Staphylococcus hyicus Virulence in Relation to Exudative Epidermitis in Pigs

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ABSTRACT

Staphylococcus hyicus **strains with different phage types, plasmid profiles, and antibiotic resistance patterns were isolated from piglets with exudative epidermitis. The strains could be divided into virulent strains, producing exudative epidermitis, and avirulent strains, producing no dermal changes when injected in experimental piglets. The results showed that both virulent and avirulent strains were present simultaneously on diseased piglets. This constitutes a diagnostic problem. Concentrated culture supernatants from nine virulent strains injected in the skin of healthy piglets produced a crusting reaction in all piglets. Acanthosis was observed in the histopathological examination of the crustaceous skin. Concentrated culture supernatants from nine avirulent strains produced no macroscopic or microscopic skin changes. Protein profiles from all virulent strains and seven out of nine avirulent strains showed a high degree of protein band homology. An approximately 30 kDa protein present in all concentrated culture supernatants capable of producing skin changes, could not be detected in samples that did not produce skin changes. No other protein showed a similar association. It is concluded that crusting reaction of piglet skin is a suitable indicator of virulence in *S. hyicus* in relation to exudative epidermitis, and that virulent strains produce a 30 kDa protein, absent in concentrated culture supernatants from avirulent strains. This 30 kDa protein might be an exfoliative toxin.**

RÉSUMÉ

Des isolats de *Staphylococcus hyicus* de phagotypes différents et ayant des profils plasmidiques et de résistance aux agents antimicrobiens différents ont été isolés de porcelets souffrant d'épidermite exsudative. Les souches pouvaient être divisées en souches virulentes produisant l'épidermite exsudative et en souches avirulentes qui ne produisaient aucun changement dermique lorsque injectées chez des porcelets. Les résultats ont démontré que les souches virulentes et avirulentes étaient présentes simultanément chez les porcelets affectés, ceci constituant un problème pour le diagnostic bactériologique. Les surnageants de concentrés de cultures provenant de neuf souches virulentes injectées dans la peau de porcelets en santé produisirent des croûtes chez tous les porcelets. L'examen histopathologique des croûtes démontrait de l'acanthose. Les surnageants de concentrés de cultures à partir de souches avirulentes ne produisirent aucune lésion cutanée autant macroscopique que microscopique. Les profils protéiniques de toutes les souches virulentes et de sept des neuf souches avirulentes ont démontré un haut degré d'homologie. Une protéine d'approximativement 30 kDa présente dans tous les surnageants de concentrés de cultures produisant des lésions cutanées n'a pu être détectée dans les échantillons qui ne produisaient pas de lésion cutanée. Il fut conclu que la formation de croûtes est un indicateur de virulence de *S. hyicus* en relation avec l'épidermite exsudative et que les souches virulentes produisent une protéine de 30 kDa, absente dans le surnageant de concentré de culture provenant des souches avirulentes. Cette protéine de 30 kDa pourrait être une toxine exfoliante. ***Traduit par Dr Manon Paradis***)

INTRODUCTION

Staphylococcus hyicus (1,2) is recognized as the causal agent of different diseases in several animal species; exudative epidermitis (EE) in suckling and weaned piglets (3,4,5), bacteriuria in pigs (6), polyarthritis in pigs (7,8), abortion in a sow (9), skin infection in horses, donkeys and cattle (10,11,12), subclinical mastitis in cows (13), osteomyelitis in heifers (14), and conjunctivitis in chickens, turkeys and ostriches (15,16). Spontaneous EE is a generalized infection of the skin, that may be presented in a peracute, acute or a subacute form. It is initially characterized by a thick brownish, greasy and odorous exudate. Later the skin becomes crustaceous (17). Piglets apparently die from dehydration or septicemia, and mortality may reach 90% (18). Diseased piglets can be treated with antibiotics locally, or systemic, and sows can be vaccinated with autogenous vaccine (18).

The pathogenicity of *S. hyicus* has not been studied intensively. *Staphylococcus hyicus* strains can be separated into virulent and avirulent strains, with regard to their ability to produce EE in piglets (19,20). Strains isolated from the skin of diseased piglets have been shown to produce substances which causes exfoliation of the skin of piglets (21,22). Furthermore isolates of *S. hyicus* from other porcine sources such as, arthritis,

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necrosis of the ear, and milk as well as from the skin of bovines have been shown not to produce such substances (21).

