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Molecular Detection and Therapeutic Management of Exudative Epidermitis in Swine

Moloy Sarmah Baruah[1]*, Arabinda Phukan[2], Rajib Kr. Sharma[3] and Razibuddin A. Hazarika[4]

[1]ICAR RC for NEHR, Basar, Arunachal Pradesh, INDIA
[2]Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, C. V. Sc., AAU, Khanapara, Guwahati, Assam, INDIA
[3]Department of Veterinary Microbiology, C. V. Sc., AAU, Khanapara, Guwahati, Assam, INDIA
[4]Department of Veterinary Public Health, C. V. Sc., AAU, Khanapara, Guwahati, Assam, INDIA

Corresponding author: MS Baruah; E-mail: drmoloysb@gmail.com

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ABSTRACT

Of 343 swine examined, 34 (9.91%) were found to be affected with exudative epidermitis from which 39 *S. hyicus* and 6 *S. aureus* isolates were recovered. Out of 39 *S. hyicus*, 34 *S. hyicus* isolates were found to be positive for virulence genes of which, 6 isolates (17.64 %) were positive for *exhA* gene and 28 isolates (82.35 %) were positive for *exhD* gene with an exhibited band size of 316 bp and 588 bp respectively in PCR. Early recovery of swine with exudative epidermitis was found with amoxyclav with supportive therapy than ceftriaxone and tazobactam combination with supportive therapy though the recovery rates with both the antibiotics were 100 per cent.

Keywords: Swine, exudative epidermitis, *Staphylococcus hyicus*, PCR, antibiogram

Exudative epidermitis (EE), also known as "greasy pig disease", is a skin infection mainly affecting neonatal and newly weaned piglets, characterized by localized lesions of a few mm in diameter to a generalized condition covering the entire body (Victor *et al.* 2013). *Staphylococcus hyicus* is a commensal found frequently on the skin of swine (Devriese, 1977b) and the exfoliative toxins producing *S. hyicus* have become concern as an etiology of porcine exudative epidermitis (EE) particularly in suckling and weaned pigs (Jones, 1956; Andresen *et al.* 1993; Watanabe *et al.* 2000; Andresen and Ahrens, 2004).

The organism enters through abrasion or cuts of the skin to cause exudative epidermitis (Wegener and Skov-Jensen, 1999) with presence of greasy exudate and hence the name "Greasy pig disease". 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. There have been subjective reports that the staphylococcal disease has become more common and difficult to treat (Park *et al.* 2013). It is frequently treated with antimicrobial agents (Penny and Muirhead, 1986), but successful treatment is complicated by the occurrence of antimicrobial resistance among the strains. Therefore, the present investigation is undertaken to study the molecular diagnosis and therapeutic management of exudative epidermitis based on antimicrobial sensitivity tests.

MATERIALS AND METHODS

Ethical Approval

The samples were collected from the lesions without affecting the healthy tissues with non invasive method. Approval was taken from ethical committee.

Collection of Samples

Skin samples were collected using sterile cotton-tipped swabs after application of 1 ml 0.9% sodium chloride to the lesions and brought to the laboratory for further processing. Samples from the distance place of the

laboratory were collected on the transport swab supplied by Hi-Media Pvt. Ltd., India and brought to the laboratory for further processing. A total of 343 pigs from 43 pig farms of Kamrup district of Assam, India were investigated.

Isolation and Identification of Bacteria

The collected material in the form of sterile swabs in 5 ml of nutrient broth or brain heart infusion broth and in the transport swab containing thioglycollate medium (Hi-Media Pvt. Ltd., India) were processed for growth and multiplication of the organism (Collins and Lyne, 1970). After primary isolation on 5% sheep blood agar plates, well isolated Gram positive suspected colonies of *Staphylococcus* species were grown in the Mannitol Salt Agar, Baird-Parker agar and selective media for *S. hyicus* (Devriese, 1977a). The isolates were initially identified and characterized on the basis of their colony characteristics, staining reactions, morphology on smear, growth pattern on selective and differential media, growth pattern on nutrient agar, 5% sheep blood agar, coagulase production, biochemical tests (Cowan and Steel, 1993) and with Hi Staph^{TM} Identification Kit (Manufactured by Hi-Media Pvt. Ltd., India). Staphylococci were distinguished from the micrococci by modified Hugh and Leifson test proposed by the international subcommittee on taxonomy of staphylococci and micrococci (Anon, 1965).

