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Experimental studies on the comparative infectivity and pathogenicity of *Streptococcus suis* type 2 I. Porcine and human isolates in pigs

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SUMMARY

Piglets between 1 and 40 days of age were inoculated with varying numbers and with different isolates of *Streptococcus suis* type 2 by the intranasal, intravenous and subarachnoid routes. Less than 100 organisms of an isolate cultured from apparently normal pigs caused subclinical infection in 1-day-old piglets after intranasal inoculation. This infection was naturally transmitted between litter mates. Intravenous inoculation of a similar isolate in 7-week-old pigs resulted in a sub-clinical bacteraemia in 3 of 8 piglets. One other piglet developed a bacteraemia 7 days after inoculation and concurrently developed signs of lameness and nervous dysfunction. Ten piglets were inoculated by the subarachnoid route with a porcine isolate and two with an isolate from a person with clinical disease. Only the latter two pigs developed the classical signs of nervous disease associated with infection by *S. suis* type 2. It is concluded that strains of *S. suis* type 2, of varying pathogenicity for both pigs and man, are endemic in New Zealand. It is suggested that the occurrence of disease is associated with both exposure to a pathogenic strain and other, as yet undetermined, secondary factors.

INTRODUCTION

Streptococcus suis type 2 can produce septicaemia, meningitis, polyarthritis and pneumonia in pigs [1]. The organism has been found in most pig rearing countries, however the losses from infection appear to vary greatly between herds and countries. Robertson and Blackmore [2] demonstrated that in infected Australian and New Zealand herds, the prevalence of infection was nearly 100% in pigs after weaning, and yet clinical disease was rarely reported. *Streptococcus suis* type 2 is normally carried within the palatine tonsils of apparently healthy pigs [3] and these subclinical carriers play an important role in the spread of infection throughout a piggery.

In the present study isolates of *S. suis* type 2 cultured from the tonsils of normal healthy carrier pigs and an isolate obtained from a human were used to investigate the infectivity and pathogenicity of the organism via intranasal, intravenous and subarachnoid routes.

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MATERIALS AND METHODS

Microorganisms

Three isolates of *S. suis* type 2 were used in these experiments. Two isolates (1 and 2) were cultured from the palatine tonsils of two apparently healthy bacon weight pigs. These pigs and the experimental ones originated from the same 60 sow herd. Isolate three was cultured from a human patient with a bacteraemia and signs of clinical disease [4]. All isolates were initially grown in Todd Hewitt broth (GIBCO) for 18 h and subsequently diluted in physiological saline to obtain the required number of organisms for inoculation.

It was assumed that the organisms subsequently isolated from the inoculated pigs were of the same strain as those that were initially inoculated.

Intranasal inoculation

Two sows, at a similar stage of gestation, were penned in a building that had previously not housed pigs. This building was thoroughly hosed out and fumigated with formaldehyde vapour prior to the introduction of the sows. There was no contact between the subsequent litters until weaning at 4.5 weeks of age, when the piglets were placed in the same pen.

When the piglets were 1 day old, two from one litter were intranasally inoculated with $5{\cdot}5 \times 10^9$ organisms of isolate 1 and two from the second litter were inoculated with approximately 100 organisms of this isolate by the same route. The organisms were infused into the right nostril in a 0·5 ml volume of Todd Hewitt broth diluted in physiological saline.

Swabs from both nasal chambers of the piglets were collected daily for the first 2 weeks of life. The swabs were plated onto 5% sheep blood agar, incubated for 18 h at 37 °C and an Indirect Fluorescent Antibody Test (IFAT) was performed on the primary growth as described by Robertson [5] and Robertson and Blackmore [6]. Blood samples (0.5 ml) were collected daily from the cephalic vein until the piglets were 8 weeks old. The blood samples were dispensed into 10 ml of Todd Hewitt broth. This suspension was incubated for 18 h at 37 °C and an IFAT performed on any bacterial growth. Samples were collected from all piglets prior to infecting the four piglets. Piglets that died were autopsied and samples taken for microbiological examination.

Swabs were also collected from the environment before and after the sows farrowed.

