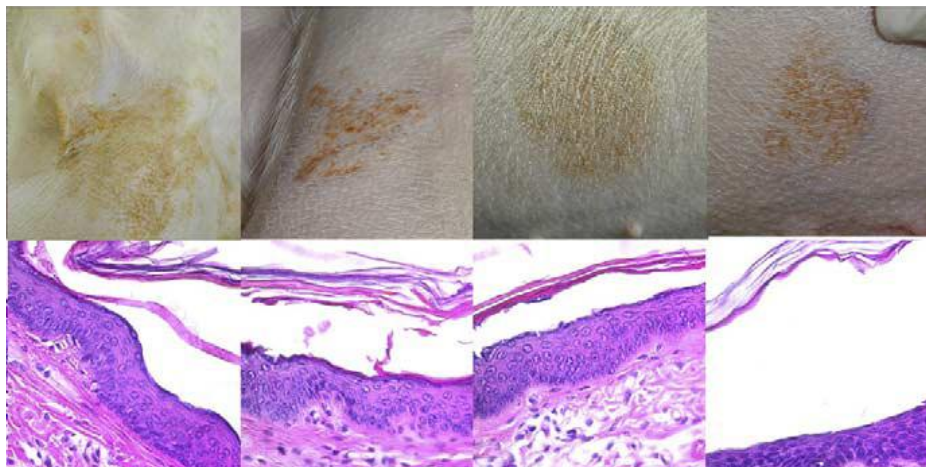Sveriges lantbruksuniversitet

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Prevalence of toxin-producing strains and antimicrobial resistance in isolates of *Staphylococcus hyicus* from pigs with exudative epidermitis and from healthy pigs

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Förekomst av antibiotikaresistens och toxinproducerande stammar i isolat av *Staphylococcus hyicus* från grisar med exsudativ epidermit och från friska grisar

Christina Björklund

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SUMMARY

Virulent strains of *Staphylococcus hyicus* produce exfoliative toxins and cause a potentially fatal skin disease in piglets known as exudative epidermitis. Vaccines and antimicrobials are used to control the disease. Antimicrobial resistance and the prevalence of several different toxin genes and toxigenic strains make it problematic. The aim of this literature study was to identify potential differences between countries and between healthy and diseased pigs regarding antimicrobial resistance and toxin-producing strains. Great differences could influence vaccine production, treatment strategies and surveillance programs.

The prevalence of virulent strains were much higher in diseased pig isolates but were not uncommon among healthy pigs. No evidence for differences in the distribution of toxin genes between healthy and diseased pigs was observed but differences between countries were obvious. Some toxin genes were found to be dominant in certain countries. Differences in resistance patterns were seen between healthy and diseased pig as well as between countries but the antimicrobials with the highest resistance rates were almost the same in all observed countries. These findings show the importance of national and outbreak-related identification of the disease-causing strains to ensure that the most effective antimicrobial is used and that vaccines are produced from the right strains. More research is needed to follow the development but also to investigate why some pigs stay healthy despite carrying toxin-producing strains. There might be natural factors involved that could be used to prevent the disease and in turn decrease the need for antimicrobials in the future.

SAMMANFATTNING

Exsudativ epidermit är en potentiellt dödlig hudsjukdom som framförallt drabbar smågrisar. Hudskadorna orsakas av exfoliativa toxiner som produceras av virulenta stammar av *Staphylococcus hyicus*. Vacciner och antibiotikabehandling används idag för att kontrollera sjukdomen men resistensutveckling och förekomst av flera olika toxingener och toxinproducerande stammar försvårar arbetet. Den här litteraturstudien syftar till att undersöka skillnader i förekomst av virulenta stammar och antibiotikaresistens mellan olika länder samt mellan friska och sjuka grisar. Stora skillnader skulle vara av betydelse för vaccinproduktion, behandlingsstrategier och övervakningsprogram.

