

The carriage of *Corynebacterium suis* in male pigs

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(Received 20 March 1984; accepted 17 April 1984)

SUMMARY

This investigation was designed to determine the prevalence of *Corynebacterium suis* in the preputial diverticulum of pigs of various age groups, located in different parts of the U.K., and to demonstrate whether the organism is transmissible between male pigs.

C. suis was isolated from almost 90% of 224 adult males, distributed among 25 pig units in widely separated regions of the country and in 96% of 66 pigs aged 5–6 months originating from six different herds in East Anglia. The rate of isolation of *C. suis* from younger pigs depended, at least in part, on the system of husbandry practised. Thus, in one herd, A, in which young pigs were maintained in comparative isolation, none of 26 pigs aged 5–8 weeks harboured *C. suis*; however, in the same herd, the organism was isolated from 77% of 64 pigs aged 9–15 weeks which were in close contact with male pigs aged up to 6 months. In another herd, B, in which individual litters were housed in the same pen from birth to 5 months of age, *C. suis* was isolated once from only one of 36 pigs in the age range 5–17 weeks, sampled on two occasions, with an interval of 5 weeks between each sampling. In a third herd, C, in which adult males had never been present, *C. suis* was not isolated from any of 26 pigs aged 4 months.

In each of two herds at the Royal Veterinary College colonization of the preputial diverticulum by *C. suis* was shown to have occurred by the age of 5 weeks in all of 38 pigs examined in a Large White herd and in all of 29 pigs in a Göttingen miniature pig herd.

Transmission experiments clearly demonstrated the ease and rapidity with which the preputial diverticulum became colonized with *C. suis* when non-infected pigs were placed in close contact with pigs which harboured the organism.

INTRODUCTION

Corynebacterium suis was first described by Soltys & Spratling in 1957 following its isolation from the bladder and kidneys of sows affected with cystitis and pyelonephritis. In a preliminary investigation into the carriage of *C. suis* in pigs (Jones, 1978) it was shown that 75% of 65 adult males harboured the organism in the preputial diverticulum. It was not found in the posterior urogenital tract (vestibule) of any of 80 adult females nor in the bladder urine of 40 adult males and 95 adult females. In that investigation, only a small number of pigs of younger age groups was examined. *C. suis* was found in 80% of 32 male pigs aged 15 weeks

Table 1. *The isolation of Corynebacterium suis from the preputial diverticulum of male pigs in various regions of the United Kingdom*

Region	No. of pigs examined	No. from which <i>C. suis</i> was isolated
Eastern (9)*	115	103 (90%)
South Eastern (4)	22	20 (91%)
South Western (1)	3	2 (67%)
East Midlands (3)	30	28 (93%)
West Midlands (3)	17	14 (82%)
East Scotland (2)	11	11 (100%)
North Scotland (1)	7	5 (71%)
North Wales (1)	15	10 (67%)
Northern Ireland (1)	4	3 (75%)
Total (25)	224	200 (89%)

* Figures in parentheses indicate number of pig units sampled in each region.

but only in 90% of 22 aged 5–10 weeks. In 32 females aged 5–10 weeks, *C. suis* was found in only two (6%).

The purpose of the present investigation was to establish (i) whether *C. suis* is present in adult males widely distributed geographically throughout the United Kingdom, (ii) the prevalence of *C. suis* in male pigs of various age groups, (iii) the age at which the urogenital tract of pigs may be colonized by *C. suis* and (iv) whether the organism is transmissible between male pigs.

MATERIALS AND METHODS

Source of animals

Live pigs. The adult males were from commercial herds, pig-testing stations and artificial insemination centres widely distributed throughout the U.K. (Table 1). The younger pigs were from herds in Bedfordshire, Essex and Gloucestershire and from the animal units of the Royal Veterinary College; further information is given in the appropriate section below.

Slaughtered pigs. These were pigs aged 4–6 months originating from six different farms in East Anglia and slaughtered at a bacon factory in Suffolk.

Sampling procedure

Live pigs. Samples of fluid from the preputial diverticulum were obtained by means of cotton-tipped swabs. The orifice of the prepuce was cleansed with tincture of iodine and a swab was inserted into the diverticulum. Over 60% of the samples obtained were examined on the same day; the remaining samples were mailed to us and examined within 48 h of collection.

Slaughtered pigs. The prepuce and contiguous superficial part of the abdominal wall were removed by a slaughterman, placed individually in plastic bags, transported to the laboratory and examined within 2 h of slaughter. The dorsal surface of the diverticulum was seared and incised and a sample of fluid obtained with a cotton-tipped swab.