So far no *in vitro* assay has been developed that can distinguish between virulent and avirulent strains.

The objective of this study was to isolate and study the ability of different strains of *S. hyicus* to produce exudative epidermitis in piglets. Furthermore, we investigated whether injection of sterile concentrated culture supernatants from the same *S. hyicus* strains into the skin of piglets could be used as an assay of bacterial virulence. The skin reactions were compared to the natural infection concerning distribution and severity of the macroscopic lesions, and histopathological changes. Finally, protein preparations from virulent and avirulent strains of *S. hyicus* were studied by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, in order to distinguish virulent from avirulent strains, and identify proteins associated with *S. hyicus* pathogenicity.

TABLE I. Phage types, plasmid profiles, and antibiogram patterns exhibited by ten isolates of *Staphylococcus hyicus* randomly recovered from the skin of each of three piglets with exudative epidermitis

Pig No.	Number of isolates (N = 10)	Phage-type	Plasmid profile (kb)	Antibiogram pattern[a] (resistance)
1403	4	A/B/C	11.5	St/Te/Pe/ Li/Er/Ol
	2	long[b]	2.5/3.7	Li/Er/Ol
	3	long[b]	2.5/3.7	sensitive
	1	Q	2.5/3.9	Li/Er/Ol
1289	1	A/B/C	11.5	St/Te/Pe/ Li/Er/Ol
	8	NT[c]	2.4/4.4/ 6.3/13	St/Te/Pe
	1	NT	2.4/3.9/4.4/ 6.3/13	St/Te/Pe/ Li/Er/Ol
842	2	long[d]	no plasmids	sensitive
	6	A/Q/R/U	2.4/3.7	Li/Er/Ol/Ka
	1	A/Q/R/U	2.4	Li/Er/Ol
	1	I/N/O/Q	2.4	sensitive

[a]Antibiotics used: streptomycin (St), tetracycline (Te), trimethoprim + sulfamethizol (Tr), penicillin (Pe), lincomycin (Li), methicillin (Me), erythromycin (Er), chloramphenicol (Ch), gentamycin (Ge), oleandomycin (Ol), kanamycin (Ka), fucidin (Fu)
[b]A/E/G/K/L/M/N/Q/R/S/T
[c]Not typable
[d]E/G/H/I/J/L/M/N/O/P/Q/R/T/U/W

TABLE II. *Staphylococcus hyicus* strains investigated, and effect of strains when inoculated in healthy 14-day-old SPF piglets

Strain No.	Piglets develop generalized EE after exp inoc	Origin			Reference
		Animal species	Country	Source[a]	
NCTC 10350	+	Pig (EE)	Denmark	1	(1,3)
P411	+	Pig (EE)	UK	2	(33)
SK170	−	Pig (EE)	UK	2	(33)
P119	+	Pig (EE)	UK	2	(34)
S3588	+	Pig (EE)	Germany	3	(21)
A2869C	+	Pig (EE)	Germany	3	(21)
A3793/76	+	Pig (arthritis)	Germany	3	(21)
A4569/73	−	Pig (necrosis)	Germany	3	(21)
A72/75	−	Pig (mastitis)	Germany	3	(21)
9390-88	+	Pig (EE)	Denmark	4	(35)
842A-88	+	Pig (EE)	Denmark	4	Present work
842G-88	−	Pig (EE)	Denmark	4	—
842J-88	−	Pig (EE)	Denmark	4	—
1403B-88	−	Pig (EE)	Denmark	4	—
1403E-88	+	Pig (EE)	Denmark	4	—
1403H-88	−	Pig (EE)	Denmark	4	—
1289D-88	+	Pig (EE)	Denmark	4	—
1289E-88	−	Pig (EE)	Denmark	4	—

