Coliform mastitis in sows: Analysis of potential influencing factors and bacterial pathogens with special emphasis on *Escherichia coli*

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# Table of Contents

General Introduction................................................................................................................ 1

Chapter 1 .................................................................................................................................. 3
  Coliform mastitis in sows: A review ...................................................................................... 3

Chapter 2 ................................................................................................................................ 32
  Comparison of virulence gene profiles of *Escherichia coli* isolates from sows with Coliform mastitis and healthy sows ................................................................................. 32

Chapter 3 ................................................................................................................................ 50
  Assessing individual sow risk factors for coliform mastitis in sows:
  A case-control study.............................................................................................................. 50

Chapter 4 ................................................................................................................................ 61
  Application of decision-tree technique to assess herd specific risk factors for coliform mastitis in sows........................................................................................................ 61

General Discussion ............................................................................................................... 75

General Summary .................................................................................................................... 82

Zusammenfassung .................................................................................................................. 84
**General Introduction**

The survival and growth of piglets in their first days of life is strongly dependent on adequate colostrum and milk production by the sow. Coliform mastitis (CM), a disease in sows occurring after farrowing, is not only characterised by fever and an inflammation of the mammary glands, but also, as a consequence, by greatly reduced milk production within 12 to 48 hours post-partum. The syndrome affects therefore the productivity of the sows as well as the growth and the preweaning mortality of the piglets. It is a serious problem for the economy and animal welfare in pig production, and has been reported worldwide. Coliform mastitis is a multifactorial disease, and most research to date has focused on the husbandry-influenced occurrence, although a single pathway is unlikely to exist. In pathogenesis, there are hints of a predominant influence of *Escherichia (E.) coli*.

The aim of this thesis was the functional phenotyping of CM-affected sows involving advanced bacteriological techniques. All bacteria isolated from milk samples of diseased and healthy sows were identified at an extensive level and compared. Special emphasis was given to *E. coli* isolates.

A further objective was the analysis of sow- and birth-related factors contributing to the occurrence of CM, which were assessed under production conditions. Different statistical approaches were applied.

The first chapter provides an insight into the disease complex according to present knowledge. Most studies on the topic were carried out between 1970 and 1990. Changes in pig production over the last few decades and the still existing economic losses demand a closer look at CM again.

**Chapter two** deals with the analysis of milk samples of sows with and without CM for the presence of *E. coli*. All identified *E. coli* isolates were subsequently investigated for particular virulence genes, including genes for adhesion factors, toxins, iron acquisition factors, lipopolysaccharides, polysaccharide capsules and invasion factors. New findings on the pathogenesis of the disease and the occurrence of different virulence factors in *E. coli* isolates associated with coliform mastitis in sows were to be attained.
The focus of **chapter three** is on identifying potential risk factors, in particular individual sow characteristics, for CM by a case-control study. In this epidemiological clinical study, diseased sows were matched with healthy ones of the same herd by conditional logistic regression. In addition, a second case-control study was conducted to investigate the risk of repeated clinical mastitides in the following lactations for sows that had already suffered mastitis before.

In **chapter four**, the application of the decision-tree technique to potential risk factors for CM, analysed in chapter three, is investigated. The aim of this data mining method was to make sow herd datasets accessible and comparable by generating graphical trees and by visualizing possible decision rules. The ability of the decision-tree technique to distinguish between sows with CM from healthy ones and to predict the outcome of the disease was analysed.
Chapter 1

Coliform mastitis in sows: A review

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Abstract
Coliform mastitis (CM) represents an economically very important disease complex in sows that also affects the health, welfare, and performance of the piglets. Most research has concentrated on the husbandry-influenced occurrence of CM. In pathogenesis, there are many hints of an outstanding influence of *Escherichia coli* and its endotoxins, although different species among the *Enterobacteriaceae* have been isolated from affected animals. Most studies on this topic were conducted between 1970 and 1990. But with particular respect to the economic damages and the lack of recent literature, it is time for research to have a closer look at this disease again. The use of body temperature as a single indicator for CM diagnosis and treatment must be regarded critically. To minimise use of antibiotics and to achieve a proper diagnosis, a combination of appropriate criteria should be applied. Additional approaches, for instance, genetic resistance, are promising tools for future prevention.

KEYWORDS
Swine, mastitis, dysgalactia, sows, endotoxins
Introduction

Postparturient disorders represent an economically important disease complex in sows world-wide (Bertschinger, 1999), incurring losses due to reduced productivity and high mortality rates. These disorders are commonly categorized under the terms mastitis-metritis-agalactia (MMA) complex (Martin et al., 1967), postpartum dysgalactia syndrome (PPDS or PDS) (Klopfenstein et al., 2006), and periparturient hypogalactia syndrome (Smith et al., 1992). Miscellaneous other names, such as agalactia complex (Penny, 1970), lactation failure (Elmore and Martin, 1980), agalactia toxemica (Ringarp, 1960), or agalactia postpartum syndrome (Hermannson et al., 1978), reflect the numerous aetiologies involved in the pathophysiology of this disease that varies in its clinical presentation. All these terms summarize the characteristic syndrome of greatly reduced milk production within 12 to 48 hours post-partum, leading rapidly to piglet starvation. However, the name MMA complex is misleading, as metritis is found only occasionally in affected animals (Heinritzi and Hagn, 1999; Waldmann and Wendt, 2001), and instead of total agalactia, sows continue to produce milk at a reduced level. Still, MMA is the commonly used term in European countries, while PPDS or PDS have become widely accepted in English-speaking areas (Waldmann and Wendt, 2001; Klopfenstein et al., 2006).

Of the variety of conditions related to puerperal disorders in sows, mastitis is one of the central clinical signs, as shown by several studies (Ross et al., 1981; Wegmann et al., 1986; Heinritzi and Hagn, 1999). Bacteria most commonly isolated from affected sows are coliforms, including the genera *Escherichia*, *Citrobacter*, *Enterobacter*, and *Klebsiella* (Ross et al., 1981; Wegmann et al., 1986; Awad Masalmeh et al., 1990; Hirsch et al., 2003; Gerjets et al., 2008). The predominant role of these organisms in mastitis in sows has been demonstrated by several infection experiments (Ross et al., 1981; Wegmann and Bertschinger, 1984; Bertschinger et al., 1990). Hence, to avoid the confusing terminology and to point out the parallels to coliform mastitis in cows, the term coliform mastitis (CM) was suggested for peripartal mastitis in sows (Bertschinger, 1999). This review will concentrate on CM as an essential part of the puerperal disease complex and as a major cause of dysgalactia in sows. Most investigations into CM were carried out between 1970 and 1990, and the scarcity of recent studies is reflected in the reference list of this review.
As shown in Sweden, udder problems are the reason for culling up to 13% of sows (Ringmar-Cederberg and Johnson, 1996), but the main adverse economic effect of CM is a high pre-weaning piglet mortality (Furniss, 1987). The piglets are totally reliant on the sow for access to colostrum and milk, and growth rate depends both on milk yield and composition (Grün et al., 1993). By lying on their mammary glands, affected sows refuse piglets access to the teats. As a result of dysgalactia in combination with pain in the mammary gland, the sow fails to meet the needs of the piglets. Mortality and growth retardation in piglets are the result (Ringarp, 1960; Penny, 1970). The first 3 days after birth are the most critical period for survival of piglets. As glycogen stores are very low in new-born piglets and glyconeogenesis is insufficient, hypoglycemia may be induced in piglets with insufficient milk intake by the rapid decrease in glycogen (Sujatha et al., 2003). Inadequate colostrum intake results in deaths primarily due to starvation and hypothermia, but also because of inadequate transfer of maternal immunoglobulins to the piglet. Due to its energy and immunoglobulin content, a sufficient intake of colostrum is essential for healthy development of piglets. Inadequate colostrum intake is often followed by severe health problems, for instance, diarrhea, poor growth, and inanition (Rooke and Bland, 2002). Thus, CM creates animal welfare issues both for the sow and her piglets.

Even though infection is not transmitted through animal-animal contact, CM may become nearly epidemic in affected herds, with up to 80% of sows affected (Waldmann, 2000). In other herds, it may be limited to a few animals and may be only sporadic. The incidence of CM at farm level is reported to vary from 0.5% to 60% (Hirsch et al., 2004) in Scandinavia and from 1.1% and 37.2% (Bäckström et al., 1984) in Illinois; but average incidence at herd level is approximately 13% (Hermannson et al., 1978; Jorsal, 1983; Bäckström et al., 1984; Madec and Leon, 1992; Thorup, 2000; Krieter and Presuhn, 2005). Herds managed using totally different hygienic practices and standards may be affected (Waldmann and Wendt, 2001; Hirsch et al., 2003); CM even occurs on excellently managed farms with optimized disinfection practices (Gerjets et al., 2008; Papadopoulos et al., 2008).
Pathological findings

In recent years, several attempts have been made to classify the wide variety of clinical syndromes affecting the sow's mammary gland diagnosed in the peripartal period, but no classification has become widely accepted. For example, classifications have been based on the number of affected glands, including uniglandular or multiglandular mastitis, or, with regard to duration and state of inflammation, mastitis has been subdivided into acute and chronic mastitis (Waldmann and Wendt, 2001). Systemic signs of disease, such as fever and anorexia, are widespread, often associated with constipation and depression (Scuka et al., 2006a). The infected glands show typical signs of inflammation, such as severe oedema and skin congestion. There may be acute induration of the mammary region, although oedema without signs of acute mastitis can be found, especially in primiparous sows (Martin et al., 1967; Nachreiner et al., 1971). Caudal glands are reported to be more affected than cranial ones (Bostedt et al., 1998b), but in contrast, a more recent study detected no differences with regard to anatomic location (Gerjets et al., 2008). Other pathological findings may include fever, constipation, vulvovaginal discharge, skin discoloration, and anorexia. Haematological findings comprise leucopenia or leucocytosis, a decrease in packed cell volume and haemoglobin concentration, and an increase in serum phosphorus concentration, while concentrations of serum calcium, magnesium, and glucose may decrease (Baer and Bilkei, 2005).

A histological study by Swarbrick (1968) revealed an accumulation of secretion in mammary glands of affected sows. These findings, and the fact that early initiation of lactation (up to 24 hours before parturition) might result in engorgement of the mammary gland, suggest that early lactation is a predisposing cause of CM (Martin et al., 1978; Gooneratne et al., 1982). Initiation of lactation is induced by a decline in plasma progesterone level (Kuhn, 1969), which may appear earlier in sows with CM (Gooneratne et al., 1982). In contrast, a delayed decline in plasma progesterone level was reported by Liptrap (1980) as a causative factor for development of clinical CM.

In piglets, reduction of milk intake causes various clinical signs. The greater tendency of the sow to lie in lateral recumbency, combined with the weakness of malnourished piglets, results in an increased incidence of crushing (Hellbrugge et al., 2008). Total
piglet mortality up to the age of 1 week in the litters of CM-affected sows varies from 5.0% (Hühn and Rehbock, 2008) to 38.6% (Bäckström et al., 1984). In a study with 46 sows, the mammary secretion of sows that subsequently developed CM within 12 to 24 hours after farrowing contained significantly higher concentrations of lactose and significantly lower concentrations of protein and Na\(^+\) compared to milk from unaffected sows, while the concentration of fat and K\(^+\) was similar (Gooneratne et al., 1982). From these results, the authors of this study suggested an analysis of colostrum to identify sows predisposed to CM, to indicate affected glands, and to monitor recovery, but as this was not put into practice, there is a lack of further evidence for this theory. Coliform mastitis is often followed by temporary or permanent infertility (Bilkei et al., 1994a) caused by direct bacterial and inflammatory effects on the genital tract that prevent conception. A direct effect on the onset of the estrus cycle may not be important for development of later infertility (ten Napel et al., 1995).

**Diagnosis**

Diagnosis of CM in commercial herds is based mainly on clinical signs. Hypogalactia within the first 3 days post-partum suggests CM (Bertschinger, 1999). Piglets make vigorous nursing efforts. Both the decrease in nursing intervals and the increase in piglets’ activity derive from absent or reduced milk ejection (Bertschinger, 1999). The piglet’s strenuous nursing efforts may cause traumatized teats. After exhaustion of their energy reserves, piglets often retreat to the warmest parts of the farrowing crate and decrease their attempts to nurse (Klopfenstein et al., 2006). In sows, mammary glands may appear normal or pathologically altered, varying from swollen, firm, and warm to the touch. In addition, the skin colour can be changed.