Molecular Identification of Isolates by PCR

DNA Extraction

For PCR study template DNA was extracted from isolates of *S. hyicus* (Titball *et al.* 1989) and was stored in a micro-centrifuge tube at -20 °C for further use.

PCR Amplification and Visualization

Molecular level identification of isolates was done by polymerase chain reaction (PCR) (Sasaki *et al.* 2010) by using the primer mentioned in the Table 1.

Polymerase chain reaction (PCR) was conducted by preparing reaction mixture consisting of 3µl DNA template, 12.5 µl 2X master mix (Xcelris genomics, Ahmedabad, India), 1 µl each of specific forward and reverse primer (Xcelris genomics, Ahmedabad, India) and making the final volume up to 25 µl with nuclease free water in a PCR tube.

Table 1: Sequences of the primer for *nuc* gene identification of *S. hyicus*

Target gene	Primer	Sequence (5/ - 3/)	Product Size (bp)
nuc gene	hy-F1	CATTATATGATTTGAACGTG	793
	hy-R1	GAATCAATATCGTAAAGTTGC	

PCR tube containing the mixture was tapped gently, followed by quick spun at 8000 rpm for 3 to 5 seconds and the mixture was subjected to Thermocycler (Applied Biosystem, USA) with an initial denaturation by 95°C for 2 minutes followed by 30 cycles of 30 sec. at 95°C, 30 sec. at 52°C and 30 sec. at 72°C. The PCR reaction was completed by 2 minute incubation at 72°C in order to ensure full extension of the PCR products. The amplified products of desired sized were visualized by submarine gel electrophoresis in 1.5% agarose gel. Amplified DNA fragments of specific sizes were visualized with UV light by Gel documentation system (Bio-Imaging system Mini Lumi, Israel) and the image was captured using Alpha imager EP software (Alpha Innotech Corparation, Multi Image System, San Leandro, CA, USA).

Characterization of *S. hyicus* for Virulence Genes

All isolates of *S. hyicus* identified by species specific PCR were further characterized in respect to virulence genes encoding for exfoliative toxins (Andresen and Ahrens, 2004) by simplex PCR targeting *exhA*, *exhB*, *exhC* and *exhD* virulence genes for locus 316, 717, 525 and 588 bp respectively with a specific primer pair (Table 2).

Polymerase chain reaction was conducted in 25 µl reaction mixture consisting of 3µl DNA template, 12.5 µl 2X master mix (Xcelris genomics, Ahmedabad, India), 1 µl each of specific forward and reverse primer (Xcelris genomics, Ahmedabad, India) and making the final volume 25 µl with nuclease free water in a PCR tube. The mixture was then tapped gently, followed by quick spun at 8000 rpm for 3 to 5 seconds and subjected to thermo-cycler (Applied Biosystems, USA) with an initial denaturation by 94 °C for 3 minutes followed by 30 cycles of 1 minute at

Table 2. Sequences of primers in molecular characterization of *S. hyicus* virulence genes encoding exfoliative toxins

Target Gene	Primer	Sequence (5/ - 3/)	Product Size (bp)
exhA	MU4FA	GCTACTGGTTTTGTAGTTTCAC	316
	MU3RA	GTAACCTACAACTCTTAGAACC	
exhB	F2EB	AACACGCCAATAGAGAATGTATCAC	717
	MU3RB	TATCAAATCTTATACCAGTTAGAATATCTCC	
exhC	MU3FC	GAATAAATATTATGGAGTCTCTCCTGATC	525
	MU4RC	CCATAGTATTTCAATCCAAAATCAGTAC	
exhD	F2ED	GAACAAATATAATGGAAGAAACCCAC	588
	MU3RD	GATTTCCCTACGTGAATACCTACAATAC	

94°C, 1 minute at 56°C and 1 minute at 72°C. The PCR reaction was completed by 10 minute incubation at 72°C in order to ensure full extension of the PCR products. The amplified products of desired sized were visualized as described earlier.

