INVESTIGATION OF
EXUDATIVE EPIDERMITIS AND EAR NECROSIS IN PIGS

by

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ABSTRACT

INVESTIGATION OF EXUDATIVE EPIDERMITIS AND EAR NECROSIS IN PIGS

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This thesis is an investigation of two common skin conditions of pigs: exudative epidermitis (EE) and ear necrosis (EN). The cause of exudative epidermitis and risk factors are well understood, however the study was prompted because of reports of treatment failure. A survey of veterinary practitioners (n=15) and pork producers (n=58) was conducted to determine which treatments are commonly used. Amongst farmer respondents topical treatments were often used and in serious cases injectable penicillin G was administered. Thirty farms with a history of EE were visited and skin samples taken from affected pigs. The antimicrobial resistance pattern for isolates of Staphylococcus hyicus and Staphylococcus aureus revealed that almost all isolates were resistant to penicillin G and ampicillin. In addition, certain isolates of S. hyicus as well as S. aureus were shown to possess the mecA gene which is associated with resistance to methicillin. The presence of widespread resistance to penicillin G among staphylococci isolates suggests a reason for poor treatment response. The presence of the mecA gene in staphylococci other than S. aureus recovered from pigs has not been reported before and is of interest from a public health standpoint.

A second study investigated EN. The causative agent(s) and the associated risk factors are not well understood. Eleven case farms were visited and skin biopsies and oral swabs taken from pigs in early, mid and late stages of the disease. Bacteriological culturing was performed for staphylococci and spirochetes as well as histological examination of the
biopsy samples. Farm-level risk factors were assessed on 14 case farms and 9 control farms. Staphylococci were generally recovered in abundance from the majority of samples but spirochetes were not cultured and only identified microscopically in a small number of tissue samples. Histology revealed that the disease appeared to occur first as a lesion on the epidermal surface that caused tissue damage and led to subsequent invasion of the dermis. This pathogenesis was consistent with the hypothesis that staphylococci colonize the skin surface and produce exfoliating toxins. Ear biting was noted to be commonly present and may be an important contributing factor.
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CHAPTER ONE

Introduction, Literature Review, and Objectives

Introduction

Skin is the largest organ in the body, representing approximately 12-24% of the pig’s body weight, and skin performs multiple functions including; the maintenance of body fluids, and the protection of the body from microbial invasion and physical trauma. Therefore, damage to skin can result in serious health consequences including dehydration and secondary infection. Lesions can be painful and are frequently linked to welfare concerns. Pig skin has economic value at slaughter in that it is an edible tissue but also can be used for leather and other purposes.

Because the pig lacks a thick hair coat, lesions and blemishes of the skin are readily visible. Skin diseases can be characterized by the location of lesions on the body as well as the colour, shape and texture. Schwartz (2002) lists the following dermatopathological terms to describe skin lesions: erythema (increased redness), hemorrhage and bruising, cyanosis (bluish colour due to lowered oxygen content of blood), icterus (yellow colour reflecting liver damage or increased red blood cell destruction), urticaria (raised plaque-like swellings), scales or flakes of keratinized skin, crusts, hyperkeratosis, erosions, ulcers, vesicles and pustules (Schwartz, 2002). A colour change or a lesion on the skin is often the first sign of a disease condition. There are a number of important diseases which primarily attack the skin, and there are diseases that attack a number of different body organs including the skin, and there are also a number of diseases where changes to the
skin are noted as secondary lesions and are indicative of a more general condition. This literature review will focus on two common conditions of young pigs where the skin appears to be the primary organ affected: exudative epidermitis and ear necrosis.

**Literature review**

**Exudative epidermitis**

Exudative epidermitis (EE), also known as “greasy pig disease”, is a skin infection mainly affecting neonatal and newly weaned piglets, characterized by lesions ranging from localized lesions of a few mm in diameter to a generalized condition covering the entire body. The condition has been recognized for over 150 years and reported worldwide. It occurs sporadically and can be of economic significance as a cause of mortality and a cause of poor growth rate (Wegener and Skov-Jensen, 2006).

*Staphylococcus hyicus* is generally considered the causal agent, and in particular, virulent strains of *S. hyicus* that produce exfoliative toxins (Andresen, et al., 1993; Wegener, et al., 1993). Both virulent and avirulent strains of *S. hyicus* can be isolated from the skin of healthy or diseased pigs (Park and Kang, 1986). There may be other factors associated with virulence as well as toxin production but these factors are not yet well defined. Other staphylococci including; *Staphylococcus aureus* (van Duijkeren, et al., 2007), *Staphylococcus chromogenes* (Andresen, et al., 2005), and *Staphylococcus sciuri* (Chen, et al., 2007), can produce exfoliative toxins and have been isolated, although rarely, from cases of EE. It is generally agreed that along with the presence of the causative bacteria there is a requirement for skin wounds which allow the bacteria to invade the epidermis. In addition, there are environmental and host factors that are
important in determining whether disease occurs or not (Wegener and Skov-Jensen, 2006).

Exudative epidermitis is found worldwide and is a common disease problem in young pigs. The highest prevalence and most severe clinical signs of the disease are generally reported in suckling pigs within the first week of life. Fighting that occurs in the first 48 hours as the piglets establishing teat order results in cuts to the face and is considered a predisposing factor in the disease occurrence. The cutting of “needle teeth” at birth is a common practice on many farms and is performed in an attempt to minimize facial cuts. The other peak time when pigs appear to be at risk of developing exudative epidermitis is shortly after weaning. Pigs from different litters are commonly mixed together at weaning and fighting occurs in order to establish a social order and again facial abrasions frequently result, which may allow for infection to occur. Pigs at weaning may also be at a vulnerable period of their development in that their immune system is still immature at 3 to 4 weeks of age when weaning generally takes place and passive immunity is waning at this age. Host immunity appears to be an important aspect in predicting the development and severity of the disease. In young breeding herds, where parity-one sows make up the majority of farrowing sows, outbreaks of severe exudative epidermitis are not infrequent. However these sporadic outbreaks tend to be self-limiting and disappear as herd immunity develops (Wegener and Skov-Jensen, 2006).

The challenge dose of bacteria may also be important in that a very large bacterial population may be able to overcome the immunity of the pig under certain conditions. Staphylococci survive well in the environment, particularly in warm humid conditions typical of farrowing rooms and nurseries and therefore sanitation appears to be important
as well as pig management and housing. For example, all-in/all-out pig flow may reduce the bacterial challenge. On the other hand, high density housing may increase the bacterial challenge as well as contribute to fighting and stress. It is unlikely that pigs can escape exposure to *S. hyicus* on a commercial pig farm, and it has been suggested that most piglets become exposed during the birth process, picking up *S. hyicus* from the vagina of the sow (Wegener and Skov-Jensen, 2006, Underdahl, et al., 1965).

Researchers were able to successfully reproduce the lesions of exudative epidermitis in susceptible piglets by applying pure cultures of *S. hyicus* to a skin wound (Underdahl, et al., 1965). Bacterial invasion triggers an inflammatory response resulting in reddening of the skin initially. The pig’s first attempt at fighting the infection is via phagocytosis; however a capsule present in all virulent types of *S. hyicus* inhibits phagocytosis (Wegener, 1990). In addition, there are several other attributes of *S. hyicus* that allow the bacteria to overcome the initial attempts by the immune system to eliminate the infection. The most important virulent factor in the pathogenesis appears to be the production of exfoliative toxins. Recently, Nishifuji et al. (2008) explained the mechanism of action of staphylococcal exfoliative toxins, which act as “molecular scissors”. Virulent strains of the bacteria produce exfoliative toxins that cause the loss of keratinocyte cell-cell adhesion in the superficial epidermis. The study indicated that the 3 isoforms of exfoliative toxins, i.e., ETA, ETB, and ETD are glutamate-specific serine proteases that specifically and efficiently cleave a single peptide bond in the extracellular region of human and mouse desmoglein 1, which is a desmosomal intercellular adhesion molecule. In addition, 4 isoforms of *S. hyicus* exfoliative toxins, ExhA, ExhB, ExhC, and ExhD, cleave swine desmoglein 1, resulting in skin exfoliation similar to that observed in pigs.
Skin exfoliation allows excess sebaceous secretion and serous exudates, causing the characteristic “greasy” appearance of the lesions. At this point the skin integrity is damaged to the point that dehydration might occur from loss of fluids and septicemia is possible because the protective barrier provided by the skin is lost.

At least 6 exfoliative toxins of *S. hyicus* have been described: ExhA, ExhB, ExhC and ExhD, and ShetA and ShetB (Andresen, 1998; Sato, et al., 2000; Andresen, 2005) and their existence are species-dependent (Takeuchi, et al., 2000). Exfoliative toxins are produced by other staphylococci strains and cause several diseases in other animal species including humans. The exfoliative toxins produced by *S. aureus* have been divided into 3 types, ETA, ETB, and sETC. ETA and ETB are known to cause staphylococcal scalded skin syndrome (SSSS) in humans and sETC has been identified in equine isolates (Arbuthnott, 1983; Andresen, 1998). Exudative epidermitis shares similar histopathology with the SSSS in terms of blister formation and exfoliation of the skin caused by splitting of the skin at the granular layer of the epidermis, which may be because of the production of similar exfoliative toxins by *S. hyicus* and *S. aureus* (Hanakawa, et al., 2002; Ahrens and Andresen, 2004). Andresen et al. (2005) showed that the exfoliative toxin from *S. chromogenes* reacted in immunoblot analysis with polyclonal and monoclonal antibodies specific to ExhB from *S. hyicus* and had an apparent molecular weight of 30kDa. They experimentally inoculated pigs with the isolates of *S. chromogenes* and produced the clinical disease of EE (Andresen, 2005). Chen et al.(2007) reported that *S. sciuri* was recovered from the pericardial fluid of EE
affected pigs and reproduced EE with inoculation of the isolates to newborn piglets. The exfoliative toxin, ExhC was found in the isolate’s genome DNA (Chen, et al., 2007).

The first clinical signs of EE include redness at the site of infection and swelling, and as the disease progresses the pig will have a reduced appetite and may appear listless. The disease is characterized by sebaceous exudation and formation of a crust. The lesion may be localized or extend to cover the entire body. Lesions usually start around the face and extend to the abdomen. Dirt and feces become encrusted to the skin. Ulcers may occur in the mouth and separation of the horn may occur in the hooves. Dehydration and inappetence leads to rapid weight loss. Pruritus is not a feature, although EE could be secondary to a disease such as mange that causes the pig to scratch and the subsequent wounds become infected by S. hyicus. Not all piglets in a litter or pen become infected. Mortality can vary greatly and may be high. When lesions are extensive, the growth rate of survivors is compromised (Pepper and Taylor, 1977).

The history and clinical signs are suggestive of a diagnosis. A post mortem examination will reveal microscopic evidence of exfoliation, exocytosis, crust formation and hyperplasia of the epidermis. Bacterial colonies may also be visualized during histological examination. S. hyicus can be readily grown from swabs of the lesions, however several different strains may be present and it is important to demonstrate the presence of virulent types. Multiplex PCR assays and ELISA methods have been used for detection of exfoliative toxins in order to determine virulence. Recently, exfoliative toxin genes of ExhA, ExhB, ExhC, ExhD and SHETA and SHETB from S. hyicus have been cloned and sequenced and then, used to determine the virulence of bacteria (Ahrens and Andresen, 2004; Andresen and Ahrens, 2004).
Other skin conditions that might be confused with EE include; mange, ringworm, swine pox and pityriasis rosea. Mixed infections of these diseases with \textit{S. hyicus} infection do occur, as well as the fact that avirulent and virulent \textit{S. hyicus} are commonly isolated from the skin of pigs and therefore may complicate the diagnosis.

In general, an emphasis is placed on preventing the disease if possible and rapid early treatment is recommended if prevention is not successful. To increase immunity autogenous vaccination of sows with bacterins made from strains isolated from affected pigs is sometimes used for prevention or reduction of severity of EE. The use of a toxoid of exfoliative toxins is possible but the method has not been evaluated in the field or has not been well documented. At present it is recommended that an autogenous vaccine with bacterial cells and the culture supernatant including exfoliative toxin be used to vaccinate sows prior to farrowing if EE is a problem (Wegener and Skov-Jensen, 2006).

One key aspect of prevention is to minimize skin abrasions and damage, particularly in the very young animal. For example, cutting the tips of needle teeth at birth, minimizing cross-fostering, or mixing of pigs at weaning, and controlling mange are considered useful. It is recommended to promptly treat skin wounds with an antiseptic to prevent infection. In addition, EE preventive programs include thorough cleaning and disinfection of pens to help reduce levels of bacteria in the environment and reduce the chance of wound infection. It is also recommended that early cases of infection be treated systemically with an appropriate antibiotic (Penny and Muirhead, 1981). Antimicrobial resistance is recognized as a potential problem. Combinations of trimethoprim and sulphonamides, as well as lincomycin and spectinomycin appear to be often effective, based on \textit{in-vitro} susceptibility tests (Wegener and Schwarz, 1993).
The following is a summary of reports from various countries regarding percentage of isolates of *S. hyicus* showing resistance to various antimicrobials. Schwarz and Blobel (1989) in Germany reported 66% of isolates resistant to tetracyclines, 100% to sulphonamides, 43% to streptomycin, 25% to penicillin and 3% to erythromycin (Schwarz and Blobel, 1989). Devriese from Belgium reported 60% of isolates resistant to tetracyclines, 60% resistant to penicillin and 74% resistant to erythromycin (Devriese, 1977). Teranishi et al. from Japan found 22% of isolates resistant to tetracyclines, 4% resistant to penicillin, 2% resistant to streptomycin and 40% resistant to erythromycin (Teranishi, et al., 1987). Aarestrup and Jensen (2002) investigated and compared resistance of *S. hyicus* isolates from EE-affected pigs to 13 different antimicrobials from 1996 to 2001 in Denmark. The percentage of isolates resistant to penicillin ranged from 54-75%, streptomycin 33-53%, and tetracyclines 21-47% (Aarestrup and Jensen, 2002).

Antimicrobial resistance patterns of *S. hyicus* are difficult to compare from one study to another because of different methods used between studies. There is a need for more research about regional antimicrobial resistance of *S. hyicus* recovered in pigs with clinical signs of EE.

**Emergence and spread of methicillin-resistance among staphylococci in pigs**

There have been reports of unexpectedly high numbers of methicillin-resistant *S. aureus* (MRSA) infections and colonization in people with contact to pigs in the Netherlands (Voss, 2005). This finding of a higher rate of pig farmers carrying MRSA compared to the general public prompted a flurry of investigative studies to examine the prevalence and significance of MRSA in pigs. The most common MRSA found from the nares of pigs has been identified as multilocus sequence type (MLST) ST 398 (Huijsdens,
et al., 2006; de Neeling, et al., 2007; van Duijkeren, et al., 2008). Similar strains have been isolated from pigs and pig farmers in the USA and Singapore (Sergio, et al., 2007; Smith, et al., 2009). However, common community-associated strains (CMRSA) transmitted, presumably, from people to pigs, have been also found. In Canada, Khanna et al. (2008) found MRSA ST 398 in most herds but found CMRSA in the noses of pigs on 2 farms (Khanna, et al., 2008).

Typing of MRSA is an essential tool for studying the epidemiology of MRSA. There are several ways to study the molecular epidemiology of MRSA; pulsed-field gel electrophoresis (PFGE) using Sma I restriction enzyme, multilocus sequence typing (MLST), spa-typing, and staphylococcal cassette chromosome (SCC) typing.

Most MRSA isolated from pigs and pig-associated human cases are non-typeable by PFGE using SmaI restriction enzyme which is the most common technique used to identify human isolates. Alternatively, researchers have used MLST to characterize the common pig strain as Sequence Type 398 which is known as non-typeable strains by PFGE. Recently, the Panel on Biological Hazards of the European Food Safety Authority (EFSA) has endorsed spa-typing for discrimination between MRSA strains from livestock as the recommended procedure. A baseline study in the UK examined ST398 MRSA strains isolated from livestock and found the most common spa types to be t01, t108, and t034 (Anon., 2009).

Typing of the methicillin-resistance gene can be further examined using PCR typing of the structure of the staphylococcal cassette chromosome (SCC) which is a mobile element encoding the mecA gene. The gene cassette chromosome mec is the most
representative SCC encoding for methicillin-resistance (Katayama, et al., 2000). A SCC\textit{mec} element is composed of two essential gene complexes: the \textit{mec} complex, containing \textit{mecA} and the cassette chromosome recombinase (ccr) complex, being responsible for the mobility of SCC\textit{mec}. So far, six structurally different types of SCC\textit{mec} I, II, III, IV, V and VI have been identified by the combination of class A, B, C of \textit{mec} gene complex and the type 1, 2, 3, 4, or 5 of the cassette chromosome recombinase (ccr) gene complex (Ito, et al., 2001; Ito, et al., 2004)(Ma, et al., 2002; Oliveira and Pijoan, 2004). It is known that many SCC\textit{mec} elements were found in methicillin-resistant non-\textit{S. aureus} staphylococci (MRNaS), so there is a need for identifying new SCC\textit{mec} elements (Takeuchi, et al., 2005) because recent SCC\textit{mec} typing is based on SCC\textit{mec} sequences found in MRSA strains of human origin. Some SCC\textit{mec} elements appear to be non-typeable with the common SCC\textit{mec} typing techniques (Vanderhaeghen, et al., 2010). Most prevalent SCC\textit{mec} types are type IV and V in ST398 MRSA.