Intravenous inoculation

Eight similar sized weaned piglets (5 weeks old), were used in this experiment. Bilateral jugular catheters, made from medical grade silicone rubber tubing (Dow Corning Corporation, Michigan, USA), were inserted whilst the pigs were under a general anaesthesia using the technique of Pento [7]. After fixing the catheters in the jugulars, the catheters were directed subcutaneously so that they emerged between the shoulder blades. This allowed easy access for the collection of blood and inoculation of organisms and prevented pigs from interfering with their catheters. A sterile 15 gauge luer-lock needle, that had previously had its point ground flat, was inserted into the cannula to provide a tight secure fit. An injection

cap (Jelco Intermittent Injection Cap, Critikon, Florida) was placed on the needle to help maintain asepsis and to allow for easy access to the cannula and consequently blood. A stretch stocking net (Surgifix 7, FRA Production, Italy) placed over the pigs forequarter, was used to stabilize and protect the cannulae. Pigs were given a single intramuscular injection of 2 ml of a streptomycin-penicillin mixture (Glaxo) following the operation.

Pigs were housed in individual cages with a vacant cage between pigs to prevent interference of catheters by neighbouring pigs. Daily samples of blood were collected and the cannulae flushed with sterile heparinized physiological saline (25 units/ml) to assist in the maintenance of patency. Seven days after surgery, three pigs were inoculated with a single dose of $3{\cdot}5 \times 10^7$ organisms of isolate 2 and three pigs with $4{\cdot}5 \times 10^3$ organisms. Two other pigs were initially inoculated with 200 organisms and then reinoculated a week later with 2×10^4 organisms. The bacteria were administered in a one ml volume followed by two ml of sterile heparinized saline.

Blood samples were collected prior to inoculation; 1 min, 10 min and 1 h after inoculation; and then at daily intervals for up to 4 weeks. On the first day after inoculation, blood was collected from the cannulae that had not been used for the inoculation. To maintain the patency of the cannula they were flushed with two ml of heparinized saline after each blood collection. Rectal temperatures were taken daily at approximately the same time.

At each sampling, the first ml of blood was discarded to reduce the dilution effect of any heparinized saline remaining in the cannulae. Two, 1 ml aliquots of blood were subsequently collected and evenly dispersed onto 5% sheep blood agar plates. These plates were incubated for 18 h at 37 °C and the average number of colonies of *S. suis* type 2 determined. An extra 5 ml of blood was collected and dispensed into a tube containing 5 mg of sodium EDTA. The total number of white blood cells was determined with an automatic white cell counter (Cell-Dyn 900, Sequoia-Turner Corporation, California) and a differential white cell count was carried out on blood smears stained with a Wright Stain (Modified) (Sigma Diagnostics, St. Louis).

All pigs were killed, autopsied, examined for gross pathological changes and segments of tissue cultured and examined for histopathological changes, within 4 weeks of the initial inoculation.

Inoculation into the cerebro-spinal fluid

Fourteen, 5-week-old pigs from four litters were used in this experiment. Pigs were fasted for 18 h prior to infection but were allowed access to water. Under general anaesthesia, a 22 gauge, two and a half inch (6·3 cm) needle was inserted between the occipital condyles and the first vertebrae to a depth of approximately three cm. Penetration of the spinal canal was indicated by the appearance of CSF from the needle. Inoculation into the spinal canal was then carried out. Five pigs were inoculated with approximately $2{\cdot}4 \times 10^5$ organisms of isolate 2 and another five with approximately 8×10^2. Two pigs were inoculated with approximately 8×10^5 organisms of isolate 3. Two pigs were injected with 0·5 ml of a 1/400 dilution of Todd Hewitt broth. These pigs were used as controls and were handled in a similar manner to the infected pigs.

After inoculation, pigs were housed in individual pens with no direct contact between neighbouring pigs. Pigs were examined at least twice daily for evidence of clinical signs of infection. All pigs were killed, autopsied and examined for gross pathological changes within 3 weeks of inoculation. Segments of brain, spinal cord, liver, spleen and kidney were fixed in 10% formalin and examined for histopathological changes. Swabs of the meninges, brain, liver, kidney and spleen were cultured aerobically on 5% sheep blood agar.

RESULTS

Intranasal inoculation

Streptococcus suis type 2 was not detected in any nasal swabs collected from the piglets prior to infection. However, after experimental infection there was rapid spread of the organism throughout the litters. One day after inoculation of two piglets with 10^9 organisms, 78% (7/9) of the piglets were found to have at least one positive nasal swab whilst 64% (7/11) were infected in the litter inoculated with approximately 100 organisms. Two days after infection, all pigs in both litters had at least one positive swab for *S. suis* type 2.

Four of the nine piglets from the litter infected with the high dose, and 3 of 11 from the litter with the low dose, developed a bacteraemia of *S. suis* type 2. Two of these bacteraemias persisted for 2 days whilst the rest were detected for only 1 day. These bacteraemias were distributed over the 8-week experimental period.