Virulenta stammar kan isoleras från både sjuka och friska grisar men isoleringsfrekvensen är mycket högre hos sjuka grisar. Skillnader i fördelningen av toxingener mellan stammar från friska och sjuka grisar kunde inte påvisas men internationella skillnader var tydliga. Olika resistensmönster har observerats för friska och sjuka grisar inom begränsade områden och mellan länder men de innefattade i stort sett samma antibiotikagrupper. Forskningsresultaten styrker behovet av både nationell och utbrottsspecifik identifiering och analys av sjukdomsorsakande stammar. Detta skulle säkerställa att den mest effektiva antibiotikabehandlingen sätts in och att vacciner produceras från rätt stammar. Fortsatt forskning behövs för att följa utvecklingen men även för att utreda varför vissa grisar förblir friska trots att de bär på toxinproducerande stammar. Det kan finnas naturligt skyddande faktorer som skulle kunna användas i förebyggande syfte vilket i sin tur skulle kunna minska behovet av antibiotikabehandling i framtiden.

INTRODUCTION

Staphylococcus hyicus (*S. hyicus*) is the causative agent of exudative epidermitis (EE), an acute dermatitis that primarily affects suckling and weaned piglets. The disease, also known as greasy pig disease, is characterized by exfoliation of the skin resulting in massive sebaceous secretions causing dehydration which may be fatal. Both morbidity and lethality range from 10 to 90% and among surviving piglets retarded growth is often seen. *S. hyicus* can be divided into virulent and avirulent strains. Virulent strains induce disease by producing exfoliative toxins and are therefore also known as toxin-producing or toxigenic strains. Exfoliative toxins specifically cleave a desmosomal intercellular adhesion molecule in the skin of pigs known as swine desmoglein 1 which results in the separation of keratinocyte cells in the epidermidis (Fudaba et al., 2005). So far, a total of six toxins have been discovered and designated ExhA, ExhB, ExhC, ExhD (Andresen, 1998; Andresen & Ahrens, 2004), SHETA and SHETB (Sato et al., 2000). Recent research has however suggested that the SHETA-encoding gene (*sheta*) is a subtype of *exhB* (Onuma et al., 2011).

To prevent outbreaks in pig herds vaccines can be used. The effect depends on the possibility to detect and separate the virulent strains from the avirulent ones and to identify the specific toxins that they produce (Wegener et al., 1993; Andresen & Ahrens, 2004). Affected animals are treated with antimicrobials but the outcome is threatened by rising resistance. The aim of this literature study is to identify potential differences between countries and between healthy and diseased pigs regarding antimicrobial resistance and toxin-producing strains. Such differences could influence the procedures when outbreaks are investigated, when choosing treatment and when producing vaccines.

MATERIAL AND METHODS

Databases used in the search for articles include Web of Knowledge, Scopus, Science Direct, and PubMed. Keywords used separately or in combination: exudative, exfoliative, epidermit*, skin, blistering, staphylococc*, Staphylococcus hyicus, S. hyicus, toxin*, toxin-produc*, prevalence, occurance, findings, surveillance. The search has been refined by "Veterinary Sciences" in Web of Knowledge and by "Animals" in PubMed. Articles have also been found using certain author names; Aarestrup, Ahrens, Andresen, Futagawa-Saito, Noble, Wegener, Tanabe, Nishifuji, and Watanabe. Some of the articles were found among references of other articles. Articles cited several times by other authors have been valued higher than those less cited.

LITERATURE REVIEW

Toxin-producing *S. hyicus*

Prevalence of toxigenic S. hyicus in different countries

In 2005 Andresen presented the results of the first multi-country observation study regarding prevalence of toxigenic *S. hyicus* in healthy and diseased pigs (pigs with EE). Samples from countries in three continents; Europe (Belgium, Germany, UK, Croatia, Slovenia), Asia (Japan, Korea and India) and North America (USA) were tested for toxin genes. The isolates were both field samples and laboratory strains collected during four decades, from the 1960's up to and through the 1990's. The isolates were screened for toxin genes *exhA, exhB, exhC* and *exhD* by multiplex PCR (polymerase chain reaction) and immunoblotting (Table 1). More than half of all isolates from cases of EE were toxigenic. The differences between countries in this aspect were slim.