After studying the relationship between elevated temperature and CM, it was proposed to use post-farrowing rectal temperature to determine whether CM was likely to become a serious problem (Larsen and Thorup, 2006). A study by Hermansson et al. (1978), comparing 71 sows affected with mastitis to 71 healthy sows, showed a significantly higher body temperature for the affected ones. The first trial to evaluate sow rectal temperature as a predictor of CM and to determine the specific time when the sow’s temperature should be taken was conducted by Furniss (1987). This study suggested that a rectal temperature of 39.4°C occurring 12 to 18
hours after farrowing is an appropriate threshold at which to give preventive treatment. Today, the most common practice used to detect an animal’s risk of CM is to measure the rectal temperature post-partum. Besides abnormal temperature, criteria for the diagnosis of CM must include the combination of clinical mammary gland changes, diminished milk production, and reduced appetite (Mirko and Bilkei, 2004). The range of critical temperature values varies between 39.3°C and 40.5°C (Waldmann and Wendt, 2001), but physiological hyperthermia is often observed in postparturient sows, leading to misinterpretations (Klopfenstein et al., 2006; Gerjets et al., 2008).

Body temperature is a non-specific parameter indicating alterations of the physiological state of warm-blooded animals. Plasma concentrations of acute phase proteins such as α1-acid-glycoprotein (AGP) and haptoglobin (Hp), which are part of the immune system, increase in stressful situations and can be used as indicators of acute CM (Mirko and Bilkei, 2004). Plasma concentrations of cortisol and 15-ketodihydroxy-PGF2α have also been suggested as inflammation indicators (Garcia et al., 1998). All of these parameters can vary substantially at the time of parturition (Magnusson and Fossum, 1992; Österlundh et al., 2002), and as collecting blood samples is much more laborious than measuring body temperature, use of such nonspecific indicators to diagnose CM is not feasible under field conditions. Another attempt to diagnose puerperal diseases in sows at a very early stage was made by Petersen (1983), who suggested the combination of several urine parameters to diagnose bacteriuria. In a further study, it has been shown that analysis of urinary concentrations of minerals, especially potassium, in urine samples collected from sows in the morning and afternoon during mid-lactation provide an acceptable estimation of milk production (Papadopoulos et al., 2007).

Baer and Bilkei (2005) investigated the use of ultrasonography for differentiating sows having suffered recurrent CM from healthy animals. It was shown that with a linear array technique and a frequency of 8.5 MHz, affected mammary glands provide hyperechogenic images. Furthermore, this study supports the theory that abdominal glands are more prone to pathological changes than the pectoral glands. The use of ultrasonography as a precautionary measure has not been integrated into herd management due to impractical handling and additional costs.
Rapid mastitis tests as applied to cows are not commercially available for sows. Diagnosis via cell count is not common and data on thresholds are rare. For instance, a threshold of $5 \times 10^6$ cells per mL was proposed by Bertschinger and Bühlmann (1990), while Persson et al. (1996a) suggested $10 \times 10^6$ cells per mL. All parameters used to detect CM are summarized in Table 1.

**Table 1: Parameters altered in CM-affected sows**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CM sows</th>
<th>Literature cited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body temperature</td>
<td>$&gt; 39.3, ^\circ C$</td>
<td>Hoy (2003)</td>
</tr>
<tr>
<td>Milk production</td>
<td>Hypogalactia, dysgalactia, agalactia</td>
<td>Kiss and Bilkei (2005)</td>
</tr>
<tr>
<td>Appetite</td>
<td>Diminished; moderate or total anorexia</td>
<td>Kiss and Bilkei (2005)</td>
</tr>
<tr>
<td>Cell count</td>
<td>$&gt; 10^7$/mL</td>
<td>Waldmann and Wendt (2001)</td>
</tr>
<tr>
<td>Milk pH</td>
<td>$&gt; 6.7$</td>
<td>Waldmann and Wendt (2001)</td>
</tr>
<tr>
<td>Urine parameters</td>
<td>Bacteriuria and proteinuria</td>
<td>Petersen (1983)</td>
</tr>
<tr>
<td>Interleukines</td>
<td>Increased IL-1β, IL-6, IL8, and TNFα</td>
<td>Zhu et al. (2007b)</td>
</tr>
</tbody>
</table>

**Factors influencing clinical CM**

The aetiology of CM seems to be inconsistent and challenging. Indeed, the occurrence of the disease is multifactorial. The anatomy of the sows’ mammary glands is different from that of cows. Two complete gland systems end in two teat orifices per teat, without muscular sphincters (Klopfenstein et al., 2006). The gland cisterns are not well-defined. During the last part of each gestation, mammogenesis recurs, which implies that new glandular tissue is produced. This results in a great ability of the sow to restore mammary health from one lactation to the next, although chronic lesions of the teat canal are usually irreversible (Hartmann et al., 1997; Hurley, 2001).

Coliform bacteria are ubiquitous, and therefore, influence the factors that determine the development of infection in the single animal. Factors contributing to clinically apparent CM include the strongly related main issues of nutrition, housing microclimate, management in general, and aspects of hygiene in particular. The
Factors identified thus far for an increase in CM prevalence are summarized in Table 2.

| Table 2: Non-infectious factors increasing the occurrence of CM |
|----------------------|------------------|
| **Factor**            | **Literature cited** |
| **At individual level** |                  |
| Sows of higher parity (> 4) | Baer and Bilkei (2005) |
| Young sows of lower parity (1.2) | Bostedt et al. (1998b), Hoy (2002), Krieter and Presuhn (2005) |
| Long gestation > 116 days | Awad Masalmeh (1990) |
| Long duration of birth (> 3 hours) | Bostedt et al. (1998b) |
| After obstetric intervention | Bostedt et al. (1998b) |
| Large litter size (> 11) | Bostedt et al. (1998b) |
| Urinary tract infections | Berner (1971), Petersen (1983) |
| Obstipation | Bostedt et al. (1998b) |
| Genetic disposition | Awad Masalmeh (1990) |
| **At herd level**                  |                  |
| Increasing herd size | Bäckström et al. (1984) |
| Smaller herd size | Ringarp (1960) |
| Change of housing | Waldmann and Wendt (2001) |
| In new herds of gilts | Waldmann and Wendt (2001) |
| Seasonal influences | Awad Masalmeh (1990) |
| Lack of crude fiber in the ration | Plonait and Bickhart (1997) |
| Rapid changes in nutrition | Plonait and Bickhart (1997) |
| Single housing, lack of exercise | Hoy (2002), Ringarp (1960) |

Information about the influence of parity number on occurrence of CM is contradictory (Berner, 1971; Bostedt et al., 1998a; Baer and Bilkei, 2005). The normal length of gestation in sows varies between 113 and 117 days, and CM often occurs in sows with a gestation of > 116 days (Awad Masalmeh et al., 1990). All factors contributing to prolonged duration of the birth process increase the prevalence of CM (Berner, 1971; Bostedt et al., 1998b; Papadopoulos et al., 2007), as does the concurrent occurrence of urinary tract infections (Bilkei et al., 1994a). Nutrition clearly impacts the fertility of sows at various points in the life of the sow. Several factors, such as imbalanced diet, lack of fibre, excessive feeding, or mycotoxins (i.e., in mouldy feed), must be taken into account (Heinritzi et al., 2006; Scuka et al., 2006a).
Obstipation due to diet and inadequate water intake creates further risk of CM, probably by increasing the endogenous transfer of bacteria and endotoxins to the mammary gland (Waldmann and Wendt, 2001; Krüger et al., 2002). The influence of nutrition on the hypothalamo-hypophysical gonadal axis was evaluated in a review by Cosgrove and Foxcroft (1996), who emphasized the importance of appropriate nutritional management to support the endocrine system and its influence on lactogenesis. Seasonal influences are largely eliminated by the circumstances of modern production (Bilkei et al., 1994b). However, high ambient temperatures may cause stress responses in sows, with a negative effect on reproductive performance. During lactation, high ambient temperature (> 27°C) may reduce voluntary food intake and enhance lactational weight loss (ten Napel et al., 1995; Prunier et al., 1997). This results in a contradiction for swine management in intensive piggeries: the ideal temperature for the sow to exploit her full lactation potential (< 24°C) is not the ideal temperature for her piglets (> 30°C) (Hartmann et al., 1997). The significance of these influences has been considered in management practices by providing heat lamps and other heating devices in the creep area. Late introduction into the farrowing pen, i.e., after the 110th day of gestation, is associated with an increase in CM prevalence (Scuka et al., 2006b). Furthermore, a tendency towards a lower prevalence of CM with increasing herd size was observed (Lingaas and Ronningen, 1991). In contrast to this, Bäckström et al. (1984) found a higher prevalence of CM with increasing herd size.

Bacteria and endotoxins causing CM
The causative agents of CM and their role in pathogenesis have been discussed controversially, as many different bacterial species have been isolated from the milk of clinically diseased animals (Awad Masalmeh et al., 1990; Kobera, 2000), including mainly coliform bacteria (Escherichia coli and other lactose-splitting bacteria), but also Streptococci, Staphylococci, Pseudomonas species, and Corynebacterium species. One problem regarding the presence of different bacterial species in the milk of affected animals is the use of inadequate methods for identification.

There are three main theories concerning the routes of infection for CM: endogenous, including the gut and the uterus, and exogenous via the mammary gland. The infectious dose for colonization of the mammary gland is extremely low at < 100
organisms (Österlundh et al., 2002; Papadopoulos et al., 2007). Causative bacteria are located free in the milk or in phagocytic cells in the ductular and alveolar lumina and are often isolated from regional lymph nodes (Armstrong et al., 1968; Bertschinger et al., 1977a; Ross et al., 1981). In a study comparing the bacterial flora of the uterus, the cecum, the ileum, and the mammary gland in order to identify a likely source of endotoxin absorption, the prevalence of only gram-negative bacteria in the mammary glands and in the ileum of CM-affected sows was remarkable (Morkoc et al., 1983). The lack of gram-negative bacterial culture growth in uterine samples supports the theory that uterine involvement in CM is of minor importance, as has been suggested (Armstrong et al., 1968; Martin, 1970; Nachreiner and Ginther, 1974). The hypothesis of a galactogenous route of infection via the teat duct is supported by experiments carried out by Bertschinger et al. (1990) and Bertschinger et al. (1977b), who found a lower prevalence of CM when the mammary gland was protected against faecal contamination. Due to repeated sampling, the time of infection could be determined in this experimental setting. More than 50% of mammary glands were infected before parturition, but no new infections appeared before the 108th day of gestation (Bertschinger et al., 1990). New infections were limited to the first 2 days after farrowing. This limitation was explained by the established teat preference of the piglets and suckling at regular intervals of three-quarters of an hour (Bertschinger et al., 1990).

All isolated gram-negative bacteria are common in the sows’ environment, depending on a combination of circumstances. For instance, the use of wood shavings as bedding material leads to an increased occurrence of pathogenic *Klebsiella pneumoniae* (Hogan and Smith, 1997), that might end in more infections of the mammary glands of the sows due to a high contamination rate in the material. The origin of bacteria in the environment may be related to the excretion of urine and faeces by the sows. In this context, it is notable that infections of the urinary tract are strongly related to puerperal diseases, even though urinary infections are not apparent clinically (Mauch and Bilkei, 2004). The most common organism associated with bacteriuria and vulval discharge was found to be *E coli* (Waller et al., 2002). The mammary gland as a source of gram-negative bacteria was first described by Elmore et al. (1978) and Jones (1979). The predominant role of coliform bacteria in pathogenesis was clearly shown by Wegmann et al. (1986); both *E coli* and *K pneumoniae* were isolated from 79% of 131 mammary complexes of CM-affected
sows. In a study with 663 sows suffering recurrent CM, bacteriological examination of mammary gland changes revealed the presence of mainly *E coli* and *Klebsiella* species, but also *Clostridium* species, *Actinobaculum suis*, *Pseudomonas aeruginosa*, *Proteus* species, gram-positive streptococci (especially *Enterococci* and *Streptococcus faecalis*), staphylococci (*Staphylococcus albus*, *Staphylococcus epidermis*, *Staphylococcus aureus*), and *Erysipelothrix rhusiopathiae* (Baer and Bilkei, 2005).

