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# Streptococcal infection in young pigs

## IV. An outbreak of streptococcal meningitis in weaned pigs

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SUMMARY

Twenty-eight pigs died in an outbreak of streptococcal meningitis in an East Anglian herd. Most were 10–14 weeks old. The outbreak lasted from January to April and was finally controlled by antibiotic therapy. A similar number of losses had occurred in the previous year though no diagnosis had then been made.

The causal agent appeared to be a haemolytic streptococcus belonging to group D and provisionally designated *Streptococcus suis* type 2. It is probably identical with de Moor's group R streptococcus which causes a similar disease in the Netherlands. It is serologically distinct from *Streptococcus suis* type 1 which causes meningitis in piglets. Type 2 infection in pigs appears to be widespread in England and Wales and to occur in animals up to the age of at least 14 weeks.

A comparison is drawn between *Str. suis* meningitis in pigs and group B streptococcal meningitis in human infants.

## INTRODUCTION

During their first few weeks of life piglets are subject to streptococcal infections seen as meningitis or arthritis (Field, Buntain & Done, 1954). Infections of this kind are fairly common in the U.K. and an identical condition occurs in the Netherlands (de Moor, 1963). The streptococci involved belong to Lancefield's group D and they have been provisionally classified as type 1 in *Streptococcus suis*, a subdivision of that group (Elliott, 1966). In the Netherlands a similar disease occurs in older pigs. In these animals the infection is also caused by group D streptococci but here the micro-organisms, though similar to *Str. suis*, are serologically distinguishable from type 1. In the Netherlands streptococci from infection of the older pigs have been designated group R and those from piglets group S (de Moor, 1963). To our knowledge such infections of older pigs have not hitherto been reported in the U.K. It is our purpose to report an outbreak of streptococcal meningitis in 12- to 14-week-old pigs that occurred recently in an East Anglian herd, here designated Herd A.

## THE OUTBREAK IN HERD A

### *History of the outbreak*

The farm on which the disease was first seen maintained a closed unit of 165 sows (Landrace and Landrace × Large White). With the exception of the boars no pigs had been bought in for more than 10 years and the boars were purchased from three sources only. The object of this enterprise was to produce heavy pork pigs for sale in the market. The breeding and fattening units were on two separate farms about half a mile apart; both units were managed by the same staff. Sows farrowed in crates where they remained until weaning at 5 weeks. Three days after weaning the piglets were moved to a 'weaner pool' where normally 2–3 litters were batched.

When they weighed 50 lb. the piglets were moved by van to the fattening unit where they were put into a Danish type fattening house in pens of 20.

Farrowing fever was a regular occurrence in the sows. It was controlled by close observation at the time of farrowing and the use of antibiotics if any sows appeared sick. With this exception there had been no disease problems until January 1973 when a 10-week-old pig was admitted to the Cambridge Veterinary Investigation Centre showing nervous signs: the pig was trembling, unable to stand and from time to time made paddling movements. The pig was destroyed and a post-mortem examination carried out. Meningitis was diagnosed and a serologically unidentified streptococcus was isolated from the brain and other organs. Throughout the spring and summer of 1973 the outbreak continued. The most usual occurrence was for a pig aged between 10 and 14 weeks to be found dead in its pen. Thirty pigs died and of these 12 were examined at the Veterinary Investigation Centre. In most cases polyserositis was diagnosed but in only two was meningitis recorded. From 8 of the 12 pigs serologically unidentified streptococci were isolated. Between August and December 1973 no losses occurred. Then, on 24 December, five pigs exhibited nervous signs and two died despite antibiotic therapy. Between December 1973 and March 1974 a total of 44 pigs aged between 10 and 15 weeks were affected, 28 died and 20 post-mortem examinations were carried out. Those pigs that recovered had all received treatment with a synthetic penicillin.

The outbreak was finally brought under control by the incorporation in the food of 400 g. oxytetracycline per ton. This medicated feed was given for 14 days after moving from the farrowing to the fattening unit. After this treatment had started only one pig died from meningitis.