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MATERIALS AND METHODS

BACTERIAL STRAINS AND CULTURING OF STRAINS

Ten *S. hyicus* isolates were obtained from the skin of each of three piglets with EE submitted to the Danish National Veterinary Laboratory (Table I). Isolation and identification were performed according to Devriese (23). Skin swabs from the piglets were inoculated on selective/indicative agar (TSA (Difco 0369-01-4) supplemented with KSCN 30 g/L, $CAC1_2$ 100 mg/L, and Tween 80 10 mL/L), and ten colonies that exhibited the characteristic properties of *S. hyicus* were picked at random. Strains identified as *S. hyicus* were further characterized with regard to phage type, antibiogram-type, and plasmid profile (Table I). Strains were stored at −80°C in tryptic digest broth (TDB, Difco, 1829-17-8) to which was added 10% glycerol. Subcultures were made on 5% bovine blood agar plates (Columbia agar base, Oxoid). Broth cultures were grown overnight in tryptic soy broth (BBL, 11768) supplemented with 10 g of yeast extract (Oxoid, L21) per liter (pH 7.2), at 37°C with moderate shaking. In addition, reference strain No. NCTC 10350 originating from Denmark and eight strains originating from Germany and U.K. respectively were included in this study (Table II).

ANTIBIOGRAMS

Sensitivity to antibiotics was measured by a standard tablet diffusion test on Danish blood agar plates (24). Tablets contained the following diffusible amount of antibiotic: 100 μg streptomycin, 80 μg tetracycline, 5.2 μg trimethoprim + 240 μg sulfamethizol, 5 μg penicillin, 19 μg lincomycin, 29 μg methicillin, 78 μg erythromycin, 60 μg chloramphenicol, 40 μg gentamycin, 30 μg oleandomycin, 100 μg kanamycin, 400 μg fusidic acid, respectively (Rosco Diagnostica, Taastrup, Denmark).

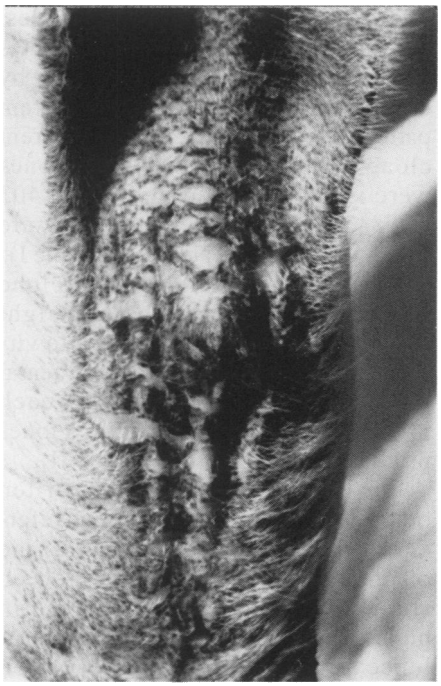

Fig. 1. Abdominal skin of piglet with generalized exudative epidermitis.

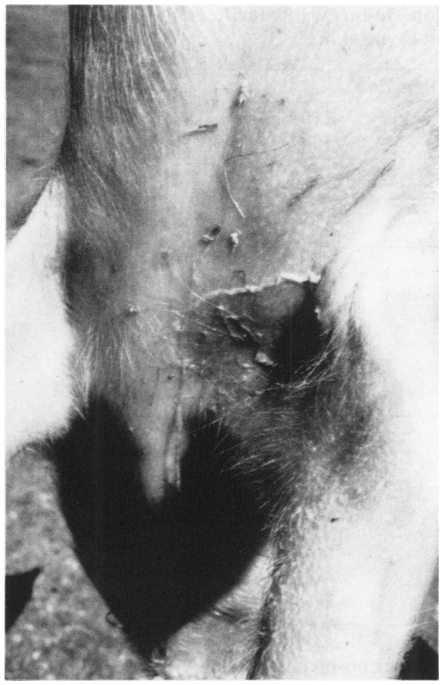

Fig. 2. Local exfoliation in skin of a 14-day-old SPF piglet caused by s.c. injection of 1 mL sterile concentrated culture supernatants from a virulent strain of *Staphylococcus hyicus* (9390-88).

Testing and interpretation were carried out according to the manufacturers guidelines for sensitivity testing (25).

PHAGE TYPING

Phage typing was performed as described by Wegener (26). In brief: Twenty-two phages with different lytic patterns isolated from *S. hyicus* by mitomycin induction according to de-Saxe and Notley (27) were used for typing. Phage-typing and interpretation of results were performed according to the criteria given for *S. aureus* (28).