Transfer of methicillin resistance among different species of Staphylococci is not well documented among pig and/or human populations. It is known that the first MRSA strain originated when SCC\textit{mec} with the \textit{mecA} gene was integrated into the chromosome of a susceptible \textit{S. aureus} strain (Ito, et al., 2001). The SCC\textit{mec} elements are common among the coagulase-negative staphylococci, e.g. \textit{S. haemolyticus}, and these are considered to be potential SCC\textit{mec} donors (Takeuchi, et al., 2005). Specifically SCC\textit{mec} typing is useful to investigate the epidemiological relationship of bacteria isolated from human and pigs.
Ear Necrosis

Ear necrosis in pigs has been reported in many countries and there are concerns of increased prevalence and the impact of this condition on animal welfare. Richardson et al. suggested the name for the disease be “porcine necrotic ear syndrome” until the pathogenesis and etiology is clearly defined (Richardson, et al., 1984). The disease has also been called “streptococcal auricular dermatitis” (Maddox, et al., 1973), and “porcine ulcerative spirochetosis” (Harcourt, 1973). The disease has also been referred to as “ear biting” (Penny and Mullen, 1976) but most researchers have concluded that ear necrosis is a complex disease and the lesions and clinical signs suggest a syndrome involving more factors than simply cannibalism (Richardson, et al., 1984; Mirt, 1999).

The cause of ear necrosis is unknown. The disease has been attributed to ear biting (Penny and Mullen, 1976; Blocks, et al., 1994). However histological and microbiological evidence indicates at least some involvement by micro-organisms. Some believe the problem is mainly caused by trauma from ear biting and that the severe lesions characterized by necrosis are a result of a cellulitis from infection by bacteria such as beta-hemolytic streptococci from the mouth of pen-mates (Maddox, et al., 1973). It has also been suggested that ear necrosis may be a form of EE since there are similarities between the two conditions with respect to histopathological findings and bacterial cultural results (Mirt, 1999). The argument that S. hyicus plays a role in causing this syndrome has been strengthened by the research work exploring the virulence factors associated with S. hyicus (Wegener, et al., 1993) and the identification of exfoliative toxins produced by certain strains of S. hyicus (Tanabe, et al., 1996).
There have been other etiological agents suggested as the primary cause. One case report described the isolation of spirochetes from the ear lesion of pigs and oral swabs of affected pigs and suggested that spirochetal bacteria, genus *Treponema*, may be the cause of the disease (Pringle, et al., 2009). Others have also observed spirochetal bacteria during microscopic examination of the lesions (Harcourt, 1973; Richardson, et al., 1984).

Many researchers believe that ear necrosis is likely the result of a combination of an infectious agent or several different agents and other contributing factors including ear biting or at least irritation from oral exploratory activity that may contribute by creating skin trauma and possibly introducing bacteria from the mouth that are important contributors to the infection. In summary, these researchers believe the lesion starts on the surface of the ear, whereas others believe the problem begins from within the ear.

There has been speculation that because ear necrosis often occurs at the tip of the ears in a bilateral manner that the condition is possibly caused by an agent that affects circulation. Pig ear tips sometimes become cyanotic and skin may become damaged due to systemic diseases such as hog cholera and salmonellosis because of a vasculitis. However, in these conditions there are always other clinical signs such as depression and respiratory disease or enteric disease, whereas the ear necrosis syndrome discussed here is not associated with other signs of illness.

Papatsiros (2011) in Greece observed that when porcine circovirus associated disease (PCVAD) is present on a farm; more pigs with ear necrosis are present (Papatsiros, 2011). Brazilian researchers reported a problem of ear necrosis involving 10 to 20 % of weanling pigs and suggested porcine circovirus type 2 (PCV2) as the cause (Zlotowski, et
al., 2008). The lesions involved the inner and outer external margins of the ears, beginning with redness and develop crusts. The lesions were usually bilateral and generally starting at the tips and extending ventrally. Other than the ear lesions there were no other skin lesions but the pigs did present with poor growth and on post-mortem examination showed lesions in multiple tissues typical of PCVAD. Skin lesions were associated with a vasculitis caused by an immune-mediated hypersensitivity reaction and PCV2 was present in epidermal epithelial cells and dermal histiocytes. Polish researchers (Pejsak, et al., 2010) have reported a reduction of ear necrosis after vaccination against PCV2. They state that ear necrosis is a consequence of small blood vessels becoming occluded by immunocomplexes. However, Lang et al. (2010a) examined a total of 96 pigs with ear necrosis from 15 farms and based on in-situ hybridization, found no evidence of PCV2 (Lang, et al., 2010a). Mycoplasma suis has also been suggested as an infectious cause of immunocomplexes that would result in occluded small vessels in the tips of the ear (Pejsak, et al., 2010). Constriction of circulation to the tips of the ear can be caused by the products of molds such as ergot. Researchers have investigated the association between moldy feed and ear necrosis and suggested mycotoxins may be important but further work needs to be done to confirm a role (Lang, et al., 2010b).

Henry and Tokach reported that in Kansas the incidence of ear necrosis is increasing (Henry and Tokach, 2006). Likewise, researchers in France claimed that the cases of ear necrosis have become more prevalent in recent years (Madec, et al., 2005). A study of the prevalence of clinical signs of disease in Danish finisher pigs between 1999 and 2001 indicated that ear necrosis was the most commonly observed condition with a mean prevalence of 4.44% (Petersen, et al., 2008; Grub, et al., 2009). In another study 70% of
the pigs were affected in a herd (Pringle, et al., 2009). Ear necrosis almost always starts in the nursery in pigs from 3 weeks of age up to 10 weeks of age but it takes several weeks for the lesions to resolve, particularly if cannibalism is associated with the outbreak.

Biting by other pigs as a response to stressful conditions in the environment was considered to be a cause of ear necrosis (Jericho and Church, 1972). However, Richardson et al. (1984) and Mirt (1999) maintained that playing with the tips of the ears alone would not cause the ear necrosis lesions (Richardson, et al., 1984; Mirt, 1999). A Danish study examined the risk factors for ear necrosis and tail lesions in weanling pigs, and found no correlation between ear necrosis and tail lesions (Busch, et al., 2008a).

Several factors may increase the risk of developing ear necrosis, including; stocking density, type of flooring, air quality, weight and age of the pigs, behaviour of pigs, and additional diseases in the barn. Overcrowding and boredom can stimulate aggression, therefore causing increased trauma to the ears and thus resulting in a higher incidence of ear necrosis. Fully-slatted floors tend to have a higher prevalence of ear necrosis than partially-slatted floors. Poor air quality and high humidity may affect behaviour as well as susceptibility to infection. Diffuse air intake through the ceilings resulted in a higher risk compared to wall inlets (Busch, et al., 2008a). It has been observed that increased body weight and age reduces the risk, presumably because older pigs are likely to have better immunity (Busch, et al., 2010).

Feed can also have an impact on ear necrosis. In one study, pigs fed dry feed were at higher risk of ear necrosis than those fed wet feed (Busch, et al., 2008b). Early weaning,
poor environment with high humidity and poor sanitation, mange, crowding and mixing are all considered possible risk factors (Penny and Mullen, 1976; Mirt, 1999).

Ear necrosis starts with the formation of superficial vesicular dermatitis. The vesicles rupture and develop into shallow moist lesions; exudation and thickening progress and a crust may form over the surface (Richardson, et al., 1984; Mirt, 1999). The early skin change is similar to skin lesions caused by S. hyicus in cases of exudative epidermitis. Deep ulceration can follow the early lesions and lead to acute cellulitis, vasculitis, thrombosis, ischemia and necrosis (Richardson, et al., 1984). Trauma from ear biting may contribute to the tissue damage and bacterial infection.

In general, pigs with mild to moderate ear necrosis appear bright and alert with a good appetite. The condition can be overlooked if only a small lesion occurs in a few pigs. However an entire pen can often be affected and it is common for both ears of a pig to be affected. The severity varies. Some pigs may lose more than half their ear. Secondary infections can cause reddening and swelling that extends beyond the base of the ear. In such severe cases the pigs will exhibit signs of pain and discomfort. In general, the first signs of necrosis occur in the early weanling stage and healing is complete by mid-grower stage.

Ear necrosis has not been found to affect average daily gain although it is suspected to influence the prevalence of other diseases. Additional studies are needed to support the fact but it is believed that ear necrosis pigs have a higher incidence of other diseases than pigs without ear necrosis (Busch, et al., 2010).
There is very little effect at slaughter; generally, the lesions heal once the pigs reach
the finisher barn. Although ear damage or scarring may be present at slaughter it is
localized and causes little impact. Potential losses may arise, however, if affected pigs
need to be sold as feeder pigs at about 10 weeks of age because their appearance may
result in a loss of a sale (Doster, 1995). Ear necrosis may be viewed as a welfare problem
and therefore under certain circumstances require intervention in order to meet animal
care standards.

A thorough diagnostic work-up as with all disease investigation should include
details about age, morbidity and mortality, distribution of lesions, appearance and
progression of the disease, as well as the presence of other clinical signs. Most pig herds
have animals of slightly different ages so that you may be able to see the early lesions as
well as a progression to the stage of healing. Doster (1995) recommended that ‘wedge’
biopsies be taken for diagnostic purposes and that these samples should represent the
lesion as well as includes some normal tissue. The area to be sampled should not be
prepared or cleaned. The biopsy should include epidermis, dermis, and subcutis. Biopsies
destined for histological examination may be placed in 10% buffered formalin, and other
fresh samples may be used for culture of bacteria or fungus (Doster, 1995).

A diagnosis is primarily based on clinical signs (i.e. the presence of necrotic lesions
involving the margins of the ears of otherwise healthy pigs). The causative agent (or
agents) is not known but bacteria may be cultured or identified in the lesions of the ear
during histological examination. Whether the bacteria associated with the lesions are
primary or secondary, an antimicrobial sensitivity report may be useful if treatment is to
be considered.
There are no reports in the literature of ear necrosis being successfully treated with antibiotics. Hansen and Busch treated pigs with trimethoprim-sulfa, and although pigs grew better, the treatment had no effect on the lesion score (Hansen and Busch, 2008). It is generally recommended to apply high levels of hygiene and to ensure the environment is appropriate with regard to temperature and humidity. Steps to reduce ear biting such as providing adequate feeder and drinker space and reduce stocking density (Luescher, 1989) are important in reducing the severity of the lesions. There are no reports of specific vaccines for ear necrosis but there is at least one report of the prevalence decreasing after the initiation of PCV2 vaccination (Pejsak, et al., 2010).

**Thesis objectives**

In general, the main goals of this research were to investigate whether or not anecdotal reports of treatment failure associated with exudative epidermitis were accurate and if so what the cause might be. In addition, a goal of this research was to investigate the cause of ear necrosis and gain a better understanding of this syndrome.

Specific objectives were:

- To determine what treatments are most commonly employed for the treatment of exudative epidermitis in Ontario pig herds.
- To isolate *S. hyicus* and *S. aureus* from cases of exudative epidermitis, and to determine their antimicrobial resistance profiles.
- To characterize staphylococcal isolates from cases of exudative epidermitis and to investigate the presence of genes associated with resistance to beta-lactam antibiotics especially methicillin resistance.
• To determine if there is an association between the presence of the mecA gene in 
*S. hyicus* and *S. aureus* at the farm level.

• To describe the lesions of ear necrosis on farms with clinical disease, and to 
determine the presence of potential pathogens, particularly staphylococci and 
spirochetes.

• To determine herd-level management practices and other factors that may be 
associated with the prevalence of ear necrosis.
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CHAPTER TWO

An investigation of exudative epidermitis (greasy pig disease) and antimicrobial resistance patterns of *Staphylococcus hyicus* and *Staphylococcus aureus* isolated from clinical cases

Abstract

Exudative epidermitis (EE) is a common skin disease of young pigs caused mainly by *Staphylococcus hyicus*. Increased prevalence of EE and poor response to treatment are reported. Common strategies used by Ontario pork producers to treat pigs with EE were determined. Antimicrobial resistance patterns of *S. hyicus* and *S. aureus* from clinical cases were determined to establish whether or not resistance was associated with poor treatment response. Penicillin G was the EE treatment preferred by farmers willing to use an injectable antimicrobial (32 of 35 respondents). Skin samples were obtained from affected pigs (approximately 6 pigs per farm in 30 case herds). Over 97% of *S. hyicus* isolates were resistant to penicillin G and ampicillin; 71% of these isolates were resistant to ceftiofur. Similar resistance was noted among *S. aureus* isolates. Antimicrobial resistance has become a problem in treatment of EE in Ontario, and therefore the choice of medication should be based on bacterial culture and antimicrobial susceptibility testing.
Introduction

Exudative epidermitis (EE), commonly known as “greasy pig disease” is a generalized or localized skin disease of piglets characterized by exfoliation, sebaceous exudation, and formation of a crust that may cover the entire body (1). The disease is most commonly caused by strains of *Staphylococcus hyicus* that produce exfoliative toxins (2). Less frequently, the disease can also be caused by toxin-producing strains of *Staphylococcus aureus* and *Staphylococcus chromogenes* (3-5). Trauma from biting (particularly newborns with unclipped needle teeth), or from scratches from rough bedding or rubbing against projections on pen walls, can expose the dermis and allow the staphylococci that are widely present on healthy pigs and in the environment to establish infection (1). However, exfoliative toxin-producing *S. hyicus* may also penetrate the epidermis directly. The exfoliative toxins act as “molecular scissors” to cut keratinocyte cell-cell connections in mammalian skin, and then destroy the barrier function of the skin, with subsequent blister formation (6).

The disease occurs worldwide and is a sporadic endemic problem on most farms, but occasionally, major outbreaks involve large numbers of piglets. The recent trend by the swine industry to discontinue the practice of cutting the tips of needle teeth at birth, coupled with the trend of increased litter size, may lead to a rise in the prevalence of EE. There have been anecdotal reports that the disease has become more common and more difficult to treat.

The main objectives of this study were to determine what treatments for EE were being used in the Ontario swine industry and to isolate *S. hyicus* and *S. aureus* from cases of EE, and to determine their antimicrobial resistance profiles.
Materials and methods

Survey

A survey of pork producers (n = 58) was conducted to obtain information regarding treatment of EE. A questionnaire was completed by the researcher by interviewing pig farmers who attended a regional trade show (28/58) or alternatively, by interviewing pork producers who participated in a cross-sectional study of farms with cases of EE (30/58). The inclusion criteria for the cross-sectional study included farms that veterinary practitioners identified as having an outbreak of EE, as well as local farms that were conveniently chosen. The questionnaire consisted of questions related to herd type, treatments, and perception of the efficacy of medication, as well as questions about other approaches to control the disease, such as improving hygiene, management changes, and autogenous vaccine use. A copy of the questionnaire template for farmers is available in Appendix 1.A of this thesis.

A survey of swine veterinarians (n = 15) was also conducted in order to obtain their opinions regarding recommendations for treatment and prevention, and whether or not they thought the disease was becoming more difficult to control. The questionnaire was distributed at a regional meeting of Ontario swine veterinarians and was completed during the meeting by all swine practitioners in attendance, showing 100% respondents rate. The responding swine veterinarians constituted 53.6% of all Ontario swine veterinarians who practiced in the region (15/28). A copy of the questionnaire template for swine veterinarians is available in Appendix 1.B of this thesis.
Cross-sectional study: bacterial culture and antimicrobial susceptibility test

Thirty pig farms from south-western Ontario (Canada) were purposively selected for the study. The inclusion criteria: were willingness to participate, and presence of local or systemic EE in suckling or weanling pigs, as reported by a herd veterinarian. One hundred and eighty-six pigs from the 30 farms were included in the study. An average of six pigs per farm was chosen for sampling. Pigs with localised or systematic clinical signs of EE were chosen. Generally, pigs with the most severe lesions were selected over pigs with mild clinical signs. When large numbers of pigs with clinical disease were present then attempts were made to select from different pens and rooms but if only a small number of affected pigs were available then multiple piglets from the same litter or the same pen were sometimes included. A single sample per pig was applied. Skin sampling from the facial lesions of pigs affected by EE with one scraping and swab taken per pig. Skin scabs from pig facial lesions were scraped into a sterile container by using a melon-baller. The melon-baller was cleaned and disinfected with 70% isopropyl alcohol between pigs. Skin swabs were collected using cotton-tipped swabs after application of 1ml 0.9% sodium chloride to the lesions. Skin scrapings were placed in empty clean tubes and cotton-tipped swabs were placed in liquid Stuart’s medium and submitted to the Animal Health Laboratory (AHL), Ontario Veterinary College, Ontario, Canada. Bacterial culture from skin samples and swabs was performed and isolates were identified as *S. hyicus* and *S. aureus* by standard laboratory techniques including colony morphology, haemolysis, Gram-stain, catalase reaction, and coagulase reaction. The recovery rates of the two pathogens were determined at the herd and pig levels. Antimicrobial susceptibility to penicillin G (pen), ampicillin (amp), ceftiofur (cef),
spectinomycin (spec), sulphonamide (sul), tetracycline (tet), tiamulin (tia), and trimethoprim/sulfamethoxazole (tri/sul) was determined by the disk diffusion method (Kirby-Bauer Procedure) defined by the Clinical and Laboratory Standards Institute (7). For the purpose of the study, intermediate strains were considered resistant.