The prominent role of *E coli* in mastitis has been emphasized in several studies (Armstrong et al., 1968; Bertschinger et al., 1977a; Ross et al., 1981; Wegmann et al., 1986). Bacteriological examinations of milk and udder biopsies and necropsy material from sows with CM have indicated that *E coli* is the causative pathogen for agalactia in the majority of cases (Persson, 1997; Pedersen Mörner et al., 1998). Typically, peripartum mastitis caused by *E coli* is acute (Bäckström et al., 1984), but postparturient mastitis has also been described in sows lacking signs of clinical CM (Persson, 1997). In sows, experimentally induced *E coli* or *K pneumoniae* mastitis provokes clinical and haematological changes comparable to natural infections (Bertschinger et al., 1977a; Ross et al., 1983). The extensive interplay between pathogen and host can cause different clinical syndromes. While some sows develop clinical signs of CM after inoculation of the mammary glands with *E coli*, others remain unaffected (Österlundh et al., 1998). A large study of 39 pairs of full siblings (Swedish Landrace × Swedish Yorkshire) over six parities demonstrated that less than half of the mammary glands with CM (diagnosed by milk bacteriology and cytology) showed clinically detectable mastitis (Persson et al., 1996b).

Nevertheless, the involvement of defined *E coli* strains and the occurrence of certain virulence determinants such as shigatoxins remain ambiguous with regard to the development of clinical appearance (Pedersen Mörner et al., 1998). A wide variety of *E coli* serotypes have been substantiated in mastitic sows’ milk in previous studies (Armstrong et al., 1968; Awad Masalmeh et al., 1990). Bostedt et al. (1998b) found a high percentage of antibiotic-resistant *E coli* in cervical swabs from sows with CM: the isolated strains were 100% sensitive only to gentamicin. Sensitivity to all other tested antibiotics was < 100%. The findings of Pedersen Mörner et al. (1998) support the theory of a galactogenous route of infection: serological homogeneity was found
in *E coli* isolates from the same teats at different times during lactation, while heterogeneity was encountered for different teats in the same sampling. On the basis of current knowledge, this may be interpreted as mastitis in sows being caused by several *E coli* strains harbouring virulence factors which are as yet unknown. Indeed, recent genome-sequencing studies of various *E coli* strains have determined a core genome of only 30% harboured by all these strains, making this possibility a challenging concept.85

Lipopolysaccharide (LPS) endotoxins, present in all gram-negative bacteria, play a major role in the etiology of CM (Hacker et al., 2004). Like bacteria, endotoxins enter via the uterus, gut, and mammary gland. The systemic clinical signs elicited by endotoxin release are complex, as various endogenous mediators are involved in pathogenesis. The relevance of *E coli* endotoxins initiating complex reactions in the animal organism has been proven before (Ramasoota et al., 2000; Magnusson et al., 2001). The administration of coliform endotoxins via intravenous, intramammary, intrauterine, or subcutaneous application causes clinical and blood chemical changes similar to those in natural CM cases (Nachreiner and Ginther, 1969, 1974; Elmore et al., 1978). For instance, subnormal serum concentrations of Ca++, Zn++, and iron is a clear indication of endotoxin exposure (Holst et al., 1993), as is a rise in serum cortisol levels (Magnusson et al., 1994). Furthermore, secretion of colostrum and milk depends on the complex and well-balanced interaction of a series of different hormones. These complex balances can be easily disturbed when LPS suppresses the release of prolactin by the anterior pituitary, increasing cortisol concentrations and decreasing circulating thyroid hormone (Smith and Wagner, 1985). Production and secretion of milk are affected adversely by these changes.

**Immune response and innate immunity**

To a large extent, the outbreak of disease is determined by the interaction between the invading microorganism and the host’s immune system. Clinical signs of CM are most often seen in the first 24 hours after parturition, indicating a strong connection to the postpartum period. In an experimental setting, Magnusson et al. (2001) found that the time of inoculation of bacteria into the mammary gland influenced the development of disease: clinical signs were seen in sows infected 48 hours, but not 96 hours, before parturition. Furthermore, the number of circulating
polymorphonuclear neutrophils was higher in sows that were more prone to develop disease. Whether this fact can be related to the presence of other microorganisms was not defined, but all sows had been diagnosed as healthy at the beginning of the infection trial (Magnusson et al., 2001). Possibly, an exaggerated response to bacterial infections, causing tissue injury, aggravates clinical signs (Magnusson et al., 2001). Moreover, lysozyme, an enzyme that non-specifically stimulates the phagocytic activity of leucocytes and the level of immunoglobulins, was present in high concentrations in sows from herds of low CM prevalence (Wawron, 1995). After experimental inoculation of *E coli* (0.5 mL of bacterial suspension per teat, $10^5$ colony forming units per mL), Österlundh et al. (2002) showed no significant differences in functional capacities of granulocytes in sows affected and non-affected by CM.

After inoculation of 12 sows with *E coli* by the intramammary route (0.5 mL of bacterial suspension per teat, $10^5$ colony forming units per mL), Zhu et al. (2007a) detected an increase in proinflammatory cytokines. The mammary glands appeared capable of producing IL-1β, IL-6, IL-8, and TNF-α, and the authors concluded that local cytokine mRNA expression differs between mammary glands of sows that do or do not develop clinical signs of mastitis. Especially TNF-α is considered to be a useful indicator to monitor the severity and course of CM (Nakajima et al., 1997; Zhu et al., 2004; Zhu et al., 2007b). Löving and Magnusson (2002) showed a significantly higher density of CD4$^+$ and CD8$^+$ cells in animals developing clinical mastitis compared to those without clinical disease, supporting the theory that massive inflammatory reactions are triggered by endotoxins. In addition, in this study by Löving and Magnusson (2002), sows developing clinical disease had a lower density of MHC class II$^+$ cells. This down-regulation can be related to the adverse effects of LPS. Therefore, the authors postulated that the outcome of mammary infection was related to sensitivity to LPS rather than to an ineffective immune response (Löving and Magnusson, 2002).

Furthermore, the immune response is modified both by cortisol and oestrogen affecting resistance to infection (Kelley et al., 1994), and both hormones vary considerably in their concentration at the time of parturition. Resistance to infection in swine is also influenced by sex hormones (Magnusson and Einarsson, 1990; Magnusson and Fossum, 1992). However, in another study by Magnusson et al.
(2001), a difference in concentration of these hormones could not be identified in sows with and without CM, suggesting that development of mastitis in sows before parturition is not modulated by cortisol and oestrogen.

**Treatment**

After diagnosis of CM, antibiotic treatment must be started as soon as possible to reduce the negative effects on both the sow and the piglets. Antibiotics are often administered immediately after diagnosis to shorten the time period of undernutrition for the piglets, but antimicrobial susceptibility is not tested. Therefore, the use of broad spectrum antimicrobials administered parenterally, for example amoxicillin (Markowska-Daniel and Kolodziejczyk, 2001), tylosin (Waldmann and Wendt, 2001), or potentiated sulphonamides (Waldmann and Wendt, 2001), is indicated. Antibiotics must reach effective levels in the mammary gland; consequently, pharmacokinetics have to be considered. Another antibiotic showing a concentration in colostrum and milk explicitly above the minimum inhibitory concentration is enrofloxacin (Oliel and Bertschinger, 1990). In several studies, its use as a highly efficient antibiotic given orally at 2.5 mg per kg body weight twice a day is recommended (Schöning and Plonait, 1990; Rose et al., 1996; Scuka et al., 2006a). In a study on the therapeutic performance of the cephalosporin cefquinom, this antibiotic, injected intramuscularly at doses of 2 mg per kg body weight every 24 hours for 3 days was more efficient than the control drug, amoxicillin (Heinritzi and Hagn, 1999).

In order to reduce inflammatory reactions, therapy with non-steroidal anti-inflammatory drugs (NSAIDs), especially meloxicam at 0.4 mg per kg body weight per sow in a single injection, has become popular in recent years (Hirsch et al., 2003; Hoy and Friton, 2005). The advantages of this treatment are better recovery rates and reduced piglet weight losses (Hoy and Friton, 2005). Use of flunixin meglumine combined with enrofloxacin achieved no advantages compared to use of enrofloxacin alone (Sterr, 2001). Occasionally, oxytocin (10 IU), injected five times at 2- to 3-hour intervals, can initiate milk production (Waldmann and Wendt, 2001). However, as routine use of oxytocin is associated with poorer herd performance (Ravel et al., 1996), overuse should be avoided.
The effect of prostaglandin F2\(\alpha\) (PGF2\(\alpha\)) injection is controversial: in some herds, the risk for periparturient disorders was minimized (Baer and Bilkei, 2005), while in others, no effect could be proven (Hansen and Jacobsen, 1976; Ehnvall et al., 1977). Prostaglandin F2\(\alpha\) has its main impact on uterine debris postpartum, and, therefore, administration in cases of CM is not indicated. As proposed by Kirkwood (Kirkwood, 1999), in the absence of vulval discharge problems, PGF2\(\alpha\) does not improve sow and litter performance. An alternative attempt to treat CM with bee venom was proposed by Choi and Kang (Choi and Kang, 2001). Animals treated with apitherapy showed significantly shorter periods of abnormal milk secretion (clots, blood traces, or discoloration) compared to animals receiving antibiotic treatment with penicillin G at 400,000 IU per animal. Besides treatment of sows, all economically reasonable efforts to save the piglets should be attempted. To save the litter, piglets can be cross-fostered or fed milk replacer (Klopfenstein et al., 2006).

From the very first recognition of CM as a problem in sows, there have been various efforts to reduce prevalence of CM by a considerable number of measures. Nutrition management is proposed as a useful tool to minimize the risk of CM (Persson et al., 1989). High-fibre diets in late gestation have been used to decrease the occurrence of early lactation problems, but it is unclear whether fibre addition or resultant protein dilution in the feed ration is the cause of a lower prevalence of CM (Klopfenstein et al., 2006). Feed reduction before parturition is a widespread practice and might reduce not only obstipation, but also the amount of faeces produced. Consequently, the exposure of the teats to contamination is reduced, and CM risk decreases as well (Klopfenstein et al., 2006). On the day before and after farrowing, provision of ad libitum drinking water is recommended (Waldmann and Wendt, 2001). Supplementation with lactulose as a prebioticum in periparturient sows results in better sow and piglet performance (Cosgrove and Foxcroft, 1996). Other measures to avoid obstipation are feeding of linseed and other laxatives and adequate exercise for the sow (Bilkei and Horn, 1991; Cosgrove and Foxcroft, 1996). Good hygiene practice with all-in, all-out management, adequate temperatures in the farrowing houses, and introduction of sows to clean farrowing houses 10 to 14 days prior to farrowing are management factors that should be taken into account (Hammerl et al., 1995). Manual interventions, eg, manual obstetrics in the peripartal period, should be reduced to a minimum. Nevertheless, neither this nor other management practices
are able to totally prevent CM. Identification and reduction of risk factors, combined with excellent hygiene management, are the only way to cope with a herd problem in the long term (Hoy, 2003).

Non-specific paramunity inducers like an immunostimulator containing inactivated *Parapovirus ovis* (Bayer AG, Leverkusen, Germany) were proved to have positive effects on sows affected by CM (Choi and Kang, 2001). However, after natural infection, mammary glands did not develop resistance to subsequent infections (Bertschinger et al., 1990). Therefore, the effect of vaccines against *E. coli* with regard to CM can be doubted. Furthermore, there must be strict adherence to subcutaneous injection of the vaccine, as the same dose administered via intramuscular or intravenous injection may cause severe endotoxemia (Garcia et al., 1998; Bertschinger, 1999). While vaccinations against infections with enterotoxigenic *E. coli* in piglets are commercially available and show positive effects (Haesebrouck et al., 2004), the current knowledge about pathogen-host-interactions in CM is still too limited to develop useful prevention tools.

**Conclusion and future approaches**

Commercial sow lines from pig breeding companies are continuously being improved in their reproductive capabilities, with large litters and high-milk-producing potential, and pigs are therefore exposed to a physiologically extreme situation during and soon after birth. Although severe forms of CM are rare, piglet mortality and failure to gain weight contribute to the outstanding economic relevance of this disease complex. The demands for sufficient growth rate of suckling piglets and greater litter size puts pressure on the lactating sow. The transition from gestation to lactation is of paramount importance to sufficient milk yield and prevalence of CM during that period. High piglet mortality, poor growth of suckling piglets, and poor average weaning weights can be prevented only when CM is approached in a holistic way. The current method to deal with postparturient disorders includes immediate antibiotic treatment of sows if body temperature is above a defined threshold. This threshold is defined rather subjectively and the use of it might be regarded critically, since increases and decreases in body temperature may appear physiological. To minimize the administration of antibiotics, it is therefore essential to diagnose CM and PPDS not only by temperature increase, but also by a combination of appropriate
criteria. A threshold of 39.5°C in the time frame 12 to 24 hours postpartum is recommended to avoid confusion of fever with physiological hyperthermia (Gerjets et al., 2008).