A wide spread of toxin genes was seen in German and Belgian isolates from diseased pigs as all four toxin genes were present (Andresen, 2005). The German isolates were dominated by *exhD* followed by *exhB*. These genes were the least prevalent in the Belgian isolates where *exhA* was the dominating gene (Table 1). Somewhat surprising, *exhD* was the most prevalent gene among toxigenic isolates from healthy Belgian pigs (5/6). Samples from healthy pigs were only available from Japan, Belgium and Croatia. The number of toxigenic isolates was much lower. Only 10% (8/83) were positive, two isolates contained *exhA* and six contained *exhD*. The same distribution of toxin genes was seen in 189 strains from 200 pig carcasses in two slaughterhouses in Switzerland (Hassler et al., 2008). Among 31 toxigenic strains six were *exhA*-positive and 25 were *exhD*-positive.

Table 1. Prevalence of toxin genes in isolates of S. hyicus from pigs affected with EE in different countries (modified from Andresen, 2005)

Country	No of toxigenic isolates[a] (%)	No of isolates positive for			
		exhA	*exhB*	*exhC*	*exhD*
Germany	24/44 (55)	4	6	4	10
Belgium	8/17 (47)	4	1	2	1
UK	3/5 (60)	2	0	1	0
Croatia	4/6 (67)	1	1	0	2
Slovenia	3/6 (50)	0	0	0	3
USA	5/8 (63)	1	4	0	0
Japan	5/10 (50)	5	0	0	0
Total	54/98 (55)	17	12	7	16

[a] number of toxigenic isolates of total number of isolates tested

The occurrence of toxin genes in Russia and Germany were investigated by Kanbar et al. (2006). The isolates from Russia were not dated but the German isolates were collected between 1978 and 2005. Three strains from healthy pigs and 26 from diseased pigs were

analysed by multiplex PCR for carriage of all four Exh-encoding genes (*exhA-D*). Toxin production was only seen in strains from diseased pigs where 77% were toxigenic. Of the 19 Russian strains from pigs with EE 90% contained toxin genes and all were *exhD*-positive. Three of the seven German strains from diseased pigs were toxigenic, two were *exhD*-positive and one was positive for *exhC*.

Prevalence of toxigenic S. hyicus in Denmark

A wide spread of toxin genes has been found in Denmark. In two connected studies (Andresen, 1998; Andresen & Ahrens, 2004), isolates from 60 pigs were analysed. Each pig represented an outbreak of EE in Denmark from late 1996 to mid 1997. A total of 584 isolates were screened for toxin production. In the first study, Andresen (1998) used ELISA and immunoblotting methods and were only able to identify production of ExhA, ExhB and ExhC (Table 2). He found 46% of the total number of isolates to be toxigenic but suspected the existence of a fourth toxin (ExhD), not detectable by the methods used, among the non-toxigenic isolates. By using a multiplex PCR, Andresen together with Ahrens (2004) identified production of ExhD in 13 of the 17 previously negative specimens. The combined results showed that toxin producing isolates of *S. hyicus* were present in 93% of the specimens. ExhA, ExhB, ExhC and ExhD were found in 12, 20, 11 and 13 specimens, respectively.

Prevalence of toxigenic S. hyicus in Japan

The first Japanese study was conducted by Tanabe et al. (1996) and included 131 strains isolated from 370 healthy pigs on two farms and one strain each from 37 diseased pigs on eight farms. For the analysis, 46 strains were randomly selected and six reference strains including the type strain for *S. hyicus* were added to the diseased pig strains. By inoculation in 1-day-old chickens and by immunodiffusion, toxin production was recognized in 81% of all strains. The frequency was very high in strains from both healthy pigs (76%) and diseased pigs (88%). Six toxin-producing strains including two strains isolated from healthy pigs were selected and experimentally inoculated into the ears of healthy piglets. All four strains from diseased pigs caused disease. The two strains from healthy pigs did not produce disease in half of the infected piglets. These results lead the authors to suspect some unidentified factors to be involved in development of EE.