PLASMID SCREENING

Plasmid DNA was prepared according to Wegener (26). In brief: Cells were lysed by 70 μg/mL lysostaphin in STET-buffer (8% sucrose, 10 mM Tris, 50 mM EDTA, and 0.5% Triton X-100, adjusted to pH 8.0). The lysate was boiled for 40 s, and chromosomal DNA and proteins were pelleted by centrifugation. The supernatant was extracted with phenol:chloroform: isoamylalcohol (25:24:1), and subjected to agarose gel electrophoresis. Two plasmid preparations from each strain were examined. Plasmids in *Escherichia coli* V517 (29), and *E.coli* 39R861 (30) served as molecular weight markers.

PRODUCTION OF EXOPROTEIN CONCENTRATES AND SDS-PAGE ANALYSIS

Supernatants from 24 h broth cultures were concentrated 20 times in an Amicon ultrafiltration cell using a Diaflo PM10 filter (10 kDa cut off) (Amicon Corporation, Danvers, Massachusetts) and subsequently concentrated 2.5 times in a Millipore CX-10 filter (10 kDa cut off) (Millipore Corporation, Bedford, Massachusetts). Twenty μL samples of the concentrated proteins were dissolved in 20 μL of Laemmli sample buffer (31), heated in a boiling water bath for 5 min, and separated in 8–18% gradient sodium dodecyl sulphate (SDS)-polyacrylamide gels (Pharmacia LKB Biotechnology, Uppsala, Sweden). Gels were stained with silver according to the method of Morissey (32). The protein sizes were determined by semilogarithmic plots against standard molecular weight marker proteins (Bio-rad). Comparison of protein profiles were performed by computerized video-image processing using CREAM 1-D software (Kem-En-Tec Software Systems, Copenhagen, DK-2100).

VIRULENCE TESTING

Experimental infection in pigs — Virulence of *S. hyicus* was tested by subcutaneous inoculation of 2 mL broth culture behind the right ear, in two-week-old specific-pathogen-free (SPF) piglets. Prior to injection OD600 of the samples was measured, and the number of cells per sample was standardized to approximately 5×10^8 per mL. The number of viable cells was evaluated by plate counting on tryptic soy agar (BBL, 11043). The piglets were kept separated from each other and observed every day for a 14 day period. Any visible changes of the skin and behavior of the piglets was noted. On the 14th day p.i. piglets were killed by exposure to CO_2. Postmortem examination included macroscopical, microscopical, and microbiological examination of the skin, liver, spleen, urether, lymph nodes, and lungs. All strains were tested twice in piglets from different litters.

Skin test in piglets — One mL sterile filtered concentrated culture supernatant (CCS) was injected subcutaneously (s.c.) in 14-day-old SPF piglets. Concentrated growth medium was used as negative control. Injections were placed at approximately 10 cm distance on each side of the ventral thorax, the first injection being proximal to the axilla. Each piglet received eight injections. The appearance of the skin at the injection sites was recorded every 24 h. Eight days p.i. the piglets were killed by exposure to CO_2. The intensity of the skin reactions; erythema, crusting, and exfoliation were graded as, −, (+), +, ++, +++.

SKIN BIOPSIES AND HISTOPATHOLOGICAL EXAMINATION

Skin biopsies were taken from all injection sites of each piglet and from unaffected abdominal skin as an internal control. Biopsies were performed by cutting a rectangular piece of the skin horizontally 1/2 to 1 centimeter from the rim of the process and vertically to under the subcutis. The skin biopsies were fixated in 4% neutral buffered

TABLE III. Macroscopic and microscopic lesions induced by sterile concentrated culture supernatants from *Staphylococcus hyicus* injected in pig skin

Strain No.	Virulence[a]	Skin Reaction[b] Erythema Pig1/Pig2	Crusting Pig1/Pig2	Exfoliation Pig1/Pig2	Histopathological observations[c]
NCTC 10350	+	++/+	−/+++	−/−	Acanthosis
P411	+	++/++	+++/+++	+/−	Acanthosis
SK170	−	+/+	−/−	−/−	Normal
P119	+	++/+	+++/+++	−/−	Acanthosis
S3588	+	+/+	+++/(+)	++/−	Acanthosis
2869C	+	+/+++	+/(+)	−/−	Acanthosis
A3793/76	−	++/(+)	−/−	−/−	Normal
A4569/73	−	(+)/++	−/−	−/−	Normal
A72/75	−	+/−	−/−	−/−	Normal
9390-88	+	(+)/++	+/+	−/−	Acanthosis
842A-88	+	+/++	+++/+++	(+)/−	Acanthosis
842J-88	−	++/+	−/−	−/−	Normal
842G-88	−	++/+	−/−	−/−	Normal
1403B-88	−	+/−	−/−	−/−	Normal
1403E-88	+	+/++	+++/++	(+)/−	Acanthosis
1403H-88	−	++/+	−/−	−/−	Normal
1289D-88	+	++/+++	++/++	−/−	Acanthosis
1289E-88	−	+/+	−/−	−/−	Normal
Conc. culture medium		−	−	−	Normal

