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The carriage of *Streptococcus suis* type 2 by pigs in Papua New Guinea

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SUMMARY

An indirect fluorescent antibody test was used to detect the presence of *Streptococcus suis* type 2 in nasal and pharyngeal swabs taken from pigs in Papua New Guinea. The rate of carriage for the two sites in domesticated indigenous village pigs was 0·5 and 2·5% respectively, compared to 39 and 43% for intensively reared pigs. These findings were supported by the results of a serological survey, using an enzyme linked immunosorbent assay, in which 87% of intensively reared pigs but only 8% of village pigs were seropositive to *S. suis* type 2.

It is proposed that in intensive piggeries *S. suis* type 2 is continually cycled between pigs. In village pigs, the low population density and harsh environmental conditions prevents this cycle of infection.

INTRODUCTION

Streptococcus suis type 2 is an important cause of disease in recently weaned pigs. It is primarily associated with septicaemia, meningitis and arthritis [1], although in some countries it is an important cause of pneumonia [2, 3]. *Streptococcus suis* type 2 has been isolated from pigs from most countries of the world [4]. The bacterium may be carried in the palatine tonsils (5, 6] and/or the nasal cavities [4, 7] of apparently healthy pigs. *Streptococcus suis* type 2 is also a rare zoonotic agent producing meningitis with permanent vestibular and auditory dysfunction in humans who have handled pigs or their products [8, 9].

Prior to this study the carriage of *S. suis* type 2 by pigs in Papua New Guinea (PNG) had not been investigated. In PNG, pigs are predominantly kept by the villagers under extensive conditions, however there is also a small but growing commercial pig industry. In villages, households may own from 1–10 pigs. Pigs interact closely with their owners, frequently occupying the same house. This close contact may expose the villagers to risk of infection with zoonotic agents such as *S. suis* type 2.

The aims of this project were firstly to establish whether *S. suis* type 2 was

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present in PNG, and secondly to compare the pattern of carriage in commercially reared, intensively housed pigs with that of domesticated indigenous village pigs.

MATERIALS AND METHODS

Source of samples

Samples were collected from live pigs at a 140 sow intensive commercial piggery situated on the outskirts of Goroka (the capital of the Eastern Highlands Province) and from pigs at three villages. Two of these villages (Korfena and Openga) were 25 km from Goroka whilst the third (Masilakaufa) was only 3 km away. Pigs are allowed to roam free throughout these villages and surrounding bush. The pigs to be sampled were restrained and two sterile swabs (Medical Wire and Equipment Ltd, England) were inserted 2–5 cm into their nasal cavities and rotated against the mucosa. The swabs were replaced in their packets, stored on ice and returned to the laboratory within 2 h of collection. Nasal swabs were collected from 151 live intensively reared and 219 live village pigs. Tonsillar swabs were also collected from live pigs. A steel Y-framed gag was used to hold the pig's mouth open and to allow access to the palatine tonsils. Twenty-one tonsillar swabs were collected from intensively reared pigs and 40 from pigs at Masilakaufa. The sex and age (weaners, growers, finishers and breeders) of each pig sampled was recorded.

Material was also collected at slaughter from the intensively reared pigs. Both palatine tonsils were excised from 91, 6-month-old pigs and from two cull sows. Nasal swabs had also been collected from 17 of these prior to slaughter. The tonsils were placed into individual containers and returned on ice to the laboratory within 2 h of collection. Samples of lungs with lesions typical of mycoplasmal pneumonia were also collected from 17 pigs. Blood from 63 pigs was collected into sterile plain tubes during exsanguination and the sera subsequently collected. Sera which had previously been collected [10] from pigs ($n = 83$) at three other villages in the Goroka area, and from 11 3-month-old pigs from the piggery were also examined.

Bacterial culture and immunofluorescence

Nasal and tonsillar swabs were plated onto both horse blood agar and horse blood agar incorporating 5 μg/ml of gentamicin. Tonsil and lung samples were flamed, sectioned, swabbed and plated onto the two media. Plates were incubated aerobically at 37 °C for 18 h. The indirect fluorescent antibody test (IFAT), as described by Robertson and Blackmore [11] was performed on the resulting mixed growth. To avoid reader bias the plates were coded and mixed so that the origin of each smear was unknown whilst being examined.