### *Post-mortem findings in 20 pigs dying from meningitis in herd A*

The incidence of the disease was unrelated to sex. Half of the pigs affected were 12 weeks old but the total age range was 8–15 weeks. Table 1 shows the main post-mortem findings in 20 pigs in which a diagnosis of streptococcal meningitis was made. All the animals appeared well nourished. Apart from congestion of the meninges and lungs there were no consistent gross abnormalities. In addition to the findings listed in Table 1 two animals showed haemorrhagic necrosis of the

Table 1. *Post-mortem findings in* 20 *pigs that died from streptococcal meningitis*

| Lesion seen | Number of animals showing the lesion |
|---|---|
| Congestion of meninges | 18 |
| Considerable excess of cerebro-spinal fluid | 8 |
| Purulent meningitis | 6 |
| Congestion of the lungs | 19 |
| Discoloration of skin | 14 |
| Congestion of the carcass lymph nodes | 11 |
| Congestion of the liver | 11 |
| Generalized reddening of carcass | 10 |
| Congestion of the turbinates | 10 |
| Polyserositis | 9 |

pancreas and 2 showed renal infarcts. Hydronephrosis was seen in 4 pigs and purulent arthritis in 2. Two others showed evidence of sanguinous pericarditis.

## *Bacteriological investigations*

### *Streptococcal cultures and extracts*

Primary post-mortem cultures were made on blood agar (5 % sheep blood in Oxoid agar CM331) and on MacConkey agar (Oxoid CM7). For serological tests the streptococci were grown in digest broth containing 0·1 % glucose. The centrifuged cocci were resuspended in a small volume of 0·03 N-HCl to give 100-fold concentration of the broth culture. The concentrated suspension of cocci was heated at 100° C. for 5 min. and the supernate neutralized with N-NaOH after adding 1 drop of phenol red indicator. de Moor (1963) has reported loss of serological reactivity of specific antigen from his group R streptococci after heating to 100° C. at pH 2. We have confirmed this with the strains examined here and have made our streptococcal extracts by heating at pH values between 2·5 and 3·0.

### *Streptococcal antisera*

Grouping antisera were kindly provided by Dr R. C. Lancefield. Typing antisera were prepared in rabbits by the method previously described (Elliott, 1960).

### *Slide agglutination tests*

The technique described by Griffith (1926) was used.

### *Precipitin tests*

These were carried out by the capillary technique (Swift, Wilson & Lancefield, 1943).

## RESULTS

Cultures were taken at autopsy from all 20 pigs referred to above under 'post-mortem findings'. In all cases samples were inoculated on 5% sheep blood and MacConkey agar and incubated aerobically at 37° C. In brain cultures from all 20 pigs streptococci predominated and in some no other micro-organisms were seen. Streptococci were also recovered from the nostrils of 11 of 15 of these pigs, from the heart blood of 8 of 15 and from the livers and spleens of 4 of 10. Streptococci were also recovered from other tissues including lungs and kidneys and from joint fluids.

### *Cultural and morphological characteristics of streptococci from Herd A*

On sheep blood agar the streptococci formed large, mucoid colonies surrounded by a zone of haemolysis incomplete after 18 hr. at 37° C. but becoming complete after 2–3 days. On horse blood agar haemolysis was complete in 18 hr. The streptococci grew readily on MacConkey's medium producing small, lenticular, red colonies. Growth in digest broth produced diffuse turbidity in 18 hr. at 37° C. Gram-stained preparations of all the streptococci isolated from Herd A showed minute Gram-positive cocci, oval or rod-shaped, arranged singly and in pairs. The morphology of these micro-organisms resembled that of streptococci associated with meningitis in piglets and described by Field *et al.* (1954).

### *Serological examination of streptococci from Herd A*

Streptococci from six pigs that died from meningitis were examined serologically. The cultures included one blood culture (strain P 72) taken several hours before death. The serological tests included slide-agglutination reactions with broth cultures and precipitin tests with acid extracts.

*Slide agglutination tests.* Eleven streptococcal cultures from six pigs in Herd A were tested by slide agglutination. The cultures included 6 from brains, 4 from other organs and 1 ante-mortem blood culture, strain P 72. Eight different streptococcal antisera were used in the tests: serum RC 26 was from a rabbit immunized with strain P 72; serum R 800 had been raised against *Str. suis* type 1 isolated from a 6-week-old piglet with meningitis (Elliott, 1966); of the 6 remaining antisera included as controls, 4 were raised against strains of unrelated streptococci and 2 against group D streptococci differing in type from *Str. suis* type 1 and from strain P 72.