Prevention is the best way to cope with CM in a population, but difficult to accomplish, as the etiology of CM is extremely variable. At the current state of knowledge, the reason for only some sows developing clinical signs of infection after contact with ubiquitous bacteria remains unknown. The immune response and the actual development of clinical signs seem to depend on the immunological reactivity of the sow. Hence, one may hypothesize that developing clinical CM is largely dependent on the individual resistance of the sow. Immune competence, including resistance to infections, is genetically determined (Mallard et al., 1992; Magnusson and Greko, 1998). The heritability for CM resistance is approximately 10% (Lingaas and Ronningen, 1991; Berg et al., 2001). As shown by Heringstad et al. (1999; 2003) for mastitis resistance in dairy cattle, it is possible to achieve a sustainable selection response for disease traits of low heritability. Thus, the analysed heritabilities for CM resistance indicate the opportunity to use this trait for selection (Lingaas and Ronningen, 1991; Berg et al., 2001). In pig production, genetic disease resistance, particularly resistance against certain *E. coli*, is applied as a breeding tool in the United States, Canada, Denmark, and Switzerland. Since infectious organisms evolve resistance against drugs used to control them, as shown for pathogens that cause CM (Acar and Rostel, 2001), and since the costs of treatment and veterinary care are increasing faster than the value of animals, breeding for enhanced disease resistance offers a number of advantages over other control measures. Additionally, on the basis of the current knowledge of *E. coli* strains involved in CM, no common virulence factor has been identified. To discover this genetic component in the involved *E. coli* strains and other bacterial species is an immense challenge for further research, as are the scientific questions relating to CM in general.
References


Chapter 2

Comparison of virulence gene profiles of *Escherichia coli* isolates from sows with Coliform mastitis and healthy sows

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Abstract
Coliform mastitis (CM) is not only a serious economical and animal welfare touching problem in dairy cattle, but also in sows after farrowing. Due to this disease, the essential adequate supply with colostrum for the growth and the health of the piglets is not ensured. Besides other influencing factors, *Escherichia* (*E.*) *coli* is of great importance as a causative agent of this multifactorial disease. In this study, *E. coli* isolates from milk samples of healthy and CM-affected sows were examined for the presence of virulence genes associated with extraintestinal *E. coli* strains, enterotoxigenic *E. coli* and other pathogenic *E. coli*. The isolated *E. coli* harbored mainly virulence genes of extraintestinal *E. coli* strains (especially *fimC, ompA, traT, hra, kpsMTII, iroN*). The virulence gene spectrum for both samples from CM-affected and healthy sows did not differ significantly. Particular virulence gene profiles of *E. coli* isolates from diseased sows were not detected.

This study provides novel insights into the role of *E. coli* in association with mastitis in sows since it is the first time *E. coli* isolates from CM-affected sows’ milk were analysed for virulence genes. Because there were no differences in the prevalence of *E. coli* and their virulence-associated genes between healthy and diseased sows, other causative factors seem to have greater influence on the pathogenesis of porcine CM.

Keywords
ETEC, ExPEC, multiplex PCR, swine, virulence factors
Introduction

‘Coliform mastitis’ (CM) is the main symptom of puerperal disorders occurring in sows after farrowing which are subsumed under the term postpartum dysgalactia syndrome (PPDS or PDS) (Gerjets and Kemper, 2009; Klopfenstein et al., 2006). The etiology of CM is multifactorial with husbandry, management, feeding and hygiene as influencing factors (Klopfenstein et al., 2006), but mainly bacteria are the causative agents for the inflammation. In bacteriological analyses, especially *Escherichia (E.) coli* was isolated, but the strains were not further investigated for virulence-associated genes. Strains of *E. coli* can be broadly classified into three groups by their location and their characteristic virulence genes: commensal *E. coli*, intestinal pathogenic *E. coli* (IPEC) colonizing the intestine, and extraintestinal pathogenic *E. coli* (ExPEC) that reach extraintestinal niches like the urinary tract (Russo and Johnson, 2000). In swine, especially enterotoxigenic *E. coli* (ETEC) as a pathotype of IPEC are well described as causal agents for severe diseases like diarrhea in neonatal and weaned piglets (Casey and Bosworth, 2009). The ExPEC pathotypes are e.g. causative for urinary tract infections (uropathogenic *E. coli* (UPEC)) or septicaemia in pigs (Daigle et al., 1997; Krag et al., 2009; Shpigel et al., 2008). A selection of virulence genes known to be associated with ETEC, ExPEC pathotypes and shiga toxin-producing *E. coli* (STEC) is listed in Table 1.

A new putative pathotype of ExPEC was proposed by Shpigel et al. (2008): mammary pathogenic *E. coli*, with as specific set of virulence genes, which are associated with mastitis in dairy animals. However, up to now epidemiological studies have not shown a common virulence gene profile for these *E. coli* so far (Kaipainen et al., 2002; Srinivasan et al., 2007; Wenz et al., 2006).

The aim of our study was to analyse the occurrence of different virulence genes in *E. coli* isolates associated with Coliform mastitis in sows.
Table 1: Prevalence of virulence-associated genes in *E. coli* isolates from healthy/diseased sows (*P*<0.05)

<table>
<thead>
<tr>
<th>Gene(s)/categories</th>
<th>prevalence of virulence-associated genes (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli isolates (n = 1,271)</td>
<td>E. coli isolates (n = 1,132)</td>
</tr>
<tr>
<td></td>
<td>of CM-negative sows</td>
<td>of CM-positive sows</td>
</tr>
<tr>
<td>Adhesins</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>afa / dra</em></td>
<td>ExPEC</td>
<td>-</td>
</tr>
<tr>
<td><em>fimC</em></td>
<td>ExPEC</td>
<td>82.30</td>
</tr>
<tr>
<td><em>hra</em></td>
<td>ExPEC</td>
<td>11.33</td>
</tr>
<tr>
<td><em>iha</em></td>
<td>ExPEC</td>
<td>0.16</td>
</tr>
<tr>
<td><em>sfa / foc</em></td>
<td>ExPEC</td>
<td>0.08</td>
</tr>
<tr>
<td><em>K99 (fanA)</em></td>
<td>ETEC</td>
<td>-</td>
</tr>
<tr>
<td><em>K88 (faeG)</em></td>
<td>ETEC</td>
<td>0.08</td>
</tr>
<tr>
<td>987P (fasA)</td>
<td>ETEC</td>
<td>0.08</td>
</tr>
<tr>
<td><em>F18 (fedA)</em></td>
<td>ETEC</td>
<td>-</td>
</tr>
<tr>
<td><em>F41 (fedA subunit)</em></td>
<td>ETEC</td>
<td>-</td>
</tr>
<tr>
<td>Iron</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>chuA</em></td>
<td>ExPEC</td>
<td>4.80</td>
</tr>
<tr>
<td><em>iron</em></td>
<td>ExPEC</td>
<td>9.28</td>
</tr>
<tr>
<td><em>sitD chr.</em></td>
<td>ExPEC</td>
<td>0.24</td>
</tr>
<tr>
<td><em>sitD ep.</em></td>
<td>ExPEC</td>
<td>5.74</td>
</tr>
<tr>
<td>Protectins</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>neuC</em></td>
<td>ExPEC</td>
<td>0.39</td>
</tr>
<tr>
<td><em>kpsMT II</em></td>
<td>ExPEC</td>
<td>9.99</td>
</tr>
<tr>
<td><em>ompA</em></td>
<td>ExPEC</td>
<td>37.61</td>
</tr>
<tr>
<td><em>traT</em></td>
<td>ExPEC</td>
<td>49.80</td>
</tr>
<tr>
<td>Toxins</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>hlyA</em></td>
<td>ExPEC</td>
<td>1.65</td>
</tr>
<tr>
<td>Enterotoxins</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>STII</em></td>
<td>ETEC</td>
<td>-</td>
</tr>
<tr>
<td><em>STI</em></td>
<td>ETEC</td>
<td>2.28</td>
</tr>
<tr>
<td><em>LT</em></td>
<td>ETEC</td>
<td>-</td>
</tr>
<tr>
<td>Shiga Toxins</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Stx2e</em></td>
<td>STEC</td>
<td>-</td>
</tr>
<tr>
<td>Invasins</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>gimB</em></td>
<td>ExPEC</td>
<td>0.08</td>
</tr>
<tr>
<td><em>ibeA</em></td>
<td>ExPEC</td>
<td>0.63</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>pic</em></td>
<td>ExPEC</td>
<td>0.63</td>
</tr>
<tr>
<td><em>malX ( RPai)</em></td>
<td>ExPEC</td>
<td>-</td>
</tr>
</tbody>
</table>

Materials and methods

Animals and study design

The investigation was carried out between April 2008 and August 2010 on five multiplication herds in Germany (A - E), supervised by PIC Germany GmbH
Schleswig (Table 2). The farms were of high health status and tested free from enzootic pneumonia, rhinitis, *Actinobacillus pleuropneumoniae* and dysentery. The number of sows housed in the farms ranged from 700 to 1,800. The sows were in different parities (1–9) and of different lines (Landrace, Large White and crossbreds, partly with Duroc).

They were identified as CM-affected when their rectal temperature was above 39.5°C 24 h post partum (Furniss, 1987) and the mammary glands showed symptoms of inflammation. In addition, the appearance and the performance of the piglets were evaluated with regard to their behavior and body condition. Healthy half- or full-sib sows from the same farrowing group that farrowed closest in time served as controls. The half-sib design was chosen due to further studies on the genetic background via genotyping (Preißler et al., unpublished data). In total, 2,005 milk samples were examined (1,026 milk samples from sows with CM and 979 from healthy sows).

Before gathering a collective sample of several teats, mammary glands were cleaned and disinfected with disinfection swabs containing 70% isopropyl-alcohol. The first streams of milk were discarded whereas the followings were milked on transport swabs with Amies medium (transwab, medical wire & equipment, Corsham, England). The milk samples were stored at 4°C before sending them to the laboratory within 72 hours.

Table 2: Number of milk samples and *E. coli* isolates of five different farms

<table>
<thead>
<tr>
<th>Farm</th>
<th>number of milk samples</th>
<th>number of <em>E. coli</em> isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CM-negative</td>
<td>CM-positive</td>
</tr>
<tr>
<td>A</td>
<td>498</td>
<td>501</td>
</tr>
<tr>
<td>B</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>C</td>
<td>276</td>
<td>323</td>
</tr>
<tr>
<td>D</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>E</td>
<td>167</td>
<td>167</td>
</tr>
<tr>
<td>total</td>
<td>979</td>
<td>1,026</td>
</tr>
</tbody>
</table>

*Bacteriological analysis*

The swabs were incubated in Caso broth for 24 h at 37°C. With a plastic loop, 10 µL of the enrichment were streaked onto Columbia blood agar and Endo agar (both Oxoid, Cambridge, United Kingdom) and incubated aerobically another 24 h at 37°C. The grown bacteria were differentiated by their morphology, haemolysis on blood agar and Gram staining. Pure cultures were grown on blood agar after another 24 h
incubation at 37°C before biochemical confirmation to species level with the identification system API (bioMérieux, Craponne, France).

*Escherichia coli* isolates were distinguished due to individual morphology on blood agar and API 20E. All isolates were selected for further investigations. Desoxyribonucleic acid of the identified *E. coli* strains was prepared by solving a few colonies in 200 µL distilled water. After boiling for 10 min and centrifugation, 3 µL of the supernatant was taken for PCR analysis. The presence of virulence genes associated with ExPEC strains, ETEC and other pathogenic *E. coli* was determined by multiplex PCR (mPCR) assays for all *E. coli* isolates, as described by Ewers et al. (2007) and Casey and Bosworth (2009).

In total, 2,403 isolates were tested for the presence of 27 virulence genes for the following virulence factors (Table 1): heat labile toxin (*LT*), heat stable toxin a and b (*STI, STII*), Shiga toxin (*Stx2e*), capsular polysaccharide (*neuC*), group II capsule antigen (*kpsMTII*), outer membrane protein (*ompA*), transfer protein (*traT*), heme receptor gene (*chuA*), catecholate siderophore receptor (*iroN*), iron transport system genes (*sitD chr., sitD ep.*), haemolysin A (*hlyA*), invasins (*gimB, ibeA*), serin protease autotransporter (*pic*), different adhesins and fimbrial genes (*afa/draB, fimC, hra, iha, stl/foCD, K88, K99, 987P, F41, F18*) and pathogenicity-associated island marker (*RPai (malX)*). Controls for molecular assays were avian pathogenic *E. coli* (APEC) strain IMT2470, UPEC strains IMT7920 and IMT9267 and ETEC strains IMT204, IMT19, IMT4830 and IMT3838 (Casey and Bosworth, 2009; Ewers et al., 2007), kindly provided by the Institute of Microbiology and Epizootics of the Free University Berlin.