To verify that organisms that fluoresced were *S. suis* type 2, colonies of typical morphology were subcultured from 10 plates that gave positive IFAT readings. Colonies were subjected to a Gram stain and tested for lack of growth in 6·5% NaCl, at pH 9·6, and at 45 °C. Colonies were also tested for sensitivity to optochin, hydrolysis of aesculin and fermentation of mannitol. The capsule was extracted as described by Clifton-Hadley and Alexander [12] and a capillary tube precipitation test performed using group R antiserum (Wellcome Reagents Limited, Becken-

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ham, England). Three Australian isolates of *S. suis* type 2 were used as positive controls.

Serology

An enzyme-linked immunosorbent assay (ELISA) was developed to detect antibodies to *S. suis* type 2 in pig sera. Antigen was prepared by extracting the capsule of an 18 h broth culture of *S. suis* type 2 [12] and then washing it three times in phosphate buffered saline (PBS). Aliquots were frozen at −70 °C and used when required. Chequerboard titrations were undertaken to standardize the dilution of antigen, sera and conjugate required. Antigen was diluted 1:16 in PBS prior to deposition of 100 μl into the appropriate wells of a polystyrene immunoplate (NUNC Maxisorb F96, Intermed, Denmark). The plate was incubated at 37 °C for 30 min and then washed five times with PBS-Tween 20 (Sigma Chemical Company, St Louis, USA) using an automatic plate washer. Next 100 μl of PBS-Tween 20–1% bovine serum albumin (BSA) was added to all wells and the plate incubated for 30 min. After washing, 100 μl of sera, diluted 1:200 in PBS-Tween 20–1% BSA, was added to the wells. The plates were incubated for a further 90 min, washed, and then 100 μl of anti-pig horseradish peroxidase conjugate (Merck, Darmstadt), diluted 1:1000 in PBS-Tween 20–1% BSA, was added. The plates were then incubated for a further 60 min at 37 °C. After washing, 100 μl of the substrate *ortho*-phenylenediamine (OPD) (Sigma Chemical Co., USA) was added and the plates kept in the dark at room temperature for 7 min. The reaction was stopped by the addition of 50 μl of 1N H_2SO_4. The plates were then read at 492 nm with an automatic plate reader (Titertek Multiskan Plus, Flow Laboratories, Finland). To account for background absorbance, two wells of antigen and two wells of 'no antigen' were used for each sera. A selected high titre sera was also included on all plates so that interplate variation could be standardized. The cut-off point between a positive and negative titre was determined by using a group of 12 village pigs at the lower end of the absorbance distribution ($< 0{\cdot}4$) as a negative control group. Three standard deviations were added to the mean absorbance for this group to obtain a value of 0·55. Then an 'indeterminate region' of 0·05 was added to either side of 0·55 so that sera giving a reading of less than 0·5 were considered seronegative while those with a reading greater than 0·6 were classed as seropositive.

Statistical analysis

The results for bacterial isolation, immunofluorescence and serology in village pigs and pigs in the commercial piggery were compared using a Chi square test for independence.

RESULTS

The proportion of pigs from which *S. suis* type 2 was isolated is displayed in Table 1. At slaughter the bacteria were detected in the palatine tonsils and pneumonic lesions of 56 and 29% of the intensively reared pigs respectively. Two pigs with positive lung samples were also carrying the organism in their tonsils.

Streptococcus suis type 2 was detected in 39% of all nasal swabs collected from intensively reared pigs. There was no significant difference in the carrier rates

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Table 1. *Isolation of* S. suis *type 2 from intensively reared pigs and from village pigs in Papua New Guinea*

	Intensively reared pigs		Village pigs	
Sample	Number sampled	Number positive (%)	Number sampled	Number positive (%)
Slaughter pigs				
Palatine tonsils				
Growers	91	51 (56)	0	—
Sows	2	0 (0)	0	—
Pneumonic lesions	17	5 (29)	0	—
Live pigs				
Nasal swabs				
Weaners	78	38 (49)	98	0 (0)
Growers	37	15 (41)	77	1 (1·3)
Finishers	36	6 (17)	0	—
Adults			44	0 (0)
Tonsillar swabs				
Weaners	21	9 (43)	4	0 (0)
Growers	0	—	29	1 (3·4)
Adults	0	—	7	0 (0)

between weaners and growers, however the difference between weaners and finishers, and growers and finishers, were significantly different ($P < 0{\cdot}01$). Eight (47%) of 17 pigs from which nasal swabs and palatine tonsils were both collected. were recognized as tonsillar carriers but only three (18%) were nasal carriers. The rate of carriage in the tonsils of live weaner pigs was not significantly different from the nasal carrier rate (43 and 49% respectively). There were no significant differences in the carrier rates between male and female pigs.

