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Carrier Rate of *Streptococcus suis* Capsular Type 2 in Palatine Tonsils of Slaughtered Pigs

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Palatine tonsils of 143 slaughtered pigs aged 4 to 6 months were investigated for the presence of *Streptococcus suis* type 2. Slices (50 μm) of frozen tonsils were cultured on a selective agar medium containing antibodies against *S. suis* type 2 in which colonies of this bacterium showed a halo of immunoprecipitation. When tonsils were sectioned in one plane *S. suis* type 2 was found in 45 of 143 pigs (32%). This percentage increased to 50% when tonsils were sectioned in more then one plane, which was done on 55 tonsils. The first 45 strains showing a ring of immunoprecipitation were studied and found to be biochemically identical to our reference strain 735 (de Moor) and to 23 isolates from human patients with meningitis. In slices incubated for 24 h at 37°C on selective agar plates and stained with hematoxylin and eosin after fixation, it could be demonstrated that *S. suis* type 2 was confined to the crypt lumen. The same was true in sections fixed directly (without incubation) that were stained by an indirect immunoperoxidase method with a rabbit anti-*S. suis* type 2 serum.

In Europe, *Streptococcus suis* type 2 (13), originally described by de Moor (3) as group R streptococcus, is a frequent cause of disease in pigs (4, 6, 7). It has been isolated from pigs with septicemia, endocarditis, arthritis, pneumonia, and meningitis. In England, a sharp rise of meningitis in pigs due to *S. suis* type 2 was noted from 1973 to 1977 (8).

Experimental studies have suggested that the nasopharynx, especially the palatine tonsils, are the main portals of entry of infection (2, 12). In healthy pigs, *S. suis* type 2 has been cultured only from the palatine tonsils. The carrier rate, determined with tonsillar swabs in two herds in England with endemic *S. suis* type 2 infections, was 24% of 122 pigs aged 3 to 8 weeks and 0% of 59 younger pigs (2). In a similar investigation in two endemic herds in Germany, *S. suis* type 2 was cultured in 15% of sectioned palatine tonsils obtained after slaughtering of 52 pigs of unspecified age (H. M. Clausen, Inaugural-dissertation, Tierärztliche Hochschule Hannover, Federal Republic of Germany, 1980).

S. suis type 2 also causes meningitis in humans (1, 10, 14). In the Netherlands, 27 cases of *S. suis* type 2 meningitis have already been registered since 1968, which is more than have been published in any other country. Most patients were pigkeepers or butchers who were probably infected by contaminated pigs or pork.

To obtain data on the carrier rate in pigs in this country, we investigated the tonsils of 143 slaughtered pigs. Identification and localization of *S. suis* type 2 colonies in the tissue are possible when sections obtained from frozen tonsils are cultured on a transparent selective poured plate containing antiserum to *S. suis* type 2. Several aspects of these techniques have been described before (9, 15).

MATERIALS AND METHODS

Palatine tonsils from 117 healthy pigs and from 26 pigs with unspecified diseases, aged 4 to 6 months, were obtained at two different slaughterhouses between October 1982 and June 1983. Tonsils were frozen in liquid nitrogen and stored at −70°C until used. Frozen tonsils (one half) were sectioned in one plane on a freeze microtome parallel to their surface and thereby perpendicular to the crypts. Three to five successive 10-μm sections were carefully placed on a transparent poured culture plate. Because only a small percentage (0.1%) of the total weight of each tonsil was sectioned, 55 tonsils were resectioned on seven additional planes.

The culture medium consisted of 10 ml of agar (isosensitest Oxoid), in which 0.5 ml of sheep antiserum directed against *S. suis* type 2, polymyxine B (15 U/ml), and nalidixic acid (15 μg/ml) were incorporated. The optimal quantity of antiserum was experimentally determined. The plates were examined after 24 h of culturing at 37°C and a further 24 h at 4°C. Tonsils were designated positive when colonies surrounded by bright immunoprecipitation rings were present (Fig. 1). Only colonies which were present in at least two successive sections, but not on the margins of the section, were counted.