Therapeutic Study

Therapeutic trial was conducted among randomly selected exudative epidermitis affected swine having 6 animals in a group based on *in-vitro* antibiotic sensitivity test (CLSI, 2008) of the identified *S. hyicus* and on keen clinical observation. The efficacy of treatment was assessed on the basis of rate and speed of recovery of the affected animals as well as bacteriological examinations. Three parenteral antimicrobial agents showing the highest sensitivity among the tested antimicrobials namely amoxyclav (intramuscularly @ 8.75 mg/kg body weight daily) in first group, ceftriaxone and tazobactam combination (intramuscularly @ 10 mg/kg body weight daily) in second group and gentamicin (intramuscularly @ 4mg/kg body weight twice daily) in third group were selected for therapeutic trial. All the groups were provided with supportive treatment with a combination of chlorpheniramine maleate intramuscularly @ 30 mg total dose, multivitamin (vitamin A 2.5 lac IU, vitamin D3 25000 IU, vitamin E 100 IU, biotin 12.5 mcg) @ 1ml intramuscularly on alternate days for 5 injections, topical application of povidone iodine antiseptic ointment twice daily and oral rehydration solution based on degree of dehydration till the complete recovery of the case or up to 15 days which one is necessary. Six exudative epidermitis infected animals were kept as infected control where only supportive therapy was provided. After completion of the therapeutic trial all animals which were not recovered and not included in the therapeutic trial, also treated with best suited medicine combinations based on the results obtained from the above trial.

RESULTS AND DISCUSSION

Thirty four numbers of swine out of 343 numbers of pigs examined were found to be affected with exudative epidermitis based on clinical examination and molecular diagnostic techniques. Initially the disease exudative epidermitis was detected based on the clinical symptoms and later stage on isolation, identification and virulence genes characterization of *S. hyicus* from the affected site. Identification was done on the basis of morphology, culture characteristics, bio-chemical properties as described in the materials and methods and finally with specific PCR. Out of 34 pigs found to be positive for exudative epidermitis; erythema and swelling at the site of infection was observed in 34 pigs (100.00 %). Twenty one pigs (61.76 %) showed the symptoms of formation of brownish, greasy and odorous exudate along with exfoliation of the skin (Figure 1).

Symptoms of catarrhal inflammation of eyes were observed in 9 (26.47 %) pigs and 32 (94.12 %) pigs were found to be dehydrated. Dullness and anorexia were found in all the

34 affected pigs (100.00 %). The prevalence of exudative epidermitis lesions were found to occur mostly on the face (52.94 %) and least on abdomen (5.88 %) region. Predominant incidences of lesions in face of exudative epidermitis affected swine in the present findings were in agreement with the previous findings (Underdahlnl, 1963).