**Data management and statistical analysis**

The survey data from the questionnaires for farmers and swine veterinarians were entered into EpiData Entry v.3 (The EpiData Association, Odense, Denmark) and verified manually for accuracy of entry. Statistical analyses were carried out using Stata10.1 (Statistics/Data Analysis, Texas, USA).

**Results**

**Survey for treatment of EE**

The most common approach to treatment of EE (41/58 farmers) was topical therapy, including mixtures of topical antibiotics, antiseptics, and/or mineral oil, mostly in the form of a spray (Table 2.1). The most frequently used topical antibiotic treatment (69%) was a mixture of procaine penicillin G and novobiocin (Novodry®, Pfizer Canada Inc, Kirkland, QC, Canada). Penicillin G (18.7%) and cephalirin benzathine (Cefa-dri®, Wyeth Animal Health, Guelph, Ontario) plus cloxacillin benzathine (Dry-Clox®, Wyeth Animal Health, Guelph, Ontario) (6.2%). In addition, 55.2% of respondents (32/58) stated that they used injectable antibiotics and if using this method most farmers (93.8%; 30/32) indicated that they preferred to use injectable penicillin G. The other injectable antibiotics chosen by a small number of producers were trimethoprim/sulfamethoxazole
(6.3%; 2/32), ceftiofur (3.1%; 1/32), and streptomycin (3.1%; 1/32). One farmer (3.1%) reported that ivermectin was used for treatment of clinical cases of EE.

Swine veterinarians commonly recommended novobiocin (66.7%; 10/15) as a topical treatment. In the case of antibiotics for injection, 40% of veterinarians (6/15) recommended penicillin G and 26.7% (4/15) of the veterinarians recommended ceftiofur, followed by 20% (3/15) for trimethoprim/sulfamethoxazole. Swine veterinarians reported that they also commonly recommended clipping needle teeth (12/15), reducing humidity (6/15), changing ventilation (4/15), and improving hygiene (14/15). Approximately a quarter of the veterinarians (4/15) recommended autogenous vaccines as an aid to controlling EE, but only 5% of the farmers (3/58) considered vaccination to be an option. Five swine practitioner respondents (33.3%) in surveys expressed some concern that response to treatment was poor.

**Cross-sectional study: bacteriology and antimicrobial susceptibility testing**

The recovery rate of *S. hyicus* from skin samples was 76.9% (143/186) and the recovery rate of *S. aureus* was 48.9% (91/186) based on parallel interpretation of the two methods of sampling, skin scraping and skin swabs. Both *S. hyicus* and *S. aureus* were cultured from 39.8% of pigs (74/186), whereas *S. hyicus* was cultured alone from 33.9% of pigs (63/186) and *S. aureus* was cultured alone from 6.5% of the pigs (12/186). At the farm level, the recovery rate of *S. hyicus* was 100% (30/30) and the recovery rate of *S. aureus* was 80% (24/30), based on at least 1 positive isolate from a farm.

The overall antimicrobial resistance profiles are presented in Table 2.2. Antimicrobial susceptibility testing revealed that most *S. hyicus* and *S. aureus* isolates
were resistant to β-lactam antibiotics such as penicillin G, ampicillin, and ceftiofur. Over 90% of isolates of *S. hyicus* and *S. aureus* were resistant to penicillin G and ampicillin. Over 70% of isolates of *S. hyicus* and *S. aureus* were resistant to ceftiofur. Resistance of *S. hyicus* (55.6%) and *S. aureus* (87.6%) to tetracycline was relatively high. Antimicrobial resistance patterns of the two pathogens were very similar, except that resistance to tetracycline was higher in *S. aureus* than in *S. hyicus*. Resistance to 1 or more antimicrobial was detected in 99.3% (142/143) of *S. hyicus* isolates. Resistance to 5 or more antimicrobials was detected in 40.6% (58/143) of *S. hyicus* isolates. The most common resistance patterns of *S. hyicus* isolates were penicillin G-ampicillin-ceftiofur (24.5%, 35/143), penicillin G-ampicillin-ceftiofur-spectinomycin-tetracycline-tiamulin (12.6%, 18/143), penicillin G-ampicillin-spectinomycin-tetracycline-tiamulin (11.2%, 16/143), and penicillinG-ampicillin-ceftiofur-tetracycline (9.1%, 13/143). Resistance to 1 or more antimicrobials was detected in 98.9% (90/91) of *S. aureus* isolates. Resistance to 5 or more antimicrobials was detected in 40.9% (36/91) of *S. aureus* isolates. The most common resistance patterns of *S. aureus* isolates were penicillin G-ampicillin-ceftiofur-tetracycline (28.6%, 26/91), penicillin G-ampicillin-ceftiofur-spectinomycin-tetracycline (22.0%, 20/91), penicillin G-ampicillin-tetracycline (8.8%, 8/91), and penicillin G-ampicillin-ceftiofur (7.7%, 7/91). The antimicrobial resistance patterns of *S. hyicus* and *S. aureus* isolates were compared between two categories of farms: “antibiotic-used farms” and “antibiotic-free farms” (Figure 2.1). “Antibiotic-free farms” did not use antibiotics in feed or water and if they had to treat an individual pig for a disease problem, the pig was removed from the production stream. Seven farms of the 30 cross-sectional study farms (23.3%) were categorized as “antibiotic-free farms”.
Discussion

Exudative epidermitis is a sporadic disease that causes significant problems and economic losses on certain farms, particularly for newly populated farms (1). Mortality and morbidity may be high during an outbreak of EE. However, even mild expressions of the disease can negatively influence the price of feeder pigs, because the readily visible skin lesions make weanling pigs with clinical signs of EE difficult to sell. It is possible that recent trends in the industry such as an increase in litter size and a move to not clip needle teeth at birth may be leading to an increase in EE.

The traditional treatment for EE has been the prompt use of antiseptics for wounds or injection of clinically affected pigs with procaine penicillin G. The surveys of pork producers and veterinarians demonstrate that penicillin G is still considered an appropriate drug to use for EE, but antimicrobial susceptibility results strongly contradict this idea. The finding that close to 20% of producer respondents replied in the survey that they did not attempt to treat may have reflected the fact that previous treatments had resulted in a poor response. Studies from other countries have also demonstrated a high level of resistance to penam penicillins among S. hyicus isolates (8-13).

Antimicrobial susceptibility information is an essential guideline to select effective antibiotics for treatment of bacterial infections. This information illustrates the benefit of promoting prudent use of antibiotics, which will then minimize the pressure of induction of bacterial antimicrobial resistance (14). However, there is a lack of timely and regional information about antimicrobial resistance profiles for EE that can be used for guidance for practitioners and farmers. Several good examples of monitoring antimicrobial resistance at country or region level are provided by government or university
laboratories. Annual antimicrobial resistance profiles of some species of animal-staphylococci are provided. Most monitoring programs for antimicrobial resistance have focused on human pathogens or indicator bacteria such as *Escherichia coli* and enterococci. The Danish national monitoring program (12) showed the antimicrobial resistance profiles of bacteria from diagnostic submissions. In the report from this program, *S. hyicus* isolates from submission of cases of skin disease (2001 to 2008) showed a moderately high resistance to penicillin G (60% to approximately 80%) (15). The present study of Ontario pigs shows a much higher proportion of resistance with over 90% of isolates from cases of EE resistant not only to penicillin G, but in most cases, resistance to other members of the β-lactam family of antibiotics, including ampicillin and ceftiofur. These results help to explain the poor response to treatment of EE reported by farmers, because penicillin G as seen in the study was the farmers preferred treatment of choice to resolve EE. The higher level of resistance in this study compared to the Danish study might also be due to differences in methods used to assess resistance and the determination of cut-points. It should be noted that isolates showing an intermediate response were classified as resistant in the current study.

Ceftiofur was the second most recommended injectable antimicrobial by veterinarians in the present study. It is considered to be resistant to penicillinases so that it would seem more likely to be effective in the treatment of a staphylococcal infection that is resistant to penam penicillins. However, ceftiofur is not a good choice for staphylococcal infection because of its relatively high MIC90 (Minimum Inhibitory Concentration) (1.0 µg/mL). In addition, the MIC90 of desfuroylceftiofur (a metabolite of ceftiofur in the body) is 4.0~8.0 µg/mL, in contrast to that of other organisms such as
*Pasteurella multocida* and *Actinobacillus pleuropneumoniae* (MIC90, 0.03µg/mL). Thus, a higher dosage of ceftiofur is required to treat a *S. hyicus* infection than to treat other bacterial infections (16, 17).

Overall the antimicrobial resistance patterns for *S. hyicus* and *S. aureus* were similar; however tetracycline resistance was more common in *S. aureus* isolates compared to *S. hyicus* isolates with the pen-amp-cef resistance pattern being most prevalent in *S. hyicus* isolates and pen-amp-cef-tet the most prevalent pattern in *S. aureus* isolates. A difference in resistance to β-lactam antibiotics was observed between *S. hyicus* and *S. aureus* isolates from farms classified as “antibiotic –free” with more isolates of *S. aureus* showing sensitivity to penicillin G and ampicillin. It appeared that reduced antibiotic pressure encouraged a reduction of resistance in the *S. aureus* population but *S. hyicus* isolates were not affected. Proliferation of resistance plasmids or chromosomally encoded resistance genes in the strains remained even with no obvious antimicrobial selection pressure. Information regarding how long farms had maintained their antibiotic-free status was not available for the present study and this knowledge might have been useful in interpreting the resistance data. In general one can conclude that whether or not antimicrobials are being used on the farm, *S. hyicus* and *S. aureus* will likely be resistant to penam penicillins, at least according to *in-vitro* testing.

In the present study, the disk diffusion method (Kirby-Bauer Procedure) was performed to test for antimicrobial susceptibility of *S. hyicus* and *S. aureus*. The antimicrobial susceptibility test results apply to the population of animals on the farm, but not necessarily to individual animals. The disk diffusion method has some limitations; for example, we cannot determine the MICs of the antimicrobial agents, which describe the
breakpoints of antimicrobial concentrations required to kill bacteria in *in-vivo* situations. When we apply the results of antimicrobial susceptibility testing to clinical cases to achieve better treatment success, pharmacokinetic-pharmacodynamic parameters should be considered: the bound versus unbound state of the agent, tissue versus plasma concentrations, drug degradation over time, variations among microorganisms, and factors associated with the specific environment at the infection site (16). Failing to consider these parameters is responsible for the discrepancy between *in-vitro* results of antimicrobial susceptibility tests and the results of using the sensitive antimicrobials in clinical cases (18). The types of antimicrobial agents that were tested in the study were limited; for example, the use of novobiocin was reported frequently, but it was not included in our antimicrobial susceptibility test. The information of antimicrobial resistance to novobiocin of *S. hyicus* and *S. aureus* would be useful for farmers and veterinarians.

In conclusion, the likely reason for the poor response to treatment of EE in the south-western Ontario region in this study was the high presence of antimicrobial resistance of *S. hyicus* and *S. aureus* isolates, especially to β-lactam antibiotics. Therefore, pork producers and swine veterinarians would benefit from performing bacterial culture and antimicrobial susceptibility tests prior to treating EE diseased pigs. Prevention needs to be emphasized and includes; minimizing wounds by minimizing cross-fostering and non-essential mixing of pigs, and possibly by clipping needle teeth, and in addition, exercising good sanitation, lowering humidity, and treating wounds promptly with an antiseptic.
Acknowledgments

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Table 2.1. Treatment options for exudative epidermitis as listed by farmers and veterinarians.

<table>
<thead>
<tr>
<th>Option of treatment</th>
<th>Farmers (%)</th>
<th>Vets (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injectable antibiotic only</td>
<td>17.2</td>
<td>6.7</td>
</tr>
<tr>
<td>Topical oil only</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>Topical antibiotic + topical oil + injectable antibiotic</td>
<td>10.3</td>
<td>13.3</td>
</tr>
<tr>
<td>Antiseptic + injectable antibiotic</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>Topical oil + injectable antibiotic</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>Topical antibiotic + antiseptic + topical oil + injectable antibiotic</td>
<td>5.2</td>
<td>66.7</td>
</tr>
<tr>
<td>Topical antibiotic + topical oil</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>Antiseptic + topical oil + injectable antibiotic</td>
<td>5.2</td>
<td>6.7</td>
</tr>
<tr>
<td>Antiseptic + topical oil</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Topical antibiotic + antiseptic + injectable antibiotic</td>
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<td></td>
</tr>
<tr>
<td>Topical antibiotic + antiseptic + topical oil</td>
<td>1.7</td>
<td>6.7</td>
</tr>
<tr>
<td>Topical antibiotic + injectable antibiotic</td>
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<td></td>
</tr>
<tr>
<td>nothing</td>
<td>17.2</td>
<td></td>
</tr>
<tr>
<td>Total (number)</td>
<td>58</td>
<td>15</td>
</tr>
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Table 2.2: Antimicrobial resistance profiles determined by the disk diffusion method for *S. hyicus* and *S. aureus* isolates from pigs with clinical signs of exudative epidermitis.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th><em>S. hyicus</em></th>
<th></th>
<th><em>S. aureus</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Resistant</td>
<td>95% CI</td>
<td>% Resistant</td>
<td>95% CI</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>97.2</td>
<td>94-100</td>
<td>92.1</td>
<td>86-98</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>97.2</td>
<td>94-100</td>
<td>92.1</td>
<td>86-98</td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>71.1</td>
<td>64-77</td>
<td>76.4</td>
<td>67-85</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>45.1</td>
<td>37-53</td>
<td>48.3</td>
<td>38-59</td>
</tr>
<tr>
<td>Sulfonamide</td>
<td>8.5</td>
<td>4-13</td>
<td>13.5</td>
<td>6-21</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>55.6</td>
<td>47-64</td>
<td>87.6</td>
<td>81-91</td>
</tr>
<tr>
<td>Tiamulin</td>
<td>31.0</td>
<td>23-39</td>
<td>15.7</td>
<td>8-23</td>
</tr>
<tr>
<td>Trimethoprim/sulfa</td>
<td>2.1</td>
<td>0-5</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>142</strong></td>
<td></td>
<td><strong>89</strong></td>
<td></td>
</tr>
</tbody>
</table>

CI: confidence interval
Figure 2.1. Antimicrobial resistance profiles of *S. hyicus* and *S. aureus* in farms with different patterns of antimicrobial usage.

Pen: penicillin G

Amp: ampicillin

Cef: ceftiofur

Spec: spectinomycin

Tet: tetracycline

Tia: tiamulin

Sul: sulphonamide

Tri/sul: trimethoprim/sulfamethoxazole
CHAPTER THREE

Beta-lactam antimicrobial resistance of *Staphylococcus hyicus*,

*Staphylococcus aureus*, and other staphylococci isolated from pigs with
clinical signs of exudative epidermitis

Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been isolated from pigs in many countries. However, little is known about antimicrobial resistance in other staphylococci commonly found on pig skin and occasionally associated with skin disease. The goal of this research was to determine if *Staphylococcus hyicus*, the cause of exudative epidermitis (EE), exhibits similar antimicrobial resistance as *S. aureus* especially pertaining to methicillin resistance. Skin swabs and skin scrapings were taken from each of 6 pigs with clinical signs of EE on 30 conveniently chosen farms in southwestern Ontario. In addition to the skin samples, nasal swabs were taken from each pig and submitted for PCR tests and PBP2a presence testing for methicillin-resistance. The methicillin-resistance gene, the *mecA* gene was demonstrated to be present in certain isolates of *S. hyicus* (11%), *S. aureus* (9.9% of skin isolates and 16.1% nasal isolates) as well as *Staphylococcus chromogenes*, *Staphylococcus pseudintermedius*, and *Staphylococcus arlettae*. The majority of SCCmec types of the *S. hyicus* isolates (66.7%) and *S. aureus* isolates (100%) was SCCmec typeV and majority of spa types of *S. aureus* from skin samples (88.9%) and nasal samples (83.3%) was spa type 539.
Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been isolated from pigs in many countries (1-4) and pigs have been considered as reservoir for MRSA spread to humans (5, 6). People working with pigs have a higher risk of being colonized with MRSA than people who have no pig contact (7). Most of the efforts regarding antimicrobial resistance associated with pig staphylococci have been directed at public health issues. However, antimicrobial resistance is also an important concern in animal health because it can lead to treatment failures (8). Treatment failure associated with exudative epidermitis (EE) has been discussed and a high prevalence of resistance to certain antibiotics has been noted (9, 10).

In a previous study of EE on 30 Ontario pig farms we reported that the prevalence of resistance to beta-lactam antibiotics including penicillin G, ampicillin and even ceftiofur was very high for both *S. hyicus* and *S. aureus* isolates. It seems possible, based on this information and the fact that MRSA can commonly be found on Ontario pig farms (4), that genetic determinants for methicillin resistance (the *mecA* gene) may be transferred between *S. hyicus* and *S. aureus* in pigs.

The research objectives of this study are to characterize staphylococci isolates from cases of EE and to investigate the presence of genes associated with resistance to beta-lactam antibiotics especially methicillin resistance. In addition, we intend to determine if there is an association between the presence of the *mecA* gene in *S. hyicus* and *S. aureus* at the pig and at the farm level and to examine the molecular types of these genes.
Materials and methods

Sample collection

Thirty pig farms from south-western Ontario (Canada) were purposively selected for the study. The inclusion criteria were willingness to participate and presence of local or systemic EE in suckling or weanling pigs, as reported by a herd veterinarian. One hundred and eighty-six pigs from the 30 farms were included in the study. Skin sampling consisted of scrapings and swabs from the face lesions of pigs affected by EE. Skin scabs from pig facial lesions were collected using a melon baller which was disinfected with 70% isopropyl alcohol between animals. Skin was also tested using a cotton-tipped swab. Skin scrapings were placed in a sterile plastic tube and cotton-tipped swabs were placed in liquid Stuart’s medium and submitted to the Animal Health Laboratory (AHL), University of Guelph Ontario, Canada. Nasal swabs were taken from each pig as well. Bacterial culture of skin samples were performed and identified as S. hyicus and S. aureus by standard laboratory techniques including colony morphology, haemolysis, Gram stain, catalase reaction, and coagulase reaction.

