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Experimental studies on the comparative infectivity and pathogenicity of *Streptococcus suis* type 2. II. Porcine and human isolates in laboratory animals

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SUMMARY

Mice, rats, guinea-pigs and rabbits were inoculated with isolates of *Streptococcus suis* type 2. An isolate cultured from the tonsils of a healthy pig, produced disease in rabbits after intravenous inoculation but not in mice, rats or guinea-pigs. An isolate of *S. suis* type 2, that was pathogenic for pigs and had been cultured from a human patient with clinical disease, produced signs of neurological disease in mice, rats and rabbits following intravenous inoculation. There was an apparent dose response in mice with 31% of mice receiving more than 10^6 organisms developing clinical disease, whilst mice receiving less than this did not develop disease. There were no detectable histopathological lesions in the brains or meninges of mice with nervous signs. It is proposed that the disease in mice may mimic that reported in humans and that mice may be a useful indicator species for determining the virulence of isolates cultured from pigs.

INTRODUCTION

Streptococcus suis type 2 has mainly been associated with disease of pigs producing a meningitic, septicaemic and arthritic condition, however the organism can occasionally infect other species. *Streptococcus suis* type 2 can produce disease in humans following infection via the percutaneous route, although disease occurs rarely [1]. The organism has also been isolated from a raccoon dog whose diet included uncooked pig meat originating from pigs which had died in piggeries with endemic streptococcal meningitis [2]. Hommez and colleagues [3] also isolated *S. suis* from cattle, sheep and goats, however only one isolate from a bovine was identified as being *S. suis* type 2.

Although numerous experiments have demonstrated the virulence of this organism for pigs, there have been few trials investigating the virulence for other species. Williams and co-workers [4] demonstrated that mice could develop clinical signs of disease after intravenous inoculation with *S. suis* type 2. They showed that the infection could be transmitted to in-contact mice and that the disease appeared to mimic that seen in pigs. They proposed that mice may play a role in the epidemiology of the infection within a piggery.

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This paper reports on experiments carried out to determine the infectivity and pathogenicity of various isolates of *S. suis* type 2 for laboratory animals.

MATERIALS AND METHODS

Experimental animals

Laboratory mice (N.O.S.), rats (Sprague–Dawley), guinea-pigs and rabbits (New Zealand white) were used in this experiment. The mice were 6–12 weeks old, the rats and guinea pigs 10 weeks old and the rabbits 12–26 weeks old. Female guinea-pigs and rats were used, however the mice and rabbits were of both sexes. All animals appeared healthy prior to experimentation. The mice, rats and guinea-pigs all originated from the conventional non-barrier maintained Small Animal Breeding Colony, Massey University, whilst the rabbits were obtained from two local breeders with similar genetic stock.

The laboratory animals were housed in a heated, isolated room and examined twice daily for evidence of clinical signs of disease. They were autopsied within 3 weeks of inoculation, except for the offspring of some of the infected mice born during the experiment, which were killed between the age of 1 day and 1 month. Mice, rats and guinea-pigs were killed by gassing in a carbon dioxide chamber, whilst rabbits were destroyed with an intravenous injection of sodium pentobarbitone. The liver, kidney, spleen, heart blood, brain and the major joints (hip, shoulder, stifle and elbow) were cultured from each animal autopsied and samples of these tissues were collected for histopathological examination.

Infection procedure

Mice were infected by either the intraperitoneal route or by an intravenous injection into the coccygeal vein. Rats and guinea-pigs were infected by intracardiac injection. The rabbits were infected by an intravenous injection into the marginal ear vein. Prior to inoculation, xylene was rubbed on the ear to produce vasodilation.

Microorganisms

A porcine strain of *S. suis* type 2 that had been cultured from the palatine tonsils of an apparently healthy pig and a human strain of *S. suis* type 2 isolated from a patient with bacteraemia [5] were used in these experiments. The isolates were grown overnight in Todd Hewitt broth (GIBCO), and either inoculated directly into the laboratory animals or initially diluted in phosphate buffered saline.

Porcine strains of S. suis

Eleven mice were inoculated intravenously with 0·1 ml containing $1{\cdot}2 \times 10^7$ organisms of the porcine strain of *S. suis* type 2. Rats and guinea-pigs were inoculated with 6×10^7 organisms in a volume of 0·5 ml and four rabbits with 6×10^7 organisms of this isolate.