[a]According to Table I
[b]The highest degree of reaction noted during the eight-day observation period
[c]Skin biopsies were taken for histopathological examination on the 8th day p.i.

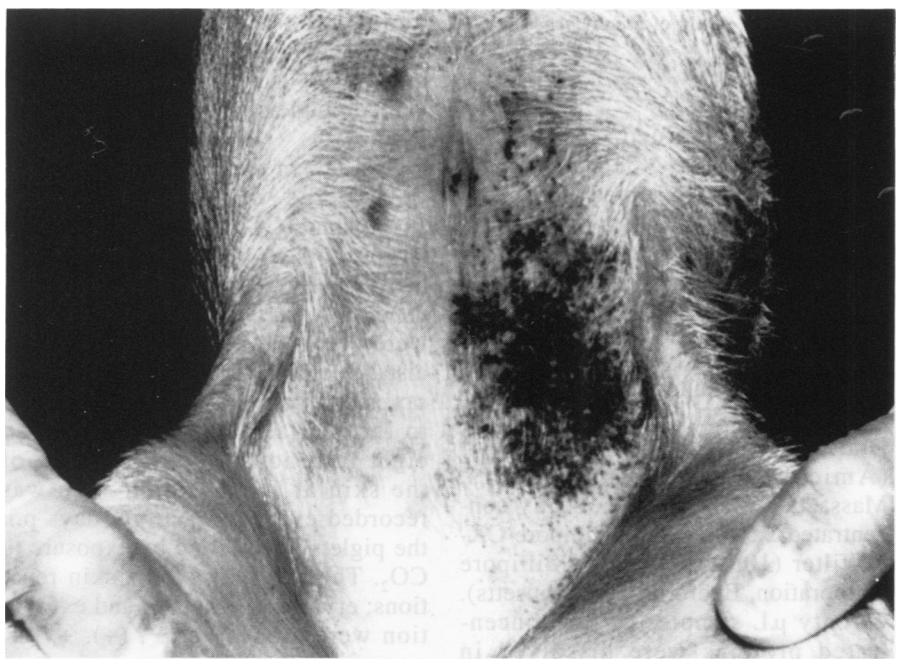

Fig. 3. Crusting reaction in skin of a 14-day-old SPF piglet caused by s.c. injection of 1 mL sterile concentrated culture supernatant from a virulent strain of *Staphylococcus hyicus* (NCTC 10350).

formaldehyde and embedded in paraffin wax. Sections were cut 5–6 μm, stained with hematoxylin and eosin and examined by light microscopy.

RESULTS

Thirty isolates of *S. hyicus*, ten from each of three piglets with EE, were investigated with regard to phage type, antibiogram type, and plasmid profile (Table I). Isolates from piglet No. 1403 exhibited three phage types, three plasmid profiles, and three antibiogram-types. Isolates from piglet No. 1289 exhibited two phage types, three plasmid profiles, and two antibiogram types. Isolates from piglet No. 842 exhibited three phage types, three plasmid profiles, and three antibiogram types. Isolates differing by phage type as well as by either plasmid profile or antibiogram pattern were considered to be different clones. Thus, three different clones were isolated from piglets No. 1403 and 842, and two different clones were isolated from piglet No. 1289 (Table I).

One representative of each clone from each piglet, altogether eight strains, was studied with regard to virulence by inoculation in experimental piglets. Only one clone from each piglet produced EE, the other clones produced no signs of EE (Table II).

Furthermore the virulence of ten other strains were studied: the reference strain NCTC 10350 and nine other strains that had previously been studied with regard to virulence in piglets (1, 3, 21, 33, 34, 35). The virulence of all previously described strains was confirmed in this study (Table II).