The origin of the pigs sampled is given in the description of the results; all were

Table 2. *The isolation of Corynebacterium suis from the preputial diverticulum of slaughtered male pigs, aged 4–6 months, originating from six herds*

Number of pigs examined in each of six different herds	Number from which <i>C. suis</i> was isolated
10	10
10	9
15	15
10	10
17*	16
7	6
69	66 (96%)

* Castrates.

clinically healthy and there was no history of *C. suis* cystitis or pyelonephritis in any of the units from which the pigs were derived.

Bacteriological examination

The fluid in the preputial diverticulum contains a complex bacterial flora. Consequently, during the course of this investigation it became necessary to develop media that supported growth of *C. suis* but inhibited the growth of organisms that would otherwise impede the isolation of *C. suis*. Initially, 5% horse blood agar containing neomycin sulphate to a final concentration of 50 mg/l was used. However, an improved medium (Dagnall & Jones, 1982) was developed and used for the greater part of this investigation. This medium consisted of Columbia CNA (Difco) containing colistin sulphate (10 mg/l) and nalidixic acid (15 mg/l) to which was added metronidazole (50 mg/l) and defibrinated horse blood to a final concentration of 5%. Following primary isolation, *C. suis* colonies were subcultured onto 5% horse blood agar and their identity confirmed according to the criteria described by Soltys (1961) and Dagnall (1982).

RESULTS

The isolation of C. suis from adult males in different regions of the United Kingdom

The results are summarized in Table 1. Boars in each of 25 pig units distributed among nine widely separated regions of the U.K. were sampled and a high carriage rate was found in boars in each unit. *C. suis* was isolated from 200 (89%) of 224 boars examined.

The prevalence of C. suis in male pigs of various ages

Further information was obtained by sampling young pigs originating from several sources.

(i) *Slaughtered pigs aged 4–6 months.* The results are summarized in Table 2, which shows that *C. suis* was isolated from 66 (96%) of 69 pigs sampled.

(ii) *Live pigs aged 5–15 weeks (Herd A, Bedfordshire).* Twenty-six of 90 pigs sampled were aged 8 weeks or less. These 26 pigs originated from seven litters and

Table 3. *The isolation of Corynebacterium suis from the preputial diverticulum of male pigs of various ages in herd A*

Age (weeks)	No. of pigs examined	No. from which <i>C. suis</i> was isolated
5	7	0
6	8	0
8	11	0
9	8	2 (25%)
10	17	13 (77%)
12	19	14 (74%)
15	20	20 (100%)

Table 4. *The isolation of Corynebacterium suis from the preputial diverticulum of male pigs in herd B*

Litter no.	No. of male pigs examined	Age (weeks) at sampling		No. from which <i>C. suis</i> was isolated
		1st sample	2nd sample	
1	3	5	10	0
2	4	5	10	0
3	4	6	11	0
4	7	7	12	1*
5	6	8	13	0
6	1	9	14	0
7	5	9	14	0
8	6	12	17	0

* Isolated at first sampling.

had been weaned at the age of 5 weeks and were housed either in the farrowing pens in which they had been born or as individual litters in a weaner house; they had not had direct contact with any pigs other than their dam and their female litter-mates. The remaining 64 pigs had been moved at 8 weeks of age into a fattening house containing about 5500 pigs aged 8–20 weeks. In this house pigs were in groups of 12, separated only by tubular steel partitions, so that contact between pigs of various age groups was close and there was ample opportunity for the spread of urine and faeces between adjacent pens. *C. suis* was not isolated from any of the 26 pigs aged 5–8 weeks but was found in 49 (77%) of 64 aged 9–15 weeks in the fattening house. Details are given in Table 3.

(iii) *Live pigs aged 5–17 weeks (Herd B, Essex)*. In this herd pigs were housed in the same pen from birth until they were marketed at 20 weeks of age; they were weaned at 5 weeks of age. There were nine boars in this herd, eight of which were known to harbour *C. suis* in the preputial diverticulum. There was no direct contact between the boars and the younger male pigs.

Samples were obtained from all male pigs in each of eight litters in the age range 5–12 weeks. They were sampled a second time 5 weeks later. The number of male pigs in each litter, the age of the litters and the results obtained are summarized in Table 4. *C. suis* was isolated from only one of the 36 pigs at the first sampling and not from any at the second sampling.