**Statistical analysis**

The statistical analysis was performed using the procedures FREQ and CORR from the Statistical Analysis System (SAS Institute Inc., 2005). Chi square-tests were used to analyse differences in virulence gene frequencies between diseased and healthy sows. Statistical significance was indicated in two levels: $P<0.05^*$ and $P<0.01^{**}$. Pearson correlation coefficients, calculated to show associations between virulence genes, were presented as heatmaps. Heatmaps representing gene prevalence were generated to allow assessment of the virulence genes regarding occurrence and distribution (R Development Core Team, 2009). Virulence genes in the heatmaps were arranged automatically according to their means. Genes with similar means are ordered close together.
Correlations and heatmaps were performed with only those virulence genes detected in more than 1% of the analysed E. coli strains.

Results

Escherichia coli strains

Escherichia coli was found in 70.6% (n=724) of the milk samples of CM-affected and in 77.9% (n=762) of the milk samples of non-infected sows. In total, 1,132 E. coli isolates from CM-positive samples and 1,271 isolates from CM-negative samples were identified and further examined by mPCR. The median number of isolates in milk samples of both diseased and healthy sows was one (Figure 1). Of the 2,403 E. coli isolates, 593 harbored one virulence gene, 983 two, 357 three and 369 four or more virulence genes. In 101 E. coli isolates, no virulence-associated genes were found. The E. coli isolates from CM-positive as well as from negative sows had a median number of two virulence genes.

![Figure 1: Number of E. coli isolates in milk samples from CM-positive and -negative sows](image)

Comparison of virulence gene profiles

A variety of virulence genes was identified consisting of mainly those associated with ExPEC (98.9% of E. coli isolates from diseased and 99.0% of E. coli isolates from healthy sows) (Table 1). The highest prevalence was found for the type 1 fimbriae fimC (in 84.7% of the isolates of diseased and 82.3% of the isolates of healthy sows).
sows) and for the protectins *ompA* and *traT* (in 35.3 % and 52.1 % of the isolates from CM-positive, and 37.6 % and 49.8 % of the isolates from CM-negative sows, respectively). Other genes identified in 9.3 to 14.8 % of the *E. coli* isolates were *hra*, *kpsMTII* and *iroN*. Almost all of the virulence-associated factors were more often detected in *E. coli* isolates of CM-affected sows than in isolates of healthy sows, except *987P*, *neuC*, *ompA* and *gimB*.

The virulence genes *hra*, *chuA*, *iroN* and *kpsMTII* occurred significantly more frequently in isolates of diseased animals. The same applied for particular combinations of these genes (Table 3), except for the profiles *chuA* - *iroN*, *kpsMTII* – *chuA* - *iroN* and *kpsMTII* – *hra* – *chuA* - *iroN*. Those combinations were also less prevalent in all *E. coli* isolates. The greatest difference between diseased and healthy sows was found for the virulence gene profile *chuA* - *hra* (2.7 % in *E. coli* of CM-positive and 0.9 % in *E. coli* of CM-negative sampled sows, respectively). In total, there were no obvious patterns specific for either diseased or healthy sows.

### Table 3: Prevalence of virulence gene profiles in *E. coli* isolates from clinically CM-diseased and healthy sows (*P*<0.05, **P*<0.01)

<table>
<thead>
<tr>
<th>Virulence gene profile</th>
<th>Prevalence of <em>E. coli</em> isolates (%) with respective gene profile from:</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>samples (n = 979) of CM-negative sows</td>
<td>samples (n = 1,026) of CM-positive sows</td>
</tr>
<tr>
<td><em>kpsMTII</em>**</td>
<td>9.99</td>
<td>13.07</td>
</tr>
<tr>
<td><em>chuA</em>**</td>
<td>4.80</td>
<td>6.71</td>
</tr>
<tr>
<td><em>hra</em>**</td>
<td>11.33</td>
<td>14.84</td>
</tr>
<tr>
<td><em>iron</em>**</td>
<td>9.28</td>
<td>12.37</td>
</tr>
<tr>
<td><em>kpsMTII, chuA</em>**</td>
<td>0.87</td>
<td>2.21</td>
</tr>
<tr>
<td><em>kpsMTII, hra</em>**</td>
<td>2.44</td>
<td>4.42</td>
</tr>
<tr>
<td><em>kpsMTII, iron</em>**</td>
<td>1.42</td>
<td>3.09</td>
</tr>
<tr>
<td><em>chuA, hra</em>**</td>
<td>0.94</td>
<td>2.65</td>
</tr>
<tr>
<td>*chuA, iron</td>
<td>0.24</td>
<td>0.44</td>
</tr>
<tr>
<td><em>hra, iron</em>**</td>
<td>1.34</td>
<td>2.65</td>
</tr>
<tr>
<td><em>kpsMTII, chuA, hra</em>**</td>
<td>0.63</td>
<td>1.59</td>
</tr>
<tr>
<td>*kpsMTII, chuA, iron</td>
<td>0.08</td>
<td>0.27</td>
</tr>
<tr>
<td><em>kpsMTII, hra, iron</em>**</td>
<td>0.31</td>
<td>1.33</td>
</tr>
<tr>
<td><em>chuA, hra, Iron</em>**</td>
<td>-</td>
<td>0.35</td>
</tr>
<tr>
<td><em>kpsMTII, chuA, hra, iroN</em></td>
<td>-</td>
<td>0.27</td>
</tr>
</tbody>
</table>
Correlations between virulence genes

Statistical analysis of associations between all virulence factors of the *E. coli* isolates is shown in Figure 2. Several similar patterns in the heatmaps were visible for virulence genes of strains from CM-positive and negative sows: the gene *hlyA* is positively associated with *chuA and pic*; *iroN* is positively associated with *ompA* and *sitDepi*, respectively. Highest positive correlations existed between the genes *iroN* and *sitDepi* for both isolates from diseased and healthy sows. The genes *traT* and *fimC* were also highly positive correlated, but only in *E. coli* isolates of CM-negative sows.
Figure 2: Statistical associations between 12 virulence-associated genes from *E. coli* isolates of CM-positive and CM-negative sows. Colours range from light grey (little associated) to dark grey (highly associated) (p<0.05). Gaping spaces indicate no significant correlation between virulence genes.
Gene prevalence with regard to different seasons and farms

The gene \textit{traT} was more often found in \textit{E. coli} isolates of samples of CM-positive sows in winter whereas \textit{STI} (heat stable toxin a) was only found in summer (Figure 3). The gene \textit{chuA} occurred more frequently in \textit{E. coli} isolates of positive sows in winter and autumn and \textit{iroN} in summer, autumn and winter as well as \textit{kpsMTII} was always more prevalent in samples of diseased sows. All virulence genes were found more often in \textit{E. coli} isolates of diseased sows in all seasons except for \textit{ompA} and \textit{traT} which were more prevalent in isolates of healthy sows in spring.

However, the differences in occurrence of the genes were greater between the seasons than between CM-positive and negative sows.

The same held true for the influence of the farms on the occurrence of virulence genes. The gene \textit{STI} was only found in \textit{E. coli} isolates sampled from farm A whereas \textit{kpsMTII} was more prevalent in samples from farm D. The gene \textit{traT} occurred more often in isolates from diseased sows. The gene prevalence on the farms differed only slightly between CM-infected and healthy sows. Differences regarding the occurrence of the mentioned virulence genes in the seasons and farms were significant (P<0.05).
Figure 3: Heatmaps representing gene prevalence in *E. coli* isolates (n=2,403) of different CM-status (neg, pos), seasons (spring, summer, autumn, winter) and farms (A, B, C, D, E). Colours range from light grey (gene found in 1 - 5 % of the isolates) to dark grey (gene found in 80 - 88 % of the isolates).
Discussion

The aim of the study was to analyse and compare virulence genes of *E. coli* isolates from milk samples of CM-positive and CM-negative sows, because virulence gene profiles of *E. coli* isolates associated with mastitis has not been described so far (Kaipainen et al., 2002; Srinivasan et al., 2007; Wenz et al., 2006). *Escherichia coli* is the pathogen most frequently isolated in association with porcine puerperal disorders (Armstrong et al., 1968; Awad Masalmeh et al., 1990; Bertschinger et al., 1977a; Ross et al., 1981). It was also isolated in high frequencies in milk samples of diseased sows in this investigation as well as in milk from healthy sows.

The detailed analysis of virulence-associated genes of the *E. coli* isolates revealed only slight differences between isolates of diseased and healthy sows \((P<0.05)\). Although there were single genes or gene combinations with a greater linkage to *E. coli* isolates from milk samples of CM-affected sows, there were no specific virulence gene patterns detectable. Heatmaps were performed to allow a visualization of correlations among virulence genes of isolates of different CM-status, seasons and farms.

The *E. coli* strains were isolated using an enrichment of the milk samples. This qualitative culture procedure was used to promote the growth of the *E. coli* strains, as described before for faecal samples (Hussain et al., 2010; Wu et al., 2010). Regarding the actual presence of virulence genes, an influence of enrichment procedures has only been described in detail for STEC (Vimont et al. 2007), but has not been proven for incubation in Caso-Broth for the applied duration. However, a possible influence on the quantitative proportion of different strains cannot be excluded though faecal contamination of the samples was minimized by a strict sampling protocol.

*Escherichia coli* strains causing acute coliform mastitis in dairy cattle originate from the animal’s faecally contaminated environment and infect the udder via the teat canal (Eberhart, 1984). Experiments by Bertschinger et al. (1990) and Bertschinger et al. (1977b), where the mammary glands of sows were protected against faecal contamination, support the theory of a galactogenic route of infection via the teat duct. Like bovine mastitis, porcine mastitis may also resemble urinary tract infection as the infection may be ascending (Kaipainen et al., 2002). Among others, causative agents of urinary tract infections (UTI) are UPEC, a pathotype of ExPEC. In contrast to commensal *E. coli* isolates, UPEC harbor more virulence genes encoding virulent
capsule antigens, iron acquisition systems, adhesions and secreted toxins (Wiles et al., 2008). The virulence genes *iroN* and *fimC* are reported as urovirulence factors (Russo et al., 2002; Wiles et al., 2008) and were also identified in high percentages in our study. In a survey by Won et al. (2009), the presence of 19 virulence-associated genes in avian pathogenic *E. coli* (APEC), another pathotype of ExPEC, was determined, and approximately 95 % of the APEC isolates possessed *fimC*. However, *fimC* has also been frequently detected in non-pathogenic *E. coli* and is proposed to be not highly associated with the pathogenesis of APEC-infections (Kawano et al., 2006). We also found the fimbrial gene *fimC* in high prevalence in isolates of healthy sows, confirming this theory.

The *traT* gene, detected in half of the examined *E. coli* strains, was found in milk of CM-affected dairy cattle, too. Out of 160 Finnish isolates from cows with mastitis, 37 %, and out of 113 Israeli isolates, 41 % harbored *traT* (Kaipainen et al., 2002). Nemeth et al. (1991) identified the gene in 43 % of *E. coli* strains isolated from the milk of cows with mastitis. In another study by Acik et al. (2004), milk samples from healthy cows and sheep were analysed and *traT* was present in 62.3 % of all isolates (62.5 % of the isolates from cows and 60 % of the isolates from sheep).

All in all, a spectrum of virulence genes was present in bovine mastitis strains of *E. coli*, but those strains do not possess specific virulence factors contributing to clinical disease. Serum resistance was the only virulence property of *E. coli* consistently associated with isolates of coliform mastitis in dairy cattle (Barrow and Hill, 1989; Fang and Pyorala, 1996). A relationship between *traT* and serum resistance, however, could not be confirmed (Nemeth et al., 1991; Vandekerchove et al., 2005).

The results and conclusions concerning the virulence genes related to bovine mastitis are comparable to the findings of our study in sows. Specific sow factors, e.g. the individual disposition of the animal, are probably more important and the host defense status is generally accepted as key factor determining the outcome of the disease (Burvenich et al., 2003). Current investigations deal with the genetic background of CM via genotyping of diseased and healthy sows (Preißler et al., unpublished data).