A larger nationwide surveillance study including pigs from 38 farms was conducted by Futagawa-Saito et al. (2007). Samples were collected from 217 healthy pigs and 207 pigs with EE between 1994 and 2002. They recovered 207 isolates from 76 piglets with the majority of isolates originating from diseased pigs (n=161). Carriage of *exhA, exhB, exhC, exhD* and *shetb* were identified by PCR, multiplex PCR and dot blot hybridization. In all, 73% were found to be toxigenic. The frequency was more than four times higher in diseased pig isolates (88%) when compared to healthy pig isolates (20%). The result for diseased pigs was almost identical to that of Tanabe et al. (1996) but the figures for healthy pigs were remarkably lower. Of the nine toxigenic isolates from healthy pigs five were *exhA*-positive, two were *exhB*-positive and two were positive for *exhD*. This corresponded well with the results for diseased pig isolates (Table 2).

Onuma et al. (2011) investigated the distribution of toxin genes among 69 toxigenic strains collected from pigs with EE during the period 1983-2009. By dot blot hybridization and multiplex PCR they could identify five toxin genes (*exhA-D* and *shetb*). In contrast to previous results from Japan (Futagawa-Saito et al., 2007) they found *exhB* to be the most prevalent toxin gene (Table 2). They also observed interesting changes in prevalence of toxin genes during the 26 years covered by their material. They compared three periods; 1983-1990 (n=11), 1991-2000 (n=34) and 2001-2009 (n=24). The ExhB-encoding gene dominated during the entire period but decreased from 64-65% during the first and second period to 38% after 2000. The ExhD-encoding gene went from being completely absent during the first period to be prevalent in 32-33% of strains in the second and third period. In the first and the third period, *exhA* were seen in around 30% but was not detected in any of the isolates from the 1990's. The almost complete absence of *exhC* and *shetb* is in accordance to previous results of Andresen (2005) and Futagawa-Saito et al. (2007). However, the results for *exhA* and *exhB* are in contrast to the results of Futagawa-Saito et al. (2007) regarding the 1990's (Table 2).

Table 2. Distribution of toxin genes in toxigenic S. hyicus isolated from pigs affected with EE in Japan during 1994-2002 (Futagawa-Saito et al., 2007) and 1983-2009 (Onuma et al., 2011) and in Denmark (Andresen, 1998)

Country	Isolates collected between (no of isolates)	Percentage of toxin genes				
		exhA	*exhB*	*exhC*	*exhD*	*shetb*
Japan	1994-2002 (141)	48,9	27,0	0,7	23,4	0,0
	1983-2009 (69)	14,5	55,1	1,5	27,5	2,9
Denmark	1996-1997 (268)	24,6	49,6	25,7	n.d.[a]	n.d.[a]

[a] not determined

Antimicrobial resistance

Resistance rates and resistance patterns

S. hyicus isolated from pigs have a remarkably higher frequency of antimicrobial resistance when compared to *S. hyicus* isolated from cattle and chicken (Teranishi et al., 1987, Schwarz & Blobel, 1989). Swine strains of *S.hyicus* also show a much higher frequence of multiple resistance. In Japan, Teranishi et al. (1987) examined resistance to 21 antimicrobials among 124 swine strains from both healthy and diseased pigs, 34 bovine strains and 54 chicken strains. They found resistance in 74% of swine strains compared to only 15% each of strains from cattle and chicken. Multiple resistance (≥2) was seen in 73% of swine strains and in 60% of cattle strains. Schwarz & Blobel (1989) observed a similar situation in German *S. hyicus* cultures from 20 cattle with skin lesions and 32 pigs with EE. Resistance to all the nine antimicrobials used in the study were seen among porcine cultures while bovine cultures only showed resistance against two. Multiple resistance (≥2) was seen in 78% of porcine cultures

compared to only 10% of bovine cultures. Resistance to three or more agents were not seen in any of the bovine cultures but in 44% of porcine cultures.