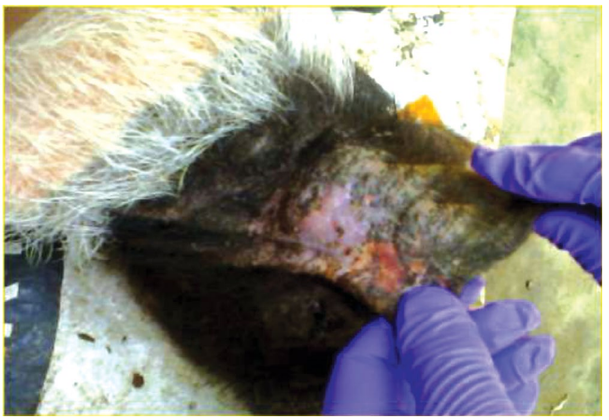

Fig. 1. Swine showing the clinical symptoms of exudative epidermitis on the ear

Injury of the skin around the mouth caused by sharp teeth of littermates while competing for teat of mother might be the predisposing cause of lesions of exudative epidermitis predominantly on the face. Significant reddening of the skin, catarrhal inflammation of the eyes as recorded in the present study was also reported earlier (Victor *et al.* 2013). Significant dehydration observed in the present investigation might be due to the fact that the affected pigs rapidly became depressed and refuse to eat. In the present investigation from the 34 numbers of clinically exudative epidermitis affected swine 39 *S. hyicus* were isolated of which 33 *S. hyicus* isolated as single isolate and 6 *S. hyicus* isolated along with *S. aureus*. Simultaneously, from the rest 309 pigs, which were not showing the clinical symptoms of exudative epidermitis, 73 numbers of *S. hyicus* were isolated singly. The *S. hyicus* were initially identified on the basis of their growth on *S. hyicus* selective medium.

During the study 110 *S. hyicus* isolates (98.21%) were found to produce white pigment on nutrient agar, while the rest two (1.78 %) could exhibit yellow pigment. None of the isolates showed zone of haemolysis on 5 per cent sheep blood agar. All the 112 isolates (100.00%) 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 °C.

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. Characteristics growth of white pigmented colonies of *S. hyicus* surrounded by a zone of precipitated lipid material on *S. hyicus* selective media observed in the present finding is of typical for *S. hyicus* (Devriese and Oeding, 1976; Sasaki *et al.* 2010). *Staphylococcus aureus* isolates were identified based on their growth on Mannitol Salt Agar (MSA) and Baird-Parker Agar (BPA).

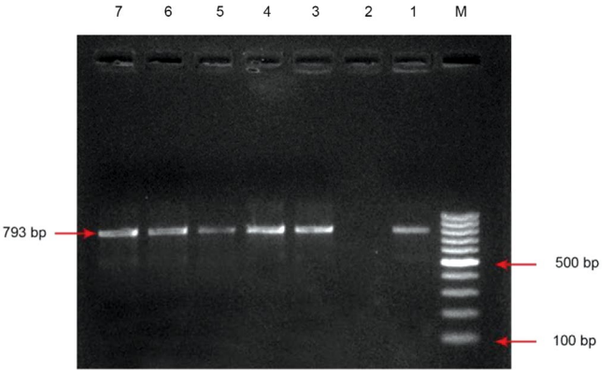

Fig. 2. Picture showing 793bp fragments of *S. hyicus nuc* gene

Lane M: 100 bp marker

Lane 1, 3, 4, 5, 6 and 7: Positive sample

Lane 2: Negative control

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 were observed surrounded by a whitish precipitate zone. In the present study, all the strains of *S. hyicus* (39) associated with clinical cases of exudative epidermitis (EE) were subjected for molecular identification by polymerase chain reaction (PCR) along with few (36) randomly selected isolates of *S. hyicus* recovered from apparently healthy animals were also tested for molecular

confirmation targeting *nuc* gene. During the molecular study, it was revealed that all the selected *S. hyicus* isolates (75), irrespective of health status of the swine showed a successful amplification of internal fragments with the expected size (793 bp) with a primer pair specific for *S. hyicus* (Figure 2).

The thermonuclease *(nuc)* gene has shown moderate diversity and has been well conserved among members of the genus staphylococcus. Therefore, this gene is to be a suitable PCR target for species identification of *S. hyicus* (Wegener *et al.* 1993). All the *nuc* gene positive *S. hyicus* isolates (39), recovered from pigs with suspected exudative epidermitis cases and few randomly selected *S. hyicus* isolates (36) of apparently healthy animals were further characterized in respect to certain virulence genes (*exhA*, *exhB*, *exhC* and *exhD*) encoding for exfoliative toxins by simplex PCR. Screening for exfoliative toxins was done to establish the association of the virulence gene positive *S. hyicus* isolates with exudative epidermitis in pig. Out of 39 *S. hyicus* isolates recovered from clinical cases of exudative epidermitis, 34 *S. hyicus* isolates were found to be positive for virulence genes of which, 6 isolates were positive for *exhA* gene and 28 isolates were positive for *exhD* gene with anexhibited band size of 316 bp and 588 bp respectively (Figure 3).