Streptococcus suis type 2 was not cultured from two stillborn piglets, one mummified foetus and two overlain piglets that died when they were 1 day old. A 4-day-old piglet (litter mate to those infected with 10^9 organisms) which also died from overlaying had positive palatine tonsils. Nasal swabs collected from this piglet prior to death had also been positive.

Swabs collected from the environment both prior to the introduction of sows and before farrowing failed to detect the presence of *S. suis* type 2. However after parturition and experimental infection, *S. suis* type 2 was detected in 4 of the 72 (5·5%) swabs collected.

Intravenous inoculation

Table 1 displays the isolation of *S. suis* type 2 from the blood of pigs after intravenous inoculation. Initially there was a rapid reduction in the number of detectable organisms, with only 10·3% being detected 1 min after infection, 1·5% after 10 min and 0·7% after 1 h. Two of the three pigs which received the high dose (10^7 organisms), (nos. 6 and 5) remained continuously bacteraemic for 6 and 7 days respectively, whilst the other pig (no. 8) was still bacteraemic 1 day after inoculation when both cannulae became occluded and further blood samples were not possible. In pigs inoculated with 10^4 organisms, *S. suis* type 2 was detected for up to 1 h following inoculation, however no organisms were detected in the blood of pigs receiving only 200 organisms. One of the pigs (no. 7) injected with 10^4 organisms, subsequently redeveloped a bacteraemia, 7 days after the initial inoculation. This bacteraemia was present for 2 days before the pig was killed. On the day before the bacteraemia was detected, the pig showed front leg lameness, anorexia and depression. These signs persisted for 2 days and were accompanied

Table 1. *Numbers of* S. suis *type 2 isolated from each ml of blood after intravenous inoculation*

	Animal number							
	1*	2*	3	4	5	6	7	8
Number of *S. suis* injected...	200, 2×10^4	200, 2×10^4	10^4	10^4	10^7	10^7	10^4	10^7
	Days after operation							
1–6	0	0	0	0	0	0	0	0
7 Days and 1 min	0	0	5	3	3×10^3	2×10^3	3	10^3
7 Days and 10 min	0	0	1	0	$1{\cdot}6 \times 10^2$	90	1	80
7 Days and 60 min	0	0	0	1	55	40	0	40
8	0	0	0	0	$1{\cdot}6 \times 10^2$	2×10^3	0	2×10^2
9	0	0	0	0	8×10^4	16×10^4	0	—
10	0	0	0	0	$6{\cdot}6 \times 10^2$	4×10^2		—
11	0	0	0	0	4×10^2	50	0	—
12	0	0	0	0	$2{\cdot}4 \times 10^2$	80	0	—
13	0	0	0	0	1×10^2	30	0	—
14 Days and 1 min	7	2	0	0	20	0	2	—
14 Days and 10 min	4	2	.	.	.	.	.	.
14 Days and 60 min	1	0	.	.	.	.	.	.
15	0	0	0	0	0	0	$2{\cdot}4 \times 10^3$†‡	—
16	0	0‖	0	0	—	0	.	—
17	0	.	0	0	—†	0	.	—
18	0	.	0	0	.	0	.	—
19	0	.	0	0	.	0	.	—
20	0	.	0	0	.	0	.	—
21	0†	.	0	0†	.	0†	.	—
22	.	.	0	.	.	.	.	—
23	.	.	0	.	.	.	.	—
24	.	.	0	.	.	.	.	—
25	.	.	0	.	.	.	.	—
26	.	.	0†	.	.	.	.	—†

* pigs infected with two doses of *S. suis* type 2.
† Euthanasia.
‡ Clinical meningitis.
‖ Death.
[—] Blocked cannula no blood sample collected.
Pigs 1 and 2 were inoculated on day 7 and 14.
Pigs 3 to 8 were inoculated on day 7.

by incoordination, staggering and lateral recumbency with paddling and opisthotonus. The following day the pig was brighter, in sternal recumbency and was eating and drinking before being subsequently killed. On histopathological examination a meningitis and encephalitis characterized by vacuolation of the white matter of the brain, mild gliosis and infiltration of the cerebellum with neutrophils were present. *Streptococcus suis* type 2 was cultured from the meninges, liver and joints of this pig. Pig 4 also developed forelimb lameness and *S. suis* type 2 was subsequently cultured from the right shoulder joint and meninges. Although nervous signs had not been present in this pig, the meninges were congested and there was spongiosis of the white and grey matter of the brain.