Denmark is the only country that includes *S. hyicus* in their surveillance program for antimicrobial resistance. The program known as DANMAP (Danish integrated antimicrobial resistance monitoring programme) started in late 1995 and has since then monitored resistance in indicator bacteria (*Escherichia coli* and *Enterococcus* spp.), zoonotic bacteria (*Campylobacter* spp.*, Salmonella* spp. and *Yersinia* spp.) and animal pathogens (*Escherichia coli, Staphylococcus aureus, S. hyicus, Actinobacillus* spp. and coagulase-negative staphylococci) isolated from pigs, cattle and chicken. Yearly data is available for the period 1996-2008. The first results, published in 1997, revealed an overall higher frequency of resistance in isolates from pigs compared to cattle and chicken (Aarestrup et al., 1998). Only *S. hyicus* isolates from diseased pigs are included in the programme, which makes a comparison between species or between healthy and diseased pigs impossible.

DANMAPs data show that major changes in resistance have taken place over the time period 1996-2008, especially for sulphonamides, trimethoprim and erythromycin. Resistance to sulphonamides drastically dropped from 30% in 1998 to 5% the year after and has since then not been detected with the exception of 4% in 2001 and 2% in 2003. Trimethoprim resistance was seen in 40-50% of isolates during the first three years of observation (1996-98) but decreased to 22% in 1999. The rate remained at around 20% the following years up to 2007-08 when it returned to approximately 40-50%. Erythromycin resistance was up at 62% in 1997 but dropped drastically in 1998 to around 20%. The level was rather stable up to 2007-2008 when a significant increase was observed. Penicillin resistance has dominated the picture during the whole time period and stood, together with streptomycin and tetracycline resistance, for the least drastic changes. However, resistance to penicillin, streptomycin and tetracycline have fluctuated within the range of 54-85%, 26-53% and 21-47%, respectively.

Differences in resistance between *S. hyicus* from healthy pigs and pigs affected with EE have been observed. Wegener and Schwarz (1993) found higher resistance rates to penicillin, tetracycline and streptomycin among diseased pig isolates when investigating isolates from 28 healthy and 72 diseased pigs on 100 Danish herds (Table 3). Teranishi et al. (1987) found the opposite, but both studies found resistance to macrolides and lincosamides to be approximately the same in strains from both sources. Teranishi et al. (1987) used strains collected from 74 healthy and 50 diseased Japanese pigs between 1979 and 1984. Resistance to at least one antimicrobial was seen in 74% of all strains, in 89% of strains from healthy pigs and in 52% of strains from diseased pigs. For ampicillin, tetracycline and streptomycin the rates were much higher in strains from healthy pigs. For erythromycin, resistance was approximately the same in strains from both sources (Table 3).

A possible connection between toxin gene carriage and resistance gene carriage was investigated by Futagawa-Saito et al. (2009). They used 207 *S. hyicus* strains and nine groups of common antimicrobials. Samples included 150 toxigenic and 57 non-toxigenic strains from healthy and diseased pigs collected between 1994 and 2002. In accordance with the theory

they found higher resistance in toxigenic strains than in non-toxigenic strains to chloramphenicol (33% vs 0%) and trimethoprim-sulphamethoxazole. Non-toxigenic strains showed higher resistance than toxigenic strains to kanamycin (47% vs 9%) and erythromycin. For penicillin/ampicillin there was no difference between the two groups (Table 3). The authors came to the conclusion that no correlation could be found. However, higher rates of multiple resistance (≥2) was seen in toxigenic strains. This was in accordance to results of Wegener and Schwarz (1993). Their isolates were not identified as toxigenic or non-toxigenic but multiple resistance (≥3) was more prevalent in isolates from pigs with EE than from healthy pigs. Only one study show opposite results (Teranishi et al., 1987) (Table 3).

