Streptococcal infection in young pigs

II. Epidemiology and experimental production of the disease

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INTRODUCTION

In their studies on piglet mortality, Field, Buntain & Done (1954) noted that outbreaks of streptococcal meningitis and arthritis were usually confined to animals in a single litter. From this they concluded that the sow was the probable source of infection. In the work reported here we found evidence supporting this theory. Furthermore, our study of the disease in the field suggests that the causative organism (PM streptococcus) is probably transmitted from the nose or throat of the sow to the upper respiratory tract of the piglet. This is supported by the results of experiments in which a condition indistinguishable from the naturally occurring disease was produced in young pigs by spraying cultures of the PM streptococcus into the nose and throat. Complete protection against such infection was afforded by the prior administration of serum from piglets convalescent from experimental infection.

METHODS

Bacteriological examination of sows and piglets

Throat and nose cultures

Throat and nose swabs were plated on 5% horse blood agar with and without the addition of crystal violet and thallous acetate (Edwards, 1933). The plates were incubated aerobically for 18 hr. at 37° C. and haemolytic streptococcal colonies subcultured in Todd Hewitt broth containing 10% horse serum.

Saline or acid extracts from these streptococci were tested by precipitin reactions with type-specific rabbit antisera prepared with PM streptococci (Elliott, 1966).

Blood cultures

Five ml. samples of blood from the anterior vena cava were mixed with 500 units of heparin. Two 1 ml. volumes of the heparinized sample were plated with 15 ml. molten nutrient agar for colony counts; the remainder was added to 15 ml. Todd Hewitt broth. Plate and broth cultures were incubated aerobically at 37° C. for 7 days.

Experimental animals

Piglets to be used in the experimental production of the disease were weaned at 7 days. Each group of animals was reared in semi-isolation in a loose-box and fed

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on a diet consisting of antibiotic-free milk substitute (Amvilac) and pellets. Convalescent serum used in protection experiments was obtained by bleeding the animals from the axillary vessels while under barbital anaesthesia.

EXPERIMENTS AND RESULTS

Incidence of PM streptococci in infected litters

Throat cultures were examined from both sick and healthy piglets of litters in which outbreaks of streptococcal meningitis or arthritis had occurred within the preceding 3 weeks. All the animals were less than 8 weeks old.

In two consecutive outbreaks of infection cultures positive for PM streptococci were obtained from the throats of five out of five animals with the overt disease, either arthritis or meningitis; blood cultures from these animals were also positive. Throat cultures were also positive in fourteen of twenty-three apparently normal piglets from five litters in which cases of infection had occurred during the preceding 3 weeks (Table 1). This probably gives a low estimate of the number actually infected for colonies of the PM streptococci are not easily identified on blood agar plates.

The finding of PM streptococci in the throats of infected piglets and their littermates suggested two possibilities: first, that the reservoir of infection might be the porcine upper respiratory tract, and secondly, that in susceptible piglets the upper respiratory tract might provide the portal of entry for the PM streptococci. Further investigations were therefore undertaken to investigate these possibilities.

The incidence of PM streptococci in the upper respiratory tract of normal sows

We were unsuccessful in preliminary attempts to isolate PM streptococci from sows in whose young streptococcal infection had recently occurred; cultures from the rectum, vagina and from the skin overlying the teats were negative. Bacteriological examination of the upper respiratory tract of forty-four normal non-pregnant sows (gilts), however, yielded positive cultures from three. From the noses of three sows streptococci serologically indistinguishable from the PM strain were recovered; in one of these animals the PM streptococcus was also recovered from the throat. Experiments described later in this report showed that at least one of these strains (C 22 N) was pathogenic for piglets up to the age of 12 days.

Experimental production of the disease in piglets by infection of the upper respiratory tract with PM streptococci

Infection with PM streptococci from diseased piglets

The same general procedure was adopted in all the following experiments. PM streptococci were grown for 12–14 hr. at 37° C. in modified Todd Hewitt broth containing 10% normal horse serum. In most experiments approx. 5 ml. of the culture were sprayed from an ordinary throat spray (atomiser) into the nose and throat of the piglets. In two cases the centrifuged deposit from 100 ml. of culture

was resuspended in milk which was rapidly gulped down by the piglets. Litter-mates serving as controls received the same dose contained in a gelatin capsule from which the cocci were subsequently liberated during digestion in the stomach and intestine. After inoculation of the animals, serial 5 ml. samples of blood were withdrawn from the anterior vena cava and cultured for PM streptococci. In some instances throat cultures were also taken and in animals dying from the infection post-mortem cultures were taken from the heart blood, joints and brain.