Molecular analysis

Nasal swabs were sent directly for molecular analysis to Dr Weese’s Laboratory, Department of Pathobiology, University of Guelph. First, the swabs were placed in enrichment broth and incubated for a day and then the broth was inoculated onto MRSA Chromogenic agar (BBL CHROMagar MRSA, Becton, Dickinson and Company, Sparks, MD) and incubated aerobically for 24-48 hours. The isolates were identified as S. aureus by Gram stain, catalase test, tube coagulase test, and the S. aureus latex agglutination assay (Pastorex Staph-plus, Bio-Rad Laboratories Ltd, Mississauga, ON). The methicillin
–resistance was screened for the presence of penicillin binding protein 2a (PBP2a) using a latex agglutination test (LAT) (Oxoid, Hants, UK). The positive isolates were tested for the mecA gene using a polymerase chain reaction test (PCR) (13). The isolates from the skin samples that were identified as S. hyicus and S. aureus by AHL were submitted for similar molecular analysis.

The mecA gene was amplified by the following primer sequence: forward (5’-GTT GTA GTT GTC GGG TTT GG-3’) and reverse (5’-CTT CCA CAT ACC ATC TTC TTT AAC-3’) (11). Positive and negative controls were used. The PCR product was viewed on 1.5% agarose gel by using ethidium bromide and UV transilluminator. In order to improve the sensitivity and specificity of detecting methicillin resistance, PBP2a test by LAT and PCR test were both used when the mecA gene band’s existence in the PCR product was questionable (band of mecA gene size is 147bp).

S. hyicus isolates were further characterized by using SCCmec typing. S. aureus isolates from skin and nasal samples were further characterized by using SCCmec types and spa types. SCCmec types were determined by identifying the type of ccr and class of mec by multiplex PCR assay using the primers according to the methods described by Zhang et al (12). Multiplex PCR discriminated SCCmec type I, II, III, IV, and V by detecting Class A, B and C of the mec gene complexes and allotype 1,2,3, 4 and 5 of the ccr gene complexes. Furthermore, MRSA isolates from skin and nasal samples were classified by spa typing (13) using eGenomics (http://tools.egenomics.com).

Twenty-five presumptive S. hyicus isolates from AHL that were positive for methicillin resistance were subjected for speciation by 16s rRNA analysis.
The subsets of *S. hyicus* and *S. aureus* that were negative for methicillin resistance but showed resistance to three β-lactam antibiotics: penicillin G, ampicillin, and ceftiofur, were examined to determine the production of β-lactamase by using an agar inhibition test to cefpodoxime and clavulanic acid/amoxicillin (Oxoid Limited, Hampshire, England).

**Statistical analysis**

**Association of methicillin-resistance and SCCmec type V between *S. hyicus* and *S. aureus* isolates from skin samples**

The statistical analysis for the association of the *mecA* presence between *S. hyicus* isolates and *S. aureus* isolates in skin samples was performed at the pig and farm level. In addition, the association of SCCmec type V between *S. hyicus* and *S. aureus* isolates in skin samples in pig and farm level was examined. In the pig-level analysis, 74 pigs that were positive for the isolation of both *S. hyicus* and *S. aureus* in skin samples were included. Contingency tables were used to evaluate associations between the presence of the *mecA* gene in *S. aureus* isolates and the presence of the *mecA* gene in *S. hyicus* using Fisher’s exact test (Table 3.1). In addition, the association between the presence of SCCmec type V in *S. aureus* isolates and the presence of SCCmec type V in *S. hyicus* isolates was evaluated with Fisher’s exact test, with statistical significance set a priori at *P*<0.05 (Table 3.2). *S. aureus* isolates from nasal samples were excluded from the statistical analysis because we identified only MRSA, not *S. hyicus* from nasal samples in the study. In addition, the diagnostic procedure was different for examination of the methicillin resistance for skin samples and nasal samples. In skin samples, first PCR test was used for screening the *mecA* gene and then PBP2a LAT was used for confirmation.
For nasal samples, PBP2a LAT test was used for screening for methicillin resistance and then the PCR test was used for confirmation. Therefore, this could lead to a misclassification bias if association analysis of the prevalence of methicillin resistance between *S. hyicus* of skin samples and *S. aureus* of combined samples from skin and nose or *S. aureus* of nasal samples were performed.

For a herd-level analysis, herds were included only if they had at least one pig positive for isolation in both *S. hyicus* and *S. aureus* isolates. Therefore, 24 farms were included in the farm level analysis. The association of the presence of the *meca* gene between farms which have at least one pig being positive for the *meca* gene in *S. aureus* and the farms which have at least one pig being positive for the *meca* gene in *S. hyicus* was examined (Table 3.1). In addition, the association of SCCmec type V between the farms which have at least one pig being positive for the SCCmec type V in *S. aureus* and the farms which have at least one pig being positive for the SCCmec type V in *S. hyicus* in contingency table using Fisher’s exact test with statistical significance at \( P < 0.05 \) was investigated (Table 3.2).

Statistical analysis for the association of other SCCmec types II and III were not done because there were not enough isolates to examine statistically.

**Results**

**Descriptive and statistical analysis for methicillin-resistance and SCCmec type V of *S. hyicus* and *S. aureus* isolates from skin samples**

The recovery rate of *S. hyicus* was 73.1% (136/186) and the recovery rate of *S. aureus* was 48.9% (91/186) from skin samples of pigs showing clinical signs of EE.
Fifteen of 136 (11.0%) *S. hyicus* isolates were positive for having the *mec*A gene based on the PCR testing and can be referred to as methicillin-resistant *Staphylococcus hyicus* (MRSH). Among *S. hyicus* isolates that were negative for the *mec*A gene by PCR but where the band of the *mec*A gene product was not clear, 32 were further confirmed to be negative by PBP2a LAT.

Nine isolates (9.9%) were positive for having the *mec*A gene by PCR testing among 91 *S. aureus* isolates from skin samples. The test results of PBP2a LAT of *S. aureus* isolates were fully concordant with the test results of the PCR test.

No pigs were found to be positive for MRSA among 8 pigs that were positive for MRSH, whereas, 5 pigs were found to be positive for MRSA in 66 pigs being negative for MRSH (7.6%) (Table 3.1). Seven pigs being positive for MRSH and 4 pigs being positive for MRSA were excluded from the statistical analysis.

Farm-level prevalence of MRSH of skin samples was 20% (6/30). Farm-level prevalence of MRSA of skin samples was 13.3% (4/30). Additionally, no farms were found to have at least one pig being positive for MRSA in 5 farms that have at least one pig being positive for MRSH, whereas, 4 farms were found to have at least one pig being positive for MRSA among 19 farms that did not have a pig being positive for MRSH (26.3%) (Table 3.1). One farm being positive for MRSH was excluded from the statistical analysis.

**SCCmec types of MRSH and MRSA of skin samples**

The majority of SCCmec types in MRSH isolates were SCCmec type V (66.7%, 10/15). All of SCCmec types in MRSA isolates were SCCmec type V (100%, 9/9). The
The overall distribution of the SCC\textit{mec} types of MRSH and MRSA of skin samples are presented in Table 3.3. There were no pigs having SCC\textit{mec} type V in MRSA among 6 pigs being positive for SCC\textit{mec} type V in MRSH whereas 5 pigs were positive for the SCC\textit{mec} type V in MRSA among 68 pigs that were negative for the SCC\textit{mec} type V in MRSH (7.4%) (Table 3.2). Four pigs being positive for SCC\textit{mec} type V in MRSH were excluded from the statistical analysis because \textit{S. aureus} was not recovered from the pigs. Four pigs being positive for SCC\textit{mec} type V in MRSA were excluded from the statistical analysis because \textit{S. hyicus} was not recovered from the pigs.

Four farms (13.3%) were positive for having the SCC\textit{mec} type V in MRSH isolates. Four farms (13.3%) were positive for having the SCC\textit{mec} type V in MRSA isolates. However, no farm was found to have at least one pig being positive for the SCC\textit{mec} type V in MRSA among 2 farms that had at least one pig being positive for the SCC\textit{mec} type V in MRSH, whereas, 2 farms were found to have at least one pig being positive for the SCC\textit{mec} type V in MRSA among 22 farms that did not have at least one pig being positive for the SCC\textit{mec} type V in MRSH (9.1%) (Table 3.2). Two farms being positive for having the SCC\textit{mec} type V in MRSH isolates were excluded from the statistical analysis. Two farms being positive for having the SCC\textit{mec} type V in MRSA were excluded from statistical analysis.

Spa types of \textit{S. aureus} from skin samples were 539(8/9), and 109 (1/9).
Prevalence of MRSA in nasal samples and their SCCmec types and spa types

Thirty isolates of 186 (16.1%) pig nasal samples were positive for PBP2a based on LAT. PCR testing for the mecA gene confirmed that 10 of these isolates were methicillin resistant. Farm level prevalence of MRSA based on nasal samples was 26.7% (8/30).

One pig had both MRSH from a skin sample and MRSA from a nasal sample. Additionally 7 pigs had MRSA from both skin and nasal samples. In addition, at the farm level, MRSH was isolated from skin samples of some pigs and MRSA from nasal samples of different pigs on one farm and on 3 farms both MRSA was isolated from skin and nasal samples of different pigs.

The majority of SCCmec types of MRSA in nasal samples were SCCmec type V (96.7%, 29/30). Seven of 8 farms (87.5%) positive for MRSA had SCCmec type V. The overall distribution of the SCCmec types of MRSA in nasal samples is presented in Table 3.3.

Spa types of S. aureus from nasal samples were 539 (25/30), 93 (1/30), t8588 (1/30), t011 (1/30), 2 (1/30), and t1298 (1/30).

Speciation of methicillin resistant S. hyicus isolates

During the molecular analysis, 25 presumptive MRSH isolates were subjected to sub-speciate testing by 16S rRNA analysis. These 25 S. hyicus were classified by AHL and proceeded to determine the presence of methicillin resistance by molecular analysis. As a result, 15 MRSH were found to be concordant with the previous results identifying them as S. hyicus but 10 MRSH isolates were subspecified into S. chromogenes (7/10), S.
pseudintermedius (2/10), and S. arlettae (1/10). The 10 isolates were also tested to determine SCCmec types. The results of SCCmec typing are shown in Table 3.3.

One pig had both S. hyicus and S. chromogenes isolates recovered from skin samples and their SCCmec types were ccr5. Two pigs had been cultured with both S. aureus from nasal samples and S. chromogenes isolated from a skin samples and their SCCmec types were both type V. One pig had been cultured with both S. arlettae from a skin sample and S. aureus from a nasal sample and their SCCmec types were both type V.

β-lactamase production of S. hyicus and S. aureus isolates

To further investigate the reason for resistance to β-lactam antibiotics, 14 of the 88 isolates of S. hyicus and 6 of the 59 isolates of S. aureus identified as being resistant to penicillin G, ampicillin, and ceftiofur but not having the mecA gene were examined for the production of β-lactamase using disk diffusion test with clavulanic acid/amoxicillin and cefpodoxime. One of 14 S. hyicus isolates was resistant to clavulanic acid/amoxicillin and resistant to cefpodoxime. The others were sensitive to clavulanic acid/amoxicillin but resistant to cefpodoxime. One of 6 S. aureus isolates was resistant to clavulanic acid/amoxicillin and resistant to cefpodoxime. The others were sensitive to clavulanic acid/amoxicillin but resistant to cefpodoxime.

Discussion

The investigation of the presence of methicillin resistance in other staphylococci as well as S. aureus is important in order to increase our understanding of the epidemiology of methicillin resistance and prevention of the spread of methicillin resistance among staphylococci family both in human and livestock populations. Pigs and other livestock
are believed to be potential reservoirs of community-associated MRSA (5, 6). Because of the potential public health risk there has been considerable work done to examine the prevalence of MRSA in the pig population and its association with human infection (7, 14, 15). However there has been little attention paid to antimicrobial resistance in other staphylococci that are commonly present on pig farms and some of which are important pathogens of pigs. The finding of the meca gene in these other staphylococci particularly S. hyicus is important for a number of reasons. From a veterinary medical standpoint this is a concern because it rules-out the use of an important family of antibiotics that have been traditionally valuable in the treatment of EE and other staphylococcal infections. From a public health point of view the findings of this study raise the concern that the genetic material conferring multiple antimicrobial resistance may be passing from species to species within the bacterial population of a farm.

The finding of the presence of meca gene in S. hyicus was predictable since colonization of MRSA has been reported to be common in pigs in Ontario (4) and transfer of the meca gene from S. aureus to other staphylococci species has been reported to occur readily in human medicine (16, 17) and the prevalence of methicillin resistance in non-S. aureus staphylococci in humans has been shown to be common (18, 19).

The prevalence of methicillin resistance in S. hyicus and S. aureus was relatively low compared with high prevalence of resistance to 3 β-lactam antibiotics: penicillin G, ampicillin, and ceftiofur. It would appear that the staphylococci isolated from these Ontario pig farms were generally resistant to penicillin. However, the very high prevalence of isolates resistant to ceftiofur might have been a result of the laboratory
choosing a cut-point for the MIC that incorrectly conferred resistance to bacteria that may have been susceptible in-vivo. Intermediate results of the resistance testing were classed as resistant in the in-vitro test. This speculation is supported by the results of the clavulanic acid/amoxicillin inhibition testing of certain of these resistant isolates. Testing suggested that the mechanism of antimicrobial resistance in the isolates was caused by the action of β-lactamase rather than the action of methicillin resistance.

Another objective of the study was to examine the possibility of the transmission of methicillin-resistance genetic material, the mecA gene, between S. hyicus and S. aureus in EE diseased pigs. The results of statistical analysis showed that there was no evidence of transfer of the mecA gene between S. hyicus and S. aureus at pig level or at the farm level. However, the possibility cannot be discarded since the similarity of major SCCmec type and spa type in S. hyicus and S. aureus isolates was shown as SCCmec type V, and as spa 539. In this study, diagnostic procedure difference between skin samples and nasal samples hinder further examination. Only S. aureus was investigated from the nasal samples so comparisons between S. aureus and S. hyicus could not be made. If nasal isolates of S. hyicus were available to examine, it may have provided information to demonstrate a possible transfer of the mecA gene between S. hyicus and S. aureus. There were very few pigs found in this study that carried two different staphylococci that both carried the mecA gene and thus giving strength to the idea that transfer of the genetic material for resistance was taking place. There were 2 pigs affected with exudative epidermitis with mecA gene-positive S. chromogenes isolated from the skin and MRSA isolated from the nose and both S. chromogenes and MRSA had the SCCmec type V. This finding supports speculation that the mecA gene is transferred between S.
chromogenes and S. aureus. The same SCCmec types among S. aureus, S. chromogenes, and S. arlettae were found from isolates from 3 pigs and from the isolates of another pig the S. hyicus and S. chromogenes were shown to have the same SCCmec type. Therefore, more investigation about whether these results are common in staphylococci populations is warranted to give more insights into the epidemiology of methicillin resistance in pig populations.

The presence of methicillin resistance in other staphylococci: S. chromogenes, S. pseudintermedius, and S. arlettae was confirmed in this study. It is known that phenotypic methods to differentiate these species are not sufficient so molecular typing can be applied for more specific differentiation (20, 21). Isolation of these other staphylococci from lesions of EE is not proof that they were the causative organism but it should be pointed out that outbreaks of EE caused by S. aureus, S. chromogenes, and S. sceuri have been previously reported (22, 23).

This investigation into the presence of methicillin resistance in S. hyicus and S. aureus in EE diseased pigs was in part conducted to determine possible reasons of treatment failure of EE in pigs in Ontario, Canada. Because the prevalence of isolates of S. hyicus with methicillin resistance was relatively low, we conclude that the emergence of MRSH is not the main explanation for treatment failure with respect to EE outbreaks. The widespread presence of staphylococci capable of producing β-lactamase is likely a more common reason and producers should be encouraged to use alternative medication rather than treat pigs with penicillin. However the identification of methicillin resistance in a variety of staphylococci from several farms does raise concerns about the spread of serious multi-drug resistance in food producing animals and warrants further study.
References


Table 3.1. Cross-tabulation of presence of *mecA* gene between *S. hyicus* isolates and *S. aureus* isolates at the pig-level and the farm-level, based on pigs and farms that were positive both for isolation of both *S. hyicus* and *S. aureus* in skin samples.

<table>
<thead>
<tr>
<th>S. hyicus</th>
<th>pig level(^a)</th>
<th>farm level(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>pos</td>
<td>neg</td>
</tr>
<tr>
<td>Positive (pos)</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Negative (neg)</td>
<td>8</td>
<td>61</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>66</td>
</tr>
</tbody>
</table>

\(^a\) Fisher’s exact test *P*=1

\(^b\) Fisher’s exact test *P*=1
Table 3.2. Cross-tabulation of presence of SCCmec type V between *S. hyicus* isolates and *S. aureus* isolates at the pig-level and farm-level, based on pigs and farms that were positive both for isolation of *S. hyicus* and *S. aureus* in skin samples.

