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Clinico – Histopathological Study of Exudative Epidermitis Caused by *S. hyicus* in Swine

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ABSTRACT

This study to determine the infection of *S. hyicus* in swine and its prevalence and associated histopathological alteration in the affected skin. The study was conducted in 349 pigs of different age groups from organized, semi organized and unorganized pig farms of Kamrup district of Assam, of which 34 numbers (9.91%) were confirmed for exudative epidermitis based on clinical signs, isolation and identification of causative organism and by protein profiling of *S. hyicus* by detecting 30 kDa virulence indicative protein band through SDS-PAGE. The histopathological examination of affected pig skin showed mild hyperplasia of the stratum corneum, as indicated by the presence of invasion of stratum germinativum by neutrophils, leading to pustule formation; acanthosis and lengthening of the interpapillary pegs. There was abscessation of the hair follicle, also degeneration and ulceration of the stratum germinativum and invasion of the dermis. The epidermal and dermal lesions involving the follicles were also shows neutrophilic invasion and the presence of clusters of bacteria. Highest prevalence of exudative epidermitis (64.70%) were observed in the piglets of age group 1 to 7 days old followed by piglets of age group 8 days to 8 weeks (29.41%).

1. Introduction

Swine is one of the most potential sources of meat and more efficient feed converter after the broiler chicken among the various livestock species. It is also an integral part of livestock farming system of almost all the tribal populations of the North Eastern Region of India. According to FAO, India's pig population is 13.84 million (FAOSTAT 2011) and it constitutes 1.47% of world pig population and out of total pig population in India, 28% are grown in this region (Anon. 2003) and 60 - 90% of rural families in the region keep a few pigs as primary source of income for livelihood (Deka and Thorpe 2008). However, swine farming in this region often experiences severe economic set back owing to high mortality due to various diseases mostly of infectious in nature.

*S. hyicus*is, a commensal found frequently on the skin of swine (Devriese 1977a) of which the exfoliative toxins-producing *S. hyicus* (toxigenic strains) enters through abrasion or cuts of the skin to cause exudative epidermitis (Wegener and Skov-Jensen 1999) which is highly contagious and spreads rapidly from one group to the other. The initial signs of the disease are listless and reddening of the skin in one or more piglets in the litter. Affected pigs become depressed and refuse to eat, body temperature may be elevated initially and become normal thereafter. Although the disease is mostly seen as an acute or peracute infection in suckling and newly weaned piglets, it may occur as a chronic infection in adult (Sato et al. 1991). If not treated with appropriate therapeutic measures, the diseased piglets may develop a generalized exudative epidermitis and may die within a few days due to dehydration.

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Histopathological study of affected skin conducted by Olivry and Linder (2009) observed acanthosis, crusting, exfoliation and exocytosis. This research in exudative epidermitis is a new approach in India and the aim of this study was to describe the clinical symptoms, associated organism detection and histopathological alteration of the affected skin.

2. Materials and Methods

In the present investigation 349 pigs of different age groups from organized, semi organized and unorganized pig farms of Kamrup district of Assam were examined. Samples (from the base of the ear, snout, vagina, subcutaneous abscess, foot lesions, conjunctiva and skin lesions of other parts of body of swine) were collected during the period of June, 2013 to May, 2015. Primary isolation of the organisms was done on 5% sheep blood agar by streak plate method. The plates were incubated at 37°C for 24 hours aerobically and well isolated colony showing the characteristics of *Staphylococcus* species were picked up for Gram's staining. Gram positive suspected colonies of *Staphylococcus* species were further grown in the selective media for *S. Hyicus* (Devriese 1977). The isolated bacteria were identified on the basis of their colony characteristics, morphology, staining reactions and biochemical characteristics (Cowan and Steel, 1993). Protein profiling of cell free supernatants of identified *S. hyicus* isolates were carried out by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) as per the method described by Andresen (1998) using 12.5% acrylamide gel concentration (Table 1). Finally, the stained gel was visualized and analyzed with Gel Documentation System (Kodak, Biostep, Germany). For histopathological examination, tissue (skin) samples from exudative epidermitis affected skin lesions were collected, preserved and fixed in 10% buffered formol saline for 3 to 5 days. After the tissues were fixed properly, the representative pieces of tissue were cut in 2 to 3 mm thickness and washed in running tap water for overnight then dehydrated in ascending grades of alcohol and cleaned in xylene and then embedded in paraffin. The paraffin sections were cut in 4 to 6 micrometer thickness and stained with routine Haematoxylin and Eosin staining. Duplicate sections were stained with Brown and Brenn method as described by Luna (1968) for demonstration of bacteria in tissue sections. Stained sections were examined under microscope for recording histopathological alterations. Detailed history of the affected swine was recorded including their age, sex, breed and site of infection and a prevalence study of exudative epidermitis infections in swine was conducted for both in organized and unorganized farm.