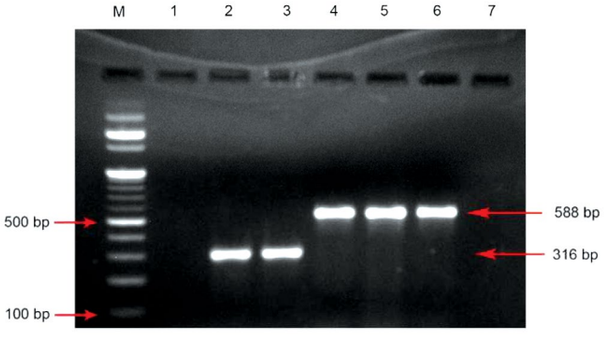

Fig. 3. Picture showing 316bp fragments of *exhA* gene and 588bp fragments of *exhD* gene of *S. hyicus*

Lane M: 100 bp marker
Lane 1: Negative sample
Lane 2 & 3: Test isolate positive for *exhA* gene
Lane 4 to 6: Test isolate positive for e*xhD* gene
Lane 7: Negative control

None of the isolates were found to be positive for exfoliative toxin producing genes *exhB* and *exhC*. However, five (5) *S. hyicus* isolates isolated from suspected exudative epidermitis pigs could not reveal the presence of any of the virulence genes. The virulence genes also could not be detected in any of the *S. hyicus,* isolated from apparently healthy animals. Exudative epidermitis affected swine may harbour both virulence gene positive *S. hyicus* along with avirulent *S. hyicus* in the same group of exudative epidermitis affected pigs (Tanabe *et al.* 1996).

But the isolation rate of *S. hyicus* positive for virulence genes from swine suffering from exudative epidermitis was higher than that of avirulant *S. hyicus* (Andresen and Ahrens, 2004). Higher incidence of *exhD* virulence genes occurrence might be due to the fact that *exhD* positive *S. hyicus* were more frequently isolated from pigs with exudative epidermitis than that of other virulence genes (Kanbar *et al.* 2006; Straw *et al.* 2006). Role of avirulent strain of *S. hyicus* along with the virulent strain found in the present study is still unknown for establishment of disease (Wegener and Skov-Jensen, 1992). Contradictory finding of *exhB* gene was the most prevalent, followed by *exhD, exhA, shetb* and *exhC* was also found in earlier studies among the virulence positive *S. hyicus* isolated from exudative epidermitis affected swine. In spite of the high colonization of *S. hyicus* in healthy pigs, no pigs developed exudative epidermitis.

The suggested reason might be that either the strains were avirulent or immunity of the pigs plays a major role for prevention of occurrence of the disease (Tanabe *et al.* 1996). Isolation of *S. hyicus* from the healthy pig (most frequently from the young piglets) might be due to the fact that *S. hyicus* is an indigenous bacterium of healthy piglets (Sha *et al.* 2013). All the 112 *S. hyicus* isolates isolated from both clinical cases of exudative epidermitis as well as from the apparently healthy animals were subjected to drug sensitivity tests by disc diffusion techniques and the results were presented in Table 3.

The most of the *S. hyicus* isolates recovered in the present study indicated a high sensitivity to most of the antimicrobial agents except neomycin and penicillin. This might be due to the fact that these antimicrobial agents have so far not been used in the treatment of swine diseases. Inhibition of *S. hyicus* by many antibiotics, including amoxicillin,

ampicillin, erythromycin, lincomycin, penicillin, tylosin, trimethoprim, sulfonamide, the aminoglycosides and cephalosporins were described earlier (Davies, 2013).