Pig no.	Age (days)	Culture inoculated by	Cultures from young pigs at intervals (days) after spraying													
			0	1	2	3				7				10		17
1	7	Spray												B+		
														† Joints+		
														Brain+		
2	"	"												B+		Joints+
																† Brain+
																Blood+
3	11	"			B- B++	B++										
					T- †	Joints+										
						CSF+										
4	"	"			B- B++	B-								† B++		
														T-		
5	17	Infected milk			B++	B++								† B+		
						T+								T+		
6	"	"			† B++											
					T+											
7	"	Gelatin capsule				B-										
					T+	T-										
8	"	"				B-								B-		
					T-	T-								T-		
			} No sign of infection →													

Fig. 1. Result of inoculating PM streptococci* into nose and throat of young pigs.

* Strain PM 23 B isolated from blood of naturally infected piglet.

† indicates death of piglet.

T, throat culture; B, blood culture; +, positive for PM streptococci; ++, positive for PM streptococci > 300 colonies per ml. blood.

The results of these experiments are summarized in Fig. 1. In all six piglets, aged 7-17 days, inoculation of the upper respiratory tract with strain PM 23 resulted in a streptococcal bacteraemia which terminated fatally in four animals; of the two remaining, one (piglet 5) died as a result of cardiac puncture and the other (piglet 2) was killed after 17 days. In the two animals which received the cocci in a gelatin capsule no bacteraemia resulted. In two additional piglets, not included in Fig. 1, an attempt to produce the disease by spraying at the age of 21 days proved unsuccessful although one of these animals yielded a positive throat culture 21 days later.

Infection with PM streptococci from a normal sow

In the next experiment the invasiveness of two different streptococcal strains of porcine origin was compared. Strain C 22 N had been isolated from the nose of a 2-year-old sow with no history of disease. This strain was serologically identical

with the PM streptococcus used in the preceding experiments. The second strain, A 227, was isolated from the throat of an 8-week-old pig which had recovered from an experimental bacteraemia produced with PM streptococcus strain PM 23. Strain A 227 belonged to group D and resembled strain PM 23 in all respects other than the possession of a capsule (Elliott, 1966).

The upper respiratory tracts of two 12-day-old pigs (S 1 and S 2) were sprayed with strain C 22 N and two litter-mates (C 1 and C 2) with strain A 227. The results are summarized in Fig. 2. Piglets S 1 and S 2 developed a streptococcal bacteraemia the duration of which was at least 14 days in S 1 and 21 days in S 2. Both animals appeared fully recovered when they were killed after 38 days. No positive blood cultures were obtained from the two piglets C 1 and C 2 sprayed with the non-capsulated streptococcus strain A 227.

From the results of this experiment it was concluded that PM streptococci in

Pig no.	Strep. sprayed in nasopharynx	Cultures at intervals after spraying (days)											
		0	1	2	7	10	11	14				21	38
S 1	C 22 N (PM strep.)			B— B++	B++ T+		B++ B++	B++				B—	B— † Killed Joint+
S 2	„			B+ B+	B++ T—		B++ B++	B+				B+	B— † Killed Joint+
C 1	A 227 (non-capsul. strep.)			B— B—	B— T—			No sign of disease					
C 2	„			B— B—	B— T+ *			No sign of disease					

Fig. 2. Result of spraying PM strep. (C 22 N)** and non-capsulated GpD strep. (A 227)* in 12-day-old piglets' nasopharynx.

** Strain C 22 N was isolated from nose of a normal sow.

* Strain A 227 was isolated from throat of convalescent piglet 9 weeks after infection.

T, throat culture; B, blood culture; +, positive for PM streptococci; ++, positive for PM streptococci > 300 colonies per ml. blood.

