in the kidney sections.

(b) Sow 149.

This sow was inoculated 110 days after mating. Leptospira appeared in the urine on the 21st day after infection and the last positive sample was obtained on the 42nd day (Fig. 1). Altogether, eight of the daily samples were positive within this period. Leptospira were never numerous and were mainly non-motile forms.

Seven days after infection the serum titre to *L. hyos* was 1/30, and after 28 days was 1/1000. Serum collected on the 145th day after infection showed no agglutinins to *L. hyos* (Table 2).

Temperatures recorded included some that were between 104 and 105·4 deg. F. This applied especially to the afternoon temperatures and appeared to occur on those days when the air temperature was also high. Panting was pronounced on these days.

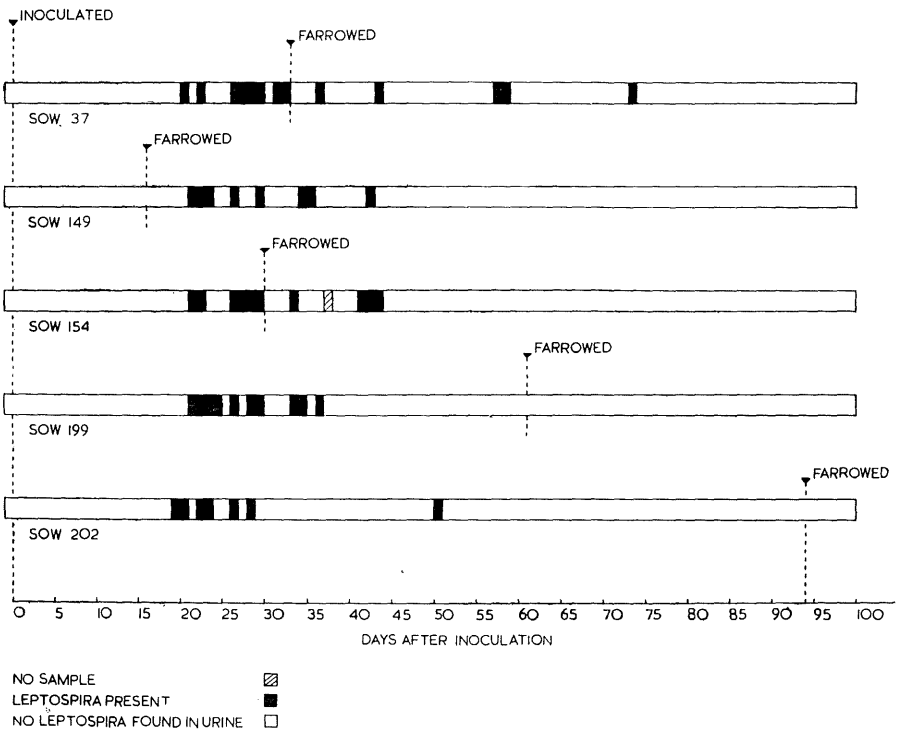

Fig. 1.

Examination of Urine for Leptospira.

Slight constipation with passing of dry faeces and inappetence lasting for several days were noticed over a period of three weeks following farrowing.

Farrowing occurred during the night after a gestation period of 116 days. Leptospiruria commenced five days after farrowing. The litter included eight live and five dead piglets. Three of the five dead piglets were mummified and weighed 1·0, 1·3, and 1·5 oz. respectively. One stillborn piglet weighing 1·5 lb. showed an excess of slightly bloodstained fluid in the thoracic and abdominal cavities and one weighing 1·3 lb. was decomposed.

The weights of the live piglets ranged from 2·5 to 3·6 lb.

Of the live piglets, one died within 24 hours of birth and one within 48 hours. On post-mortem, the former piglet showed five sharply defined

bright red areas on the surface of the liver. These areas were 3–10 mm. in diameter and about 3 mm. deep. The lungs, kidneys and edges of the liver lobes were congested.

Histological examination showed congestion of the liver with dilatation of sinusoids and portal vessels and some fragmentation of the portal cells. In the red areas mentioned above the hepatic cells had undergone liquefaction necrosis and the resulting spaces had filled with red blood cells, together with cellular debris, Kupfer cells, polymorphs and some mononuclear cells. Bile duct proliferation was prevalent throughout.

The piglet that survived 48 hours showed bruising under the throat and the lower jaw. Death was attributed to overlaying.

No Leptospira were demonstrated in any of the dead piglets by histological examination, culture or guinea pig inoculation. The three mummified foetuses were not examined.

Agglutination tests done on sera collected from the piglets the day after farrowing showed agglutinins for *L. hyos.*

The sow was slaughtered five months after infection. No gross abnormalities were seen and no Leptospira were detected by histological examination of kidney tissue.

(c) Sow 154.

This sow was inoculated 86 days after mating.

Leptospiruria was first detected on the 21st day after infection and last observed on the 43rd day (Fig. 1). The urine samples showed Leptospira on 10 days during this period. No sample was obtained on one day.

The serum titre was 1/100 when the sow was bled seven days after inoculation, rose to 1/1000 on the 28th day, and was 1/30 when the sow was slaughtered 145 days after inoculation (Table 2).

The sow showed no clinical evidence of ill-health apart from slight inappetence over a period of seven days immediately after farrowing. The sow's temperature varied from 99 to 104 deg. F. for most of the period of observation, with an occasional temperature above 104 deg. F. being recorded on afternoons of particularly hot days. The highest recorded was 105·6 deg. F. on the 66th day after infection.