<table>
<thead>
<tr>
<th></th>
<th>S. hyicus</th>
<th>pig level&lt;sup&gt;a&lt;/sup&gt;</th>
<th>farm level&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td></td>
<td>pos</td>
<td>neg</td>
</tr>
<tr>
<td>Positive (pos)</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Negative (neg)</td>
<td>6</td>
<td>63</td>
<td>69</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>68</td>
<td>74</td>
</tr>
</tbody>
</table>

<sup>a</sup> Fisher’s exact test *P*=1  
<sup>b</sup> Fisher’s exact test *P*=1
Table 3.3. The distribution of SCCmec types in staphylococci isolates in pigs with clinical exudative epidermitis.

<table>
<thead>
<tr>
<th>Species type</th>
<th>S. hyicus (skin)</th>
<th>S. aureus (nose)</th>
<th>S. chromogenes</th>
<th>S. pseudintermidius</th>
<th>S. arlettae</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type II a</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Type III b</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Type V c</td>
<td>10</td>
<td>9</td>
<td>29</td>
<td>2</td>
<td>1</td>
<td>52</td>
</tr>
<tr>
<td>Class A d</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>ccr5 e</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>9</td>
<td>30</td>
<td>7</td>
<td>2</td>
<td>64</td>
</tr>
</tbody>
</table>

a SCCmec type II which is the combination of allotype 2 of ccr gene complex and class A of mec gene complex

b SCCmec type III which is the combination of allotype 3 of ccr gene complex and class A of mec gene complex

c SCCmec type V which is the combination of allotype 5 of ccr gene complex and class C of mec gene complex

d Only detection of class A of mec gene complex

e Only detection of allotype 5 of ccr gene complex
CHAPTER FOUR

Investigation of ear necrosis in pigs

Abstract

Ear necrosis is commonly seen on swine farms. The cause of the condition is not well understood and the factors that influence severity have not been well documented. The objectives of this study were to investigate possible causative agents through bacterial culture and histological examination of lesions and to determine farm-level risk factors. Eleven case farms were visited and tissue biopsies and oral swabs taken from pigs in early, mid and late stages of the disease. Bacteriology was performed specifically for: Staphylococcus hyicus, Staphylococcus aureus and spirochetes. Formalin-fixed tissues were examined histologically. The management and environment were assessed and clinical signs of diseases and behavioral vices were noted on 14 case farms and 9 control farms. S. aureus and S. hyicus were recovered from 88.6% and 68.6% of pigs affected by ear necrosis, respectively. Spirochetes were identified in 8.6% of formalin-fixed tissue samples but were not successfully cultured in tissue samples. Histological examination consistently showed that the disease began as damage from the outer surface of the skin and not as vascular damage from within. We speculate that the disease may be initially caused by toxins produced by certain staphylococci and that spirochetes, if present, are likely secondary invaders. It appeared that ear necrosis and ear biting were closely associated and we speculate that lesions of ear necrosis may attract chewing by pen mates resulting in trauma and contamination that lead to infection of secondary bacteria and more severe lesions.
Introduction

Ear necrosis has been recognized as a common problem on Canadian pig farms, however the cause is not known and little is understood regarding the risk factors that influence the severity and prevalence. The condition has been referred to as “porcine necrotic ear syndrome” (1), “streptococcal auricular dermatitis” (2), “porcine ulcerative spirochetosis” (3), and even “ear biting” (4). In general, ear necrosis does not affect the growth rate of pigs at least when the lesions are mild to moderate (5). The major economic impact of the syndrome is usually related to the visual appearance of the pigs making it difficult to sell them. Ear necrosis is mostly seen in young pigs in the nursery or early grower stage (4-7). It is characterized by necrotic lesions of the tips, base and/or margins of the ear. Lesions of ear necrosis usually become obvious around 4-6 weeks of age and may remain visible until approximately 14-16 weeks of age. When first noticed there is usually nothing more than a black greasy deposit on the ear tip. Over a matter of a few weeks the ear tip slowly erodes with a blackened edge. Sometimes there is evidence of trauma and bleeding but this may be a result of ear biting occurring in conjunction with ear necrosis. If there is no secondary trauma or infection, healing will occur but part of the ear may be missing by the time this occurs.

The causative organism(s) and risk factors are unknown or poorly understood. It appears to be an infectious disease that is influenced by environmental factors (1). Some researchers have noted the similarity in histopathological findings and bacterial cultural results with exudative epidermitis and have suggested that S. hyicus is the causative agent (6). The role of S. hyicus in causing ear necrosis has been supported by research associated with exfoliative toxins produced by S. hyicus. These toxins can act as
molecular scissors to damage the epidermal surface of pigs (8). Human skin disease, staphylococcal scalded skin syndrome (SSSS) caused by *S. aureus* and its exfoliative toxins are also similar to histopathological lesions of exudative epidermitis and ear necrosis in pigs (8). Therefore *S. aureus* might also be considered as a potential cause. Other authors have suggested that ear necrosis is caused by trauma from ear biting and that the severe lesions characterized by necrosis are a result of a cellulitis from infection by bacteria such as β-hemolytic streptococci from the mouth of pen mates (2). Spirochetes are also discussed as the causative bacteria of ear necrosis (3, 7). Pringle et al. successfully cultured and identified spirochetal bacteria (genus *Treponema*) from ear lesions and mouth swabs of pigs with ear necrosis. These researchers suggested that spirochetal infection may occur through ear biting (7). Several reports of sporadically finding spirochetes by histological examination of ear necrosis lesions have been noted (1, 3).

An argument has been made that ear necrosis is an expression of circulatory disturbance by systemic disease or toxins, or a manifestation of immunocomplexes caused by diseases such as porcine circovirus associated disease (PCVAD) caused by porcine circovirus type 2 (PCV2) (9). The lesions often occur at the tips of the ear affecting the part of ear supplied by the smallest blood vessels and vulnerable to disruptions in vascular supply by the immunocomplexes.

It is generally agreed that ear necrosis is a multifactorial disease and that there are likely important management and environmental risk factors that play important roles. Ear biting is commonly linked to ear necrosis and possibly the factors triggering ear biting such as overcrowding, mixing causing fighting for social hierarchy, competition
for the drinker (10), high environmental temperatures, inadequate feeder space, slatted flooring, and poor sanitation (11), are also important in ear necrosis (4, 12). Poor air quality and high humidity have also been implicated in ear necrosis (13). Feed has also been suggested as a risk factor in outbreaks of ear necrosis, with dry-feed being associated with a higher risk than wet-feed (14). Management risk factors that have been considered in previous work include; early weaning, poor sanitation, the presence of mange (4, 6).

This study has two objectives; firstly, to describe the lesions of ear necrosis, and determine the presence of potential pathogens, particularly staphylococci and spirochetes, and secondly, to determine herd-level management practices and other factors that may be associated with the presence of ear necrosis.

**Materials and Methods**

**Investigation of causative organisms**

A total of 11 swine operations in southern Ontario reporting the presence of ear necrosis were visited once between May 19th and July 14th, 2010. These case farms were conveniently selected from herds known to the researchers. On each farm, samples were taken from 3 pigs at each of 3 different stages of disease (early, mid, and late). Wedge-shaped tissue biopsies were collected from the margin of an affected ear by using an ear notcher which was cleaned and disinfected with 70% isopropyl alcohol after each pig. One piece of tissue was placed in a sterile plastic tube and submitted for bacterial culture of *S. hyicus* and *S. aureus*. Another piece of ear tissue was placed in a tube with fastidious anaerobe broth (FAB), (LAB 71, Lab M, Lancashire, UK) for bacterial culture
of spirochetes. A third piece of ear tissue was transported in containers filled with 10% formalin solution and submitted for histological examination. Cotton swabs were used to sample the mouth and gums of the same pig and these swabs were also transported in tubes with FAB and submitted for bacterial culture of spirochetes. All samples were transported directly to the Animal Health Laboratory (AHL) (University of Guelph, Guelph, Ontario, Canada) for bacterial culture or histological examination. Bacterial culture was performed and identification of *S. hyicus* and *S. aureus* was by standard laboratory techniques including colony morphology, haemolysis, Gram stain, catalase reaction, and coagulase reaction. Antimicrobial susceptibility testing to penicillin G (pen), ampicillin (amp), ceftiofur (cef), spectinomycin (spec), sulphonamide (sul), tetracycline (tet), tiamulin (tia), and trimethoprim/sulfamethoxazole (tri/sul) was determined by the disk diffusion method (Kirby-Bauer Procedure) defined by the Clinical and Laboratory Standards Institute (15). For the purpose of the study, intermediate results were considered to indicate resistance.

Bacterial culture of spirochetes was performed following the method described by Pringle et al. (7). The samples with FAB were incubated at 37°C, in an anaerobic chambers and purified through membrane filters with pore size 0.22µm. The filter was placed on fastidious anaerobe agar with 10% horse blood and inoculated with a drop of culture broth. The filter was removed after 2-3 days and the growth under the filter was checked through phase contrast microscopy.
Ear tissue in 10% formalin, after fixation, was embedded in paraffin and sectioning was performed by standard methods. Histological examination was performed on 5-7µm sections stained using hematoxylin and eosin (HE) and Warthin-Starry silver stain.

**Investigation of risk factors**

A total of 14 case farms, including the 11 farms used for sampling in the previous study, and 9 control farms were visited. All farms were conveniently chosen. The criteria for classifying a herd as a case or a control farm was based, firstly, on a phone call asking the producer if ear necrosis was ongoing at that time on the farm and secondly, on inspection of the pigs for lesions at the time of the visit to the farm. In 3 circumstances, herds that were thought to be free from ear necrosis according to the farmer were positive for the condition based on inspection and therefore classed as case farms.

A questionnaire and observation template were developed and pre-tested on colleagues. A revised questionnaire was administered to the farm owners or managers during a face-to-face interview at the time of each visit. The observation template was completed by the researchers during the farm visit. The questionnaire included farm demographics, farm management such as weaning age, pig flow, cleaning procedures, vaccination and medication regimens, feeding information, and source of pigs. Observations that were made included: the prevalence of ear necrosis of each stage (early, mid, and late), a description of the lesions, the presence of clinical signs of other diseases and behavioral vices, a description of the facilities noting number and size of pens and stocking density, flooring, feeder and water space availability, temperature and a perception of the environment including humidity and air quality. Observation was performed in 3 different stages. For farms with clinical signs of ear necrosis, the
observation notes were made corresponding to the stages of disease (early, mid, and late) that samples were taken. In the control farms, 3 different groups were observed approximating the ages used for the case farms. Copies of the questionnaire and observation template are available in Appendix 2.A and 2.B of this thesis.

**Data management and statistical analysis**

Information from survey questionnaires and observation notes for each stage group were entered into Epi-data Software (EpiData Entry version 2.0, The EpiData Association, Odense M, Denmark) and imported to Stata software (Stata Intercooled, version 10; Stata Corporation, College Station, Texas, USA) for further processing and analysis. Initially, they were checked for accuracy, consistency, and missing values. Within-group prevalence of ear necrosis was estimated as the number of pigs affected with ear necrosis divided by total number of pigs in the pen.

Association between ear necrosis status of farms and factors, obtained from the questionnaire and observation notes were evaluated using a chi-square test or Mann-Whitney test, as appropriate ($P<0.05$). Then multivariable models were built using logistic regression. The dependent variable was the presence or absence of lesions of ear necrosis on the farm. Independent variables tested included factors from the questionnaire pertaining to feed factors, management factors, and demographical factors and from observation notes pertaining to farm facility factors, farm environmental factors, behavioral factors and general health factors. Variables in survey questionnaires were measured at the farm level and the variables in observation notes were measured at farm level in a different age group. Initially, a total of 16 variables obtained from the
questionnaire (Table 4.1) and a total of 21 variables obtained from the observation notes (Table 4.2, 4.3, and 4.4) were examined in a univariable screen using a 20% significance level \( (P<0.2) \). Correlation analysis was performed using the Spearman’s rank correlation statistic to identify variables that may be collinear. Manual model building, which combined forward selection, was employed in our multivariable model building process. Independent variables associated with the dependant variable at a 20% significance level \( (P<0.2) \) in the univariable analysis were considered primary predictors of interest and were included in our multivariable main effects model. Mainly two primary predictors were paired for assessment in the main effects models and possible confounding effects were checked. Interaction terms were created between the statistically significant main effects from the multivariable main effects model. Significance of variables in the final model was evaluated using the likelihood ratio test. To assess general model fit, Pearson Goodness-of-fit (GOF) tests and Deviance GOF tests were used. In the final model, standardized Pearson residuals and influence statistics were examined for extreme values, and then the final model was refitted without them to examine influence of these extreme values on significance and interpretation of coefficients.

**Results**

**Investigation of potential causative organisms**

1. *S. hyicus* and *S. aureus* culture and antimicrobial resistance testing

   In total, 11 farms (105 pigs) were selected for sampling from nursery and grower barns. Usually 3 pigs in each stage of disease (early, mid and late) were selected from a farm. The mean \% of pigs with signs of ear necrosis at each disease stage was: 31.6\%
(sd:32.8, min 2 to max: 99%), 44.2 % (sd:36.1, min:0 to max:99%) and 54.8% (sd: 38.1, min:0 to max:99%) for early, mid and late disease stage, respectively.

The recovery rate of S. hyicus from ear tissue biopsies was 67.6% (71/105) and the recovery rate of S. aureus from ear tissue biopsies was 87.6% (92/105). The recovery rate of these 2 bacterial species is not statistically different ($P=0.54$). Both S. aureus and S. hyicus were cultured from 58.1% of pigs (61/105), whereas S.aureus was isolated alone from 29.5% (31/105) of pigs and only S. hyicus was recovered from skin lesions of 9.5% (10/105) of the pigs. At the farm level, the recovery rate of S. hyicus was 90.9% (10/11) and 100% for S. aureus, based on at least 1 positive isolate from a farm. The recovery rate of S. hyicus was 76.9%, 66.7%, 66.7%, in early, mid and late disease stage, respectively. The recovery rate of S. aureus was 82.0%, 93.3%, and 90.0%, in early, mid and late stage, respectively. A total of 8 antimicrobials, from 5 classes, were used for antimicrobial susceptibility testing. Separate antimicrobial resistance profiles of S. hyicus and S. aureus in each disease stage group are presented in Table 4.5 and Table 4.6 and Figure 4.1. Resistance of S. hyicus to 1 or more antimicrobials was detected in 95% of the isolates and resistance to 5 or more antimicrobials was detected in 26.7% of the isolates. The most common resistance patterns of S. hyicus isolates were penicillin G-ampicillin-ceftiofur (27.8%), penicillin G-ampicillin-ceftiofur-tetracycline (26.4%), penicillin G-ampicillin-ceftiofur-spectinomycin-tetracycline-tiamulin (20.8%).

Resistance of S. aureus to 1 or more antimicrobials was detected in 97.8% (91/93) of isolates and resistance to 5 or more antimicrobials was detected in 20.4% (19/93) of isolates. The most common resistance patterns of S. aureus isolates were penicillin G-
ampicillin-ceftiofur-tetracycline (20.4%), penicillin G-ampicillin-tetracycline (20.4%), penicillin G-ampicillin (11.8%), and penicillin G-ampicillin-ceftiofur (11.8%).

2. Spirochetes

Attempts to culture spirochetes from fresh biopsy material and from mouth swabs were unsuccessful. Histological examination of formalin-fixed tissue from ear lesions using silver staining revealed the presence of spirochetes in 8.6% (9/105) of the samples. Based on stage of disease, 5.7% (6/105) of formalin-fixed tissue samples from the early stages were positive for the presence of spirochetes, 2.9% (3/105) were positive from pigs in the mid-stage of the disease, and in the late stage, no positives were found. Three pigs were positive for recovery of S. hyicus and S. aureus and positive for presence of spirochetes in the tissue, 5 pigs were positive for S. aureus and positive for the presence of spirochetes in the tissue, and 1 pig was only positive for presence of spirochetes in the tissue. Four of 11 case farms were positive for the presence of spirochetes based on at least one positive histological finding. The prevalence of ear necrosis in the groups with at least one animal being positive for the presence of spirochetes was varied from a minimum of 12% to a maximum of 90% and an average of 48.6%.

3. Histological examination of lesions

The vast majority of lesions were consistent with an “outside-in” lesion, or in other words lesions beginning on the epidermal surface, with eventual extension to underlying dermis. In support of this pathogenesis, it was noted that lesions were typically more severe superficially than at deeper sites (i.e. severe epidermal erosion/ulceration/crusting, with relatively mild dermal inflammation). Segmental to diffuse epidermal hyperplasia in many sections was severe and bordered on pseudocarcinomatous hyperplasia. Foci of
erosion and ulceration were typically very discrete, with a sharp transition to adjacent, intact and relatively normal epidermis. The morphologic diagnosis for the majority of cases was crusting erosive to ulcerative pinna dermatitis with variably severe neutrophilic dermatitis and large numbers of intra-lesional bacterial cocci. In the few cases where vasculitis or overt thrombosis was evident, vascular lesions were associated with foci of epidermal ulceration and dermal necrosis/inflammation, suggesting that vascular lesions may have developed secondarily to more superficial (primary) lesions. More detailed information of pathological findings and clinical lesion sites on ear are presented in Table 4.7 and 4.8.