Table 3. *In-vitro* antibiotic sensitivity pattern of *S. hyicus* (n = 112) isolates

Name of antimicrobials	No. of isolates sensitive to
Amoxyclav	112 (100.00)
Amoxicillin & Sulbactum	68 (60.71)
Ceftriazone & Tezobactam	112 (100.00)
Clindamycin	13 (11.61)
Cefixime	11 (9.82)
Ceftriaxone & Sulbactum	101 (90.18)
Cefpodoxime	92 (82.14)
Enrofloxacin	73 (65.18)
Gentamicin	102 (91.07)
Lincomycin	28 (25.00)
Mupirocin	107 (95.53)
Neomycin	10 (8.93)
Novobiocin	90 (80.36)
Penicillin-G	7 (6.25)

(Figure in the parenthesis indicate percentage)

The efficacy of amoxicillin and clavulanic acid in bacterial dermatitis were also described earlier (Beco *et al.* 2013). The high sensitivity to amoxyclav, combination of ceftriazone and tazobactam and amoxicillin and sulbactam combination was probably because these antibiotics were less frequently used in swine treatment in this region and the isolates had no chance of developing drug resistance. On the basis of *in-vitro* drug sensitivity test, three antimicrobial agents were selected for therapeutic trial and on that basis suitable line of treatment with other supportive therapy was recommended for the treatment of exudative epidermitis of pigs. From the results of the treatment trial conducted in the present study, it was observed that amoxyclav could induce recovery earlier than ceftriaxone and tazobactam combination though the recovery rates in both the cases were 100.00 per cent. Therefore, based on the prevailed situations like availability of antibiotics in the local market, price of the medicines (ceftriaxone and tazobactam combination is costlier than amoxyclav) either amoxyclav or ceftriaxone and tazobactam combination with supportive therapies could be suggested for the treatment of exudative epidermitis in swine.

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REFERENCES

Andresen, L.O. and Ahrens, P. 2004. A multiplex PCR for detection of genes encoding exfoliative toxins from *S. hyicus*. *J. Applied Microbiol.,* **96**: 1265-1270.

Andresen, L.O., Wegener, H.C. and Bille-Hansen, V. 1993. *Staphylococcus hyicus* skin reactions in piglets caused by crude extracellular products and by partially purified exfoliative toxin. *Microb. Path.,***15**: 217–225.

Anon. 1965. International Subcommittee on Texonomy of Staphylococci and Micrococci. Minutes of first meeting. *IBBNT*., **15**: 109-110.

Beco, L., Guaguere, C., Mendez, L., Noli, C. and Vroom, M. 2013. Suggested guidelines for using systemic antimicrobials in bacterial skin infections (2): antimicrobial choice, treatment regiments and compliance. *Vet. Rec.*, **172**(6): 156-160.

CLSI/Clinical and Laboratory Standards Institute. 2008. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. 3rd edn. Approved standard M31-A3. Clinical and Laboratory Standards Institute, Wayne, P.A.

Collins, C.H. and Lyne, P.M. 1970. Microbiological Methods, Laboratory techniques series. 4th ed. Butterworths, London.

Cowan, S.T. and Steel, J.K. 1993. Manual for Identification of Medical Bacteria. 3rd edn. 1993 edited and revised by Barrow, GI, Feltham RKA. London, UK. The Syndics of the Cambridge University Press.

Davies, P.R. 2013. Overview of Exudative Epidermitis. 2010-2014 Merck Sharp and Dohme Corp., a subsidiary of Merck and Co., Inc., Whitehouse Station, N. J., U. S. A. Privacy Terms of Use Permissions. Merck Veterinary Manual.

Devriese, L.A. 1977a. Antibiotic susceptibility of *S. hyicus* strains isolated from exudative epidermitis cases in Belgium. *Vlaams Diergen Tijds*., **46**: 143–144.

Devriese, L.A. 1977b. Isolation and identification of *S. hyicus*. *Am. J. Vet. Res.,* **38**(6):787-792.

Devriese, L.A. and Oeding, P. 1976. Characteristics of *S. aureus* strains isolated from different animal species. *Res. Vet. Sci*., **21**: 284-291.

Jones, L.D. 1956. Exudative epidermitis of pigs. *Am. J. Vet. Res.,* **17**: 179–193.

