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Occupational exposure to *Streptococcus suis* type 2

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SUMMARY

Antibody titres to *Streptococcus suis* type 2 were measured with an enzyme linked immunosorbent assay (ELISA) in four occupational groups in New Zealand. No veterinary students, 9% of dairy farmers, 10% of meat inspectors and 21% of pig farmers were seropositive to *S. suis* type 2. The development of antibody to *S. suis* type 2 was associated with occupational contact with pigs or their meat products. Subclinical infection with *S. suis* type 2 appears to occur in humans and the antibody produced is of only short duration. The annual incidence of subclinical infection and seroconversion in pig farmers may approach 28%. Thus *S. suis* type 2 may be one of the most infectious potentially zoonotic agents present in New Zealand, although very rarely resulting in clinical disease.

INTRODUCTION

Human infection by group R streptococci (*Streptococcus suis* type 2) was first reported in 1968, when three cases of meningitis with concurrent septicaemia were diagnosed in Denmark (1). Subsequently cases have also been reported from Holland, France, England, Wales, Hong Kong, Canada and New Zealand (2–7). Although *S. suis* type 2 is endemic in most pig-rearing countries including New Zealand (8), the disease in humans has not been reported from many countries with fewer than 100 cases having been reported in the world literature (9).

Infection of humans with *S. suis* type 2 results in a meningitic/septicaemic condition similar to that in pigs. The meningitis is usually accompanied by permanent vestibular and auditory dysfunction (10). Early loss of hearing is a prominent feature of the disease and is believed to be caused by a specific ototoxin (6). Chattopadhyay (11) proposed that *S. suis* type 2 had a special affinity for the meninges and especially the cochlear division of the eighth cranial nerve. Other complications of infection of humans with *S. suis* type 2 have included arthritis (12,13), uveitis and endophthalmitis (14, 15). Although the major clinical signs are meningitis and septicaemia, a case of septicaemia without meningitis has been recorded in New Zealand (7). Although only a few affected people have died (1,10), the permanent hearing loss and problems of balance are serious sequelae of infection with *S. suis* type 2.

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Historically, streptococcal meningitis in adults has been considered uncommon (16). However, Chau *et al.* (6) recognized that *S. suis* type 2 was the most frequent cause of bacterial meningitis in adults in Hong Kong. The majority of reported cases of infection with *S. suis* type 2 in humans have involved adults who kept or handled pigs or their meat products. Injuries, such as minor cuts and abrasions, have frequently been recorded as having occurred 2–3 days prior to the onset of clinical signs (6, 10).

In the present study the enzyme linked immunosorbent assay (ELISA) was used to investigate the prevalence of antibodies to *S. suis* type 2 in sera from humans of different occupational groups. This test was designed to compare the antibody levels between groups of humans and not to specifically quantify the level of antibody of one person. The test measured the total amount of immunoglobulin present (IgA, IgM, and IgG) and did not differentiate between antibody of different classes.

MATERIALS AND METHODS

ELISA test

A formalin inactivated preparation of *S. suis* type 2, similar to that used for the production of grouping antisera for streptococci (17) was used as the antigen. Antigen was diluted 1:32 with ammonium acetate/carbonate buffer prior to deposition in the wells of polystyrene microplates (NUNC Immuno Plate 1, Cat. No. 23454, Intermed, Denmark). A conjugate (antihuman IgA, IgG and IgM immunoglobulin peroxidase labelled conjugate, Cappel Laboratories Inc., Cochranville, PA, USA) dilution of 1:2000 was used with four doubling dilutions of sera from 1:50 to 1:400. These dilutions produced absorbance values within the range detected by the ELISA reader, reduced non-specific reactions and allowed economic use of reagents. Using these dilutions 24 sera could be tested per plate. On each day of testing a positive control sample of human serum was also included as one of the sera to be tested. This was done to check on the precision of the test.

The techniques used for the ELISA was similar to that described by Voller *et al.* (18). The reaction was stopped with a 1 M solution of sulphuric acid and the absorbance measured using a micro-ELISA autor reader (SLT 210, SLT Labinstruments Ges. m.b.h. Grodig). This reader passed two beams of light through each well; the first was at the peak absorbance wavelength of the substrate reaction product (486 nm) and the second at a wavelength (620 nm) at which the substrate product showed no absorbance. The difference between the absorbance for the substrate product and the non-specific background absorbance was automatically calculated to provide an accurate measure of the absorbance of the reaction product.