Altogether 9 of the 18 strains investigated in this study produced EE in the SPF-piglets, whereas the remaining nine strains produced no signs of local or generalized dermatitis (Table II).

The course of disease was identical for all virulent strains. The first signs of disease appeared within two to three days p.i. The skin of the abdomen, head, thighs, groin, and flanks became red with a clear exudate, and the abdominal skin could be peeled off by slight rubbing. Within 24 h the exudate dried up forming brown crusts. Approximately day 6 p.i. all of the skin was affected. The abdominal skin and the skin in the regions around ears, eyes, and mouth became most severely affected showing deep cracks and fissures in the generalized stage of disease (Fig. 1). At postmortem examination *S. hyicus* could be isolated from blood and liver of all diseased animals. From one apparently healthy animal *S. hyicus* could be isolated from the liver.

Piglets that did not develop EE showed no other signs of disease. Three animals however, had an abscess of approximately 5 by 5 cm at the injection site. Although *S. hyicus* could be isolated from the pus it was not considered indicative of virulence in relation to EE.

Concentrated culture supernatant from all 18 strains studied were

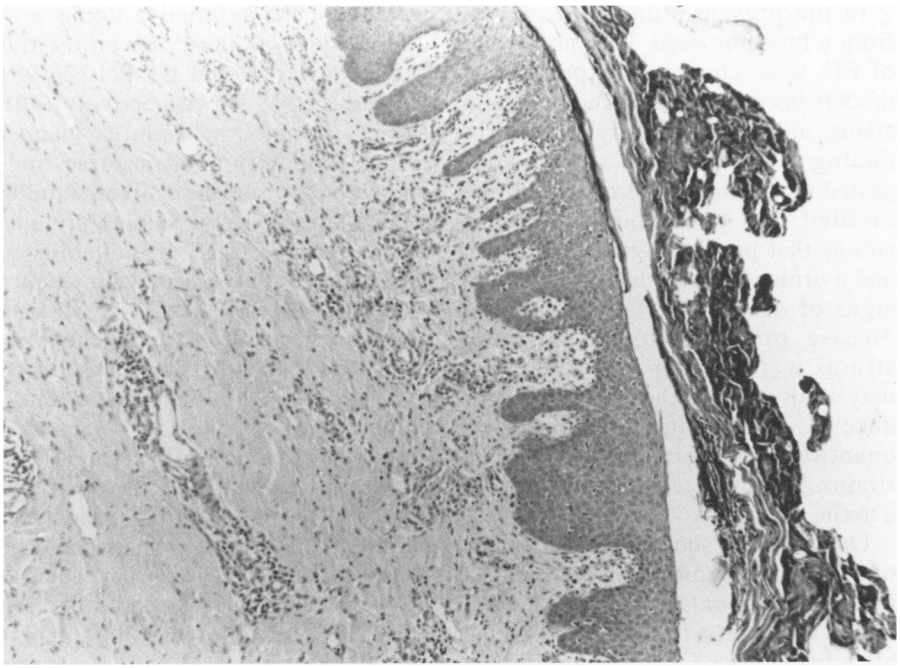

Fig. 4. Acanthosis, formation and enlargement of rete pegs due to hyperplasia of the stratum spinosum, was observed eight days p.i. in all skin biopsies where sterile concentrated culture supernatants (CCS) from virulent strains had been injected. No changes were seen in skin biopsies from sites where CCS from avirulent strains had been injected.