Table 1. Composition of gel for protein profiling of the crude toxin of *S. hyicus* by SDS-PAGE

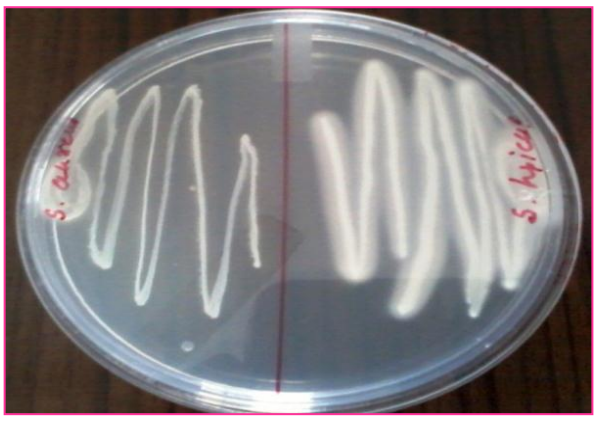
Reagents	Separating gel (12.5% acrylamide)	Stacking gel (5% acrylamide)
Distilled water	0.53ml	2.8ml
30% Acrylamide	4.17ml	0.7ml
1.5M Tris HCL, pH 8.8	3.3ml	-
0.5% Tris HCL, pH 6.8	-	1.0ml
10% SDS	1.0ml	0.5ml
50% glycerol	1.0ml	1.0ml
10% APS	100µl	50 µl
TEMED	10µl	10 µl

3. Results and Discussion

3.1 Isolation and identification of the organism

The isolates were initially identified on the basis of their morphology, colony characteristics and staining reactions. Out of the 360 Staphylococci isolates isolated, 112 (31.11%) were initially identified as *S. hyicus* on the basis of their growth on *S. hyicus* selective medium (Figure 1).

Figure 1. *S. hyicus* and *S. aureus* on *S. hyicus* selective media.



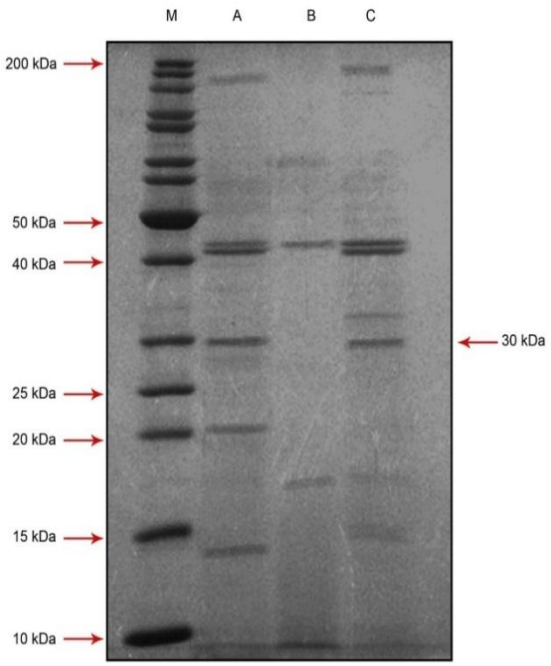
White pigmented colonies of *S. Hyicus* surrounded by a zone of precipitated lipid material on selective media was also described by Devriese (1977). Similarly, 243 (67.50%) isolates were identified as *S. aureus* considering their growth on Mannitol Salt Agar (MSA) and Baird-Parker Agar (BPA). On Mannitol Salt Agar *S. aureus* has grown with small to large zone of yellowish discoloration of the medium and on the Baird-Parker agar characteristics black colour colonies surrounded by a whitish precipitate zone were observed. While the rest five (1.39%) were categorized as other *Staphylococcus* species.