Determination of antibody titre

The antibody titre of each serum sample was calculated by the following procedure. Using linear regression, a straight line was calculated for each sample that followed the relationship of the log of the inverse dilution of the sample with the absorbance reading at that diluton. This line was extrapolated to intersect with an absorbance reading at that dilution. This line was extrapolated to

intersect with an absorbance reading of 0·1. The antibody titre at this point was then calculated by taking the inverse of the anti-log of the extrapolated value. The average titre of a number of sera was determined by taking the geometric mean of these calculated titres.

The cut-off point between a positive and negative titre was determined by studying the pattern of titres in sera collected from people with no exposure to pigs and therefore presumed to have little or no contact with *S. suis* type 2. As all sera of these people had titres below 400, this antibody level was used as the cut-off point for further studies.

Sera

The sera tested in this trial were obtained from frozen stocks collected by Blackmore & Schollum for studies on occupational exposure to leptospiral infection in New Zealand (19–21). Sera from 70 pig farmers, 96 dairy farmers, 107 meat inspectors and 16 veterinary students were examined. The sex, age and duration of occupational contact with pigs were available for all groups. Additional information was available for pig farmers on the type and size of piggery, whether or not pigs were killed at home and if pigs were introduced from other farms. Information was also available from dairy farmers on whether or not pigs were kept on the farm and if they were killed for home consumption. Information on meat inspectors included whether or not they inspected pigs and if they had contact with domestic or feral pigs outside their official occupation.

RESULTS

Figure 1 is a histogram of the percentage of sera with different titres for each of the four occupational groups. Most titres were below the 400 level selected as the cut-off value. Five meat inspectors and one dairy farmer had titres greater than 1000 whilst no pig farmers or veterinary students had titres as high as this. The meat inspectors who had these high titres all had occupational contact with pigs or their products and the dairy farmer kept pigs on his farm.

Table 1 shows the overall seroprevalence to *S. suis* type 2 in the four groups studied and Table 2, the seroprevalence in sub-occupational groups dependent on their degree of contact with pigs. Although 21·4% of pig farmers were seropositive, no other people living on the farm without pig contact had titres of 400 or greater. Similarly, although 10·4% of meat inspectors were seropositive, 15% of those with regular pig contact were positive compared to only 4% of those without such regular occupational exposure. Very similar results were obtained for dairy farmers with an overall prevalence rate of 9·3%, with a prevalence of 14% in those who kept pigs and only 4% in those who did not. No veterinary students had titres of 400 or greater.

There was a significant association between direct contact with pigs and the presence of a titre of 400 or greater for residents on pig farms and for meat inspectors ($P < 0·05$). The killing of pigs by pig farmers did not appear to create an extra risk. Although a greater number of dairy farmers who kept pigs had titres to *S. suis* than those who did not, the difference was not significant at the 0·05 level. However, the only two seropositive dairy farmers who did not keep pigs had

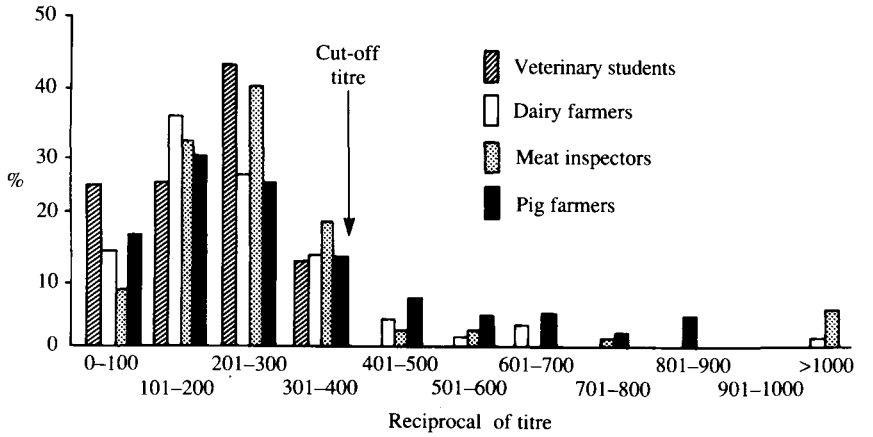

Fig. 1. Distribution of ELISA titres to *S. suis* type 2 in different occupational groups.