4. Questionnaire results and observations from case and control farms

Fourteen case farms and 9 control farms were investigated for risk factors through a survey questionnaire and observational notes. On case farms, the average age of pigs categorized as early mid or late stage disease varied from farm to farm. On a farm basis, the mean age of early-stage-disease pigs was 6.6 wk but ranged from a minimum of 3 wk to a maximum of 12 wk. The mean age of the mid-stage-disease pigs was 7.7 wk but ranged from pigs as young as 5 weeks of age on 1 farm to as old as 13 weeks of age on another. The mean age of the late-stage-disease pigs was 10 wk with a range from 5 wk to 16 wk.

On control farms, the age of pigs chosen to represent comparable animals to early-stage-disease group on case farms varied from a minimum of 3 wk of age to a maximum of 12 wk of age with a mean of 5.7 (sd:2.8). Age distribution of pigs corresponding to mid-stage-disease group varied from a minimum of 6 wk to a maximum of 12 wk with a mean of 8.4 wk (sd:2.6). The age of pigs corresponding to late-stage-disease group
varied from a minimum of 8 wk, and a maximum of 20 wk with a mean of 14 wk (sd: 4.9).

The difference in age from early-disease stage groups to late-disease stage groups was on average 3.7 wk for case farms and 8.6 wk for control farms.

Variables in the questionnaire that were significantly associated with the presence of ear necrosis on farms with univariable analysis were: earlier minimum weaning age, earlier average weaning age, and age of farm ($P<0.05$). Variables in the observations that were significantly associated with the presence of ear necrosis with univariable analysis were: perception of high humidity in early-disease stage groups, lower drinker availability, perception of high humidity and the presence of ear biting in mid-disease stage groups, and high temperature and the presence of ear biting and tail biting in late-disease stage groups ($P<0.05$). In the multivariable model, variables that were significantly associated with the presence of ear necrosis in pens were: perception of high humidity and the presence of ear biting (Table 4.9). Perception of humidity was categorized into high, medium and low in the observation report but in the final model, high and medium levels were combined into high level and low level remained unchanged. No evidence of confounding was found with the variables. Similarly, no statistically significant interaction effects were identified ($P>0.05$). Visual assessment of residuals identified that covariate 2 was an outlier so the model without covariate 2 was run and coefficient changes were not considered to be a problem. The multivariable model was considered not fitted with the data according to Pearson GOF test ($P=0.001$).
The final multivariable model based on survey questionnaire analysis could not be identified by a manual forward selection process because there were no statistically significant model with \( P<0.05 \).

The survey results of source of semen and genetic composition of piglets, sows and replacement gilts showed a wide variety of genetic sources of pigs that were affected by ear necrosis. Thus, only descriptive analysis was performed (Tables 4.10, 4.11, and 4.12). Small purebred breeders and a number of multinational genetics companies contributed to supply semen and replacement gilts to both case and control farms. The distribution of types of farms between case farms and control farms are shown in Table 4.13. Cleaning procedures in case farms and control farms are shown in Table 4.14.

**Discussion**

In this study samples were cultured for staphylococci and for spirochetes because there are reports in the literature claiming that ear necrosis is caused by *S. hyicus* (1) and there are other reports asserting that spirochetes and in particular *Treponema* sp. are the primary agents involved (1, 7). No spirochetes were cultured from any of the samples including directly from the lesions and from the mouths of the pigs. This may be due to the difficulty in growing these anaerobic bacteria. Evidence from histological examination proves that in certain cases spirochetes were present. Although spirochetes can not be ruled out completely, it does seem unlikely that they are the primary cause of the lesions because these bacteria were identified in only a few samples from pigs with ear necrosis. The scarcity of spirochetes in the current study is similar to the findings of
Richardson et al. who observed spirochetes in only 1 of 68 samples of formalin-fixed tissue using Warthin-Starry silver staining (1).

This infrequent finding of spirochetes from clinically affected pigs is suggestive of a role of secondary invader rather than of a causative agent. However, spirochetes were more commonly found in samples from pigs characterized as early-disease stage animals than mid-disease stage animals and not found in samples from the pigs in the late stages of the disease. If the opposite distribution was found it would have more strongly supported the theory of a secondary invader. Perhaps better culturing techniques or more sensitive diagnostic methods are needed to further explore the role of spirochetes. In contrast, both *S. aureus* and *S. hyicus* were frequently isolated from lesions. These bacteria are commonly found on the skin of healthy pigs as well, so this finding is not a convincing argument for their role as the primary causative agents. However, histological results demonstrating that the lesions tended to occur on the surface of the epidermis and over time extended inward to involve the dermis layer, and the description that the pathological damage is similar to the description of exudative epidermitis, is supportive of staphylococci causing the lesion. Richardson et al (1) have reproduced ear necrosis by scratching the ear and inoculating the wound with *S. hyicus*. Further work needs to be done to examine whether or not exfoliative toxins are present in the early lesions and whether certain of the staphylococci isolated in this study are able to produce toxins capable of damaging the skin in a manner noted in the histological examination.

One of the limitations of this study was the fact that although attempts were made to sample a third of the pigs at the very early stages of the disease, histology revealed that even in the early-disease stage group there were lesions that showed advanced pathology.
The disease appears to begin as a small vesicle on the surface of the skin (1). The very first lesions may have been too subtle to be recognized when pigs were chosen for sampling in this study.

The role of an infectious cause has been argued. Ear biting was shown to be associated with the presence of ear necrosis in the current study. It has been suggested that trauma from ear nibbling may be the triggering factor in allowing infectious agents to invade the skin and cause necrosis (2, 4). Similarly it is likely that pigs with ear lesions will attract attention from pen mates resulting in chewing which increases the severity of the lesions. It is difficult to say for certain whether ear necrosis leads to biting or biting is a necessary prerequisite for necrosis. At least on certain case farms ear biting did not appear to be occurring despite the presence of ear necrosis, but the observation period was short. One argument that has been raised for the lack of support for the theory of an infectious cause is that the condition often does not appear to respond to antibiotic therapy. In the current study the staphylococci isolated from the ear necrosis lesions showed a high degree of resistance to some of the commonly used antibiotics and this might explain a lack of response to treatment. The pattern of antimicrobial resistance is similar to findings in the previous two chapters where staphylococci were isolated from lesions of exudative epidermitis.

The histological findings of an “outside-in” lesion helps to discredit the argument that ear necrosis might be the result of a systematic disease such as porcine circovirus associated disease (PCVAD) or porcine reproductive and respiratory syndrome (PRRS) that may cause vasculitis or immunocomplexes that interfere with circulation through the small capillaries at the tip of the ear (9). In addition, these conditions usually result in
other clinical signs such as depression and respiratory disease or enteric disease in addition to skin lesions, whereas the ear necrosis observed on the farms in the current study was not associated with other clinical disease. The advent of widespread PCV2 vaccination has not eliminated or reduced the occurrence of ear necrosis in the study. Lang et al. did not detect in ear necrosis lesions using PCV2 in-situ hybridization testing (16). Busch et al. found that the presence of PRRSV infection was associated with a sparing effect for ear necrosis (13).

Since the current investigation is an observational study based on prevalent cases of disease, it is not possible to assign causation. There was an association between not only ear biting but also tail biting in this study. One can speculate that ear lesions may attract pigs to develop a taste for blood and serum and therefore could lead to not only ear biting but also tail biting (17). This finding is contrary to Busch et al. who found no correlation between ear necrosis and tail biting (13). There is an age difference between the present study and the Danish study in that the current study included a late-disease stage group that generally involved grower-finisher pigs whereas most of the other field studies of clinical disease only examined pigs in the nursery barn (13). It is known that pigs are more likely to have tail biting problems in the grower and finisher stage rather than the nursery stage (18). Previous studies suggested that biting and cannibalism are also thought to be the cause of ear necrosis (19). However, the lesions and clinical signs indicate the complex process of infectious disease rather than only physical damage causing ear necrosis (4, 7, 20).

In the present study, case farms did wean pigs earlier than control farms. This finding is similar to other research findings. Early weaning, an unsatisfied suckling reflex,
poor housing conditions, fighting, food and dirt on the tips of ears, bacteria and scabies have been mentioned as possible risk factors of ear necrosis (18).

Farm age was shown to be counter correlated with having ear necrosis in the current study. The most likely explanation for this finding is that convenience sampling in the present study led to a group of older farms being selected as controls. Small and older farms are possibly more likely to participate in research because of relatively low biosecurity and previous experience in research trials and therefore this may have resulted in a bias in the selection of control farms. Similarly, control farms were more likely to report that they scraped the pens as a method of cleaning compared to high pressure washing in case farms. This might reflect the selection of control farms with older barn designs where solid concrete floors require an initial scraping, and not reflect a true risk factor. Floor types are significantly different between control farms and case farms in the early-disease stage groups. The same caution in interpreting the results is required because the farms were not randomly chosen but other researchers have noted that fully slatted floors are a risk factor for ear necrosis (4, 12).

The perception of poor air quality and high humidity was found to be associated with ear necrosis in the current study and this is in agreement with other reports (13). Bacterial infections, particularly staphylococci are greatly helped with high humidity and reduced hygiene. Busch et al. (2008) has observed that diffuse air intake through the ceilings resulted in a higher risk compared to wall inlets because of associated poor ventilation resulting in high humidity (4, 6).
There were numerous sources of semen, replacement of gilts, and sows in the present study and the genetics of the pigs were too varied to make a comparison. It has been suggested that pigs with floppy ears are more prone to ear biting and possibly more vulnerable to necrosis compared to pigs with upright ears (13). However no such association was noted in this study and because of the variety of genetic type across the case farms it suggests that there isn’t a strong genetic component to this syndrome.

In the current study it was noted that control farms were more likely to supplement weanling rations with very high levels (3000ppm) of zinc oxide and high levels (>100ppm) of copper sulfate. These levels of zinc oxide are used as a treatment or control of post-weaning *Escherichia coli* diarrhea and copper sulfate is sometimes used at these high levels as a growth promotant. The association found in this study may again reflect a bias with regard to the selection of controls but may warrant more investigation because zinc is known for its positive effects on skin health (21).

In conclusion, this study provided evidence that supports the theory that ear necrosis is an infectious disease that begins with lesions to the outer skin surface. Ear biting likely plays a role but it is difficult to determine whether the necrosis is a result or a cause for pigs biting ears. Spirochetes were only occasionally observed during histological examination and therefore are not likely a primary cause, whereas staphylococci are commonly found and potential causative agents but further studies are necessary to explore this more fully. Ear necrosis is in all likelihood a disease that requires multiple contributing factors. To determine the importance of these factors a much larger observational study is required.
References


Table 4.1. Descriptive statistics of variables in survey questionnaires found to be univariably associated with ear necrosis status of the farm ($P<0.2$)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case (SD)</th>
<th>Control (SD)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc level in starter ration in feed tag (ppm)</td>
<td>191.3 (41.7)</td>
<td>1009.8 (465.7)</td>
<td>0.196</td>
</tr>
<tr>
<td>Experience of mold issue in feed (% positive farms/total farm)</td>
<td>28.6</td>
<td>0</td>
<td>0.127</td>
</tr>
<tr>
<td>Minimum of weaning age (days)</td>
<td>16.9 (1.2)</td>
<td>27 (1.7)</td>
<td>0.048</td>
</tr>
<tr>
<td>Average weaning age (days)</td>
<td>20.7 (1)</td>
<td>28.6 (3.3)</td>
<td>0.059</td>
</tr>
<tr>
<td>Average downtime after washing (days)</td>
<td>1.9 (0.3)</td>
<td>1.1 (0.4)</td>
<td>0.204</td>
</tr>
<tr>
<td>Number of sows on farm</td>
<td>871.1 (287.8)</td>
<td>357.4 (121.2)</td>
<td>0.161</td>
</tr>
<tr>
<td>Age of farm (years)</td>
<td>17.2 (3)</td>
<td>40.1 (8.8)</td>
<td>0.007</td>
</tr>
</tbody>
</table>
Table 4.2. Descriptive statistics of variables recorded in the observation notes for the group of **early-diseased pigs**, found to be univariably associated with ear necrosis status of the farms ($P<0.2$)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case (n=15)</th>
<th>Control (n=9)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Space unit (±SD$^a$)</td>
<td>4.6(0.4)</td>
<td>3.5(0.2)</td>
<td>0.089</td>
</tr>
<tr>
<td>Feeder unit (±SD)</td>
<td>0.2(0.02)</td>
<td>0.5(0.1)</td>
<td>0.061</td>
</tr>
<tr>
<td>Temperature (℃) (±SD)</td>
<td>26.6(0.9)</td>
<td>23.7(1.8)</td>
<td>0.145</td>
</tr>
<tr>
<td>Humidity level (%)</td>
<td></td>
<td></td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>33.3</td>
<td>referent</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>40</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>26.7</td>
<td>88.9</td>
</tr>
<tr>
<td>Drinker type (%)</td>
<td></td>
<td></td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>Bowl</td>
<td>57.1</td>
<td>referent</td>
</tr>
<tr>
<td></td>
<td>Bowl &amp; Nipple</td>
<td>7.1</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td>Nipple</td>
<td>35.7</td>
<td>66.7</td>
</tr>
<tr>
<td>Floor type (%)</td>
<td></td>
<td></td>
<td>0.105</td>
</tr>
<tr>
<td></td>
<td>Concrete solid</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>Half slatted</td>
<td>26.7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Wholly slatted</td>
<td>73.3</td>
<td>88.9</td>
</tr>
<tr>
<td>Ear biting (%)</td>
<td>46.7</td>
<td>11.1</td>
<td>0.178</td>
</tr>
</tbody>
</table>

$^a$ SD: Standard deviation
Table 4.3. Descriptive statistics of variables recorded in the observation notes for the group of **mid-diseased pigs**, found to be univariably associated with ear necrosis status of the farms ($P<0.2$)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case (n=14)</th>
<th>Control (n=9)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinker unit ($\pm SD^a$)</td>
<td>0.07(0.01)</td>
<td>0.14(0.02)</td>
<td>0.007</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td></td>
<td></td>
<td>0.033</td>
</tr>
<tr>
<td>High</td>
<td>28.6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>42.9</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>28.6</td>
<td>87.5</td>
<td></td>
</tr>
<tr>
<td>Floor type (%)</td>
<td></td>
<td></td>
<td>0.069</td>
</tr>
<tr>
<td>Concrete solid</td>
<td>21.4</td>
<td>37.5</td>
<td>referent</td>
</tr>
<tr>
<td>Half slatted</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Wholly slatted</td>
<td>7.1</td>
<td>37.5</td>
<td></td>
</tr>
<tr>
<td>Ear biting (%)</td>
<td>50</td>
<td>0</td>
<td>0.022</td>
</tr>
<tr>
<td>Tail biting (%)</td>
<td>35.7</td>
<td>0</td>
<td>0.115</td>
</tr>
</tbody>
</table>

$^a$SD: Standard deviation
Table 4.4. Descriptive statistics of variables recorded in the observation notes for the group of **late-diseased pigs**, found to be univariably associated with ear necrosis status of the farms ($P<0.2$)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case (n=13)</th>
<th>Control (n=8)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinker unit (±SDa)</td>
<td>0.1(0.01)</td>
<td>0.1(0.02)</td>
<td>0.144</td>
</tr>
<tr>
<td>Age (week) (±SD)</td>
<td>10 (0.9)</td>
<td>14 (1.7)</td>
<td>0.057</td>
</tr>
<tr>
<td>Temperature(°C)</td>
<td>25.8(1.0)</td>
<td>21.8(0.9)</td>
<td>0.05</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td></td>
<td></td>
<td>0.122</td>
</tr>
<tr>
<td>High</td>
<td>23.1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>38.5</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>38.5</td>
<td>87.5</td>
<td></td>
</tr>
<tr>
<td>Drinker type (%)</td>
<td></td>
<td></td>
<td>0.121</td>
</tr>
<tr>
<td>Bowl</td>
<td>46.2</td>
<td>12.5</td>
<td>referent</td>
</tr>
<tr>
<td>Bowl &amp; nipple</td>
<td>0</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Nipple</td>
<td>53.9</td>
<td>62.5</td>
<td></td>
</tr>
<tr>
<td>Ear biting (%)</td>
<td>69.2</td>
<td>0</td>
<td>0.005</td>
</tr>
<tr>
<td>Tail biting (%)</td>
<td>61.5</td>
<td>0</td>
<td>0.007</td>
</tr>
<tr>
<td>Scratches (%)</td>
<td>84.6</td>
<td>37.5</td>
<td>0.055</td>
</tr>
</tbody>
</table>

*a* SD: Standard deviation
Table 4.5. Antimicrobial resistance patterns of *S. hyicus* from three different stages of ear necrosis (n=71 isolates)

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Early (%)</th>
<th>Mid (%)</th>
<th>Late (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>86.7</td>
<td>85</td>
<td>90</td>
<td>87.7</td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>86.7</td>
<td>85</td>
<td>70</td>
<td>82.2</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>86.7</td>
<td>85</td>
<td>90</td>
<td>87.7</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>36.7</td>
<td>45</td>
<td>30</td>
<td>35.6</td>
</tr>
<tr>
<td>Sulphonamide</td>
<td>3.3</td>
<td>0</td>
<td>5</td>
<td>2.7</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>66.7</td>
<td>65</td>
<td>60</td>
<td>65.8</td>
</tr>
<tr>
<td>Tiamulin</td>
<td>36.7</td>
<td>40</td>
<td>30</td>
<td>34.3</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 beta lactam</td>
<td>86.7</td>
<td>85</td>
<td>70</td>
<td>82.2</td>
</tr>
<tr>
<td><strong>Total(number of pigs)</strong></td>
<td><strong>30</strong></td>
<td><strong>20</strong></td>
<td><strong>20</strong></td>
<td><strong>70</strong></td>
</tr>
</tbody>
</table>
Table 4.6. Antimicrobial resistance patterns of *S. aureus* from three different stages of ear necrosis (n=92 isolates)