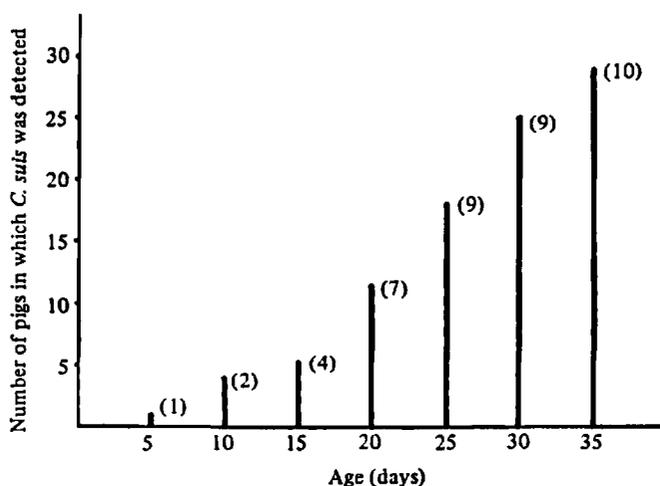


Fig. 1. The age at which *C. suis* was detected in the preputial diverticulum of 29 Göttingen miniature pigs in 10 litters. Numbers in parentheses are numbers of litters.

(iv) *Live pigs aged 4 months (Herd C, Gloucestershire)*. Since most adult males harbour *C. suis*, they may be an important source of infection for younger males, especially if husbandry systems are conducive to contact between pigs of various ages. It was therefore considered necessary to examine male pigs in a herd in which there were no adult boars and in which breeding was solely by artificial insemination. Herd C, which consisted of 100 sows and their progeny, had been established in 1968 by artificially inseminating one gilt. Subsequently all pigs in this herd had been derived from the female progeny of this gilt, inseminated artificially. There were no adult boars. The oldest males in the herd were aged 4 months and 26 of them were sampled; *C. suis* was not isolated from any.

The age at which the preputial diverticulum of male pigs may become colonized by C. suis

As it had been shown that most male pigs over 4 months of age and many aged 9–15 weeks harbour *C. suis*, it was decided to examine very young pigs to determine the age at which the preputial diverticulum may become colonized.

Two herds at the Royal Veterinary College were available for investigation. One was a herd of Large White pigs (LW) and the other was one of Göttingen miniature pigs (GM); they were housed separately but in close proximity and the same personnel attended both herds. Young LW pigs had no direct contact with older pigs other than their dam. Young GM pigs were in pens near to those housing older male pigs, many of which harboured *C. suis*.

In the LW herd, 38 male pigs distributed among seven litters (range 1–7 males/litter) and in the GM herd 29 male pigs in 10 litters (range 1–6 males/litter) were sampled at approximately 5-day intervals from birth to 35 days of age. In the LW herd, colonization was demonstrated in five pigs among two litters at 28 days of age and in all pigs at 35 days. In the GM herd, one pig in one litter was colonized at 5 days; thereafter, colonization was demonstrated in an increasing number of pigs and litters until, at 35 days, *C. suis* was present in every male pig

Table 5. *The transmission of Corynebacterium suis from an artificially infected pig (P1) to uninfected pigs*

Days post-inoculation	<i>C. suis</i> isolated from the diverticulum of pigs in					
	Group E			Group F (control)		
	P1	P2	P3	P4	P5	P6
1	+	-	-	-	-	-
2	+	-	-	-	-	-
3	}			Not tested		
4						
5	+	+	-	-	-	-
6	+	+	+	-	-	-
7	+	+	+	-	-	-

in every litter (Fig. 1). Vaginal (vestibule) samples were taken from all female pigs in all litters (27 LW, 26 GM) at the same time as the male pigs were sampled; *C. suis* was not isolated from any of them.

Transmission of C. suis infection between male pigs

Four experiments were conducted in an attempt to demonstrate whether *C. suis* could be transmitted from infected to non-infected males. Twenty-one Large White male pigs, aged 8–10 weeks, known not to harbour *C. suis* in the preputial diverticulum were purchased from Herd B. The diverticulum of each pig was shown to be free of *C. suis* on several occasions during the 10 days preceding the experiments. The pigs were housed in groups of three in steel isolators or in pens which had been disinfected and then left empty for 3 weeks.

C. suis was grown in Tryptic soy broth containing 1.2% urea for 18 h at 37 °C anaerobically. Each pig to be deliberately infected was inoculated with 1.0 ml of broth containing approximately 10^7 c.f.u. of *C. suis* into the diverticulum. Following inoculation the fluid in the diverticulum of each pig in the group was examined daily for the presence of *C. suis*.