The results of these slide agglutination tests are set out in the upper part of Table 2. All the streptococci isolated from the Herd A pigs were agglutinated by serum RC 26, specific for strain P 72. None were agglutinated by serum R 800, specific for *Str. suis* type 1 (see below). None of the streptococci were agglutinated by the 6 control antisera.

*Precipitin tests.* Acid extracts were prepared from the 11 streptococcal cultures previously tested by slide agglutination. The extracts were first tested against Lancefield grouping antisera (groups A–O). They were then tested against the type-specific antisera RC 26 and R 800 described above under 'Slide Agglutination

Table 2. *Slide agglutination reactions with streptococci from pigs with meningitis in Herd A in five unrelated herds*

| Pigs: No., Origin, Age | Cultures tested†: No. and source | Slide agglutination reactions with streptococcus serum of stated type: Strain P 72 (serum RC 26*) | *Str. suis* type 1 (serum R 800) |
|---|---|---|---|
| 6, Herd A, 12–14 weeks | 6 brain, 4 other organs, 1 blood culture (strain P 72) | + | – |
| 3, Herds B, C, 12–14 weeks | 3 brain, 3 other organs | + | – |
| 2, Herds D, E, 3–6 weeks | 2 brain, 3 other organs | – | + |
| 1, Herd F, 3 weeks | 1 brain | + | – |

* Serum RC 26 was raised in a rabbit against strain P 72 (blood culture).

† None of these cultures agglutinated in six control antisera raised against streptococci different in type from strain P 72 and *Str. suis* type 1.

Tests'. Finally, the extracts were tested against de Moor's group R antiserum (de Moor, 1963); this serum had been prepared with a streptococcus isolated in the Netherlands from a 12 to 14-week-old pig with meningitis.

The results of these precipitin tests are set out in Table 3. It can be seen that all the extracts precipitated in the group D antiserum (R 1996) raised against a strain of enterococci. They also precipitated in another group D antiserum, R 822, not included in Table 3. Serum R 822 was raised against a non-capsulated strain of *Str. suis* described in a previous report (Elliott, 1966). All the cultures from the Herd A pigs yielded extracts that precipitated with the type-specific antiserum (RC 26) raised against one of these cultures (strain P 72). These extracts also precipitated in the 'group R' antiserum. They failed to react with a type-specific serum (R 800) raised against *Str. suis* type 1 previously isolated from a 6-week-old piglet with meningitis.

It was concluded from these results that all the streptococci isolated from Herd A belonged to a single type in group D and that this type was probably identical with de Moor's 'group R'.

### *Examination of streptococci from pigs with meningitis in herds unconnected with Herd A*

During the course of the epidemic in herd A (January to April 1974) five smaller outbreaks of pig meningitis were investigated in unrelated East Anglian herds (herds B–F). Streptococci were recovered from the brains of six animals in most of which the same streptococci were also isolated from other organs. All these streptococci were examined serologically. The results are shown in Tables 2

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Table 3. *Precipitin reactions with acid extracts of streptococci from pigs with meningitis in Herd A and five unrelated herds*

| Pigs: No. Origin Age | Cultures tested: No. and source | Precipitin reactions of streptococcal extracts with sera of stated group or type: Group D (serum R 1996) | Strain P 72 (serum RC 26) | *Str. suis* type 1 (serum R 800) | Group R* (de Moor) |
|---|---|---|---|---|---|
| 6 Herd A 12–14 weeks | 6 brain, 4 other organs, 1 blood culture (strain P 72) | + | + | − | + |
| 3 Herds B, C 12–14 weeks | 3 brain, 3 other organs | + | + | − | + |
| 2 Herds D, E 3–6 weeks | 2 brain, 3 other organs | + | − | + | − |
| 1 Herd F 3 weeks | 1 brain | + | + | − | + |

All the streptococcal extracts reacted with gpD antisera but failed to react with antisera of other Lancefield groups A–O.