The very high prevalence of staphylococcifrom the pig skin sample might be due to the fact that staphylococciare one of the major groups of bacteria residing in the skin of pigs as commensal and opportunistic organisms (Schwartz 2002). Again staphylococci are ubiquitous, they present on every pig farm and involved a wide range of lesions in pigs of all ages (Straw et al. 2006). During the study majority of the *S. hyicus* isolates (98.21%) were found to produce white pigment on nutrient agar, while the rest two could exhibit yellow pigment. None of the isolates showed zone of haemolysis on 5% sheep blood agar. All the 112 isolates were negative for slide coagulase test but 34 (30.36%) isolates of them were showed positive reaction in tube coagulase test after 48 hours of incubation at 37^0C. All the isolated *S. hyicus* (112) further subjected to biochemical characterization with certain biochemical tests. The 112 isolates (100.00%) of *S. hyicus* showed positive results to catalase, alkaline phosphatase, arginine utilization, liquefaction of gelatin and fermentative on modified Hugh and Leifson's test. They also ferment sucrose, lactose, trehalose whereas none of the isolates fermened mannitol, arbinose and raffinose.The isolates showed negative reaction to Vokses Proskauer's (VP) and ortho-Nitrophenyl-β-galactoside (ONPG) reaction. Among these 112 isolates, 64 (57.14%) were urease positive. Biochemical characteristics of *S. hyicus, S. aureus* and other coagulase negative staphylococcus found in present findings were in close agreement with the findings of Gogoi (2014).

3.2 Protein profiling of the supernatant extracted from S. hyicus isolates by SDS-PAGE (Sodium Dodecyl Sulfate-Poly Acrylamide Gel Electrophoresis)

Cell free concentrated culture supernatants extracted from randomly selected 55 *S. hyicus* isolated from the clinically affected exudative epidermitisand apparently healthy swine were subjected for protein profiling by SDS-PAGE (Figure 2). The concentrated culture supernatant of *S. hyicus* strains contained approximately 13 protein bands ranging from < 200 kDa to < 10 kDa in molecular weight. A band corresponding to a protein with a molecular weight of approximately 30 kDa was observed in protein profiles from 34 strains of *S. hyicus* isolated from the clinical cases of exudative epidermitis. This band was not observed in protein profiles from 21 *S. hyicus* strains isolated from the apparently healthy pigs. The 30 kDa protein band was thus considered as indicative of *S. hyicus* virulence. Protein profiles corresponding to a protein with a molecular weight of approximately 30 kDa, present in all virulent strains of *S. hyicus* could be expected to involve in *S. hyicus* pathogenicity (Wegener et al. 1993).

Figure 2. Protein profiling of the crude *S. hyicus* toxin in 12.5% Tris-glycine SDS PAGE



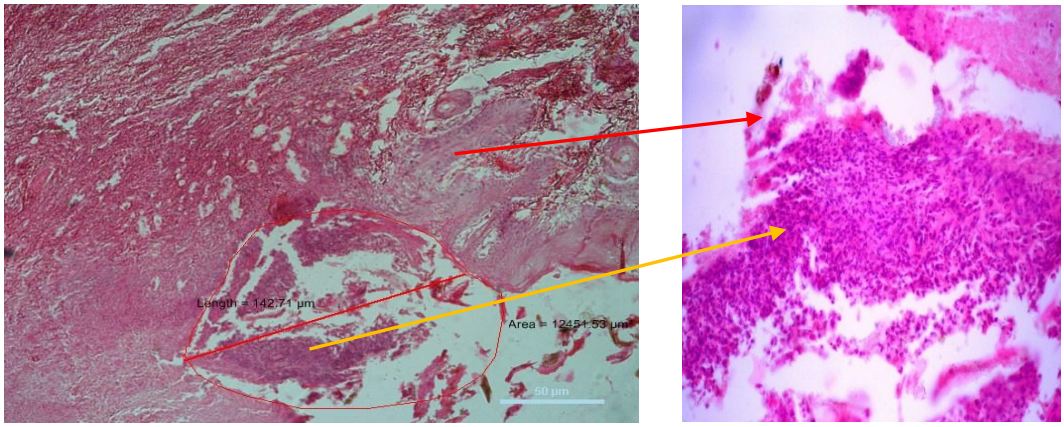
Lane M : Protein ladder
Lane A & C : Crude *S. hyicus* toxin of virulent *S. hyicus*
Lane B : Negative sample i.e. avirulent*S. hyicus*

3.3 Histopathology of exudative epidermitis affected skin

The histopathological examination of affected pig skin showed mild hyperplasia of the stratum corneum, as indicated by the presence of invasion of stratum germinativum by neutrophils, leading to pustule formation; acanthosis and lengthening of the interpapillary pegs. There was abscessation of the hair follicle ranging from 142.65 ± 0.50µm in diameter also degeneration and ulceration of the stratum germinativum and invasion of the dermis (Figure 3a and b). The epidermal and dermal lesions involving the follicles were also shows neutrophilic invasion and the presence of clusters of bacteria (Figure 4).