To obtain information on the localization of *S. suis* type 2 in the tonsillar sections, two procedures were followed. After growth, plates with sections showing positive colonies were fixed with 2% glutaraldehyde and stained with hematoxylin and eosin to visualize the histological structure of the tonsil. In a second procedure, sections of five positive tonsils were directly fixed with glutaraldehyde. In these sections, the *S. suis* type 2 bacteria were visualized by an immunoperoxidase method and counterstained with hematoxylin and eosin (5). A rabbit antiserum against *S. suis* type 2 was used as primary antibody.

Both sheep and rabbit antisera were obtained by immunizing with our reference *S. suis* strain type 2 (77628) obtained from a patient with meningitis. Bacteria for immunization were inactivated as follows: 30 min at 60°C for immunizing the rabbit; overnight at 4°C with 0.2% formalin for immunizing the sheep. The immunization scheme for the rabbit consisted of daily intravenous injections for 10 days; that of the sheep consisted of intramuscular and subcutaneous injections at 2-week intervals with either complete (first injection) or incomplete (subsequent injections) Freund adjuvant until the antibody level was satisfactory.

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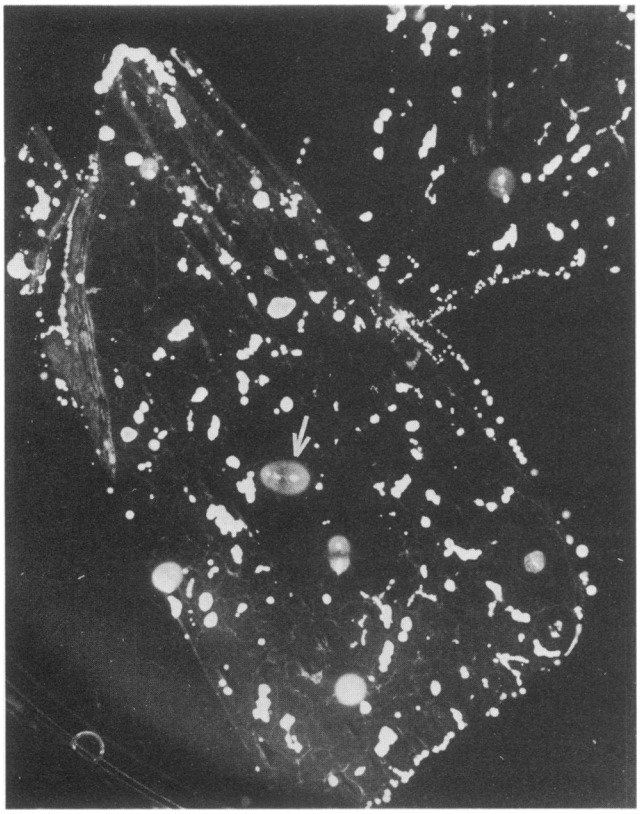

FIG. 1. *S. suis* type 2 colonies grown on the transparent antibody-containing, selective medium show white rings of immunoprecipitation (arrow).

The nomenclature proposed by Perch et al. (11) for *S. suis* was used. Strains 735/56, 1545/57, and 2651/59 were obtained from de Moor; strains 4961, 6407, 11538, 8074, 14636, and A 227 were obtained from Perch. Strain A 227 was originally described by Elliott.

RESULTS

The reference strain 735 of de Moor and 23 human isolates all showed colonies with bright rings of immunoprecipitation. Strain 2651/59 (*S. suis* type 1/2 or group RS streptococcus of de Moor), known to have antigenic determinants of *S. suis* types 1 and 2, was also positive. Of the other *S. suis* types recently described (11), strain A227 (type 6), strain 14636 (type 8), and strain 73/38 (group T streptococcus of de Moor) gave weak precipitation rings, which could easily be differentiated from S. suis type 2. *S. suis* types 1, 3, 4, 5, and 7, *Streptococcus faecalis*, and *Streptococcus faecium* showed no rings of immunoprecipitation.

In sections of 143 palatine tonsils, colonies showing precipitation rings (Fig. 1) were present in 45 (32%) when tonsils were sectioned at one level (Table 1). This percentage increased to 50% when 55 tonsils from March and June were resectioned at seven more planes. No differences were noted in the number of positive tonsils from the two slaughterhouses nor among the various time periods in which the tonsils were collected. There was also no difference in the frequency of positive cultures between the healthy and the diseased animals.