Human strain of S. suis *type 2*

Nine mice were inoculated via the intravenous route with 23×10^7 organisms in 0·5 ml, 14 mice with $4{\cdot}6 \times 10^7$ and 10 each with $7{\cdot}2 \times 10^6$, $1{\cdot}8 \times 10^4$ and 45 organisms

respectively in a volume of 0·1 ml. The intraperitoneal route was also used with eight mice receiving 23×10^7 organisms (0·5 ml), 10 with $4{\cdot}6 \times 10^7$, seven with $7{\cdot}2 \times 10^6$, 14 with $1{\cdot}8 \times 10^4$ and 10 with 45 organisms in 0·1 ml. Four rats and three rabbits were also infected with 23×10^7 organisms of the human strain in a volume of 0·5 ml.

RESULTS

Mice, rats and guinea-pigs

In Table 1 the morbidity, mortality and cultural findings are reported for the laboratory animals inoculated with *S. suis* type 2. No clinical signs were observed in the mice, rats and guinea-pigs that received the porcine strain of *S. suis* type 2. However, two rats died within 2 min of being inoculated. One had a haemopericardium and the other was believed to have died from anaphylactic shock. *Streptococcus suis* type 2 was cultured from the heart blood and liver of both these rats. The bacterium was not cultured from any of the other mice, guinea-pigs and rats inoculated with this porcine strain.

Clinical disease and mortalities occurred in the mice inoculated with the human isolate. Signs of twitching, head bobbing and violent head movements were recorded from some (31%) of the mice infected with more than 10^6 organisms by the intravenous or intraperitoneal routes. *Streptococcus suis* type 2 was cultured from the blood and/or brain of 15 of the 18 (83%) mice with clinical disease and 12 of the 84 (14%) apparently normal mice. Eight mice (two inoculated with the porcine isolate of *S. suis* type 2 and six with the human isolate) which were pregnant at the time of inoculation had apparently normal litters. These pregnant mice included two which had developed mild neurological signs after intraperitoneal inoculation with the human isolate. The offspring of these mice appeared healthy and developed in a normal manner. *Streptococcus suis* type 2 was not cultured from these offspring. There was no significant difference between the number of mice developing clinical disease in the intraperitoneal inoculated group and the intravenous inoculated group ($P > 0{\cdot}4$). No histopathological abnormalities were detected in the brains or meninges of the mice with clinical signs.

One of the rats infected with the human isolate died within 1 h of inoculation. On autopsy it was found that this rat also had a haemopericardium. However, another rat developed signs of lethargy and depression 2 days after inoculation, and *S. suis* type 2 was cultured from all tissues and joints examined.

Rabbits

Two of the four rabbits inoculated with the porcine isolate were depressed, anorectic and not drinking 2 days after inoculation. These rabbits subsequently died 3 and 4 days after inoculation. The first rabbit to die had congested lymph nodes, parenchymatous organs and subcutaneous tissues, an excess of peritoneal fluid and petechial haemorrhages on the epicardium and myocardium. The stifle joints were hyperaemic with excessive joint fluid, however the other joints appeared normal. The second rabbit to die had congested hyperaemic lymph nodes with excessive pericardial and pleural fluid. One hip joint showed signs of congestion and hyperaemia, whilst the other joints appeared normal. *Streptococcus*

Table 1. *A summary of the findings in laboratory animals inoculated with* Streptococcus suis *type 2*

Species	Number of animals	Number of organisms inoculated	Route of administration	Number with clinical disease (%)	Number of deaths (%)	Number with positive cultures (%)
Porcine *S. suis* type 2						
Rabbits	4	6×10^7	Intravenous	4 (100)	2 (50)	3 (75)
Rats	4	6×10^7	Intracardiac	0 (0)	2* (50)	2* (50)
Guinea pigs	4	6×10^7	Intracardiac	0 (0)	0 (0)	0 (0)
Mice	11	$1{\cdot}2 \times 10^7$	Intravenous	0 (0)	0 (0)	0 (0)
Human *S. suis* type 2						
Rabbits	4	23×10^7	Intravenous	4 (100)	3 (75)	4 (100)
Rats	4	23×10^7	Intracardiac	1 (25)	2* (50)	2* (50)
Mice	9	23×10^7	Intravenous	4 (44)	1 (11)	8 (89)
Mice	8	23×10^7	Intraperitoneal	6 (75)	1 (12)	4 (50)
Mice	14	$4{\cdot}6 \times 10^7$	Intravenous	3 (21)	0 (0)	7 (50)
Mice	10	$4{\cdot}6 \times 10^7$	Intraperitoneal	3 (30)	0 (0)	2 (20)
Mice	10	$7{\cdot}2 \times 10^6$	Intravenous	1 (10)	0 (0)	4 (40)
Mice	7	$7{\cdot}2 \times 10^6$	Intraperitoneal	1 (10)	0 (0)	2 (20)
Mice	10	$1{\cdot}8 \times 10^4$	Intravenous	0 (0)	0 (0)	2 (20)
Mice	14	$1{\cdot}8 \times 10^4$	Intraperitoneal	0 (0)	0 (0)	0 (0)
Mice	10	45	Intravenous	0 (0)	0 (0)	0 (0)
Mice	10	45	Intravenous	0 (0)	0 (0)	0 (0)