Table 1. *Prevalence of titres (≥ 400) to* S. suis *type 2 in different occupational groups*

Occupation	Number positive	Number negative	Total number tested
Pig farmers	15 (21·4%)	55 (78·6%)	70
Meat inspectors	11 (10·3%)	96 (89·7%)	107
Dairy farmers	9 0·3%)	87 (90·7%)	96
Veterinary students	0 (0%)	16 (100%)	16

Table 2. *The relationship between potential risk factors and titres (≥ 400) to* S. suis *type 2*

Variable	Number positive (%)	Number negative	Significance
Residents on pig farms with pig contact	15 (21·4)	42	$P < 0·05$
Residents on pig farms without pig contact	0	13	
Pig farmers killing pigs	4 (27)	11	NS ($P > 0·2$)
Pig farmers not killing pigs	8 (15)	47	
Meat inspectors with pig contact	9 (15)	52	$P > 0·05$)
Meat inspectors without pig contact	2 (4)	44	
Dairy farmers with pigs	7 (14)	43	$P > 0·01$
Dairy farmers without pigs	2* (4)	44	

* Low titres of 406 and 417.

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Table 3. *Association between prevalence of titres* ($\geqslant$ *400*) *to* S. suis *type 2 and duration of occupation*

Variable	Number examined	Number positive	Percentage positive
Period of pig farming (years)			
0–5	31	6	19
6–10	14	3	21
> 11	25	6	24
Period of meat inspection (years)			
0–5	57	6	10
6–10	30	2	7
> 11	20	3	15

titres only just above the cut off point of 400 (406 and 417). There were no significant correlations between the presence of a titre of 400 or greater in pig farmers who kept more or less than 400 pigs, who purchased or did not purchase weaner pigs and farmed pigs by either intensive or extensive systems of husbandry. In dairy farmers and meat inspectors there was no correlation between the presence of a titre and whether or not they had contact with feral pigs through pig hunting.

Table 3 shows the number of pig farmers and meat inspectors with positive titres according to the duration of their occupation. There were no significant differences or apparent trends between the different groups within each occupation. Analysis of age specific seroprevalence rates of all occupational groups in 10-year intervals showed no significant differences between groups from below 20 to above 60 years of age.

DISCUSSION

A titre of 400 was used as a cut-off for distinguishing between positive and negative sera. This titre was slightly greater than the highest value recorded from the group without exposure to pigs (308). This cut-off point appeared to be sufficiently specific as people who lived on pig farms and yet had no contact with the pigs also had titres lower than this. The difference between the titres for the four occupational groups appears to be related to the different degree of contact with pigs and recent exposure to *S. suis* type 2. This is supported by the finding that pig farmers had a higher prevalence of titres than any other occupational group. Although the proportion of seropositive dairy farmers who owned pigs was greater than those who did not, the difference was not significant at the 0·05 level. However, the two dairy farmers who had titres and did not keep pigs, had low titres of only 406 and 417, values that were just above the cut-off point. This may suggest that the 'cut-off' value was too high. However reducing this value, although increasing the sensitivity of the test, would have reduced the specificity of the test. It is believed that these two cases could have been false positives and if this assumption is correct, there would be a significant difference ($P < 0{\cdot}05$) between dairy farmers who kept pigs and those who did not.

Most cases of clinical disease have been reported in meat workers (11), however

in this study pig farmers had a greater proportion of positive titres than did meat inspectors who worked in plants processing pigs who coincidentally had some of the highest titres to *S. suis* type 2 recorded in this study. As meat inspectors routinely incise lymph nodes including those draining the tonsils, they would occasionally have direct contact with *S. suis* type 2. Breton *et al.* (22) found that meat workers involved in the evisceration of the carcasses, including the removal of the larynx and lungs, had a significantly higher risk of exposure to *S. suis* type 2 than did other abattoir workers. In the present investigation, there was a strong correlation between positive titres and contact with pigs. This would support the hypothesis that when infection of humans occurs, it originates from pigs. The apparent lack of any other identified maintenance host for *S. suis* type 2 would also support this belief.