Table 3. Occurrence of resistance to common antimicrobials in S. hyicus isolated from healthy and diseased pigs in Denmark (Wegener & Schwarz, 1993) and Japan (A: Teranishi et al., 1987; B: Futagawa-Saito et al., 2009)

Period	Percentage resistant *S. hyicus* (healthy/diseased)				Percentage of multiple resistance (healthy/diseased)	
	PC[a]	TC[a]	EM[a]	SM[a]	≥ 2	≥ 3
Japan A	62/4	76/22	42/40	37/2	61/44	45/12
Japan B[b]	72/79	2/1	79/47	5/37[c]	75/89	-
Denmark	18/54	32/53	57/61	18/43	-	36/49

[a] PC; penicillin, TC; tetracycline, EM; erythromycin, SM; streptomycin.

[b] non-toxigenic strains are presented as healthy and toxigenic as diseased.

[c] sulphamethoxazole together with trimethoprim.

Futagawa-Saito et al. (2009) also observed differences regarding resistance patterns in multiple resistant strains. Five patterns exhibiting resistance to three or more antimicrobials were found to be dominant among identified patterns. Three of these five patterns were only seen in toxigenic strains. The other two patterns were seen in strains from both sources but with higher frequencies in healthy pig strains. Similar findings were reported by Teranishi et al. (1987). The dominant patterns in strains from diseased and healthy pigs included two and five antimicrobials, respectively. The dominant pattern in strains from one source were absent in the other. In a Danish study the dominating pattern with four antimicrobials was seen in 39% of strains from pigs with EE but in just 7% of strains from healthy pigs (Wegener & Schwarz, 1993).

Usage of antimicrobials and the development of resistance

In pig farming antimicrobials have long been used in feed for growth-promotion. The effect of this type of routine use on the development of resistance in *S. hyicus* became obvious when drastic measures were taken in Denmark and Japan in the late 1990's (Aarestrup & Jensen, 2002; Futagawa-Saito et al., 2009). Tylosin, a macrolide commonly used in pig feed in Denmark was banned for use as a growth-promoter in 1999. The association between consumption and resistance was apparent (DANMAP, 1999, 2002) (Figure 1). Between 1997 and 1998, consumption of tylosin dropped from 62 to 13 tons and the percentage of resistant *S. hyicus* decreased from 62 to 26%. After the ban, resistance has landed on around 15%. In

Japan, chloramphenicol was used in the same way as tylosin in Denmark. Resistance to chloramphenicol peaked at 82% in 1998, the same year it was banned as a growth-promoter. Four years later, in 2002, the rate was down at 18%.