Table 1. *Streptococcal infection in five litters of piglets.*
Throat cultures from healthy litter-mates 3 weeks after onset

Piglets in five litters		
Piglets in litter	Survivors	Survivors with positive throat cultures*
5	3	3
8	3	2
10	4	1
10	7	2
7	6	6

* Positive throat cultures were those from which PM streptococci were isolated.

the upper respiratory tract of normal sows are capable of infecting young piglets. It seems possible that the invasiveness of these streptococci is associated, in part at least, with their possession of a capsule.

Expt. 1. Nine 12-day-old litter-mates					Expt. 2. Ten 12-day-old litter-mates					
Pig no.	Serum*	Cultures at intervals after spraying (days)				Pig no.	Serum*	Cultures at intervals after spraying (days)		
		0	7	14	0			7	10	
	From convalescent piglets (S1+S2)					From convalescent piglets (S1+S2)				
1		B—B—	B—	B—	11		B—B—	B—		
2	"	T+			12	"	T—			
3	"	B—B—	B—	B—	13	"	B—B—	B—		
4	From refractory piglets	T+			14	"	T—			
5	"	B—B—	B—	B—	15	"	B—B—	B—		
6	"	T—			16	No serum	T—			
7	No serum	B+B++	B++	B+	17	"	B—B+B++	B++	†	
8	"	T+			18	"	T—lame		CSF+	
9	"	B++B++	B++	B+	19	"	B++B++	B++		
		T+			20	"	B++B++	B++		
							B+lame	†		

Fig. 3. Effect of convalescent serum on piglets sprayed in nasopharynx with PM streptococci (strain C 22N isolated from nose of normal sow).

* 20 ml. convalescent serum was given subcutaneously 24 hr. before spraying nasopharynx.

† Indicates death of piglet;

T, throat culture; B, blood culture; +, positive for PM streptococci; ++, positive for PM streptococci > 300 colonies per ml. blood; ?, contaminated blood sample.

Passive immunization with serum from convalescent piglets

Two experiments were carried out to determine whether, by the prior administration of serum from convalescent piglets, susceptible animals could be protected from infection with PM streptococci (strain C 22 N). The convalescent serum was a pool of samples taken from piglets S 1 and S 2 38 days after experimental infection with strain C 22 N (Fig. 2).

In the first experiment nine 12-day-old litter-mates were divided into three groups: piglets 1 to 3 each received subcutaneously 20 ml. of convalescent serum; piglets 4 to 6 received 20 ml. of pooled serum from three 38-day-old piglets, litter

mates which, for some unknown reason, had proved refractory to infection with strain C 22 N; piglets 7 to 9 received no serum.

In the second experiment, ten 12-day-old litter-mates were divided into two groups each of five piglets. Those of one group each received subcutaneously 20 ml. of the convalescent serum; those of the other received no serum.

On the day following the administration of serum all the piglets were sprayed with PM streptococci, strain C 22 N. The results are summarized in Fig. 3 from which it can be seen that none of the animals that had received convalescent serum developed a bacteraemia. Of the animals that did not receive convalescent serum, in the first experiment four out of six and in the second, five out of five developed a streptococcal bacteraemia. In most cases this occurred within 24 hr. of spraying.

DISCUSSION

Streptococcal infections are a common hazard of the neonatal period. In addition to causing the disease of pigs here described, streptococci are responsible for neonatal infections in babies, lambs and foals. In babies, group B streptococci cause meningitis and have also been isolated from the blood (Hood, Janney & Dameron, 1961). At Boston City Hospital they were the commonest single cause of neonatal sepsis during the period December 1961 to June 1963 (Eikhoff *et al.* 1964). In lambs, group C streptococci cause 'joint-ill' a neonatal septicaemia with involvement of the joints and, less commonly, the heart valves (Blakemore, Elliott & Hart Mercer, 1941).

In none of these conditions is there definite information concerning the source or primary focus of the infection, although in foals it is thought that the invading micro-organisms gain entry by way of the umbilicus (Gunning, 1947). An investigation of streptococcal 'joint-ill' in lambs produced no evidence of umbilical infection (Elliott, unpublished observation). In the present report on neonatal infection in pigs we give reasons for considering the throat of the piglet a likely portal of entry and the upper respiratory tract of the sow a possible source of the streptococci.