After receiving the second inoculation of *S. suis* type 2, pigs nos. 1 and 2 showed signs of rapid respiration, dyspnoea, anorexia and a raised temperature of 41 °C.

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Table 2. *Clinical, microbiological and pathological findings after subarachnoid inoculation of 14 pigs*

Isolate and dose of *S. suis* type 2	Time of euthanasia after infection	Clinical signs	Tissue *S. suis* cultured from	Histopathological lesions
Porcine strain $2{\cdot}4 \times 10^5$	5	Normal	Brain, meninges	Occasional glial nodule with infiltration of MN cells Subacute interstitial nephritis
Porcine strain $2{\cdot}4 \times 10^5$	3	Slight incoordination	Meninges	Occasional glial nodule Congestion of meninges
Porcine strain $2{\cdot}4 \times 10^5$	19	Normal	Brain	Occasional MN infiltration into portal sinusoids
Porcine strain $2{\cdot}4 \times 10^5$	19	Normal	—	Occasional glial nodule with infiltration of MN cells Subacute interstitial nephritis
Porcine strain $2{\cdot}4 \times 10^5$	5	Normal	Meninges	Occasional glial nodule with infiltration of MN cells Subacute interstitial nephritis
Porcine strain 8×10^2	3	Initial excitability	CSF, meninges, spleen, blood	Mild infiltration of MN cells into subarachnoid space
Porcine strain 8×10^2	10	Normal	Brain	Mild infiltration of MN cells into subarachnoid space Occasional glial nodule Subacute interstitial nephritis
Porcine strain 8×10^2	10	Initial excitability	Brain, meninges	Occasional glial nodule with infiltration of MN cells Subacute interstitial nephritis

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Table 2. (*cont.*)

Isolate and dose of *S. suis* type 2	Time of euthanasia after infection	Clinical signs	Tissue *S. suis* cultured from	Histopathological lesions
Porcine strain 8×10^2	5	Normal	Brain, meninges, kidney, blood	Occasional glial nodule with infiltration of MN cells Subacute interstitial nephritis
Porcine strain 8×10^2	19	Initial excitability	—	Subacute interstitial nephritis
Control 1/400 broth	19	Initial excitability	—	Subacute interstitial nephritis
Control 1/400 broth	5	Normal	—	Occasional glial nodule Subacute interstitial nephritis
Human strain 8×10^5	1	Initially normal 8 hr depression 12 hr lateral recumbency 24 hr opisthotonus paddling, tetanic spasms	Brain, CSF, meninges	Lepto/pachymeningeal infiltration by NP and MN cells. Laminar necrosis of superficial cerebral cortex Inflammation of cerebellum*
Human strain 8×10^5	5	Initially normal 8 hr incoordination 24 hr splayed legs, tremor incoordination 48–72 hr head tremor, slight improvement	Meninges, CSF	Lepto/pachymeningeal infiltration by NP and MN cells. Severe destruction of molecular layer of cerebellum with vacuole formation*

Pig no. 2 subsequently died whilst pig no. 1 was killed 7 days after the second inoculation. There was a purulent bronchopneumonia with an acute necrotizing bronchitis in these pigs. Necrotic tissue and a jugular phlebitis was evident in sections of the cannula sites. *Actinomyces pyogenes* was cultured from the pneumonic areas of the lungs.

Three pigs (nos. 3, 5, 8) did not show obvious clinical signs, had no significant histopathological changes and *S. suis* type 2 was not isolated from any of the organs cultured.

There was no obvious correlation between the body temperature (range 38·9–41·1 °C) and the presence of clinical disease in these pigs. There was also no apparent pattern in the total white cell count when compared with either body

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temperature or the presence of clinical signs. Only two samples were outside the 'normal' range of $10–30 \times 10^9$ cells per ml of blood [8].

Inoculation into the cerebro-spinal fluid

Table 2 summarizes the clinical signs, microbiological results and pathological findings for the 14 pigs used in this experiment. Although several pigs (including one control pig) showed signs of excitability, 2–3 h after inoculation, only the two pigs that received the human strain developed severe neurological signs. These latter pigs showed signs of opisthotonus, paddling and tetanic spasms within 24 h of inoculation. One other pig, injected with 10^5 organisms, showed signs of slight hind leg incoordination and depression 2 days following inoculation. *Streptococcus suis* type 2 was cultured from the brain or meninges of all pigs other than the two control pigs and one from each of the groups receiving the high and low dose.