There was a highly significant difference ($P < 0{\cdot}001$) between the detected nasal carrier rate in intensively reared pigs and village pigs. The single positive village pig was from Masilakaufa. Only one of the nasal swabs from this pig was positive and the number of *S. suis* type 2 seen on IFAT was small. Attempts to isolate these organisms in pure culture were unsuccessful. Two tonsillar swabs collected from this pig were also negative. There were no significant differences in the nasal carrier rates between the three villages.

The carrier rate in the tonsils of village pigs (2·5%) was significantly lower than it was in intensively reared pigs (43%) ($P < 0{\cdot}005$). Nasal swabs from the sole village pig with a positive tonsillar swab were both negative for *S. suis* type 2.

The biochemical and physiological properties exhibited by isolates of *S. suis* type 2 from PNG were typical for the organism and were identical to the reactions exhibited by Australian isolates. Serological examination (slide agglutination and capillary tube precipitation) of the isolates confirmed their identity as *S. suis* type 2.

The distribution of absorbance readings obtained with the ELISA is displayed in Fig. 1. Eighty-seven percent of intensively reared pigs and 8·4% of village pigs were seropositive. In intensively reared pigs that were slaughtered there was no significant difference in the absorbance readings for pigs that were tonsillar

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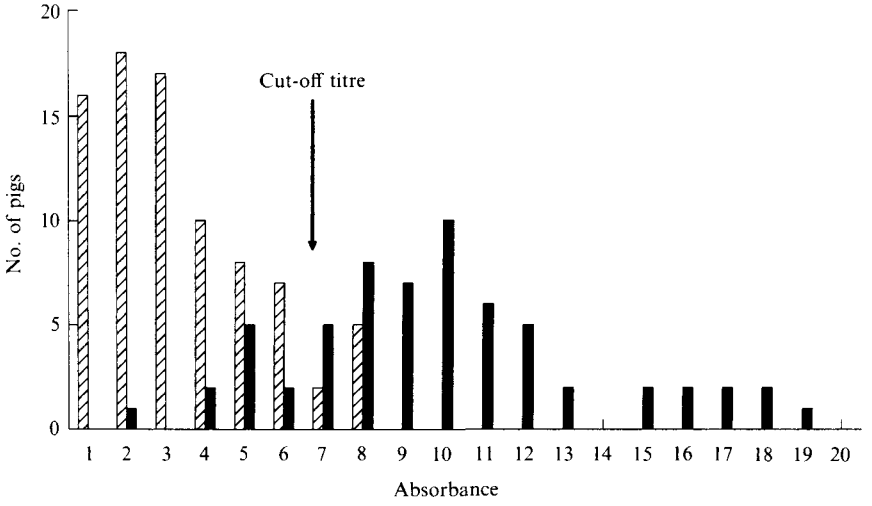

Fig. 1. Distribution of ELISA titres to *S. suis* type 2 in intensively reared pigs (■) and village pigs (▨) in Papua New Guinea.

carriers and those that were not. Absorbance values from village pigs less than 12 months old were not different from those of older village pigs.

DISCUSSION

The prevalence of tonsillar (56%) and nasal carriers (39%) of *S. suis* type 2 in intensively reared pigs in Goroka was comparable to that found by Robertson and Blackmore [4] in Australian and New Zealand herds (73 and 54% respectively). Although the Goroka piggery is older than most Australian piggeries, it would appear that the similar management procedures ensures comparable carriage rates of *S. suis* type 2.

More of the 17 pigs from which both nasal swabs and tonsils were collected were tonsillar carriers (47%) than nasal carriers (17%). This suggests that either the bacterium is able to persist for a longer period in the tonsils or that fewer organisms are present in the nasal cavities. Moreau and co-workers [13] found that whether or not a pig was a nasal carrier of *S. suis* type 2 was independent of whether it had a positive tonsillar swab. They concluded from this that the nasal cavity was as important a site for the carriage of *S. suis* type 2 as the tonsil. If the bacterium is carried in the nose it is likely that it will be shed in nasal exudates, particularly during sneezing. Thus nasal carriage may be more important than tonsillar carriage for the spread of the bacterium in densely populated groups.