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Early (%)</th>
<th>Mid (%)</th>
<th>Late (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>96.9</td>
<td>98.3</td>
<td>96.3</td>
<td>94.6</td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>43.8</td>
<td>32.1</td>
<td>37</td>
<td>41.9</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>96.9</td>
<td>89.3</td>
<td>96.3</td>
<td>94.6</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>28.1</td>
<td>35.7</td>
<td>33.3</td>
<td>31.2</td>
</tr>
<tr>
<td>Sulphonamide</td>
<td>0</td>
<td>7.1</td>
<td>11.1</td>
<td>5.4</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>75</td>
<td>71.4</td>
<td>70.4</td>
<td>74.2</td>
</tr>
<tr>
<td>Tiamulin</td>
<td>15.6</td>
<td>21.4</td>
<td>11.1</td>
<td>15.1</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 beta lactam</td>
<td>43.8</td>
<td>32.1</td>
<td>37</td>
<td>41.9</td>
</tr>
<tr>
<td>Total(number of pigs)</td>
<td>32</td>
<td>28</td>
<td>27</td>
<td>87</td>
</tr>
</tbody>
</table>
Table 4.7. Histological findings from three different stages of ear necrosis (%)

<table>
<thead>
<tr>
<th></th>
<th>Early</th>
<th>Mid</th>
<th>Late</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serocellular crusts with cocci</td>
<td>87.2</td>
<td>76.7</td>
<td>73.3</td>
<td>75.2</td>
</tr>
<tr>
<td>Intracorneal pustules</td>
<td>48.7</td>
<td>36.7</td>
<td>20</td>
<td>34.3</td>
</tr>
<tr>
<td>Subcorneal / subepithelial pustules</td>
<td>10.3</td>
<td>3.3</td>
<td>0</td>
<td>4.8</td>
</tr>
<tr>
<td>Hyperkeratosis</td>
<td>82.1</td>
<td>90</td>
<td>60</td>
<td>73.3</td>
</tr>
<tr>
<td>Parakeratosis</td>
<td>35.9</td>
<td>33.3</td>
<td>23.3</td>
<td>29.5</td>
</tr>
<tr>
<td>Epidermal hyperplasia</td>
<td>97.4</td>
<td>93.3</td>
<td>76.7</td>
<td>84.8</td>
</tr>
<tr>
<td>Erosion</td>
<td>30.8</td>
<td>23.3</td>
<td>13.3</td>
<td>21.9</td>
</tr>
<tr>
<td>Ulceration</td>
<td>69.2</td>
<td>60</td>
<td>63.3</td>
<td>61.0</td>
</tr>
<tr>
<td>Basal cell vacuolation</td>
<td>2.6</td>
<td>0</td>
<td>3.3</td>
<td>1.9</td>
</tr>
<tr>
<td>*spic_neutrophils</td>
<td>89.7</td>
<td>83.3</td>
<td>93.3</td>
<td>83.8</td>
</tr>
<tr>
<td>*spic_eosinophils</td>
<td>10.3</td>
<td>16.7</td>
<td>20</td>
<td>14.3</td>
</tr>
<tr>
<td>*spic_mastcells</td>
<td>7.7</td>
<td>6.7</td>
<td>3.3</td>
<td>5.7</td>
</tr>
<tr>
<td>*spic_lymphocytes</td>
<td>46.2</td>
<td>36.7</td>
<td>33.3</td>
<td>37.1</td>
</tr>
<tr>
<td>*spic_histiocytes</td>
<td>18.0</td>
<td>13.3</td>
<td>16.7</td>
<td>15.2</td>
</tr>
<tr>
<td>dermal interstitial inflammatory cells</td>
<td>33.3</td>
<td>36.7</td>
<td>23.3</td>
<td>29.5</td>
</tr>
<tr>
<td>dermal vasculitis</td>
<td>8.0</td>
<td>3.5</td>
<td>3.3</td>
<td>4.8</td>
</tr>
<tr>
<td>Total (number of pigs)</td>
<td>39</td>
<td>30</td>
<td>30</td>
<td>99</td>
</tr>
</tbody>
</table>

*spic: superficial inflammatory cell
Table 4.8. Distribution of the sites showing clinical lesions of ear necrosis (%).

<table>
<thead>
<tr>
<th></th>
<th>Early</th>
<th>Mid</th>
<th>Late</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip</td>
<td>66.67</td>
<td>78.57</td>
<td>92.31</td>
<td>78.6</td>
</tr>
<tr>
<td>Margin</td>
<td>13.33</td>
<td>14.29</td>
<td>23.08</td>
<td>16.7</td>
</tr>
<tr>
<td>Bottom (lobe)</td>
<td>33.33</td>
<td>14.29</td>
<td>15.38</td>
<td>21.4</td>
</tr>
<tr>
<td>Total (number of groups)</td>
<td>15</td>
<td>14</td>
<td>13</td>
<td>42</td>
</tr>
</tbody>
</table>
| Variable                  | OR    | OR (95% CI)    | P-value  *
|---------------------------|-------|----------------|----------
| Presence of ear biting    | 22.34 | 1.09 - 459.49  | 0.044    |
| High humidity level       | 52.63 | 2.81-1000      | 0.01     |

Number of observations: 24  
AUC: 0.90  
AIC: 22.61 BIC: 26.14

* Wald test
Table 4.10. Genetic composition of piglets in ear necrosis case and control farms (%)

<table>
<thead>
<tr>
<th>Breed/Mix</th>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yorkshire</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Landrace</td>
<td>0</td>
<td>12.5</td>
</tr>
<tr>
<td>Yorkshire x Landrace</td>
<td>30.8</td>
<td>25</td>
</tr>
<tr>
<td>Yorkshire x Duroc</td>
<td>15.4</td>
<td>0</td>
</tr>
<tr>
<td>3 breed-mix</td>
<td>7.7</td>
<td>12.5</td>
</tr>
<tr>
<td>4 breed-mix</td>
<td>15.4</td>
<td>0</td>
</tr>
<tr>
<td>Berkshire x Duroc</td>
<td>0</td>
<td>12.5</td>
</tr>
<tr>
<td>Others</td>
<td>30.8</td>
<td>12.5</td>
</tr>
<tr>
<td><strong>Total (number of farms)</strong></td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>Genetic composition</td>
<td>Sows</td>
<td></td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>------------</td>
<td>----------</td>
</tr>
<tr>
<td></td>
<td>case</td>
<td>control</td>
</tr>
<tr>
<td>Bodmin</td>
<td>14.3</td>
<td>0</td>
</tr>
<tr>
<td>DanBred</td>
<td>14.3</td>
<td>0</td>
</tr>
<tr>
<td>Arkell Research Unit</td>
<td>0</td>
<td>22.2</td>
</tr>
<tr>
<td>Genex</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Lakeview Swine Genetics</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td>Vista Villa</td>
<td>14.3</td>
<td>0</td>
</tr>
<tr>
<td>FI</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Genetipork</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td>Newsham</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td>PIC</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td>PIC L42 (Camboro)</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td>Stardoby Farms</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td>Thamesbend Farms with</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Vista Villa 50% + Ko</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Yorkshire</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Yorkshire + Landrace</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td>Lee Ridge Pork</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Judge</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Closed</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No information</td>
<td>7.1</td>
<td>22.2</td>
</tr>
<tr>
<td>Total (number of farms)</td>
<td>14</td>
<td>9</td>
</tr>
</tbody>
</table>
Table 4.12. Source of semen in ear necrosis case and control farms (%)

<table>
<thead>
<tr>
<th>Source of semen</th>
<th>case</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSI (Ontario Swine Improvement)</td>
<td>7.1</td>
<td>44.4</td>
</tr>
<tr>
<td>OSI + Total Swine Genetics</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td>OSI + Vista Villa</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Onsite Duroc</td>
<td>14.3</td>
<td>0</td>
</tr>
<tr>
<td>Onsite Yorkshire or duroc (90%) +Arnold Ympa (10%)</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td>Onsite Boars (Arnold Ypma)</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td>DanBred</td>
<td>14.3</td>
<td>0</td>
</tr>
<tr>
<td>Genex Duroc</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Kaslo Bay</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td>Kraayen Brink Genetics</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Total Swine Genetics</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td>No information</td>
<td>28.6</td>
<td>22.2</td>
</tr>
<tr>
<td>Total (number of farms)</td>
<td>14</td>
<td>9</td>
</tr>
</tbody>
</table>
Table 4.13. Farm types of ear necrosis case and control farms (%)

<table>
<thead>
<tr>
<th>Type of farm</th>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farrow to finish</td>
<td>28.6</td>
<td>66.7</td>
</tr>
<tr>
<td>Farrow to nursery</td>
<td>14.3</td>
<td>11.1</td>
</tr>
<tr>
<td>Farrow to weaning</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Off-site nursery</td>
<td>14.3</td>
<td>0</td>
</tr>
<tr>
<td>Grower to finisher</td>
<td>14.3</td>
<td>11.1</td>
</tr>
<tr>
<td>Farrow to nursery + grower to finisher (separate barn)</td>
<td>14.3</td>
<td>0</td>
</tr>
<tr>
<td>Farrow to weaning + off-site nursery + grower to finisher</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td>Off-site nursery + grower to finisher</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td>Farrow to finish + grower to finisher</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Total (number of farms)</td>
<td>14</td>
<td>9</td>
</tr>
</tbody>
</table>
Table 4.14. Cleaning methods used on farms with ear necrosis (case farms) and on farms without ear necrosis (control farms) (%)

<table>
<thead>
<tr>
<th>Method</th>
<th>Case (%)</th>
<th>Control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not mentioned</td>
<td>7.1</td>
<td>22.2</td>
</tr>
<tr>
<td>Pressure cold water + disinfectant</td>
<td>14.3</td>
<td>11.1</td>
</tr>
<tr>
<td>Pressure cold water + detergent</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td>Pressure cold water + detergent + disinfectant</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td>Pressure cold water + disinfectant</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pressure hot water + disinfectant</td>
<td>28.6</td>
<td>11.1</td>
</tr>
<tr>
<td>Pressure hot water + detergent + dry agent</td>
<td>14.3</td>
<td>0</td>
</tr>
<tr>
<td>Pressure hot water + detergent + disinfectant</td>
<td>14.3</td>
<td>0</td>
</tr>
<tr>
<td>Pressure hot + pressure cold + disinfectant</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td>Scraping</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Scraping + pressure cold water</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Scraping + pressure hot water</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Scraping + pressure hot water + detergent + disinfectant</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Scraping + pressure hot water + detergent + disinfectant + dry agent</td>
<td>0</td>
<td>11.1</td>
</tr>
<tr>
<td>Total (number of farms)</td>
<td>14</td>
<td>9</td>
</tr>
</tbody>
</table>
Figure 4.1. Antimicrobial resistance profiles of *S. hyicus* and *S. aureus* from clinical cases of ear necrosis.

pen = penicillin G
amp = ampicillin
cef = ceftiofur
spec = spectinomycin
tet = tetracycline
tia = tiamulin
sul = sulphonamide
trisul = trimethoprim-sulfamethoxazole

*Number of *S. hyicus* isolates is 71; number of *S. aureus* isolates is 92.*
CHAPTER FIVE

Summary Discussion and Conclusions

For treatment and control of infectious diseases, pork producers and veterinarians rely on antibiotics, possibly to the point of taking for granted that these products will continue to be available and efficacious. However, the development and spread of antimicrobial resistance among bacterial pathogens in the pig population is becoming a concern. This issue has tended to be investigated from a public health point of view. There has been widespread attention of multi-drug resistant strains of Salmonella emerging as a food safety threat and MRSA associated with pigs as a source of community-associated disease. An important goal of the research included in this thesis was to look at an endemic pig disease to determine if the development of antimicrobial resistance might be an important cause of treatment failure on pig farms.

The reason exudative epidermitis was chosen for this study was that it is a very common and potentially economically important disease that tends to be overlooked. At the start of this thesis, in conversation with veterinarians and producers we heard comments suggesting that this disease was increasing in prevalence and was becoming harder to treat. Recent changes in the industry such as the larger litters being born and an industry trend away from clipping needle teeth at birth can be considered possible reasons why exudative epidermitis may be becoming a more common problem, however historically, if caught early the disease has been responsive to antibiotic treatment and this approach has been used to prevent spread to healthy litter mates. Therefore, the question of why exudative epidermitis has become difficult to control prompted the initiation of the first part of this research project.
Exudative epidermitis is an old disease problem and much of the work to establish the etiology and pathogenesis was performed decades earlier before molecular techniques were available to help distinguish bacterial species and sub-species and investigate genes associated with virulence. It has been long accepted that *Staphylococcus hyicus* is the causative agent of exudative epidermitis, and that certain strains of *S. hyicus* are non-pathogenic and a normal inhabitant on the skin of pigs and there are some strains of *S. hyicus* that can produce exfoliating toxin and lead to serious skin disease. Over the years other staphylococci such as *Staphylococcus aureus* and *Staphylococcus chromogenes* have been shown to be also associated with causing exudative epidermitis. The epidemiology of staphylococci on the skin of pigs has not been thoroughly explored. In human medicine, because of the importance of staphylococcal wound infections in hospitals this has become an area of active research. The ready transmission of genes associated with antibiotic resistance between staphylococci species in hospitals, for example, has been documented. In this thesis we attempted to examine this area in pigs more closely.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been previously demonstrated to be present on many Ontario pig farms and was frequently isolated from nasal samples taken from farm workers. We speculated that *S. hyicus*, associated with exudative epidermitis cases that showed poor response to treatment, may carry multiple resistance characteristics similar to MRSA. In a survey of pork producers, almost all the respondents that used an injectable antibiotic to treat exudative epidermitis used procaine penicillin G. Possibly more surprising, many of the veterinarians selected penicillin as well, even though there are several reports in the literature of widespread resistance of *S.
hyicus to penicillin G. The results of our work revealed that almost all isolates of S. hyicus and S. aureus recovered from clinical cases of exudative epidermitis were resistant to penam penicillins. The poor treatment response can be easily explained by this finding. This specific message of using a different antibiotic to treat exudative epidermitis needs to be communicated to farmers and veterinarians. More importantly these findings emphasize the need to perform culture and susceptibility testing routinely to verify appropriate therapy in the case of all bacterial diseases. One interesting observation was that S. hyicus isolated from farms that produced pigs for an “antibiotic-free” market were similar in their resistance pattern to S. hyicus from conventional farms. The absence of routine use of antibiotics on a farm, particularly as a growth promotant in the feed, was not associated with less resistance.

Because the antimicrobial resistance patterns of S. hyicus and S. aureus were similar and that isolates of both species showed a high prevalence of resistance to beta-lactam antibiotics, we thought that it was important to explore these findings more fully. As a result of molecular testing of isolates and further speciation it was discovered that certain isolates of S. hyicus and other staphylococci such as Staphylococcus chromogenes, Staphylococcus pseudintermedius, and Staphylococcus arlettae carried the mecA gene which is associated with methicillin resistance. This is the first time this has been reported. However, the prevalence of the mecA gene in the isolates studied was low and was not likely the primary reason for the high level of resistance to beta-lactam antibiotics. However, the presence of the mecA gene in various staphylococci species recovered from pigs is a concern in that this finding suggests transmission of the resistance gene from one bacterial species to another. This issue needs to be investigated.
further because it has significant implication as far as controlling the spread of antibiotic resistance from pigs to humans and within the pig population from commensal bacteria to pathogens. The hypothesis of transfer of the mecA gene between *S. hyicus* and *S. aureus* was rejected in the statistical analysis of the isolates but this may have simply reflected the limited number of samples available to study. The mecA gene carried by *S. hyicus* appeared to be similar to the mecA gene found in other staphylococci species.

Ear necrosis was also studied because there are some researchers that believe ear necrosis is just a different clinical expression of exudative epidermitis. The crusty skin lesion on the ear resembles exudative epidermitis grossly and microscopically. We attempted to culture staphylococci from ear necrosis lesions and frequently were able to find *S. hyicus* and/or *S. aureus*. Histological examination supported the hypothesis that the disease began on the skin surface and spread deeper rather than beginning as a vasculitis. Spirochetes were only rarely identified and so may play a role as secondary invaders in some cases. Ear chewing was noted as a common finding on farms with ear necrosis and because of the design of the study, one can not say whether ear biting was a necessary cause or whether it was a common sequelae. One weakness of this study was that the farms were not randomly selected. It was difficult to find control farms where ear necrosis has not been seen, and so the farms that were used may not have been appropriately matched to case farms. Further work needs to be done to determine whether the staphylococci isolated from early lesions of ear necrosis are pathogenic and capable of producing lesions. This disease undoubtedly requires various cofactors which will make it difficult to satisfactorily reproduce in a controlled experimental setting and
therefore there is a need for a large observational study designed to identify and quantify the influence of various contributing factors.
Survey of greasy pig disease on pig farms in Ontario

Survey #____________________________

Interview date ______________________ (M/D/Y)

Herd owner________________________ Telephone number
________________________________

Farm address
__________________________________________________________ (postal code)______________

1. Type of operation: (check one or more if applicable)

   1) Farrow to finish □

   2) Farrowing □

   3) Farrowing & nursery □

   4) Nursery □

   5) Grow to finish □

   6) Other □

2. When was the swine unit (herd/site) first established?
   _____month _____year
3. Do you have or have had greasy pig disease in your unit?