In conclusion, a variety of virulence genes was detected among the *E. coli* isolates for both samples from CM-positive and negative sows. The identified virulence genes belonged mainly to the large group of genes related to ExPEC, but a categorization into the pathotype ExPEC only by virulence gene typing was not possible. Many
virulence-associated factors (e.g. for iron-uptake systems, fimbriae and other adhesions) are fitness factors which help the bacteria to adapt to and successfully colonize the host so that the line between virulence and fitness properties of E. coli strains is very thin (Dobrindt, 2005).

The results of our study support the hypothesis that any given E. coli strain, even those considered to be non-pathogenic, can cause coliform mastitis in sows, if further adversely environmental, genetic or other influencing factors promoting infection are present.

Acknowledgements
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References


Chapter 3

Assessing individual sow risk factors for coliform mastitis in sows: A case-control study

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Abstract
In order to investigate sow-specific risk factors associated with coliform mastitis, a case-control study was performed over the course of 28 months. Data of three farms were collected under production conditions. Sows suffering from coliform mastitis after farrowing served as cases, and healthy half- or full-sib sows from the same farm served as controls. Individual sow characteristics and the seasonal influence were analysed by conditional logistic regression. The final multivariate model identified four risk factors: the risk of suffering from coliform mastitis increased with a higher number of piglets born alive and stillborn piglets. Gilts had an increased risk for the disease, and birth intervention was also associated with a higher prevalence of mastitis. Birth induction and season had no significant influence on the occurrence of coliform mastitis.

The time during and soon after farrowing is a very sensitive period in pig production demanding great attention by the farmer. With respect to the economic losses, monitoring of potentially endangered sows as well as detailed documentation and selection of disease cases are of particular importance when coping with coliform mastitis.

Keywords
Birth intervention, fever, litter size, mastitis, post-parturient sow
Introduction

Coliform mastitis (CM) is an economically relevant disease in sows (Bertschinger and Fairbrother, 2006). The average prevalence in herds is about 13 %, but also prevalence up to 60 % has been reported (Bäckström et al., 1984; Hirsch et al., 2003; Krieter and Presuhn, 2009). After farrowing, the infection of the mammary gland results in reduced productivity of the sows and increased mortality of the piglets. The affected animals suffer from fever and an inflammation of the glands mostly followed by decreased milk secretion 24 to 48 hours post partum. Therefore, the sows fail to meet the needs of their piglets. A detailed description of the disease is given in a recent review (Gerjets and Kemper, 2009).

Coliform mastitis is a multifactorial disease, i.e. several factors influence the prevalence of mastitis among herds. The term ‘coliform mastitis’ refers to a clinical mastitis due to coliform bacteria (Escherichia species (spp.), Klebsiella spp., Enterobacter spp. and Citrobacter spp.) which have been found to be associated with the disease complex in many studies (Awad Masalmeh et al., 1990; Bertschinger and Fairbrother, 2006; Hirsch et al., 2003; Ross et al., 1981).

Most studies concerning the identification of risk factors were carried out between 1970 and 1990. Potential factors were mostly related to housing, management and feeding practices, and tested in univariate analyses. Changes in housing (Waldmann and Wendt, 2001), single housing and lack of exercise (Ringarp, 1960; Hoy, 2002) as well as overfeeding in late gestation (Göransson, 1989) are only some factors reported to increase the occurrence of CM.

A previous study by Papadopoulos et al. (2010) dealt with management and strategy-related risk factors, acquired via questionnaires, in a multivariable analysis, but focused on the herd level.

The main objective of this study was to identify potential risk factors for CM, in particular individual sow characteristics related to production parameters, for CM by performing a case-control study.
Material and methods

Data collection and study design

Data were collected from three multiplication herds in Germany, supervised by PIC Germany GmbH Schleswig, from April 2008 to August 2010 within the scope of a microbiological study (Gerjets et al., 2010, submitted).

The farms were chosen because of their similar high health status and the available documented reproduction data. They were tested free from porcine reproductive and respiratory syndrome-virus, rhinitis, *Actinobacillus pleuropneumoniae* dysentery and enzootic pneumonia. The number of sows housed on the farms varied between 1,000 and 1,800. The sows were of different parities (1 to 9) and lines (Landrace, Large White and crossbreds, partly with Duroc). Information about cross-fostering was not documented.

All sows were examined after farrowing and considered as CM cases when their rectal temperature was above the threshold of 39.5°C 24 h post partum (Furniss, 1987) and the mammary glands showed definite signs of inflammation.

Healthy half- or full-sib sows from the same herd and, if possible, the same farrowing group served as controls. The half-sib design was chosen due to further studies on the genetic background via genotyping (Preißler et al., unpublished data).

In total, data of 1,337 sows were analysed (683 CM-affected and 654 healthy sows). The investigation was carried out as *m:n* matched case-control study, i.e. one or more cases were matched to one or more controls due to their respective herd and relationship. Therefore, the number of cases and controls within one group varied from 1 to 57 and 1 to 62, respectively.

The case-control study investigated factors associated with CM, especially reproduction parameters of the sows. Information of the ‘sire’, the ‘number of piglets born alive’ and ‘stillborn piglets’, the ‘parity number’, ‘birth induction’, ‘birth intervention’ and the ‘season’ was recorded for the clinical cases and for the controls.

Statistical analyses

The unit of analysis was the sow. The dependent variable was the occurrence of CM as a binary outcome (present or absent). Independent variables (potential risk factors) were categorised by checking the distribution of the observations. A $\chi^2$-test was calculated pairwise to determine whether independent variables were correlated. The relation between potential risk factors and the occurrence of CM was analysed by conditional logistic regression with the procedure LOGISTIC (SAS Institute Inc.,
in which the sire and the farm served as strata. First, risk factors were tested in univariate analysis. Those that were associated with the outcome variable at \( P<0.25 \) were then included in the multivariate analysis. \( P \)-values for the variables were based on the Wald statistic. The Akaike information criterion and the Schwartz criterion evaluated the goodness of fit of the final models.

Results
Depending on the CM status, the average number of piglets born alive of the analysed sows varied between 11.9 and 12.5, and the average number of stillborn piglets ranged from 0.9 to 1.6. The mean number of weaned piglets varied between 10.1 and 10.6 (Table 1).

Table 1: Means (standard deviations) of reproduction traits of the investigated sows

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case-control study</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (n=683)</td>
<td>Controls (n=654)</td>
</tr>
<tr>
<td>No. of piglets born alive</td>
<td>12.1 (3.1)</td>
<td>11.9 (3.0)</td>
</tr>
<tr>
<td>No. of stillborn piglets</td>
<td>1.1 (1.6)</td>
<td>0.9 (1.3)</td>
</tr>
<tr>
<td>No. of weaned piglets</td>
<td>10.5 (2.1)</td>
<td>10.5 (1.9)</td>
</tr>
</tbody>
</table>

An overview of the levels of the collected animal-specific parameters and their respective number of cases and controls is given in Table 2.

Results of univariate and multivariate analyses of the case-control study are shown in Table 2 and 3. The risk factors ‘number of piglets born alive’ and ‘stillborn piglets’, the ‘parity number’ and ‘birth intervention’ were associated with the occurrence of CM. The parameters ‘birth induction’ and ‘season’ did not influence the occurrence of CM significantly and were not included in the multivariate analysis.

The chance of suffering CM significantly increased when the number of piglets born alive was higher than 13 (OR = 1.65). The Odds Ratio was also higher when there were more than one stillborn piglets (OR = 1.45). Primiparous sows had a twofold higher chance of contracting the disease than older ones. Birth intervention increased the chance of suffering CM (OR = 1.72).
Table 2: Levels and univariate conditional logistic regression analyses* of potential risk factors for CM in sows (n = 1337). OR = odds ratio; CI = 95% confidence interval; P = P-value.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>Case-control study</th>
<th>Univariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cases / %</td>
<td>No. of controls / %</td>
<td>OR</td>
</tr>
<tr>
<td></td>
<td>(n=683)</td>
<td>(n=654)</td>
<td>95% CI</td>
</tr>
<tr>
<td>No. of piglets born alive</td>
<td>&lt; 12</td>
<td>257 (37.6)</td>
<td>263 (40.2)</td>
</tr>
<tr>
<td></td>
<td>12-13</td>
<td>195 (28.6)</td>
<td>194 (29.7)</td>
</tr>
<tr>
<td></td>
<td>&gt; 13</td>
<td>231 (33.8)</td>
<td>197 (30.1)</td>
</tr>
<tr>
<td>No. of stillborn piglets</td>
<td>0</td>
<td>318 (46.5)</td>
<td>340 (52.0)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>165 (24.2)</td>
<td>160 (24.4)</td>
</tr>
<tr>
<td></td>
<td>&gt; 1</td>
<td>200 (29.3)</td>
<td>154 (23.6)</td>
</tr>
<tr>
<td>No. of parity</td>
<td>1</td>
<td>156 (22.8)</td>
<td>98 (15.0)</td>
</tr>
<tr>
<td></td>
<td>2-3</td>
<td>265 (38.8)</td>
<td>287 (43.9)</td>
</tr>
<tr>
<td></td>
<td>4-5</td>
<td>142 (20.8)</td>
<td>173 (26.5)</td>
</tr>
<tr>
<td></td>
<td>&gt; 5</td>
<td>120 (17.6)</td>
<td>96 (14.6)</td>
</tr>
<tr>
<td>Birth intervention</td>
<td>No</td>
<td>507 (74.3)</td>
<td>547 (83.6)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>175 (25.7)</td>
<td>107 (16.4)</td>
</tr>
<tr>
<td>Birth induction</td>
<td>No</td>
<td>274 (40.1)</td>
<td>278 (42.5)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>409 (59.9)</td>
<td>376 (57.5)</td>
</tr>
<tr>
<td>Season</td>
<td>Spring</td>
<td>183 (26.7)</td>
<td>169 (25.8)</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>226 (33.1)</td>
<td>222 (34.0)</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>182 (26.7)</td>
<td>168 (25.7)</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>92 (13.5)</td>
<td>95 (14.5)</td>
</tr>
</tbody>
</table>

*Matched on farm and sire
⁰ⁱ Different letters within an effect show significant differences between categories
Table 3: Multivariate conditional logistic regression analyses of potential risk factors for CM in sows (n = 1337). OR = odds ratio; CI = 95% confidence interval; P = P-value.

<table>
<thead>
<tr>
<th>Variable/ Levels</th>
<th>Multivariate</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of piglets born alive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 12</td>
<td>1.00</td>
<td>-</td>
<td>0.0032</td>
</tr>
<tr>
<td>12-13</td>
<td>1.07</td>
<td>0.80-1.45</td>
<td>0.57</td>
</tr>
<tr>
<td>&gt; 13</td>
<td>1.65</td>
<td>1.21-2.24</td>
<td>0.0009</td>
</tr>
<tr>
<td>No. of stillborn piglets</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.00</td>
<td>-</td>
<td>0.0595</td>
</tr>
<tr>
<td>1</td>
<td>1.04</td>
<td>0.77-1.40</td>
<td>0.37</td>
</tr>
<tr>
<td>&gt; 1</td>
<td>1.45</td>
<td>1.06-1.99</td>
<td>0.0146</td>
</tr>
<tr>
<td>No. of parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.00</td>
<td>-</td>
<td>0.0146</td>
</tr>
<tr>
<td>2-3</td>
<td>0.59</td>
<td>0.40-0.85</td>
<td>0.51</td>
</tr>
<tr>
<td>4-5</td>
<td>0.51</td>
<td>0.32-0.81</td>
<td>0.0146</td>
</tr>
<tr>
<td>&gt; 5</td>
<td>0.67</td>
<td>0.37-1.21</td>
<td>0.27</td>
</tr>
<tr>
<td>Birth intervention</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00</td>
<td>-</td>
<td>0.0008</td>
</tr>
<tr>
<td>Yes</td>
<td>1.72</td>
<td>1.25-2.37</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

Matched on farm and sire

Different letters within an effect show significant differences between categories

Discussion

The aim of the study was to identify potential sow-specific risk factors via a matched case-control study. Data were collected from three herds, possibly limiting the generalizability of the study, but allowing to focus on the single animal. Environmental influences were standardised through the recording of data on these three farms with their high health standards. According to the microbiological and genetic study design, and in contrast to previous investigations, the emphasis was put on analysing the individual sow. Milk samples of cases and controls were taken for bacteriological analysis, confirming *Escherichia coli* as main pathogen associated with CM (Gerjets et al., unpublished data).

