Production of exfoliative toxin by isolates of *Staphylococcus hyicus* from different countries

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A total of 218 isolates of *Staphylococcus hyicus* from pigs in eight countries (Belgium, Croatia, Germany, Japan, Korea, Slovenia, the UK and the USA) and 44 isolates from other animals in Belgium, India, Japan and the USA were examined for the genes encoding the exfoliative toxins ExhA, ExhB, ExhC and ExhD by multiplex PCR. The expression of the toxins was confirmed by immunoblot analysis, using monoclonal or polyclonal antibodies specific for each of the toxins. The porcine isolates were from pigs with exudative epidermitis, pigs with other lesions and from healthy pigs, and one or more of the toxins could be found among the isolates from the pigs in all the countries. Toxigenic strains of *S. hyicus* were isolated from both healthy and diseased pigs, but the chance of isolating toxigenic strains from pigs with exudative epidermitis was greater than from pigs with other lesions or healthy pigs. Of the 44 isolates from other animal species, only one isolate, from a hare from Belgium, produced ExhB, and one isolate, from a cow with mastitis from Japan, produced ExhA.

**MATERIALS AND METHODS**

**Bacteria**

Two hundred and eighteen isolates of *S. hyicus* from pigs (Table 1) and 44 isolates from other animal species were examined. The collection was obtained by the receipt of isolates from laboratories in Belgium, Croatia, Denmark, Germany, Japan, Slovenia and the USA and, in addition, isolates from India, Korea and the UK. The isolates were collected without any system; most of them were field isolates, some were laboratory strains and they had been isolated during the period from the 1960s to the 1990s. Ninety-eight of the isolates were from pigs with other types of lesions, for example, flank wounds, necrotic ear lesions or metritis, and 88 were from healthy pigs; the other isolates were seven of the 14 isolates from Korea, and from pigs with exudative epidermitis or from healthy pigs. The 44 isolates from other animal species were from a hare, a guinea pig, a rabbit, two cattle with skin lesions, two canaries, two ostriches and two chickens from Belgium; four cows with mastitis from the USA and six from Japan; two cattle with skin lesions from India; 11 goats from India; and 10 chickens from Japan. The isolates were identified as *S. hyicus* as described by Andresen (1998). The *S. hyicus* strains NCTC 10350 (Devriese and others 1978), 1289D-88, 842A-88 (Wegener and others 1993) and A2896C (Amtsberg 1979) were used as reference strains for the production of the exfoliative toxins ExhA, ExhB, ExhC and ExhD, respectively, and the presence of the corresponding genes.

**Culture conditions**

*S. hyicus* was cultivated on plates of Columbia blood agar base (Oxoid) containing 5 per cent bovine blood (C-blood agar). The liquid growth medium was 30 g/l trypticase soy broth (4311768; Becton Dickinson) supplemented with 0.5 mM cobalt chloride and 0.5 mM zinc sulphate after it had been autoclaved. For optimal expression of the exfoliative toxins, the liquid growth medium was supplemented with 0.5 mM cobalt chloride and 0.5 mM zinc sulphate after it had been autoclaved. The liquid cultures were incubated at 37°C in tightly closed 10 ml tubes containing 5 ml liquid growth medium inoculated with a single colony from an overnight culture on C-blood agar.

**PCR**

A multiplex PCR assay for the presence of the genes encoding the exfoliative toxins of *S. hyicus* was carried out as described by Andresen and Ahrens (2004). The reaction used 0.5 U of Taq polymerase per sample in a total volume of 50 µl containing 2.0 µl of template DNA preparation, 10 mM Tris-HCl, pH 8.3, 50 mM potassium chloride, 250 µM magnesium chloride.
The identity of the isolates was confirmed by biochemical and microbiological methods. Isolates that were positive for lipase, hyaluronidase, heat-stable nuclease and catalase, non-haemolytic on C-blood agar and with a negative reaction for oxidase were considered to be \textit{S. hyicus}. These isolates were assayed for the presence of genes encoding the exfoliative toxins by multiplex PCR. Fig 1 shows the results of the multiplex PCR on selected non-toxigenic and toxigenic isolates of \textit{S. hyicus}. The expression of the exfoliative toxins \textit{ExhA}, \textit{ExhB}, \textit{ExhC} and \textit{ExhD} was confirmed by immunoblot analysis of exo-proteins from the supernatant of the liquid cultures. All the isolates that contained a toxin gene expressed the corresponding exfoliative toxin, except for one \textit{exhD}-positive field strain from a healthy pig.

The results of the investigation of the 218 isolates of \textit{S. hyicus} from pigs are summarised in Table 1: 69 (32 per cent) of them were toxigenic; of the 98 isolates from cases of exudative epidermitis, 54 (55 per cent) were toxigenic. Six toxigenic isolates were detected among the 25 isolates from pigs with other types of lesions, and nine toxigenic isolates were detected among the 88 isolates from healthy pigs. All but two of the 44 isolates from other animal species were non-toxigenic; one isolate from a hare possessed and expressed the gene encoding \textit{ExhB}, and one isolate from a cow with mastitis from Japan produced \textit{ExhA}.

**RESULTS**

These results show that one or more of the different types of exfoliative toxin can be found among the isolates of \textit{S. hyicus} from pigs in many countries (Table 1). The methods used were designed to detect \textit{ExhA}, \textit{ExhB}, \textit{ExhC} and \textit{ExhD}. Other exfoliative toxins, \textit{SHETA} and \textit{SHETB}, have been reported (Sato and others 2000), but the methods for their detection were not available. The isolates included cannot be considered as representative of the population of \textit{S. hyicus} in all the countries, but the results show which of the four types of toxin can be found. In the countries from which larger numbers of isolates from cases of exudative epidermitis were tested, more different types of toxin were detected. Presumably, this trend would be even more evident if more epidemiologically independent isolates from cases of the disease were tested for toxin production. Previous studies have shown that both toxigenic and non-toxigenic strains of \textit{S. hyicus} can be isolated from pigs with the disease (Tanabe and others 1996, Andresen 1998). A study by Park and Kang (1986) showed that isolates of \textit{S. hyicus} from healthy pigs could induce the disease when they were inoculated into pigs. The present results were in agreement with the findings of these earlier studies, because toxigenic isolates of \textit{S. hyicus} were more prevalent in the animal populations in all the countries, and healthy pigs, although a higher percentage of toxigenic \textit{S. hyicus} was isolated from the pigs with the disease than from pigs with other lesions or from healthy pigs. The results also indicate that toxigenic strains of \textit{S. hyicus} were more prevalent among the isolates from pigs than among those from other animal species. However, the sampling of the isolates investigated was not designed to test the true prevalence of toxigenic and non-toxigenic \textit{S. hyicus} in the animal populations statistically, and further studies are therefore needed to investigate this conclusion.

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Papers & Articles

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