* de Moor's 'group R' antiserum supplied by Central Public Health Lab., Colindale.

and 3 from which it can be seen that two outbreaks were caused by *Str. suis* type 1 and three by streptococci related to strain P 72.

Through the co-operation of Veterinary Investigation Centres post-mortem cultures of streptococci have also been obtained from the brains of pigs dying from meningitis in 21 unrelated herds in other parts of England and Wales. In most of these outbreaks only one or two pigs were affected but in some up to 20 animals were affected. From each outbreak one representative strain of streptococci was obtained for serological examination either by slide agglutination or by precipitin tests. The results are set out in Table 4 which also includes the strains from the five East Anglian outbreaks referred to above. It can be seen that group D streptococci appeared to be the causal agent in 20 of the 26 outbreaks. Streptococci of group C and group E were each responsible for one outbreak and cultures from 4 outbreaks were not identified. Of the 20 group D strains 4 belonged to *Str. suis* type 1, and 14 were related to strain P 72. The type of the 2 remaining group D strains could not be identified. In the type 1 outbreaks none of the affected animals was more than 6 weeks old whereas in the 'P 72' outbreaks pigs aged from 1 to 14 weeks were infected.

### *Experimental production of the disease*

It has previously been shown that meningitis may be produced in piglets by spraying into their upper respiratory tract broth cultures of *Str. suis* type 1 (Elliott, Alexander & Thomas, 1966). Pigs over the age of 8 weeks are usually

Table 4. *Serological classification of streptococci from pigs with meningitis from 26 unrelated outbreaks in England and Wales*

| Classification of strep. causing outbreak of meningitis | | No. of outbreaks |
|---|---|---|
| Group (Lancefield) | Type | |
| D | *Str. suis* type 1* | 4 |
| | P 72† | 14 |
| | Not identified | 2 |
| C | Not identified | 1 |
| E | Not identified | 1 |
| Not identified | Not identified | 4 |

* de Moor's group S.
† Provisionally designated *Str. suis* type 2 (de Moor's group R).

resistant to this type of infection. An attempt was now made to produce meningitis in such animals with streptococci (strain P 72) isolated from a pig that had succumbed to natural infection in herd A.

Four pigs, 8–10 weeks of age, and 22–60 lb. in weight, were used in this experiment. Two, aged 9 weeks, were from herd A; the other two, aged 8 and 10 weeks respectively, were of the same breed but from different herds. Two attempts at infection were made at 10-day intervals in each of the four pigs. At each attempt an overnight blood-broth culture of streptococci of strain P 72 was sprayed from an ordinary throat atomizer into the nose and throat; approximately 2 ml. was sprayed into the throat and 1 ml. into the nose. At the first attempt the dose of streptococci given to each pig approximated $10^6$ colony-forming units. At the second attempt the number of streptococci sprayed in the same volume of fluid was increased one thousandfold (i.e. $10^9$ colony forming units).

No colonization of the nose or throat could be demonstrated by swabbing 5 days after the first spraying. From the second spraying meningitis resulted in the youngest of the four pigs, 10 weeks old at the time of death. Five days after spraying its temperature rose to 106° F. and it lay on its side paddling. It died within 2 hr. of first showing signs of illness.

Post-mortem examination revealed much effusion into the serous cavities and congestion of the lungs and meninges. The right hock, right stifle and left knee joints contained purulent exudate and there was associated tenosynovitis. Streptococci, serologically identical with those inoculated (strain P 72), were isolated from the brain, heart blood, peritoneum, pericardium and from the inflamed joints, but not from those joints that appeared normal.

The remaining three pigs were kept under observation for 4 weeks after the second spraying, during which time they showed no signs of disease.

## DISCUSSION

*Streptococcus suis* was established as a subdivision of Lancefield's group D to accommodate strains isolated from meningitis and arthritis in piglets. Such

strains were classified as *Str. suis* capsular type 1 (Elliott, 1966). Group D streptococci here reported as the cause of meningitis in older pigs resemble type 1 but are serologically distinct. It seems appropriate to classify these strains as *Str. suis* type 2. Their distinctive antigenic component is probably a capsular polysaccharide. It is generally agreed that porcine streptococci corresponding to *Str. suis* share the group antigen characteristic of Lancefield's group D. Streptococci here classified as *Str. suis* type 1 have, in the Netherlands, been designated 'group S'; those here provisionally classified as *Str. suis* type 2 have there been designated 'group R' (de Moor, 1963).