A higher number of piglets born alive was associated with a higher risk for the sows of becoming diseased. This is in accordance with findings of Bostedt et al. (1998), in which gilts with 1.1 piglets more than healthy sows suffered significantly more often from feverish puerperal illness, and also showed an increased stillbirth rate. Concerning the number of stillborn piglets, our study also supports these results. However, other researchers did not find differences in the number of stillborn piglets between diseased and healthy sows (Mirko and Bilkei, 2004; Van Gelder and Bilkei, 2005).
Literature about the effect of the parity number on the occurrence of mastitis is contradictory. While Baer and Bilkei (2005) found sows of higher parity (>4) having an increased risk of suffering mastitis, other studies have described a greater mastitis risk for lower parity sows (1. and 2. parity) (Bostedt et al., 1998; Hoy, 2003; Krieter and Presuhn, 2009). We also found a higher risk for primiparous sows, leading to the interpretation that those sows were more prone to disease. Explanations for this might be their not fully developed immune system (Wendt, 2000; Hoy, 2006), or that sows suffering mastitis in their first parity might be culled. Physiological hyperthermia is also often observed in postparturient sows, especially primiparous ones, leading to misinterpretations (Klopfenstein et al., 2006; Gerjets et al., 2008).

However, most of the sow reproduction parameters identified are interrelated naturally, as shown by Bostedt et al. (1998). According to the available documented parameters of this study, practical recommendations for the prevention of CM by management measures are limited.

The litter size, the rate of stillborn piglets and the parity may give hints when dealing with CM. For the single sow, these parameters, obtained around the time of farrowing and therefore the time of possible CM, are less informative for the current litter. However, considered over following parities and on herd level, these data stress the need for a precise documentation and monitoring.

Commercial sow lines from pig breeding companies are continuously improved in their reproductive capabilities, with large litters and high-milk-producing potential. The sows are therefore exposed to a physiologically extreme situation during and soon after birth. Prevention is the best way to cope with CM in a population, but difficult to accomplish, as the etiology of CM is extremely variable. The investigated factor birth intervention may be helpful in order to prevent CM in sows because it is associated with a higher risk for mastitis and can be regarded in the management. This fact has also been reported by Bostedt et al. (1998). Papadopoulos et al. (2010) pointed out that frequent supervision of farrowing by the stockpersons may reduce the incidence of postpartum dysgalactia syndrome. These contradictory findings could be explained by different interpretations of the terms birth intervention and supervision. Frequent supervision might be positive because all factors prolonging the duration of the birth process increase the prevalence of CM (Berner, 1971; Petersen, 1983; Bostedt et al., 1998) and therefore, supervision, resulting in a reduced birth duration, decreases the risk of CM. On the contrary, manual intervention leading to a manipulation of the birth
process might have a negative influence, especially if accompanied by insufficient hygiene.

**Conclusions**
The identification of potential risk factors is the key element in preventing diseases. We found that the risk of CM in sows increased with a higher number of piglets born alive and stillborn piglets, with a lower parity and the application of birth intervention. These results should be taken into account when coping with problem herds, e.g. by the detailed documentation of disease on a single animal basis and the careful selection of non-diseased sows for further production cycles.

**Acknowledgements**
This research project was funded by the German Federal Ministry of Education and Research (BMBF) in the research programme “FUGATO – Functional Genome Analysis in Animal Organisms,” project “geMMA – structural and functional analysis of the genetic variation of the MMA-syndrome” (FKZ0315138).

**References**


Chapter 4

Application of decision-tree technique to assess herd specific risk factors for coliform mastitis in sows

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Abstract
The aim of the study was to investigate factors associated with coliform mastitis in sows, determined at herd level, by applying the decision-tree technique. Coliform mastitis represents an economically important disease in sows after farrowing that also affects the health, welfare and performance of the piglets. The decision-tree technique, a data mining method, may be an effective tool for making large datasets accessible and different sow herd information comparable. It is based on the C4.5-algorithm which generates trees in a top-down recursive strategy. The technique can be used to detect weak points in farm management.
Two datasets of two farms in Germany, consisting of sow-related parameters, were analysed and compared by decision-tree algorithms. Data were collected over the period of April 2007 to August 2010 from 987 sows (499 CM-positive sows and 488 CM-negative sows) and 596 sows (322 CM-positive sows and 274 CM-negative sows), respectively.
Depending on the dataset, different graphical trees were built showing relevant factors at the herd level which may lead to coliform mastitis. The application of birth intervention and a higher number of piglets born alive and stillborn ones were the main risk factors identified by the decision-tree technique to be associated with coliform mastitis.
Herd specific risk factors for the disease were illustrated what could prove beneficial in disease and herd management. The application of decision trees may be a possibility of analysing critical points and decisions in management on an individual farm basis.

Keywords
decision-tree modeling; management tool; mastitis; sows
**Introduction**

Coliform mastitis (CM) is an important infection in sows after farrowing followed by serious economic losses due to lower productivities of the affected sows and higher preweaning piglet mortalities (Bertschinger and Fairbrother, 2006). The diseased animals suffer from fever and an inflammation of the mammary glands that often leads to a decreased milk secretion 24 to 48 hours post partum. The average prevalence in herds is about 13 %, but also a prevalence up to 60 % has been reported (Bäckström et al., 1984; Hirsch et al., 2003; Krieter and Presuhn, 2009).

The term ‘coliform mastitis’ refers to a clinical mastitis due to coliform bacteria (*Escherichia* species (spp.), *Klebsiella* spp., *Enterobacter* spp. and *Citrobacter* spp.) which have been found to be associated with the disease complex in many studies (Awad Masalmeh et al., 1990; Bertschinger and Fairbrother, 2006; Hirsch et al., 2003; Ross et al., 1981). As a multifactorial disease, CM is influenced by the strongly related main issues of management, feeding and hygiene as well as individual sow-related parameters (Klopfenstein et al., 2006). It is generally assumed that optimal herd management including the detection of weak points is a key element in reducing the prevalence of diseases in general and CM as multifactorial infection in herds in particular (Papadopoulos et al., 2010).

With the aid of management information technology, farmers are able to collect, process and interpret data based at individual animal level (van Asseldonk et al., 1999). Data mining methods are special statistical instruments which are applied to detect relationships between attributes in datasets. The decision-tree technique, a data mining method, has been proven as an effective tool to make large farm datasets accessible and different sow herd information comparable (Kirchner et al., 2004).

The aim of this study was to investigate the application of the decision-tree technique to assess potential risk factors associated with CM-infected sows. Decision-trees which allow deduction of association rules could support the comparison and assessment of herd data and thereby the establishment of optimal and individual management strategies.
**Materials and methods**

*Datasets*

The study was based on datasets from two rearing herds in Germany with 1,200 (Farm A) and 1,800 sows (Farm B) collected from April 2008 to August 2010 within the scope of a microbiological study (Gerjets et al., 2010, submitted). The farms were of high health status and tested free from porcine reproductive and respiratory syndrome-virus, rhinitis, *Actinobacillus pleuropneumoniae* dysentery and enzootic pneumonia.

The datasets comprised individual reproduction traits of the sows (Table 1) and a respective binary record of the occurrence of CM (present or absent). All sows were examined after farrowing and considered as mastitis cases when their rectal temperature was above the threshold of 39.5°C 24 h post-partum (Furniss, 1987) and the mammary glands showed definite signs of inflammation. Healthy half- or full-sib sows from the same farrowing group served as controls. The half-sib design was chosen due to further studies on the genetic background via genotyping (Preißler et al., unpublished data). Manual obstetric measures after the beginning of birth were defined as the trait ‘birth intervention’. ‘Birth induction’ was the hormonal induction of birth after the 115. day of gestation in order to get the birth process started.

The first dataset (Farm A) consisted of a total of 987 observations – 499 observations from CM-positive sows and 488 observations from CM-negative sows. The second dataset (Farm B) contained 596 observations whereas 322 observations distinguished CM-positive sows and 274 observations CM-negative sows. The mean number of parities per sow was 4.0 for Dataset A and 3.2 for Dataset B (Table 1). The average number of piglets born alive was 12.1 (Dataset A) and 12.3 (Dataset B) and the average number of stillborn piglets 1.2 (A) and 1.0 (B), respectively. The mean number of weaned piglets was 10.6 for both datasets.
Table 1: Means (standard deviations) and frequencies (yes/no) of reproductive traits for Farms A and B

<table>
<thead>
<tr>
<th>Variable (abbreviation)</th>
<th>Farm A (n = 987)</th>
<th>Farm B (n = 596)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of parities per sow (np)</td>
<td>4.0 (1.9)</td>
<td>3.2 (1.9)</td>
</tr>
<tr>
<td>Piglets born alive per litter (pba)</td>
<td>12.1 (3.0)</td>
<td>12.3 (3.1)</td>
</tr>
<tr>
<td>Piglets born dead per litter (pbd)</td>
<td>1.2 (1.6)</td>
<td>1.0 (1.5)</td>
</tr>
<tr>
<td>Birth intervention (biv)</td>
<td>212/ 775</td>
<td>143/ 453</td>
</tr>
<tr>
<td>Birth induction (bid)</td>
<td>409/ 578</td>
<td>356/ 240</td>
</tr>
</tbody>
</table>

**Decision tree algorithm**

The C4.5-algorithm was used to generate decision trees by employing the top-down and recursive-splitting technique (WEKA, 3-6-2, 2010). Every decision-tree consisted of a root node and internal nodes representing the attributes, and branches that characterized the attribute values. In this study, the reproduction parameters and the information of birth intervention (biv) and birth induction (bid) served as attributes. The leaves (leaf node of the decision tree) expressed the binary decision (presence or absence of CM) and indicated the classification of either positive (CM-positive sow) or negative (CM-negative sow) examples.

Therefore, the classification was performed by starting from the root node until arriving at a leaf node. The descending order of the attributes within the decision-tree and the threshold values of the branches were calculated by the algorithm with the gain ratio criterion whereas the root of the tree represented the attribute with the highest information gain.

In order to reduce the chance of overfitting, the C4.5-algorithm simplifies very highly and complexly generated trees by the error-based pruning method (Quinlan, 1993). The C4.5-algorithm is described in detail by Quinlan (1993) and Mitchell (1997).

The classification accuracy of the algorithm was tested with the stratified 10-fold cross-validation method which analyses the number of correctly and incorrectly classified instances (observations) (Kohavi, 1995). The whole dataset was randomly divided into ten subsets, nine parts being dedicated to the training and one for the test. The training set learned the algorithm and generated the tree and the test set estimated the classification parameters. Then the algorithm ran ten times, each time with a different training and test set, and the results were validated.
The classification accuracy assessment was calculated with a two-dimensional confusion matrix consisting of the numbers of true positive (TP), false negative (FN), true negative (TN) and false positive (FP) classified examples. Sows with CM described the positive instances and healthy sows represented the negative instances in this study. The classification accuracy of the C4.5-algorithm was expressed by specific evaluation parameters (Table 2). The overall classification accuracy described the number of correctly classified instances in total. The proportion of correctly classified CM-positive sows in relation to all CM-positive sows was represented by the sensitivity. In addition, the specificity was defined by correctly classified CM-negative sows in relation to all CM-negative sows. The Kappa value reflected the degree of agreement for classifying the sows in the CM-positive or CM-negative classes. The error rate indicated the falsely classified CM-positive sows in proportion to all positively classified sows.

Table 2: Evaluation parameters of the classification accuracy of the C4.5-algorithm

<table>
<thead>
<tr>
<th>Evaluation parameters</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classification accuracy</td>
<td>( \frac{TP+TN}{TN+FP+FN+TP} \times 100 )</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>( \frac{TP}{TP+FN} \times 100 )</td>
</tr>
<tr>
<td>Specificity</td>
<td>( \frac{TN}{TN+FP} \times 100 )</td>
</tr>
<tr>
<td>Kappa value</td>
<td>( \frac{(TP+TN) - \left[ \frac{(TP+FN) \times (TP+FP) + (FP+TN) \times (FN+TN)}{N} \right]}{N - \left[ \frac{(TP+FN) \times (TP+FP) + (FP+TN) \times (FN+TN)}{N} \right]} \times 100 )</td>
</tr>
<tr>
<td>Error Rate</td>
<td>( \frac{FP}{FP+TP} \times 100 )</td>
</tr>
</tbody>
</table>

\( TP = \) true positive; \( TN = \) true negative; \( FP = \) false positive; \( FN = \) false negative, \( N = \) total number of instances.

