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<th>Experimental Staphylococcus hyicus Infection in Piglets (Staphylococcus hyicus のブタへの感染実験)</th>
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EXPERIMENTAL STAPHYLOCOCCUS HYICUS
INFECTION IN PIGLETS

Akira SHIMIZU, Kōsuke OKADA, Junichi KAWANO, Haruka TERANISHI, Shige KIMURA, and Kazumi SUGIHAHA

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Abstract

Two strains (phage types S9/S39/S188 and S9/S188) of Staphylococcus hyicus isolated from pigs with exudative epidermitis (EE) were used in this experiment in order to study the pathogenesis of EE in pigs. The disease was reproduced in the piglets of 30 days old when the cultures of the 2 strains were inoculated subcutaneously or by scarification. All of the 3 pigs showed mild exudative gross lesions of EE. Histologically, accumulation of cellular debris including coccoid organisms was observed on the epidermal surface. The fact that an infection was experimentally induced by inoculation proves that S. hyicus is responsible for pathogenesis of swine EE.

Introduction

Exudative epidermitis (EE) is a skin disease that affects mainly suckling pigs of one month and downward, and is commonly referred to as greasy pig disease. The disease is said to be introduced first by SPINOLA in 1842 as the name of “Hautauschlag” in Germany6,7). After that, its occurrence has been reported in each of European countries, Australia and the United States. In Japan11), ever since the occurrence in Niigata Prefecture in 1966, which seemed to be the first. Numerous investigators15,16) have dealt with this disease in their reports. We12) also encountered an outbreak of EE in a litter of 7 newborn pigs of 2 - 3 days old in a farm in Hyogo Prefecture, and isolated Staphylococcus hyicus in pure culture from the skin lesions of the affected pigs, and furthermore, from the parenchymatous organs such as heart, liver, kidneys and spleen of the pig died.

Some investigators1-3,6,8,14) in Europe have attempted experimental infection of S. hyicus in piglets. In 1953, SOMPOLINSKY13) isolated a gram-positive coccus from the pigs with EE, and he succeeded in reproducing the disease by inoculating culture suspension of this organism into the apparently healthy pigs. He named this gram-positive coccus Micrococcus hyicus. Later in 1978, the organism was classified as the genus Staphylococcus, and was designated Staphylococcus hyicus by DEVRIESE et al.1)

This paper describes an experimentally induced infection in piglets by using strains of S. hyicus isolated from pigs with EE.

Materials and Methods

S. hyicus strains and cultivation

Two strains of S. hyicus (phage types of S9/S39/S188 and S9/S188) isolated from pigs with EE12) were used in this experiment. The strains had been preserved in trypto-soy agar (Nissui) stab cultures at ambient temperature until they were used. In order to prepare the inoculum, the stock cultures of S. hyicus were transplanted to 5% sheep blood agar and incubated at 37°C for 18 hours, and then they were cultivated in brain heart infusion
(BHI) broth (Nissui) with shaking at 37°C for 18 hours.

Experimental pigs

Three Landrace piglets of 30 days old weighing about 5 kg were selected from a litter and used for this experiment. Prior to the experiment, nasal and skin swab samples were obtained from each piglet to bacteriologically ascertain that the pigs were not infected with S. hyicus.

Experimental infection

Experimental procedure is summarized in Table 1. A volume of 1 ml of the 18-hour BHI broth culture was subcutaneously inoculated into the right ear of each piglet, while the same amount was inoculated by scarification into the left ear of each piglet. Pig No. 1 was given a strain of phage type 89/839/8188 only and pig No. 2 was given 89/8188 only. Pig No. 3 was given a mixture of equal measures of phage types 89/839/8188 and 89/8188. All the piglets were examined for clinical signs daily for 7 postinoculation days.

Recovery of organisms

Recovery of organisms was conducted from the nasal cavity and skin surface of body of each piglet using cotton swab on postinoculation day 2. The cotton swab samples taken from each piglet was directly transplanted to the S. hyicus selective agar medium and cultured at 37°C for 48 hours. Identification of the recovered organisms was done conforming to the criteria described by DEVRIESE et al.

All the piglets were exsanguinated for autopsy on the postinoculation day 7. Recovery of the inoculum organisms was attempted even from the internal organs (heart, liver, lungs, spleen, and kidneys) and superficial lymph nodes of each piglet.

Histopathological examination

Histopathological examination of the skin lesions of each piglet was conducted on the postinoculation day 7. Pieces of skin tissue were fixed in 10% neutral formalin solution, and then embedded in paraffin. Sections were stained with hematoxylin and eosin (HE).

Results

Clinical findings

All the piglets inoculated with S. hyicus strains had mild clinical signs of EE. Exudation, hyperemia, and swelling were observed in the inoculated sites of the skin in each piglet 1 - 2 days after inoculation. The superficial layer of the skin was so frail as to be peeled even by rubbing with cotton swab. Three to four days after inoculation, the
lesions were found taking a turn for restoration and drying up. Furthermore, thin firm scabs were observed covering the inoculated sites 5–6 days after inoculation.

**Autopsy**

No gross lesions were recognized in the internal organs in either of the inoculated piglets.