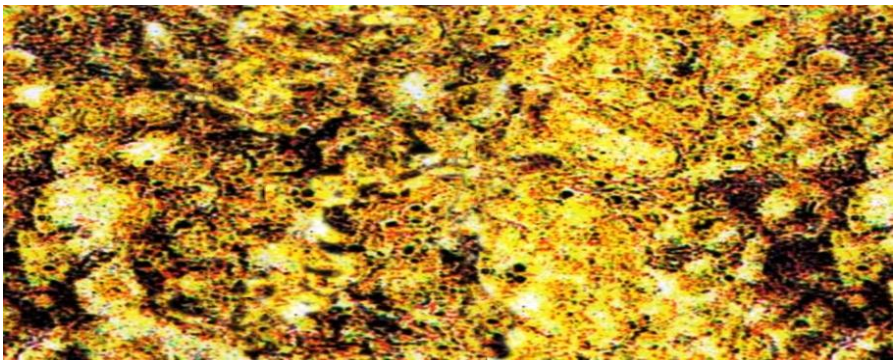
The *S. hyicus* organism multiplies on the abraded skin surface and then grows between the corneocytes of the epidermis, where microcolonies developed. Inflammation, marked hyperplasia of the stratum corneum, and invasion by neutrophils might be the prime cause of increasing thickness of epidermis and follows its erosion (Straw et al. 2006). Pustule formation, neutrophil invasion in epidermal and dermal layer and presence of bacterial clusters found in the present study is in agreement with the findings of Foster (2012).

Figure 3. Photomicrograph of skin showing abscessation with neutrophilic infiltration (⟶) and hyperplasia of stratum cornium (⟶) X 100, H&E (a) AND X 40, H&E (b).



(a) (b)

Figure 4. Photomicrograph of skin showing bacterial clumps x 100 (Brown and Brenn stain)



3.4 Prevalence of exudative epidermitis

Thirty four (9.91%) numbers of swine out of 343 numbers of pigs examined were found to be affected with exudative epidermitis based on clinical examination (Figure 5) and molecular diagnostic techniques. Highest prevalence of exudative epidermitis (64.70%) were observed in the piglets of age group 1 to 7 days old followed by piglets of age group 8 days to 8 weeks (29.41%). Two (5.88%) cases of milder form of exudative epidermitis were observed in adult pigs. Sex wise analysis showed, higher incidences of exudative epidermitis in females (73.53%) than the males (26.47%). Reason behind the higher prevalence in females might be due to the large proportion of females in the tested samples.

Figure 5. Swine showing the clinical symptoms of exudative epidermitis on the face along with conjunctivitis.

During the study it was found that breed variations did not have any significant value for the occurrence of diseases. All the exudative epidermitis cases were recorded from the semi organized and unorganized farms where the hygiene and sanitation was not scientifically maintained. The higher prevalence of exudative epidermitis in young groups might be due to the fact that, before farrowing the organisms multiply in the sow's vagina and infect the piglets during the process of birth or soon after. The maternal *S. hyicus* strains persisted on the skin of the offspring in the first three weeks of the piglet life - the critical period with regard to outbreaks of exudative epidermitis (Wegener and Skov-Jensen 1992). Prevalence of exudative epidermitis (EE) both in young suckling piglets as well as milder form of disease in adult pigs observed in the present investigation were corroborated with the findings of Taylor (1995). The suckling piglets are usually infected by their dams, but cross infection occurs after mixing at weaning. Other contributing factors include sharp teeth cut in the skin around the mouth while competing for teat and fighting at weaning, abrasion on knees from suckling and from poor concrete surfaces or metals, faulty injection, as most of the farmer used to give iron injection to the piglets without proper sterilization. Environmental stress of various kinds including agalactia and intercurrent infection also may the predisposing cause for higher prevalence of disease in young one (Thapa 2001). Again weaning piglets are very vulnerable for infection as in that age their immune system is still immature. That might be an another cause of higher prevalence of exudative epidermitis in young suckling groups of pigs (Wegener and Skov-Jensen 2006). Rajkhowa et al. (2014) also described prevalence of exudative epidermitis in young suckling groups of pigs.

Finally it is concluded that as this study on exudative epidermitis in swine is a new approach in India, further research is required for detection of detail virulence genes associated with the disease, mobility and mortality patterns, disease prevalence and transmissions in broader fields.

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