Only the two pigs with severe nervous signs showed gross pathological changes of the brain with opaque, oedematous meninges. On histopathological examination, these pigs also had acute suppurative meningo-encephalitis. There was extensive leptomeningeal and pachymeningeal infiltration by neutrophils and some mononuclear cells, with severe destruction of the molecular layer and Purkinje layer in the cerebellum. Most other infected pigs were found to have occasional glial nodule formation and infiltration of mononuclear cells into the brain. Some glial nodules were also found in one of the control pigs.

DISCUSSION

The results of the present study are similar to those reported by others [9] in illustrating the difficulty in consistently reproducing clinical disease in pigs after experimental infection with *S. suis* type 2. The use of hysterectomy-derived, colostrum-deprived pigs has led to more repeatable results, however, these pigs are costly to produce and maintain. Most workers have found that subarachnoid and intravenous inoculation of *S. suis* type 2 produces clinical disease more frequently than does intranasal, subcutaneous or intradermal inoculations. Similar findings were found in the present experiments where no clinical disease was evident in 22 piglets following intranasal infection, whilst 1 of 8 and 2 of 16 weaner pigs developed clinical signs following intravenous and subarachnoid inoculation respectively. Only the isolate cultured from a human appeared capable of reproducing the 'classical' clinical disease after subarachnoid inoculation. Pedersen and co-workers [10] also demonstrated that a human isolate of *S. suis* type 2 was pathogenic for pigs, when administered by the intradermal and subcutaneous routes. As in the present study, porcine isolates of *S. suis* type 2 appeared non-pathogenic, even when administered in comparable numbers to the human isolate, it would indicate that the pathogenicity of isolates varies. The human strain was presumed to have originated from pigs as the patient would frequently cut up pig heads. However, there is no evidence to suggest that pigs in the region from where this person originated have a higher morbidity rate of nervous or arthritic disease than pigs from other regions of New Zealand. This may indicate that certain pathogenic strains of *S. suis* type 2 may not be carried by 100% of pigs unlike the non-pathogenic strains, or that if pathogenic strains

are present in all pigs, secondary factors as proposed by Clausen [11] are needed for the development of clinical disease.

There was no apparent dose response for the development of clinical disease after inoculation. In fact, the pigs that did show clinical signs received the smaller number of organisms. Similarly after intranasal or intravenous inoculation there was no apparent pattern between the presence of a bacteraemia and the time of initial inoculation. Similar results were reported by Clifton-Hadley and Alexander [9] who found that one pig subsequently developed a bacteraemia 18 days after intranasal inoculation. However, in the present experiment, the sudden onset of clinical signs in the pigs inoculated with a human strain and the lack of clinical disease inoculated with porcine strains would support the belief that some strains of *S. suis* type 2 are more virulent than others. The high proportion of bacteraemias of *S. suis* type 2 in young pigs (35% in the litters infected via the intranasal route) may account for the high prevalence of disease reported in humans in Hong Kong [12]. As these people have a preference for eating suckling and weaner pigs, those involved in the slaughtering and handling of the carcasses of such young pigs would have a greater risk of contracting an infection with *S. suis* type 2.

In the present investigation, the total and differential white cell counts did not appear to have any correlation with either the development of clinical signs or the presence of *S. suis* type 2 in the blood. This may indicate that humoral rather than cellular components initially played an important role in the inactivation of viable *S. suis* type 2.

Robertson [13] found that 100% of pigs, when raised in a commercial piggery, were naturally infected with *S. suis* type 2 by the age of 26 days. However in the present study, after intranasal inoculation of two piglets, there was a faster spread so that all piglets were infected by the age of 3 days. It would therefore appear that the minimum infective dose for *S. suis* type 2, when administered by the intranasal route, is less than 100 organisms. This rapid spread of infection is probably due to the frequent nasal contact that occurs both between piglets and between piglets and sows [14].

As *S. suis* type 2 was detected in environmental swabs taken from the pens housing both litters, it may indicate that environmental contamination is associated with the population density of pigs rather than contamination resulting from the inoculation. Due to the susceptibility of the organism to disinfectants [15], destocking of a piggery should enable the total removal of the organism from the environment. The problem of keeping a piggery free from infection would then depend on the reintroduction of stock free from infection. It has generally been assumed that restocking with Specific Pathogen Free (SPF) stock will ensure initial freedom from infection. However, Robertson and Blackmore [6] demonstrated the presence of infection in four of seven SPF herds in Australia which suggests that freedom from infection may be difficult to maintain.

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