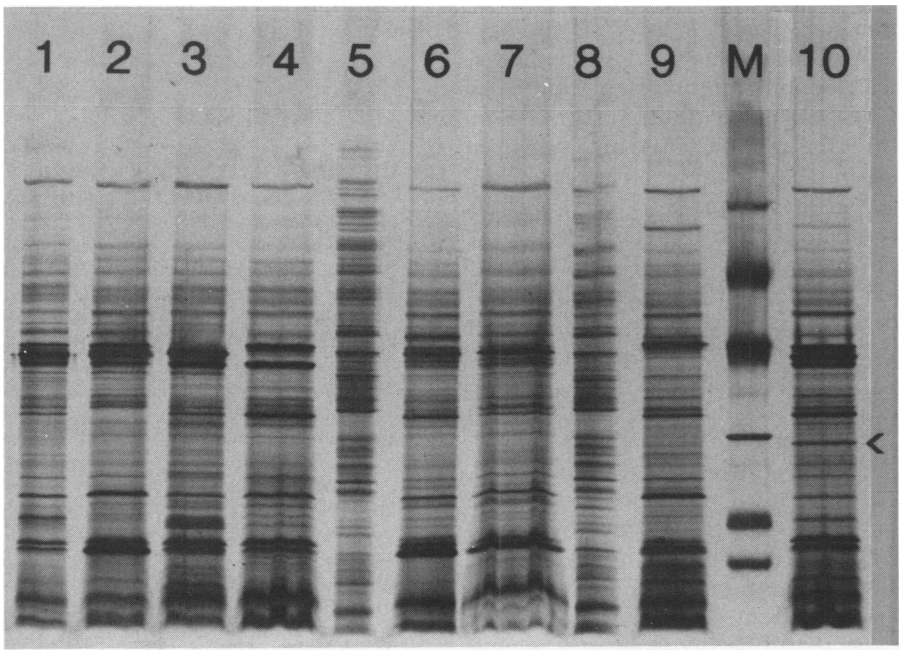

Fig. 5. Protein profiles of sterile concentrated culture supernatants from nine avirulent strains of *Staphylococcus hyicus* (3793/76, lane 1; A4596/73, lane 2; A72/75, lane 3; 842G-88, lane 4, 842J-88, lane 5; 1403B-88, lane 6; 1403H-88, lane 7, 1289E-88, lane 8; SK170, lane 9) and a virulent strain of *Staphylococcus hyicus* (NCTC 10350, lane 10) analyzed in a (8–18%) polyacryl amide gel electrophoresis and stained with silver. Lane M is proteins for molecular weight determination (from top to bottom, 97, 66, 45, 31, 21 and 14 kDa). A band corresponding to a protein with a molecular weight of approximately 30 kDa (arrow), which could not be observed in culture supernatants from avirulent strains, was observed in culture supernatants from all virulent strains investigated.

injected s.c. in the abdominal skin of 14-day-old SPF-piglets. The CCS from virulent strains produced three different skin reactions: Erythema, exfoliation, and crust reaction. All reactions were local reactions at the injection site, varying from 5 to 10 cm in diameter. Erythema and exfoliation (Fig. 2) appeared within 24 hours p.i. whereas crusting (Fig. 3) appeared within three to six days p.i. The CCS from all virulent strains produced a crust reaction. Erythema and exfoliation was produced by some, but not all CCS from virulent strains. The CCS from some avirulent strains produced a transient erythema. This reaction usually disappeared within three days p.i. No other reactions were observed. Consequently a crust reaction at the site of injection four to six days p.i. was considered indicative of virulence (Table III).

Histopathological examination of skin biopsies, eight days p.i., only showed minor variations from normal conditions. Acanthosis (enlargement of the rete pegs) was observed at the injection sites with a macroscopically identified crusting reaction (Fig. 4). Tissue samples from the injection sites of CCS from avirulent strains showed no variations at all. Thus, acanthosis was considered indicative of *S. hyicus* virulence (Table III).

Proteins in CCS from all strains were electrophoretically separated by SDS-PAGE and stained with silver (Fig. 5). The CCS contained approximately 65 protein bands ranging from > 100 kDa to < 10 kDa in molecular weight. Protein profiles of two strains (842J-88 and 1289E-88) differed significantly from all other strains. Computer analysis of the protein profiles showed 67% homology among the two strains, but only <45% homology to all other strains. All other *S. hyicus* strains showed a high degree of protein-band-homology (>65%). A band corresponding to a protein with a molecular weight of approximately 30 kDa was observed in all protein profiles from virulent strains. This band was not observed in protein profiles from avirulent strains. The 30 kDa protein band, which was the only band showing this association, was thus considered indicative of *S. hyicus* virulence.