Experiment 1

One of a group of three pigs housed in a steel isolator was inoculated and 6 days later all three pigs were infected.

Experiment 2

There were two groups, A and B, housed in steel isolators. One pig in each group was inoculated. All pigs in group A were shown to be infected 4 days later and all pigs in group B 6 days later.

Experiment 3

There were two groups, C and D. They were housed in adjacent pens with a common floor area and separated only by tubular steel partitions. One pig in group C was inoculated. Group D was an uninoculated control group. Twelve days later one of the uninoculated pigs in group C had become infected; on day 15 all pigs in both groups harboured *C. suis* in the diverticulum.

Experiment 4

As the control group in Expt 3 had become infected, two groups of three pigs (P1, P2 and P3 in group E and P4, P5 and P6 in group F) were housed in pens similar to those used in Expt 3 but the two groups were separated by three empty pens and scrupulous care was taken to avoid cross-contamination. One pig (P1) in group E was inoculated. The results are given in Table 5.

In each of these four experiments *C. suis* was shown to be present in the diverticulum for at least 30 days in the pigs which had been inoculated and in those to which infection had been transmitted. The control pigs used in Expt 4 were maintained in isolation and were shown to be free of *C. suis* for 60 days after the end of the experiment.

DISCUSSION

At the time of mating, *C. suis* may be transmitted from the prepuce of the boar into the urogenital tract of the sow, and if it becomes established there, cystitis and pyelonephritis may subsequently develop. The results of this investigation show that, throughout the U.K., most male pigs over 4 months of age harbour *C. suis* in the preputial diverticulum. Culling of infected boars has sometimes been practised in attempts to prevent the occurrence of *C. suis* cystitis in sows, but this seems unlikely to be worthwhile because there is a high probability that replacement boars will also harbour this organism. Culling might be of value if there are 'pathogenic' and 'non-pathogenic' strains of *C. suis* but at the present time there is no evidence that such different strains exist.

The carriage of *C. suis* by young pigs seems to be governed, at least in part, by the degree of contact they have with older, infected pigs. Thus in herd A, pigs under 8 weeks of age, housed in the pens in which they were born, were not carriers; however, when they were moved into an environment densely populated by pigs ranging in age from 8 to 20 weeks they soon became infected (Table 3). The contents of the preputial diverticulum of older pigs, a rich source of *C. suis*, voided onto the floor of the pens, would constitute a source of infection; spread of infection would be facilitated by the existence of a common floor area and the absence of solid partitions between the pens and by movement of personnel between pens.

The results obtained in herd B were quite different from those in herd A and are a reflexion of different systems of husbandry. In herd B, *C. suis* was isolated only in one of 36 pigs at the first sampling and in none at the second sampling. These pigs had no direct contact with the adult boars, the only known carriers of *C. suis* in the herd. It is difficult to account for the presence of *C. suis* in the one pig unless an error was made in the laboratory. If the result was correct, it is surprising that *C. suis* was not isolated at the second sampling and that infections did not spread to the other male pigs in the litter. Towards the end of our investigation in herd B, the preputial fluid of 12 male pigs aged 20 weeks, and of the 21 pigs aged 8–10 weeks, used in the transmission experiments, was examined but *C. suis* was not isolated; this result provides further evidence of the absence of *C. suis* in male pigs other than the adult boars in this herd.

The results of the investigation in herd C are of particular interest. In this herd, none of the male pigs, aged 4 months, harboured *C. suis*. Judging from the results

obtained in herd A and in the slaughtered pigs, it could have been anticipated that 90% of pigs of this age would harbour *C. suis*. Failure to isolate *C. suis* demonstrates that in the absence of adult male carriers, young males do not become infected.

Colonization of the preputial diverticulum by *C. suis* had occurred in both the LW and GM pigs in the RVC herds at the age of 4–5 weeks. In the GM pigs this is not surprising because they were kept in close proximity to carrier boars. The LW were not in direct contact with other males and it is unlikely that they were infected from their dams as we have never isolated *C. suis* from sows in the RVC herds despite many attempts to do so. There is no evidence that *C. suis* occurs anywhere outside the urogenital tract of the pig, other than on the floor of pens occupied by infected males. It seems that the likeliest way in which they became infected was by contamination from the footwear of animal attendants and by cleaning equipment which had been in contact with floors of pens occupied by other male pigs.

The transmission experiments show the ease with which male pigs, free from *C. suis*, become infected if they are in close contact with carrier males. Clearly, in the commonly employed husbandry systems the prevention of the transmission of infection would be an impracticable task.

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