* Includes rats dying from injection technique.

suis type 2 was isolated from the joints and all tissues examined from these two rabbits. Five days after inoculation, the surviving two rabbits were lame, depressed and not eating or drinking. *Streptococcus suis* type 2 was not cultured from samples of blood collected 4, 5 and 6 days after inoculation. One rabbit was subsequently killed 7 days after experimental infection. On autopsy, a purulent arthritis was found in one stifle joint and *S. suis* type 2 was cultured from it. All other tissues and joints were sterile. The remaining rabbit was lame for another 2 weeks, however its appetite returned 2 days after the onset of clinical signs. *Streptococcus suis* type 2 was not cultured from any tissues or joints.

All four rabbits inoculated with a human isolate of *S. suis* type 2 developed signs of clinical disease. After intravenous inoculation of four rabbits with the human isolate of *S. suis* type 2, all developed clinical signs. Three of these rabbits died at 1, 2 and 4 days after inoculation respectively. All rabbits showed extensive subcutaneous haemorrhages and congestion of the parenchymatous organs and *S. suis* type 2 was cultured from all organs and joints sampled. Four days after inoculation, the surviving rabbit appeared to recover and continued to eat and drink normally until autopsied. No gross pathological changes could be detected in this rabbit and *S. suis* type 2 was not cultured from any tissues or joints.

DISCUSSION

Although the pathogenicity of *S. suis* type 2 has been widely reported for pigs, there are only a few studies on the pathogenicity of this organism for laboratory

animals. In the present study it was found that a porcine isolate was non-pathogenic for mice, rats and guinea-pigs when administered via the intravenous route. However, a human isolate was pathogenic for mice when administered via the intravenous or intraperitoneal route and for rats when administered by the intracardiac route. In mice inoculated with the human isolate, there appeared to be a dose response for the development of clinical signs with only mice receiving more than 10^6 organisms showing signs of disease. Similar findings were reported by Williams and co-workers [4] who showed that at least 10^8 organisms had to be inoculated intravenously for meningitis to develop in mice. These findings differ from those reported in pigs where there was no apparent dose response for the development of clinical disease following intranasal, intravenous or intracerebral inoculation with the same isolates [6]. Both porcine and human isolates of *S. suis* type 2 were pathogenic for rabbits when administered by the intravenous route.

Inoculation with *S. suis* type 2 and the subsequent development of clinical signs in pregnant mice, had no effect on the survival of their subsequent litter. There was no detectable spread across the placenta, via the milk or from direct contact. These findings differ from those of Williams and co-workers [4] who demonstrated spread from infected to non-infected in-contact mice.

No inflammatory response was detected in histological sections taken from the brains of mice with nervous signs. Again this differs from the findings of Williams and colleagues [4] who reported a generalized diffuse meningitis similar to that recorded in clinically affected pigs. The findings in the present study may indicate that the organism produces a specific lesion in one region of the brain, rather than a generalized pathological change. The sections taken in the present study may not have included these specific areas and hence the brain would appear normal. In humans, it has been proposed that *S. suis* type 2 produces a neurotoxin that acts specifically against the eighth cranial nerve [7]. If this is the case for mice, a generalized inflammatory response would not be expected to be found as was the case in the present study.

In the present study, it was shown that *S. suis* type 2 could be cultured from the blood and/or brain of 14% of clinically normal mice following intravenous or intraperitoneal inoculation with the human pathogenic isolate. These findings are similar to those reported by Robertson and Blackmore [6] who demonstrated that *S. suis* type 2 could be cultured from the blood or brain of clinical normal pigs. However as *S. suis* type 2 was not cultured from mice inoculated with non-pathogenic porcine isolates, it would appear that these isolates were not capable of lodging and multiplying within the mouse.

In a previous paper [6], it was shown that the human isolate was capable of producing disease in pigs after intracerebral inoculation, whilst the porcine isolate could not. In the present study, the variable responses found after artificial infection of laboratory animals would indicate some differences in the pathogenicity of isolates. As rabbits appeared to be susceptible to infection with both pathogenic and non-pathogenic (for pigs) isolates of *S. suis* type 2, they would not be a good indicator for determining the pathogenicity of isolates for pigs. However as disease was only produced in mice after inoculation with isolates pathogenic for pigs, it is probable that mice are a better indicator species for determining the pathogenicity of isolates for pigs.

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