Farrowing, which occurred after a gestation period of 116 days, lasted one hour. Leptospiruria occurred before and after farrowing.

Ten live piglets were produced. Nine of these were well developed, weighing 3·0–3·9 lb. The tenth piglet, weighing 2·1 lb., was crushed by the sow one day after birth. No Leptospira were isolated by culture or guinea pig inoculation from liver, lung or kidney tissue of this piglet. Serum obtained from the 10 piglets after they had suckled all showed agglutinins to *L. hyos.*

After slaughtering, no abnormality was seen in the sow, and no Leptospira were detected on histological examination of kidney sections.

(d) Sow 199.

This sow was inoculated 61 days after mating. Leptospiruria commenced on the 21st day and the last positive sample was collected on the 36th day. The urine showed Leptospira on 10 days during this period.

Seven days after inoculation the serum agglutinated *L. hyos* at a dilution of 1/30; the titre rose to 1/1000 on the 28th day. The last sample taken 145 days after infection had a titre of 1/100 (Table 2).

Frequent afternoon temperatures of more than 104 deg. F. were recorded, usually recurring on days of high atmospheric temperature. No ill-health was noted.

Farrowing occurred after a gestation period of 116 days, 19 days after the cessation of leptospiruria.

Six live and three dead piglets were born during the night. Two of the three dead piglets were mummified and each weighed approximately 0·5 lb. The third dead piglet, weighing 2·8 lb., was partly covered with foetal membranes and on post-mortem showed some degree of decomposition. The thoracic and abdominal cavities were filled with blood and the hindquarters were severely bruised. The lungs were not inflated and the stomach was filled with brown mucus. No Leptospira were isolated by culture or by guinea pig inoculation from stomach contents, liver or kidney.

All six surviving piglets showed agglutinins to *L. hyos* when bled on the day of farrowing. The piglets had suckled before being bled.

The sow was slaughtered five months after infection. The only gross macroscopic abnormality was several lesions in the kidneys similar to those in Sow 37. Histological examination showed that these lesions were also accumulations of lymphocytes. No Leptospira were seen in the kidneys.

(e) Sow 202.

This sow was inoculated 25 days after mating. Leptospiruria was first detected 19 days after inoculation and the last positive urine sample was collected on the 50th day. Leptospira were seen in only seven samples during this period.

The serum titre was 1/100 on the seventh day after inoculation and rose to 1/300 on the 28th day. However, the titre was still 1/100 145 days after inoculation (Table 2).

The temperature of the sow was between 99 and 103 deg. F. until she farrowed on the 94th day after infection. During the following four weeks, most of the afternoon temperatures ranged from 103 to 105·2 deg. F. These high temperatures occurred on days of high air temperatures. On a few

occasions, the morning temperatures of the sow were also high, but she showed no evidence of ill-health. Ten days before farrowing, the sow developed a slight lameness of the left hind leg and walked stiffly. This lameness disappeared a few days after farrowing.

Farrowing, which lasted 1½ hours, occurred after a gestation period of 119 days. Leptospiruria had ceased 44 days previously. Nine live and one dead piglet were produced. The dead piglet was not fully developed and was slightly decomposed. No Leptospira were isolated by culture or guinea pig inoculation from stomach contents, liver or kidneys. One of the live piglets was killed by the sow soon after birth. No Leptospira were isolated from the liver, lung or kidney tissue of this piglet by culture or by guinea pig inoculation.

All nine piglets had serum agglutinins to *L. hyos* after suckling.

IV. DISCUSSION.

The results described above are similar to those reported by Ryley and Simmons (1954) and Tammemagi and Simmons (1956) in that infection of pregnant sows with *L. hyos* did not result in abortion, death of piglets or poor breeding performance.

The five sows did not exhibit, at any time during the period of observation, any symptoms referable to the *L. hyos* infection.

Circumscribed haemorrhages in the liver of one piglet and small lesions in the kidneys of two sows were the only abnormalities seen. These lesions were not considered to be the result of *L. hyos* infection. The small lesions in the sow's kidneys were suggestive of being leucotic in origin. According to Englert (1955) leucosis in pigs is often inapparent macroscopically, but histologically foci of lymphocytic aggregations can be seen in various organs.

The excretion of Leptospira by the five sows was even less marked than previously reported (Tammemagi and Simmons 1956). The relatively short period of leptospiruria, the few and abnormal organisms excreted, and the intermittent character of excretion revealed in these results, suggest that diagnosis of this infection by urine examination is not very efficient. However, this procedure must remain an important diagnostic method because alternative methods are equally unsatisfactory.

The serum titre results were similar to those obtained previously (Tammemagi and Simmons 1956) and emphasise that the very high titres of 1/10,000 or more commonly reported in other leptospiral infections are not usual in *L. hyos* infection of pigs. The presence of agglutinins in sera from three of four sows on the 145th day after infection suggests that results of agglutination tests must be interpreted with caution when assessing whether disease present at time of sampling was the result of *L. hyos* infection. Retesting of pigs showing no or low titres about 14 days after first sampling may be of value, for the appearance of titre or an increase in titre could be taken as indicative of active *L. hyos* infection.

The failure to recover Leptospira from the piglets cultured at or within a few days of birth indicates that infection of the foetus is not usual.

Kemenes (1956) reported *L. hyos* as occasionally causing abortion in pigs, and field evidence in Queensland supports this. However, there is no evidence from the experiments reported here to suggest that *L. hyos* does cause abortion or neonatal loss when it infects the pregnant sow.

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