Noble and Allaker (1992) investigated the prevalence of antimicrobial resistance in staphylococci isolated from pigs in two farms. Between 1987 and 1989 samples from piglets, weaners and sows were collected during four visits to each farm. The farms were only eight kilometres apart but differed regarding antibiotic policies. The first farm used antibiotic feed-stuff on a regular basis, mainly tylosin for growth-promotion but also trimethoprim and sulphadiazine for treatment. From this farm researchers collected an unusually high number of *S. hyicus* and clinical cases of exudative epidermitis were observed in weaners during the first three visits. In the second farm antimicrobial feed-stuffs were only seen during one visit, no cases of exudative epidermitis were seen and no *S. hyicius* were found. Of all staphylococci isolated from the two farms, the *S. hyicus* strains from the first farm showed the highest resistance rates. One strain resistant to penicillin, tetracycline, erythromycin, dalacin, streptomycin and chloramphenicol was found to be the cause of the cases of exudative epidermitis seen in the first farm.
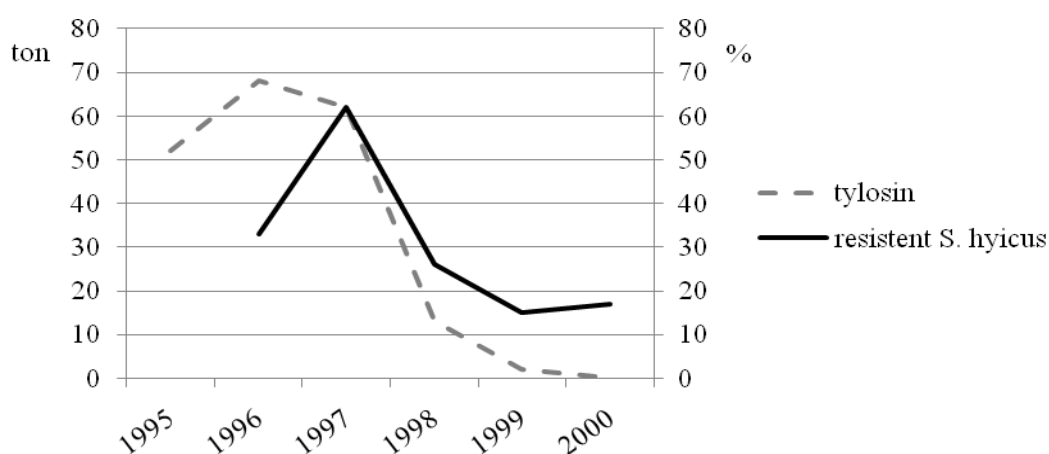

Figure 1. Occurrence of S. hyicus resistant to erythromycin and total consumption of tylosin for growth-promotion during 1996-2000 (modified from Aarestrup and Jensen (2002) and DANMAP (2002))

DISCUSSION

Research conducted during last two decades has drastically increased the knowledge in the field. Methods are now available to detect specific toxin genes and resistance patterns in each isolated *S. hyicus* strain. This has significantly improved the possibility to control this disease. Articles about prevalence of toxin production and antimicrobial resistance are still relatively few and research is mainly conducted in Japan and Denmark. It is therefore difficult if not impossible to provide an overview on the situation worldwide. Fluctuations as well as more dramatic changes in the prevalence of different toxin genes and resistance have been shown to occur over relatively short time periods (Aarestrup & Jensen, 2002; Onuma et al., 2011). This

makes comparisons of studies difficult since samples often date to different time periods (Aarestrup & Jensen, 2002). Different sample sizes and different methods in similar studies contribute to this difficulty. The occurrence of different toxin genes clearly depends on the amount of isolates and this is also commented on by Andresen (2005). Modern methods identify toxin-producing *S. hyicus* by detecting known toxin genes. Compared to the old method of experimental infection this new technology might result in misleading figures when conducting surveillance studies. This should be considered when comparing results between studies dating to different times.

Toxin-producing strains can be isolated from healthy as well as diseased pigs but the prevalence of virulent strains is higher in pigs with EE, sometimes as much as four times higher (Tanabe et al., 1996; Andresen, 2005; Kanbar, 2006; Futagawa-Saito et al., 2007). Despite small and uneven sample sizes in the only multi-country surveillance available (Andresen, 2005), an unexpected similarity in the total percentage of toxigenic strains in diseased pig isolates was displayed with an average of 55%. The existence of non-toxigenic strains (i.e. strains without any of the known toxin genes) in the skin of pigs affected with EE could easily be explained by new toxins yet to be discovered. To explain why toxin production is found among strains isolated from healthy pigs is more complicated. There must be an explanation to why 76% of 46 healthy pig isolates are toxigenic without causing symptoms in their hosts (Tanabe et al., 1996).