Kanbar, T., Voytenko, A.V., Alber, J., Lammler, C., Weiss, R., Zschock, M., Shilov, I.A., Dmitrenko, O.A. and Gintsburg, A.L. 2006. Prevalence of genes encoding exfoliative toxins among *S. hyicus* isolated in Russia and Germany. *J. Vet. Med. B Infect. Dis. Vet. Public Health*, **53**(9): 429-433.

Park, J., Friendship, R.M., Poljak, Z., Weese, J.S. and Dewey, C.E. 2013. An investigation of exudative epidermitis (greasy pig disease) and antimicrobial resistance patterns of *S. hyicus* and *S. aureus* isolated from clinical cases. *Can. Vet. J*., **54**: 139–144.

Penny, R.H.C. and Muirhead, M.R. 1986. Skin. In: Leman, A.D., Straw, R.D., Glock, W.L., Mengeling, W.L., Penny, R.H.C. and Scholl, E., eds. Diseases of Swine. Ames, Iowa: Iowa State University Press. 82-101.

Sasaki, T., Tsubakishita, S., Tanaka, Y., Sakusabe, A., Ohtsuka, M., Hirotaki, S., Kawakami, T., Fukata, T. and Hiramatsu, K. 2010. Multiplex- PCR Method for Species Identification of Coagulase- Positive Staphylococci. *J. Cl. Microbiol.* **48**: 765-769.

Sha, A.A., Atony, L. and Illayraja, S. 2013. Bacterial inter digital pyoderma in a sumatran tiger (*Panthera trgris sumatrae*). *Intas Polivet*, **14**(II): 407-408.

Straw, B.E., Zimmerman, J.J., D'Allaire, S. and Taylor, D.J. (2006). Diseases of swine. 9th ed. Blackwell Publishing. 835-837.

Tanabe, T., Sato, H., Watanabe, K., Hirano, M., Hirose, K., Kurokawa, S., Nakano, K., Saito H. and Maehara, N. 1996. Correlation between occurrence of exudative epidermitis and exfoliative toxin-producing ability of *S. hyicus*. *Vet. Microbiol*., **48**: 9-17.

Thapa, N.K. 2001. Investigation of "Greasy Pig Disease**"** Bhutan. *J. Renewable Natural Resources,* **1**(3): 1-7.

Titball, R.W.; Hunter, S.E.C.; Martin, K.L.; Moris, A.D.; Shuttle worth, A.D.; Rubdige, T.; Anderson, D.W. and Kelly, D.C. 1989. Molecular cloning and nucleotide sequence of the alpha toxin of *Clostridium perfringens. Infect. Immunol*., **57**(2): 367-376.

Underdahlnl, N.R.; Graceo, D. and Youngg, A. 1963. Experimental transmission of exudative epidermitis of pigs. *J. Am. Vet. Med. Assoc*., **142**:754-762.

Victor, I., Akwuobu, C.A., Akinleye, O.A., Tyagher, J.A. and Buba, E. 2013. Management of exudative epidermitis (greasy pig disease) in 4 week old piglets. *J. Vet. Med. Animal Health.,* **5**(7): 180-185.

Watanabe, T., Sato, H., Hatakeyama, Y., Matsuzawa, T., Kawai, M., Aizawa, C., Danbara, H. and Maehara, N. 2000. Cloning of the gene coding for *S. hyicus* exfoliative toxin B and its expression in *E. coli*. *J. Bacteriol.,* **182**(14): 4101-4103.

Wegener, H.C. and Skov-Jensen, E.W. 1999. Exudative epidermitis. In: Straw, B.E., D'Allaire, S., Mengeling, W.L. and Taylor, D.J. (Eds.), Diseases of Swine. 8th ed. Iowa State University Press, Ames. 469-474.

Wegener, H.C. and Skov-Jensen, E.W.1992. A longitudinal study of *S. hyicus* colonization of vagina of gilts and transmission to piglets. *Epidemiol. Infect.,***109**: 433-444.

Wegener, H.C., Andresen, L.O. and Bille-Hansen, V. 1993. *Staphylococcus hyicus* virulence in relation to exudative epidermitis in pigs. *Can. J. Vet. Res.,* **57**: 119-125.