Bacteraemia is a finding common to the human, ovine and porcine varieties of streptococcal infection in the newborn. In experimental infections of the upper respiratory tract of piglets we were impressed by the speed and regularity with which the PM streptococci invaded the blood stream: positive blood cultures were usually obtained within 24 hr. of spraying the naso-pharynx. Of equal interest was the observation that the presence of bacteria in the blood stream did not, of itself, appear to incapacitate the piglets. In poorly developed animals, diarrhoea was sometimes an early sequel to infection but usually, unless the joints or brain were involved, the animals continued to thrive regardless of the large numbers of streptococci circulating in the blood. After 2-3 weeks, the circulating bacteria gradually diminished in number and eventually disappeared, presumably in response to a developing active immunity. As shown here, serum taken from convalescent animals 3-4 weeks after infection and administered to susceptible piglets afforded complete protection against subsequent infection with the PM streptococcus. The results of one experiment (Fig. 3, Expt. 2) suggested that infection of

the naso-pharynx by spray was more difficult to achieve in passively immunized than in normal piglets: attempts to recover PM streptococci from the throats of passively immunized piglets 3 days after spraying were unsuccessful, although by then all of their non-immune litter-mates infected at the same time had developed a bacteraemia. In the previous experiment no difficulty had been experienced in recovering the PM streptococci from the throats of passively immunized piglets 48 hr. after spraying. These results are inconclusive owing to the small number of animals involved but they suggest that passive immunity prevents not only invasion of the blood stream but also infection of the naso-pharynx by streptococci sprayed into the upper respiratory tract. Of the nature of this immunity we have at present no information but we assume it to be directed against the type-specific, capsular polysaccharide of the PM streptococcus (Elliott, 1966).

The implication of relatively non-pathogenic micro-organisms to the exclusion of more invasive bacteria in the examples of neonatal infection cited above merits consideration. Although sometimes associated with subacute endocarditis (Fry, 1938), the group B streptococci concerned in human neonatal infections are non-pathogenic for normal human adults, the strain of group C streptococci that causes 'joint-ill' in lambs is not pathogenic for adult sheep or for laboratory animals and the PM streptococci isolated from piglets resembles other members of group D in its lack of invasiveness for adult pigs, mice, guinea-pigs and rabbits (Field *et al.* 1954). Clearly, lack of immunity in the newborn animal is a factor in the causation of disease by these relatively harmless bacteria. Is it possible that such passive immunity as the young animal receives from its mother is directed, not against these relatively non-pathogenic micro-organisms, but against agents responsible for infectious diseases of adult life? If such were indeed the case, the newborn animal might be vulnerable to micro-organisms of borderline invasiveness but immune to common pathogens. This reasoning affords no explanation for our experience that all group D streptococcal infections of the newborn pig have been caused by cocci of a single serological type. A similar observation was made in 'joint-ill' of lambs caused by group C streptococci; one serological type, only, was implicated (Blakemore *et al.* 1941). In both porcine and ovine diseases our experience has been drawn almost exclusively from outbreaks in East Anglia, but in so far as the piglet infection is concerned, streptococci of identical serological type have been isolated in Holland (de Moor, 1963). The small number of types—one only, in our experience—incriminated in the disease together with the protective effect of convalescent serum against experimental infection suggests that active immunization of the sow with PM streptococci might be effective in preventing this form of neonatal infection in piglets.

SUMMARY

1. In streptococcal infection of piglets the causative agent (PM streptococcus) was isolated from the throats of a high proportion of infected animals and from their apparently healthy litter-mates.

2. The PM streptococcus was isolated from the noses of three out of forty-three normal sows. In one sow the streptococcus was also isolated from the throat.

3. Bacteraemia was induced in piglets up to the age of 17 days by inoculating into the nose and throat broth cultures of the PM streptococcus. Blood cultures were usually positive within 24 hr., and secondary involvement of the joints and meninges frequently occurred during the ensuing few days.

4. Serum taken from piglets 5 weeks after experimental infection and administered subcutaneously protected susceptible piglets against subsequent infection with PM streptococci.

5. The possibility of preventing streptococcal infection in piglets by active immunization of the sow is discussed.

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