Village pigs had a significantly lower rate of nasal and tonsillar carriage of *S. suis* type 2 than did intensively-reared pigs. In an intensive piggery conditions are suitable for the spread of a bacterium due to the frequent nose-to-nose contact between pigs and the close proximity to sneezing and coughing pen mates. In addition transfer of *S. suis* type 2 between pigs could occur indirectly via environmental contamination [4]. Conditions in villages are far more extensive, with pigs often roaming large distances into the bush in search of food. Clifton-Hadley and Enright [14] demonstrated that *S. suis* type 2 survived for only 8–

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9 days when inoculated into faeces kept at room temperature. Therefore it would be expected that any *S. suis* type 2 shed into the environment by village pigs would have little chance of surviving before contact with another pig. Nose-to-nose contact between pigs from different households was also noted to be infrequent.

Since both of the village pigs that were carrying *S. suis* type 2 were from Masilakaufa it is possible that the bacterium has never been introduced to pigs at Korfena and Openga. However this is unlikely because in all villages, many pigs had characteristics of European breeds. Villagers also occasionally visited the Goroka piggery to buy pigs, and, although the pigs were usually slaughtered soon after arrival at the villages, it is likely that some would interact with the indigenous pigs and transmit *S. suis* type 2. As Masilakaufa is the closest village to Goroka, it is also probable that there has been more frequent introduction of European pigs than at Korfena and Openga. Therefore it is likely that although *S. suis* type 2 has been introduced to the other two villages, the extensive conditions and infrequent interactions between pigs means that it has not been able to persist. Rates of carriage of some other infectious pathogens such as rotavirus [10] and *Salmonella* spp. [15] have also been found to be significantly lower in village pigs than in pigs reared intensively. However the opposite occurs in the case of lungworm infection [16] where the earthworm (the intermediate host of *Metastrongylus* sp.) is an important part of the diet of village pigs.

Clifton-Hadley and co-workers [7] found that *S. suis* type 2 could be carried in the tonsils for up to 500 days, and Robertson (unpublished) found that the carrier rate of *S. suis* type 2 in feral pigs in Queensland was almost as high as that in intensively-reared pigs. These results have led to the suggestion that *S. suis* type 2 can be carried for a long period. If this is true, a low population density, and therefore infrequent contact between pigs, should have little consequence on the carrier rates. This apparent anomaly with the current results could occur if in fact pigs carried *S. suis* type 2 for only a short period. In the study of Clifton-Hadley and co-workers [7], pigs were kept in small groups and it is possible that infection may have cycled between them. The bacterium could also be cycling amongst feral pigs in Queensland as they frequently congregate in mobs of 50 or more pigs. Davies and Ossowicz [17] also found that the carrier rate of *S. suis* type 2 in South Australia was significantly lower in small herds than in large herds, suggesting that population density may be important in determining the carrier rate.

The results of the ELISA supported the IFAT findings by detecting the presence of two distinct populations of pigs (village pigs with low titres and intensively reared pigs with high titres). In an intensive piggery, pigs are either long-term carriers or they are continually being reinfected. Either way the immune system would receive continual stimulation and titres to *S. suis* type 2 would therefore remain high. In the indigenous village pigs there is no evidence for continual reinfection and therefore a high proportion of seronegative pigs are present. Although the sera from village pigs came from three villages not studied in this survey [10], it would appear that the pattern of infection with *S. suis* type 2 in these villages is similar to that of the three villages sampled.

This project studied the carriage of *S. suis* type 2 by healthy pigs and did not investigate the relationship between carrier status and disease. Other work has suggested that many strains of *S. suis* type 2 are avirulent [18, 19]. It is not known

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if the strains present in PNG are capable of causing disease, although there have been reports of meningitis and arthritis in weaners in one other piggery from which an organism biochemically similar to *S. suis* type 2 was isolated (L. Dasanyake, personal communication).

In another study by the authors it was found that none of 157 sera from villagers in the Eastern Highlands Province had detectable antibody titres to *S. suis* type 2, even though they interact very closely with their pigs (unpublished data). This further supports the findings of a low rate of carriage in village pigs. Although there have been adequate opportunities for the introduction of *S. suis* type 2 into Highland villages, the extensive conditions and infrequent contact between pigs prevented successful establishment in populations of village pigs. This project is the first to conclusively demonstrate the presence of *S. suis* type 2 in pigs in PNG, and adds further evidence of the worldwide distribution of this pathogen in intensively reared pigs.

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