Yes □ No □

If so, describe the problem (age group, number affected, mortality etc.).

How often do outbreaks occur?

4. How do you treat greasy pigs?

1) Do you use topical treatment? If so, what is your choice and how often do you use it
(Please circle your answer among Always (A), Usually (U), Sometimes (S), and Never (N).)

<table>
<thead>
<tr>
<th>1. Topical antibiotics</th>
<th>1) 1st choice:</th>
<th>A / U / S / N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2) 2nd choice:</td>
<td>A / U / S / N</td>
</tr>
<tr>
<td>2. Topical antiseptics</td>
<td>1) 1st choice:</td>
<td>A / U / S / N</td>
</tr>
<tr>
<td></td>
<td>2) 2nd choice:</td>
<td>A / U / S / N</td>
</tr>
<tr>
<td>3. Topical application of oil</td>
<td>Types of oil:</td>
<td>A / U / S / N</td>
</tr>
<tr>
<td>4. Other</td>
<td></td>
<td>A / U / S / N</td>
</tr>
</tbody>
</table>

2) Do you treat greasy pigs with injectable antibiotics?

Yes □ No □

If so, with what and how often?
(Please circle your answer among Always (A), Usually (U), Sometimes (S), and Never (N).)

<table>
<thead>
<tr>
<th>1st choice:</th>
<th>A / U / S / N</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd choice:</td>
<td>A / U / S / N</td>
</tr>
</tbody>
</table>

5. Do you change hygiene to help reduce the clinical signs of greasy pig disease?

Yes □ No □

What do you change?


6. Do you use or have you used autogenous vaccines for greasy pigs? Did they work?


7. Do you clip needle teeth?

Yes □ No □

8. Is there anything else you do to control or treat greasy pig disease?


9. In your opinion, do you think your choice of treatment works well?

(Please circle your answer among Always (A), Usually (U), Sometimes (S), and Never (N).)

<table>
<thead>
<tr>
<th>Topical treatment</th>
<th>Topical antibiotics</th>
<th>A / U / S / N</th>
</tr>
</thead>
</table>

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### Table

<table>
<thead>
<tr>
<th>Category</th>
<th>Options</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical antiseptics</td>
<td>A / U / S / N</td>
<td></td>
</tr>
<tr>
<td>Topical oil</td>
<td>A / U / S / N</td>
<td></td>
</tr>
<tr>
<td>other</td>
<td>A / U / S / N</td>
<td></td>
</tr>
<tr>
<td>Injectable antibiotics</td>
<td>A / U / S / N</td>
<td></td>
</tr>
<tr>
<td>Improving hygiene status</td>
<td>A / U / S / N</td>
<td></td>
</tr>
<tr>
<td>Autogenous vaccines</td>
<td>A / U / S / N</td>
<td></td>
</tr>
<tr>
<td>Improving management</td>
<td>Cutting needle teeth</td>
<td>A / U / S / N</td>
</tr>
<tr>
<td>other</td>
<td>A / U / S / N</td>
<td></td>
</tr>
</tbody>
</table>

10. Do you think that greasy pigs are becoming more common than before?

   Yes □  No □

   Are they harder to treat than before?

   Yes □  No □

11. Could we visit your farm to sample the greasy pigs?

   (It will be no charge. We will submit the sample to the lab to confirm the disease and do sensitivity test of antibiotics. The results may tell you which antibiotics are useful for treatment of greasy pigs.)

   Yes □  No □

   Thank you very much for the time and effort you spent to complete this survey.
**Survey of exudative epidermitis in swine in Ontario**

Survey # ___________ Interview Date (y/m/d) ____________________________

**Veterinarian**’s name __________________________ Telephone number __________________________

Clinic address ____________________________ (postal code) _____________

1. **Treatment of Exudative Epidermitis** (Greasy pig disease)

   1) How often do you recommend topical treatment and if so, what’s your choice?

   (Please circle your choice among Always (A) Frequently (F) Occasionally (O) Never (N).)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>A / F / O / N</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; choice:</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; choice:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical antibiotics</td>
<td>A / F / O / N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Topical antiseptics</td>
<td>A / F / O / N</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; choice:</td>
<td></td>
</tr>
<tr>
<td>Topical oil application</td>
<td>A / F / O / N</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; choice:</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>A / F / O / N</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

   Type of oil?

2) How often do you recommend injectable antibiotics and then if so, what is your choice?

<table>
<thead>
<tr>
<th>A / F / O / N</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; choice:</th>
</tr>
</thead>
<tbody>
<tr>
<td>A / F / O / N</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; choice:</td>
</tr>
</tbody>
</table>

2. Do you recommend autogenous vaccines? If so, under what circumstances?
3. Do you recommend changes regarding hygiene? If so, what protocols do you suggest?

4. Do you recommend changes regarding management? If so, what protocols do you suggest?

5. Is there anything else you recommend for the control or treatment of greasy pig disease?

6. In your opinion, do you think your choice of recommendation works well? (Please circle your choice among Always (A) Frequently (F) Occasionally (O) Never (N).)

<table>
<thead>
<tr>
<th>Topical treatment</th>
<th>Topical antibiotics</th>
<th>A / F / O / N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Topical antiseptics</td>
<td>A / F / O / N</td>
</tr>
<tr>
<td></td>
<td>Topical oil</td>
<td>A / F / O / N</td>
</tr>
<tr>
<td></td>
<td>other</td>
<td>A / F / O / N</td>
</tr>
<tr>
<td>Injectable antibiotics</td>
<td></td>
<td>A / F / O / N</td>
</tr>
<tr>
<td>Autogenous vaccines</td>
<td></td>
<td>A / F / O / N</td>
</tr>
<tr>
<td>Changing hygiene level</td>
<td></td>
<td>A / F / O / N</td>
</tr>
<tr>
<td>Changing management</td>
<td></td>
<td>A / F / O / N</td>
</tr>
</tbody>
</table>
7. Do you submit greasy pig disease samples to confirm the disease, and for antimicrobial susceptibility testing?

   Yes ☐ No ☐

1) If you check yes, what type of sample do you submit?

   

2) What resistance pattern are you commonly seeing? i.e. Penicillin etc.?

   

3) If possible, could you pull these cases from your records and forward copies to us?

   Yes ☐ No ☐

4) In your opinion, is *Staphylococcus hyicus* resistance to antibiotics becoming more a problem and causing treatment failure?

   Yes ☐ No ☐

8. In your opinion, is greasy pig disease becoming more prevalent?

   Yes ☐ No ☐

9. What age group of pigs are most commonly affected?

   Suckling piglets ☐ Weaners ☐
10. Do you think that the disease has become more difficult to treat?

   Yes □  No □

11. In your opinion, how big a problem is greasy pig disease when you compare it to other swine diseases? (Please list more (〉), less (<) or similar (〓) when you compare other diseases to greasy pig disease. For example if you think that Glässers Disease is less problem than Greasy pig disease, you put <.)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcine Reproductive and Respiratory Disease (PRRS)</td>
<td>▼</td>
</tr>
<tr>
<td>Pocine Circo Virus Associated Disease (PCVAD)</td>
<td>▼</td>
</tr>
<tr>
<td>Swine Influenza(Swine Influenza Virus:SIV)</td>
<td>▼</td>
</tr>
<tr>
<td>Pluropneumonia (<em>Actinobacillus pluropneumoniae</em>:APP)</td>
<td>▼</td>
</tr>
<tr>
<td>Enzootic pneumonia(<em>Mycoplasma hyopneumoniae</em>)</td>
<td>▼</td>
</tr>
<tr>
<td>Postweaning E. coli Diarrhea(PWECD)</td>
<td></td>
</tr>
<tr>
<td>Proliferative enteropathies (Ileitis)(<em>Lawsonia intracellularis</em>)</td>
<td></td>
</tr>
<tr>
<td>Salmonellosis</td>
<td></td>
</tr>
<tr>
<td>Swine dysentery (<em>Brachyspira hyodysenteriae</em>)</td>
<td></td>
</tr>
<tr>
<td>Streptococcal meningitis (<em>Streptococcus suis</em>)</td>
<td></td>
</tr>
<tr>
<td>Glässers Disease(<em>Haemophilus parasuis</em>)</td>
<td></td>
</tr>
</tbody>
</table>

Thank you very much for the time and effort you spent to complete this survey.
APPENDIX 2 A. EN Questionnaire

Survey of herds with ear necrosis

Survey #____________________ Interview Date
(M/D/Y)__________________________________________

Farm name ___________________________ Herd owner____________________________

Directions to the farm
________________________________________________________________________

__________________________________

Telephone, fax or e-mail
___________________________________

Farm address____________________________
Postal code_______________

1. Type of operation (check one or more if applicable)
   1) Farrow –to – Finish (one site)
   2) Farrow –to – Feeder pig (one site)
   3) Farrow -to – wean
   4) Off – site nursery
   5) Grower- to –finisher
   6) Other

2. The average weaning age is _________________days with a weaning age range of
   _________________to _______________days.

3. When was the swine unit (herd/site) first established? i.e. old (>20y) or newish
   _______________________________________________________________________

4. How many animals on the site?
5. What is the pig flow in each of the stages of production at this site?
   1) Farrowing: Continuous flow, All-in/all-out by pen AIAO by room, AIAO by barn
   2) Weaners: Continuous flow, All-in/all-out by pen AIAO by room, AIAO by barn
   3) Grower/ finishers: Continuous flow, All-in/all-out by pen AIAO by room, AIAO by barn

6. What is original genetics of sows?

   ____________________________________________________________________________

7. Where do you obtain replacement gilts?

   ____________________________________________________________________________

8. Describe the genetic make-up (or breeds) of the weaner or grower pigs

   ____________________________________________________________________________

9. What is source of semen? (which breed, which company)

   ____________________________________________________________________________

10. Describe the vaccination program for weaner pigs

    1) PRRS (Porcine Reproductive Respiratory Syndrome)
    2) PCVAD (Porcine Circo Virus Associated Disease)
    3) Mycoplasma
    4) Others, Please specify_____________________________________________________
    5) None

11. List medications in:

    1) Creep
       ration : ____________________________________________________________________

   __________
2) Stage 1
   starter:___________________________________________________________

   3) Stage 2
   starter:___________________________________________________________

   4) Later starter or grower
   feeds:___________________________________________________________

   5) Are there high levels of ZnO or copper sulfate in these
   feeds?:__________________________________________________________

   6) Is water medication ever used?....describe
  __________________________________________________________________
   __________________

   12. Could you provide feed tags or details?
   _________________________________________________________________
   ______________________________

   13. Which age group is first affected by ear necrosis and how long does it last?
   __________________________________________________________________
   __________________________________________________________________
   __________________________________________________________________
   __________________________________________________________________
   __________________________________________________________________

   14. Are there times of the year when clinical signs are worse than other times?
   ____________________________________________________________________
   ____________________________________________________________________

   15. At this time do you consider the prevalence and severity to be worse or better than usual?
16. What have you tried as far as treatment or prevention and has anything helped?

_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________

17. What method is used to clean nursery pens or finishing pens when they are emptied, circle all that apply?
   1) Scrapping
   2) Pressure wash with hot water
   3) Pressure wash with cold water
   4) Detergent
   5) Disinfectant (which one?)
   6) Drying agents
   7) Others, please specify
      ___________________________________________________________________
   8) No cleaning

18. How long is the room or pen empty after washing before new pigs are moved in?
   ____________________ days

19. Have you had any problem with mold or toxins in the feed that you are aware of?
   1) Yes (describe)
   2) No

20. Does ear necrosis result in any significant losses? For example do affected pigs grow slower, have other problems like secondary infections, or any deaths?

_____________________________________________________________________
_____________________________________________________________________

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21. Do you see behavior problems such as ear biting, tail biting or flank sucking, and if so do these behaviours seem to be linked with a higher prevalence of ear necrosis?

22. Any comments you would like to add?

Thank you very much for the time and effort you spent to complete this survey
Observation Notes for ear necrosis

Survey #________________________ Farm

____________________________________________________________________________________

Surveyor’s name: __________________________ Date (M/D/Y): ______________________________

❖ Facility

Description of barn in general:

____________________________________________________________________________________

____________________________________________________________________________________

____________________________________________________________________________________

Details of housing for the 3 groups to be sampled (where barns are all-in/all-out return visits are required)

<table>
<thead>
<tr>
<th></th>
<th>Age 1 group</th>
<th>Age 2 group</th>
<th>Age 3 group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room Temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Humidity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air quality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of pens per room</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Width of average pen (feet)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of average pen (feet)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average number of pigs/pen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average age of pigs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average weight of pigs/pen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of feeder</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N^*) of feeder spaces/pen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N^*) of drinkers /pen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of drinker/pen</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Ear Necrosis**

For the section of the survey health status is described in the phase of pigs affected with ear necrosis by the surveyors own observations by the following schedule.

Neurological disease include: lateral recumbence with paddling, ataxia, incoordination, or convulsions.

Polyarthritis can be recognized by swollen joints, inability to rise, or severe lameness.

Skin diseases: Greasy pig disease, flank necrosis, tail necrosis, mange

<table>
<thead>
<tr>
<th>N° of pigs with ear lesions</th>
<th>Age1 group</th>
<th>Age 2 group</th>
<th>Age 3 group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Which part of ear is affected &amp; how may pigs involved?</td>
<td>Tip:</td>
<td>Tip:</td>
<td>Tip:</td>
</tr>
<tr>
<td></td>
<td>Margin:</td>
<td>Margin:</td>
<td>Margin:</td>
</tr>
<tr>
<td></td>
<td>Bottom(flank):</td>
<td>Bottom(flank):</td>
<td>Bottom(flank):</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tip</th>
<th>health status (please put N° of animal affected)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Scours ( )</td>
<td>Coughing( )</td>
<td>Neurological signs( )</td>
</tr>
<tr>
<td></td>
<td>Polyarthritis ( )</td>
<td>Neurological signs( )</td>
<td>Polyarthritis ( )</td>
</tr>
<tr>
<td></td>
<td>Twisted noses( )</td>
<td>Polyarthritis ( )</td>
<td>Twisted noses( )</td>
</tr>
<tr>
<td></td>
<td>Chronic Wasting( )</td>
<td>Chronic Wasting( )</td>
<td>Chronic</td>
</tr>
<tr>
<td></td>
<td>Breathing( )</td>
<td>Breathing( )</td>
<td>Wasting( )</td>
</tr>
<tr>
<td></td>
<td>Skin diseases( )</td>
<td>Skin diseases( )</td>
<td>Skin diseases( )</td>
</tr>
<tr>
<td></td>
<td>Other. Please specify</td>
<td>Other. Please specify</td>
<td>Other. Please specify</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Behavioral problem (please put N° of animal affected)</th>
<th>Ear biting ( )</th>
<th>Scratches from fighting ( )</th>
<th>Tail biting ( )</th>
<th>Other. Please specify</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ear biting ( )</td>
<td>Scratches from fighting ( )</td>
<td>Tail biting ( )</td>
<td>Other. Please specify</td>
</tr>
<tr>
<td></td>
<td>Ear biting ( )</td>
<td>Scratches from fighting ( )</td>
<td>Tail biting ( )</td>
<td>Other. Please specify</td>
</tr>
<tr>
<td></td>
<td>Ear biting ( )</td>
<td>Scratches from fighting ( )</td>
<td>Tail biting ( )</td>
<td>Other. Please specify</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Health status (please put N° of animal affected)</th>
<th>Scours ( )</th>
<th>Coughing( )</th>
<th>Neurological signs( )</th>
<th>Polyarthritis ( )</th>
<th>Twisted noses( )</th>
<th>Chronic Wasting( )</th>
<th>Breathing( )</th>
<th>Skin diseases( )</th>
<th>Other. Please specify</th>
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<tbody>
<tr>
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<td>Neurological signs( )</td>
<td>Polyarthritis ( )</td>
<td>Twisted noses( )</td>
<td>Chronic Wasting( )</td>
<td>Breathing( )</td>
<td>Skin diseases( )</td>
<td>Other. Please specify</td>
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<tr>
<td></td>
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<td>Coughing( )</td>
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<td>Breathing( )</td>
<td>Skin diseases( )</td>
<td>Other. Please specify</td>
</tr>
<tr>
<td></td>
<td>Scours( )</td>
<td>Coughing( )</td>
<td>Neurological signs( )</td>
<td>Polyarthritis ( )</td>
<td>Twisted noses( )</td>
<td>Chronic Wasting( )</td>
<td>Breathing( )</td>
<td>Skin diseases( )</td>
<td>Other. Please specify</td>
</tr>
</tbody>
</table>

Margin
<table>
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<tr>
<th>Bottom (Flank)</th>
<th>Health status (please put N°of animal affected)</th>
<th>Scours( )</th>
<th>Coughing( )</th>
<th>Neurological signs( )</th>
<th>Polyarthritis ( )</th>
<th>Twisted noses( )</th>
<th>Chronic Wasting( )</th>
<th>Breathing( )</th>
<th>Skin diseases( )</th>
<th>Other. Please specify</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behavioral problem (please put N°of animal affected)</td>
<td>Ear biting ( )</td>
<td>Scratches from fighting ( )</td>
<td>Tail biting ( )</td>
<td>Other. Please specify</td>
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