The effect of purchasing weaner stock, the type of piggery and the size of the pig herd had no effect on the prevalence of titres in pig farmers. These results were expected as previous studies (8, 23) have shown that virtually all pigs are infected before 6 weeks of age and thus the potential for exposure by the farmer to *S. suis* is present from all pigs over 4–6 weeks of age. However, if a pig herd is very small, risks of exposure would be less and the probability of infection would be reduced. This could account for the low prevalence of titres recorded in dairy farmers and meat inspectors whose only contact with pigs was the hunting of feral pigs.

The classical zoonosis, occupationally acquired from pigs in New Zealand, is leptospirosis association with serovars *pomona* and *tarassovi* (20, 21). With this disease, there is a definite correlation between duration of occupation and seroprevalance in workers. For example the seroprevalence in meat inspectors working for 2 or less years was 4·7% compared with 14·4% in those who had been in the same occupation for 5–10 years (19). Similarly with pig farmers the seroprevalence in those who had farmed pigs for less than 10 years was 29% compared to 41% in those farming for more than 10 years (21). This increasing prevalence of titres of leptospiral antibody in groups working for a longer duration was thought to be associated with the long duration of convalescent titres of more than 15 years and not by a series of reinfections (24). In the present study no correlation between duration of occupation and seroprevalence of titres to *S. suis* was demonstrated. This indicates that the duration of titres to *S. suis* must be much shorter than those to *Leptospira interrogans*. This is compatible with the short duration of titres to group A streptococci in humans which is considered to be approximately 9 months (25). Thus the high seroprevalence rate recorded in pig farmers of 21% irrespective of duration of occupation, suggests that subclinical infections are common, immunity is of a short duration, and reinfection frequently occurs.

On the concept that *S. suis* infection of pig and man is stable in an ecological sense, the prevalence (P) of seropositive individuals will equal the product of incidence (I) of seroconversion and duration (D) of a titre (26). Thus, if it is assumed that the durations of titres to *S. suis* are similar to group A streptococci, the duration of titres will be 9 months or 0·75 of a year (25). Based on the formula $P = I \times D$, the annual incidence of seroconversion would be approximately 28% ($21 \div 0{\cdot}75 = 28$). Subclinical infections could lead to the development of antibodies to *S. suis* type 2 similar to that recorded for subclinical throat infections of

humans by group A streptococci (27). Based on similar calculations, this incidence rate of 28% compares with an annual seroconversion rate for dairy farmers for leptospiral infection of 3% (26).

When the medical history of people with positive titres was investigated there were no consistent findings of previous illness, except for that associated with leptospirosis. However, Ayanwale (28) reported symptoms of mild pyrexia in patients believed to be affected with *S. suis* type 2. Thus, although infection by *S. suis* is almost invariably subclinical, it would appear to be a very common cause of human infection. If more virulent strains of *S. suis* type 2 became endemic in the pig population of New Zealand, this could have clinical and public health implications.

It is highly probable that strains of *S. suis* type 2 of differing virulence for humans exist. From other work performed by the authors it appears that strains of different virulence for pigs and laboratory animals are endemic in New Zealand. If most strains are infective but avirulent for humans, they would cause seroconversion without the development of clinical disease, as was demonstrated in the present investigation.

From the present study, it would be reasonable to assume that contact with pigs is the major factor leading to antibody development to *S. suis* type 2 in humans. Robertson & Blackmore (8) found that 3% of normal pigs slaughtered at a meatworks in New Zealand were carrying *S. suis* type 2 in the blood. As there is residual blood in all body tissues after slaughter and *S. suis* type 2 can survive in chilled and frozen pig products, a similar percentage of carcasses and their resultant meat cuts may also be infected. However, the number of recorded cases of disease from *S. suis* type 2 in humans, other than those involved with pig farming and the slaughtering and processing of pig carcasses is less than five (6, 1, 10). The public health dangers from handling meat infected with *S. suis* type 2 therefore appears to be extremely small.

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