Beyond identifying the causal agent as *Str. suis* type 2 we can offer no definite information concerning the epidemiology of the outbreak of pig meningitis here described. As with type 1 infection in piglets (Elliott *et al.* 1966; Williams, Lawson & Rowland, 1973) so with type 2 infection in older animals the nasopharynx is the probable portal of entry for *Str. suis*: these micro-organisms were recovered *post mortem* from the nose in 11 out of 15 cases of meningitis in herd A and they provoked the typical disease in 1 out of 4 pigs when sprayed into the upper respiratory tract. During the outbreak of disease from January 1973 to April 1974, 60 pigs died; 28 of these were considered to have died of meningitis and in retrospect it seems likely that most of the 60 deaths were attributable to this condition. Three pigs died at the farrowing unit, the other 57 died at the fattening unit. Two possible explanations may account for the localization of the outbreak of streptococcal meningitis to the fattening unit in herd A. It is possible that there was some continuing source of infection in this unit during the period under review. The continuous introduction of fresh, susceptible pigs into an infected milieu would maintain an outbreak such as is here described. Secondly, one can speculate that when the pigs on farm A were moved they were at their most susceptible: they had possibly lost their maternal immunity but not acquired an active resistance. Movement at a different age might result in a lower incidence of the disease.

With the collaboration of Veterinary Investigation Officers in various parts of the U.K. we have found that *Str. suis* type 2 infection is widespread in pigs and occurs in animals of an age considered to be immune to type 1 infection. Previous work has shown that most conventionally reared pigs older than 6–8 weeks are immune to type 1 (Agarwal, Elliott & Lachmann, 1969). Their apparent lack of immunity to type 2 is puzzling but a similar association of particular serological types with different age groups has been noted in human neonatal infection caused by group B streptococci (Baker & Barrett, 1973, 1974; Wilkinson, Facklam & Worthem, 1973). In infants less than 5 days old with group B streptococcal septicaemia ('early onset' type) Baker and Barrett found no significant difference in the incidence of type II and type III cocci (44% and 33% respectively). In infants more than 10 days old ('late onset' type) type III cocci were incriminated in all cases of septicaemia and were responsible for 93% of streptococcal meningitis. Type II was never the cause of infection in this age group. Baker & Barrett (1974) postulate a different epidemiology for the cases of early and late onset. The former are probably infected from the mother but the source of 'late onset'

infection is unknown. It will be recalled that in piglets *Str. suis* type 1 infection is probably derived from the sow but in older pigs the source of type 2 infection is unknown.

It has previously been shown that serum of piglets convalescent from *Str. suis* type 1 infections protects susceptible animals from infection with homologous streptococci (Elliott *et al.* 1966). At that time only one serological type appeared to be responsible for much of this infection in piglets and therefore vaccination was suggested as a practicable means of control. From the observation of de Moor in the Netherlands (1963) and from our own more limited experience in the U.K. it appears that *Str. suis* type 2 is responsible for most streptococcal meningitis in older pigs as well as for some in piglets. It seems reasonable to conclude that vaccination with a combination of type 1 and type 2 streptococci could help to reduce the incidence of *Str. suis* infection in pigs of all ages.

We thank Mr N. F. Tebbutt, MRCVS, for bringing this problem to our attention and we are grateful to him and to the pig manager at farm A for their co-operation in the investigation. We are most grateful to Dr R. C. Lancefield for providing us with streptococcal grouping antisera and for her comments. We are also grateful to the Veterinary Investigation Officers who sent cultures and information to us and to Mr P. T. Edwards for his skilled technical assistance.

*Note added in proof* 16 *May* 1975. A case of human 'group R' streptococcal meningitis has recently been reported by Dr M. T. Parker of the Central Public Health Laboratory, Colindale (personal communication). The patient, a man aged 61, developed meningitis within 24 hr of cutting his finger while at work in a bacon factory. He was successfully treated with penicillin. 'Group R' streptococci were isolated from his blood and cerebro-spinal fluid.

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