After subculturing, the first 45 positive colonies were biochemically tested. Biochemical reactions (Table 2) of the tonsillar isolates were identical to our reference strain 735 and to 23 isolates from human patients with *S. suis* type 2 meningitis.

TABLE 1. Isolation frequency of *S. suis* type 2 in palatine tonsils of slaughtered pigs

Time of culture	No. of positive tonsils (*n*)[a]	Percentage
October 1982[b]	9 (26)	35
November 1982	11 (37)	30
March 1983	16 (56)	29
June 1983	9 (24)	37

[a] Results shown were obtained after sectioning tonsils in one plane.
[b] Diseased group of pigs.

In the sections, *S. suis* type 2 colonies were found in small groups. The number of these groups in each positive tonsil varied from 1 to more than 50 but was generally low (median, 2). When plates with sections were stained with hematoxylin and eosin, it was found that the *S. suis* type 2 colonies were situated in the tonsillar crypts. This could be further demonstrated by immunoperoxidase staining of sections which were fixed before growth. *S. suis* type 2 was sometimes found in the wall of the crypt but never in the tonsillar lymphoid tissue surrounding the crypts.

DISCUSSION

We have observed a high carrier rate of *S. suis* type 2 in pigs brought to slaughter in the Netherlands. This percentage is higher than has been found in other studies, in which the investigations were done in endemic herds (2; H. M. Clausen, Inaugural dissertation, Tierärztliche Hochschule Hannover, Federal Republic of Germany, 1980).

There are several possible explanations for the difference between our results and those of these two other studies. The pigs we investigated were older, and there are some indications that the carrier rate rises with age (2). We used a very sensitive immunological technique to detect the *S. suis* type 2 colonies, whereas the others used only the colony morphology as primary screening. Since the number of positive colonies in each section was small, it would be difficult to detect them with less sensitive techniques. It is unlikely that we overestimated the number of positive colonies, because we checked colonies from each tonsil by biochemical reactions. On the other hand, it is possible that we underestimated the carrier rate, because we took only a

TABLE 2. Biochemical identification of *S. suis* type 2 isolated from pig tonsils and from human patients

Test, substrate	Results[a]		
	Reference strain 735	Patients	Tonsils
Fermentation			
D-Glucose, glycogen inulin, raffinose	+	+	+
Sorbitol, glycerol, ribose, L-arabinose	0	0	0
Amygdalin	0	3 of 23	8 of 45
Mannitol	0	2 of 21	1 of 45
Hydrolysis			
Aesculin	+	+	+
β-D-glucopyranosid uronic acid	+	+	+
α-D-galactopyranosid uronic acid	+	+	+

[a] For patients, $n = 23$; for pig tonsils, $n = 45$. Symbols: +, 100% positive; 0, 100% negative.

small sample from the tonsils (0.1% [wt/wt]), and the percentage of positive tonsils rose from 31 to 50% when the number of planes at which the tonsils were sectioned was increased.

Colonies and immunoperoxidase-stained bacteria were confined to the crypts. This is in contrast with the observations of Williams et al. (12), who found these organisms also in adjacent lymphoid tissue after experimental infection of 10-day-old pigs.

It is interesting that many pigs harbored *S. suis* type 2 in their tonsils without being ill. This would indicate that these animals were, at least at that time, resistant to invasive disease, possibly by immune mechanisms.

No differences between the tonsillar isolates from pigs and meningeal isolates from human patients were found with respect to colony morphology and color, rings of immunoprecipitation, or biochemical reactions. This indicates that the pig strains are probably identical to the human strains, and that tonsillar tissue of slaughtered pigs might be a source of human infection. In the Netherlands, palatine tonsils are cut away during slaughtering and are destroyed.

No *S. suis* type 2 was isolated from tonsillar swabs of 34 men connected with a slaughter house who were in frequent contact with pigs or pork because of their jobs (unpublished data). A low concentration of *S. suis* type 2 deep in the tonsillar crypts could have been missed however.

The high carrier rate of *S. suis* type 2 in pigs aged 4 to 6 months, which we have detected in this study, could be one of the explanations for the comparatively high incidence of *S. suis* type 2 meningitis in humans in the Netherlands.

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