DISCUSSION

Our findings showed that diseased piglets harbored different strains of *S. hyicus* with regard to phage type,

antibiogram pattern, and plasmid profiles. Furthermore, both virulent and avirulent strains were isolated simultaneously from the diseased animals. Isolation of avirulent strains from healthy piglets, from cases of polyarthritis or gangrenous ears, have been reported (36,37). Furthermore virulent strains have been isolated from the skin of healthy piglets (38). Our results indicate that avirulent strains of *S. hyicus* might be frequently present on the skin of piglets with EE — all investigated piglets harbored avirulent strains. The frequency with which avirulent strains were isolated from diseased piglets indicated that the prevalence of such avirulent strains equals the prevalence of virulent strains in the generalized stage of infection. Whether such avirulent strains play any active role in the development and course of infection remains to be investigated.

In the diagnosis of EE in piglets there is a problem with differentiating virulent *S. hyicus* strains from avirulent *S. hyicus* strains in clinical material. Selecting a nonpathogenic *S. hyicus* bears the risk of prescribing wrong antibiotics, as well as preparing an inefficient autogenous vaccine. In both cases it might have severe economical consequences for the pig producer.

Serotyping have been attempted, and proposed, as a method to differentiate virulent strains from avirulent strains (19). Subsequent studies (20,39) however, revealed the presence of several different serotypes among virulent strains, none of which could identify all known virulent strains. Nonetheless, subtyping of isolates might be the only practical solution to the diagnostic problems until a satisfactory *in vitro* virulence assay is developed. Serotyping could be one method for subtyping, but other methods are available. At the moment ten isolates of *S. hyicus* are picked at random from each case of EE admitted to the Danish National Veterinary Laboratory. Phage types and antibiogram patterns of all these isolates are established. Only those antibiotics active against all the isolated strains are prescribed for therapy. One representative of each phage type are used for production of mixed autogenous vaccine.

In the present study SPF piglets from a breeding herd, with no history of EE, were chosen as experimental models because of well known health status, and low immunity to EE producing *S. hyicus*. The results suggested that *S. hyicus* strains could be divided into two groups: Virulent strains that produced generalized EE, and avirulent strains that produced no signs of dermatitis. The severity of disease produced by all virulent strains were almost identical. This may indicate that the ability to produce EE is a qualitative more than a quantitative difference in *S. hyicus* strains, for instance the production of a toxin.

Our findings showed that injection of sterile CCS from virulent *S. hyicus* produced a local crusting reaction, which appeared within six days p.i. at all sites where CCS from virulent strains were injected. In contrast a brief erythema appeared within 24 hours p.i., or no reaction at all, was observed after injection with CCS from avirulent strains (Table III). Thus, crust formation correlated to *S. hyicus* virulence.

Acanthosis was seen histopathologically in all skin biopsies, exposed to CCS from virulent strains. Skin injected with CCS from avirulent strains showed no such changes.

Variations in individual piglets response to CCS from the same *S. hyicus* strains (Table III) might be explained by differences in thickness of the skin at the injection site, or variation in the administration of the CCS. Differences in content of specific proteins might also account for some of the observed variation.

Protein profiles showed one band, corresponding to a protein with a molecular weight of approximately 30 kDa, present in all virulent *S. hyicus* strains. This band was not detected in any avirulent strains, and could be expected to be involved in *S. hyicus* pathogenicity. No other bands showed the same association. Sato *et al* (22) recently described a fraction of approximately five proteins obtained from *S. hyicus* CCS capable of producing exfoliation in a piglet. The molecular weight of the proteins ranged from 24 to 35 kDa. The authors propose that a 27 kDa protein is the most likely candidate for an exfoliative toxin, considering the molecular weights of the exfoliative toxins produced by *S. aureus*: the molecular weight of ET-A and ET-B is 26.951 Da and 27.318 Da respectively (40). Results obtained in a similar manner in our laboratory confirm these findings although sensitive silver staining methods reveal eight to nine proteins in the reactive fraction (unpublished observation). Purification and characterization of the 27 kDa or 30 kDa protein is necessary to verify the identity of an exfoliative toxin produced by *S. hyicus*. This work is currently being carried out.

In conclusion: Both virulent and avirulent strains could be isolated from piglets suffering from EE. Virulent and avirulent strains of *S. hyicus* could be distinguished *in vivo* by s.c. inoculation of live bacteria, or s.c. injection of sterile concentrated culture supernatants, in piglets. Virulent strains produced a 30 kDa protein, as determined by SDS-PAGE, absent in culture supernatants from avirulent strains. This protein might be an exfoliative toxin and a potential component in a vaccine.

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