**Recovery of organisms**

In each of the 3 piglets, the inoculum organisms were recovered in large numbers from the skin of the left ear, but not from the skin of the right ear (Table 2). The inoculum organisms were also recovered from the hypogastric skin of pigs No. 1 and No. 2 in small numbers. Table 3 indicates the results of recovery of the inoculum organisms from the internal organs and superficial lymph nodes. The inoculum organisms were not recovered from the internal organs such as heart, lungs, spleen, kidneys, and liver in either of the inoculated piglets, while considerable numbers of them were isolated from the parotid lymph nodes in all the piglets.

**Histopathology**

Locations and severity of histopathological skin lesions in all the pigs are summarized in Table 4.

Site of inoculation: There was no remarkable difference in severity of the skin lesions of the ear between the pigs inoculated with phage types S9/S39/S188 and those inoculated with S9/S188. Similar histopathological findings were obtained from the lesions in the right and left ear skin, and the ear lesions were characterized by the following findings. Accumulation of cellular debris including coccoid organisms was observed on the parakeratotic epidermal surface (Figs. 1, 2).
Table 4. Histological severity of exudative epidermitis in pigs inoculated with *S. hyicus*.

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Strain No. (Phage type)</th>
<th>Ear</th>
<th>Eyelid</th>
<th>Dorsum</th>
<th>Abdomen</th>
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<tbody>
<tr>
<td>1</td>
<td>No. 9 (S9/S39/S188)</td>
<td>++</td>
<td>+++****</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>No. 38 (S9/S188)</td>
<td>+++</td>
<td>+++****</td>
<td>ND</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>No. 9 + No. 38</td>
<td>+++</td>
<td>+++****</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

** Inoculated by scarification.  
*** Subcutaneous inoculation.  
**** Accompanied with abscess formation at site of inoculation.

There was acanthosis of the epidermis. The dermis was infiltrated by histiocyte, lymphocyte, and eosinophil with abscesses formation at the site of inoculation (Fig. 3).

Other skin: Pastular lesions were observed on the epidermal surface of the skin of lower abdomen (Fig. 4).

**Discussion**

EE is experimentally reproducible by inoculating piglets with *S. hyicus* \(^{1,3,6,8,14}\). In this study, two strains of *S. hyicus* isolated from pigs with EE were used as inocula in order to reproduce the disease. The pigs inoculated with each of the strains developed both gross and histological lesions in the inoculated sites. These gross and histological lesions observed in this study were similar to those reported by \(^{9}\) Jones, \(^{9}\) L'Ecuyer and \(^{9}\) Jericho, and \(^{10}\) Mebus et al.

Concerning the pathogenicity of swine *S. hyicus* strains for pigs, \(^{5}\) Hunter et al. stated in their report that the strains isolated from pigs with EE showed pathogenicity when it was inoculated into piglets, but the strains isolated from apparently healthy pigs did not. On the other hand, \(^{3}\) Devries stated that there was no remarkable difference in pathogenicity for pigs between the inoculum strains of *S. hyicus* isolated from pigs with EE and those isolated from pigs without EE.

It is well known that EE frequently attacks suckling pigs between 1 and 20 days of age. Therefore, we attempted to experimentally induce an infection using two piglets of 2 days of age in our previous study (Data are not shown). Although the inoculum strains used were different from those in the present study, none of the piglets given the strains showed clinical signs of EE.

From these results, it was estimated that there are several factors involved in infection of EE such as virulence of strains, and susceptibility of pigs. At all events, it is certain that *S. hyicus* has pathogenic effect on pigs.

**References**


Experimental S. hyicus Infection in Piglets

12) Shimizu, A., K. Okada, J. Kawano, H.


Explanation of figures

Fig. 1. Inoculated site 7 days after scarification (left ear skin of No. 1). Accumulation of parakeratotic cellular debris including coccoid organisms is seen on the epidermal surface with acanthosis of the epidermis. Infiltration of histocytic cell, lymphocyte and eosinophil is seen in the dermis. HE stain, X 60.

Fig. 2. Inoculated site 7 days after subcutaneous inoculation (right ear skin of No. 2). Accumulation of degenerated neutrophils and cellular debris including coccoid organisms on the epidermal surface, acanthosis of the epidermis, and infiltration of inflammatory cell in the dermis are observed. HE stain, X 60.

Fig. 3. Inoculated site 7 days after subcutaneous inoculation (right ear skin of No. 3). Abscess is formed in inoculated site of the dermis. There are necrotic areas with colony of organisms in the center. The lesion is surround by infiltration of neutrophil and granular tissue. HE stain, X 30.

Fig. 4. Abdominal skin 7 days postinoculation (No. 2). Pustules are observed on the epidermal surface of the lower abdominal skin. HE stain, X 60.

Staphylococcus hyicus のプタへの感染実験

清水 晃 ・ 岡田 幸助 ・ 河野 潤一 ・ 寺西 永
木村 重 ・ 杉原 一三

要 約

Staphylococcus hyicus のプタに対する病原性について検討した。プタ漏出性表皮炎から分離された S. hyicus 権株（フィージ型 S9/S39/S188 と S9/S188 の 2 株）の培養菌を、30日齢のプタ 3 頭に皮下接種あるいは皮膚挿入接種して感染実験を行ったところ、3 頭いずれも接種部位に自然発生例と同様の漏出性皮膚病変が形成された。しかし、その皮膚病変像は自然発生例のそれに対比すると軽度であった。病理組織学には、皮膚の表皮表層に球菌塊を伴う細胞崩壊物の堆積が認められた。以上のことより、S. hyicus とプタの漏出性皮炎との関連が示唆された。