Toxin-producing *S. hyicus* has been found to be widely spread in several countries but some toxin genes seem to be more dominant in certain countries. In Japan, *exhA* and *exhB* have been found to be the most prevalent toxin genes followed by *exhD*. Different figures have been presented for *exhA* and *exhB* for the same time period but the collected results clearly prove their dominating position among toxigenic *S. hyicus* in Japanese pigs (Andresen, 2005; Futagawa-Saito et al., 2007; Onuma et al., 2011). Research from Denmark and Germany show a high prevalence of toxigenic *S. hyicus* with all four Exh-encoding genes circulating in both countries. In Germany *exhD* seems to be dominant followed by *exhB* (Andresen, 2005; Kanbar et al., 2006) and in Denmark *exhB* dominates followed by almost equally distributed *exhA, exhC* and *exhD* (Andresen, 1998; Andresen & Ahrens, 2004). These results are from a limited time period and for Denmark there is no other data available for comparison. Results from Russia (Kanbar et al., 2006) showed that 90% (17/19) of diseased pig isolates were *exhD*-positive. Even though the sample size was small, the high figure for one specific toxin gene still makes the result interesting. Distribution of toxin genes in healthy pig isolates often correspond to that of diseased pig isolates from the same country but *exhA* and *exhD* have been more frequently observed in the results available (Andresen, 2005; Hassler et al., 2008).

Antimicrobial resistance in swine strains of *S. hyicus* is widespread in the big pig producing countries covered by this literature study. In general, research shows a high prevalence of resistance towards mainly penicillin, tetracycline, streptomycin, macrolides/lincosamides and sulphonamides (Wegener & Schwarz, 1993; Aarestrup & Jensen, 2002; Futagawa-Saito et al., 2009;). Within this group of agents variations are seen between countries and between farms as a result of different antimicrobial usage. The use of tylosin and chloramphenicol in pig feed

for growth-promotion in Denmark and Japan resulted in elevated levels of resistance to these two antimicrobials (Aarestrup & Jensen, 2002; Futagawa-Saito et al., 2009). The fact that resistance levels dropped radically after the ban of growth-promotion usage gives hope for the future. It shows that drastic measures can and must be taken against undiscriminated antimicrobial use in pig farming and that these measures can pay off fast.

No evidence for any difference in antimicrobial resistance rates between isolates from healthy and diseased pigs have been found in the literature. The two studies available have produced almost completely opposite results (Teranishi et al., 1987; Wegener & Schwarz, 1993). The results of a similar study with known toxigenic and non-toxigenic strains were of no help in untangling this problem since their results also pointed in two directions (Futagawa-Saito et al., 2009). Their main aim was to investigate the possible association between toxin genes and antimicrobial resistance but no such association could be found. A higher prevalence in diseased pigs would not be too unexpected, if it would have been the case, since most diseased pig isolates often originate from pigs undergoing treatment or have a history of treatment with antimicrobials (DANMAP, 2004).

Multiple resistance (resistance to ≥2 or ≥3 antimicrobials depending on the authors) is common among swine strains of *S. hyicus* and is seen in isolates from both healthy and diseased pigs (Schwarz & Blobel, 1989, Futagawa-Saito et al., 2009, Wegener & Schwarz, 1993). Two studies show a higher prevalence among diseased pig isolates and toxigenic strains, while a third show the opposite (Table 3). The results of Schwarz and Blobel (1989) matched the results of the two first studies results for diseased pig isolates and therefore the tendency towards higher prevalence in *S. hyicus* from cases of EE can be regarded as strong. A more distinct difference is seen for resistance patterns. The dominating patterns among healthy pig isolates and diseased pig isolates are often different and the dominating pattern in one source can be completely absent in the other (Teranishi et al., 1987; Futagawa-Saito et al., 2009).

The differences observed in this literature study calls for more outbreak-related and nationally directed identification of toxin-producing strains, their specific toxin genes and their resistance patterns to improve vaccines and treatments. Resistance rates in *S. hyicus* are much higher in pigs than in other farm animals. Therefore it should be included in surveillance programs as an important pathogen. Findings of toxin-producing strains among isolates from healthy pigs imply unknown naturally protecting factors. A discovery of such factors might be of great importance since it could be used to control the disease in a more natural way and in turn decrease the need for antimicrobial treatment. Research and surveillance programs are urgent needs to control this disease and the antimicrobial resistance in its causative agent *S. hyicus*.

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