In this study, the minimum number of instances per class varied between 20, 50 and 100, i.e. a new branch was created by the C4.5-algorithm only when it contained a number of instances greater or equal to the adjusted values of 20, 50 and 100. The results, calculated with the different minimum number of instances per class, were named according to the datasets \( A_{20}, A_{50}, A_{100} \) and \( B_{20}, B_{50}, B_{100} \), respectively.
Results
The evaluation parameters varied between the two datasets and due to the specified number of instances per class (Table 3). The best values were achieved for Dataset A when the number of instances was set to the minimum of 100 instances per class and for Dataset B when the number of instances was set to the minimum of 20 instances per class. The evaluation parameters for B_{20} showed a better fit compared to A_{100}: The classification accuracy (61.2 %) and the sensitivity (65.8 %) of B_{20} were higher than for A_{100} (55.0 %; 58.1 %) and the error rate of B_{20} was 8.5 % points lower. The Kappa value (21.7 %) of B_{20} reached higher values compared to A_{100} (10.0 %). The specificity of B_{20} (44.2 %) was lower than for A_{100} (48.2 %).

Table 3: Evaluation parameters for Farms A (n = 987) and B (n = 596) with varied adjusted minimum number of instances per class

<table>
<thead>
<tr>
<th>Dataset^a</th>
<th>Classification accuracy (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Error rate (%)</th>
<th>Kappa statistic (%)</th>
<th>No. of leaves</th>
<th>No. of nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A_{20}</td>
<td>53.2</td>
<td>54.7</td>
<td>48.4</td>
<td>46.4</td>
<td>6.4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>A_{50}</td>
<td>54.2</td>
<td>55.9</td>
<td>47.5</td>
<td>45.4</td>
<td>8.4</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>A_{100}</td>
<td>55.0</td>
<td>58.1</td>
<td>48.2</td>
<td>44.8</td>
<td>10.0</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>B_{20}</td>
<td>61.2</td>
<td>65.8</td>
<td>44.2</td>
<td>36.3</td>
<td>21.7</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>B_{50}</td>
<td>60.2</td>
<td>65.5</td>
<td>46.0</td>
<td>37.4</td>
<td>19.6</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>B_{100}</td>
<td>56.4</td>
<td>64.0</td>
<td>52.6</td>
<td>41.1</td>
<td>11.5</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

^a_{20, 50, 100} = at least 20, 50 or 100 instances per class

Graphical trees are presented for A_{20}, A_{100}, B_{20} and B_{100} (Figures 1, 2, 3 and 4). The decision-trees of both datasets showed differences, although the available attributes (‘parity number’, ‘piglets born alive’, ‘piglets born dead’, ‘birth intervention’, ‘birth induction’) were the same for all trees. The attribute ‘birth induction’ did not appear in any of the trees showing that the other parameters are more important for the occurrence of coliform mastitis. The attribute ‘parity number’ was not chosen in the trees of Dataset A. The trees of A_{20}, A_{100} and B_{20} started with the attribute ‘birth intervention’ as the root node which, therefore, was identified as the most influencing attribute.

In Dataset A_{20}, sows with no ‘birth intervention’ but ‘piglets born dead’ greater than zero and ‘piglets born alive’ greater than 14 were CM-positive. In Dataset B_{20}, sows
with no ‘birth intervention’, but a ‘parity number’ less than or equal to three, ‘piglets born alive’ greater than twelve and ‘piglets born dead’ with at least one were CM-positive. The right sub-tree demonstrated that sows with ‘birth intervention’ and ‘piglets born alive’ greater than nine were CM-positive. The decision-trees of $A_{100}$ and $B_{100}$ were pruned, which made the decision steps clearer and more generic. Therefore, attributes with a smaller information gain ratio were dropped by the algorithm; important parameters endured. The tree size of $A_{100}$ was decreased by one leaf and two nodes in comparison to $A_{20}$. The tree of $B_{100}$ had five leaves and ten nodes less than $B_{20}$. In Dataset $A_{100}$, sows were CM-positive when ‘birth intervention’ was applied, with more than one ‘piglet born dead’ or more than 14 ‘piglets born alive’. In Dataset $B_{100}$, sows with ‘piglets born alive’ greater than ten and a ‘parity number’ less than or equal to three were CM-positive.
Figure 1: Decision tree showing the detected parameters and threshold values associated with CM of dataset $A_{20}$ ($n = 987$; minimum number of 20 instances per class); $biv =$ birth intervention; $pbd =$ piglets born dead; $pda =$ piglets born alive.

Figure 2: Decision tree showing the detected parameters and threshold values associated with CM of dataset $A_{100}$ ($n = 987$; minimum number of 100 instances per class); $biv =$ birth intervention; $pbd =$ piglets born dead; $pda =$ piglets born alive.
Figure 3: Decision tree showing the detected parameters and threshold values associated with CM of dataset B\textsubscript{20} \((n = 596; \text{minimum number of 20 instances per class}); \ biv = \text{birth intervention}; \ np = \text{parity number}; \ pbd = \text{piglets born dead}; \ pda = \text{piglets born alive.}

Figure 4: Decision tree showing the detected parameters and threshold values associated with CM of dataset B\textsubscript{100} \((n = 596; \text{minimum number of 100 instances per class}); \ biv = \text{birth intervention}; \ np = \text{parity number}; \ pbd = \text{piglets born dead}; \ pda = \text{piglets born alive.}
Discussion

The main objective of the study was the analysis of potential risk factors associated with sows suffering from coliform mastitis, determined on farm basis, by applying the C4.5-algorithm of the decision-tree technique. Environmental influences were standardised through the recording of data on these three farms with their high health standards.

According to the microbiological and genetic study design, only clear cases of CM-positive and selected cases of CM-negative sows were used for the analysis. Therefore, it is not possible to make statements of the real prevalence of CM on the farms where a large grey area of diseased sows exist.

The values of the evaluation parameters of the C4.5-algorithm were not acceptable compared to other studies. The sensitivity and specificity were too low and the error rate was too high. Kirchner et al. (2004) analysed culling strategies in swine breeding data by using the decision-tree technique and reached a classification accuracy value of about 85%. The specificity was around 97% and the error rate on average 15%. Those datasets, however, consisted of 14,897 and 21,818 observations, much more than used in this study. Using more observations for model building improves the evaluation accuracy. With lower prevalences and therewith more skewed data, it is easier to reach higher accuracies.

The potential risk factors identified for CM by the decision-tree induction have also been described in other studies (Bostedt et al., 1998; Hoy, 2002; Krieter and Presuhn, 2009). A higher number of piglets born alive was associated with a higher risk for the sows of becoming diseased. This is in accordance with findings of Bostedt et al. (1998), in which gilts with 1.1 piglets more than healthy sows suffered significantly more often from feverish puerperal illness, and also showed an increased stillbirth rate. Concerning the number of stillborn piglets, our study also supports these results. However, other researchers did not find differences in the number of stillborn piglets between diseased and healthy sows (Mirko and Bilkei, 2004; Van Gelder and Bilkei, 2005).

Literature about the effect of the parity number on the occurrence of mastitis is contradictory. While Baer and Bilkei (2005) found sows of higher parity (>4) having an increased risk of suffering mastitis, other studies have described a greater mastitis risk for lower parity sows (1. and 2. parity) (Bostedt et al., 1998; Hoy, 2003; Krieter and Presuhn, 2009). We also found a higher risk for primiparous sows, leading to the
interpretation that those sows were more prone to disease. Explanations for this might be their not fully developed immune system (Wendt, 2000; Hoy, 2006), or that sows suffering mastitis in their first parity might be culled. Physiological hyperthermia is also often observed in postparturient sows, especially primiparous ones, leading to misinterpretations (Klopfenstein et al., 2006; Gerjets et al., 2008). The investigated factor birth intervention may be helpful in order to prevent CM in sows because it is associated with a higher risk for mastitis and can be regarded in the management. This fact has also been reported by Bostedt et al. (1998). Manual intervention leading to a manipulation of the birth process might have a negative influence, especially if accompanied by insufficient hygiene.

In our study, the decision-tree technique was shown to have the ability to illustrate confirmed influencing factors for CM. In addition, the technique was able to weight those factors on farm basis. Individual herd and management differences were made clear by a different order of the attributes and different threshold values of the branches in the trees. Decision-trees, therefore, may allow exposure of individual weak points in the management of and comparisons between farms. In the context of multifactorial diseases, the utilisation of such a technique is shown feasible when certain conditions are fulfilled. For practical use, graphical trees should be smaller with clearly arranged decision steps to simplify interpretations for farmers and consultants. The minimum number of instances per branch has to be adjusted to the total number of instances, i.e. a small number of instances in total requires a small minimum number of instances per branch. The quality of the classification might be improved by including more information about management and hygiene in the decision-tree algorithm.

Acknowledgements

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References


General Discussion

Coliform mastitis is a multifactorial disease: the underlying causes of CM are complex and its pathophysiology has not yet been fully elucidated. Factors contributing to the occurrence of CM can broadly be distinguished into environmental and sow-related ones (Figure 1). This thesis focused on the one hand on bacterial pathogens involved in the pathogenesis of CM, especially *Escherichia coli* (chapter two), and on the other hand on selected sow and birth characteristics as potential, predisposing factors (chapters three and four).

**Environmental factors**
- Management
- Climate
- Nutrition
- Water intake
- Pathogens
- Mycotoxins

**Sow-related factors**
- Body condition
- Parity number
- Duration of birth
- Obstetric intervention
- Litter size
- Urinary tract infections
- Obstipation
- Hormones
- Immune defence
- Genetics

Figure 1: The multifactorial nature of Coliform mastitis (CM)

**Bacterial pathogens**

The term “Coliform mastitis” refers to coliform bacteria which have been isolated from the milk of diseased sows (Awad Masalmeh et al., 1990; Kobera, 2000). Coliform bacteria include *E. coli* and other lactose-splitting pathogens (*Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp.), physiologically occurring in the intestinal tract. *Escherichia coli* was the organism most often found in milk as well as in mammary tissue of sows with mastitis (Armstrong et al., 1968; Bertschinger et al., 1977; Ross et al., 1981).
Considering the age of the published studies, there is a lack of recent investigations into the topic, particularly with regard to the enormous improvements in the methodology of the bacteriological identification of genera, species and pathotypes over the last few decades.

In chapter two, the bacteriological analysis of milk samples, gathered from cleaned and disinfected glands, was performed with advanced methods including the PCR technique. The biochemical identification system of API (bioMérieux, Craponne, France) enabled a classification and confirmation of the analysed bacteria at subspecies level. A corresponding software package determined probabilities and T-values for each bacterium (apíweb, bioMérieux).

*Escherichia coli* was identified in over 70% of the milk samples of CM-infected sows, which is in accordance with findings of other studies (Ross et al., 1981; Awad Masalmeh et al., 1990; Bertschinger, 1999; Hirsch et al., 2003). However, *E. coli* was also found in similar percentages in milk samples of non-infected sows – a result which has not yet been described in literature and is difficult to interpret. To our knowledge, the milk of healthy sows has not been subject to detailed bacteriological analysis up to now.

A detailed analysis of virulence-associated genes of the *E. coli* isolates should provide novel insights into the role of *E. coli* in association with mastitis in sows. Therefore, *E. coli* isolates from CM-affected – and also healthy – sows’ milk were analysed per multiplex PCR assays for the first time.

Virulence factors, expressed by virulence genes, facilitate the survival of *E. coli* and their reproduction in the respective environment. Characteristic virulence genes are possessed by different strains of *E. coli*.

In this study, the analysis of the virulence gene spectrum revealed only slight differences between *E. coli* isolates from CM-infected and healthy sows. Particular virulence gene profiles or specific mammary pathogenic *E. coli* were not detected. Further investigations should classify *E. coli* isolates by phylogeny with the aid of multilocus sequence typing. This sensitive method provides new information and a better understanding of pathogenic *E. coli* strains and their phylogenetic relationship.

Previous studies on the functional analysis of *E. coli* strains from animals suffering mastitis are very limited, which is in great contrast to the analysis of other *E. coli* pathotypes. Finally, the respective tools and methodologies in the laboratory have now been available for some time (Shpigel et al., 2008).
The bacteriological involvement in CM remains ambiguous (chapter two): Coliform bacteria causing inflammation of the mammary glands originate from the sow’s faecally contaminated environment and infect the glands via the teat canal (Elmore et al., 1978; Jones, 1979; Wegmann et al., 1986; Baer and Bilkei, 2005). The results of this thesis support the hypothesis that sows only develop clinical signs of CM if further adversely environmental, genetic or other influencing factors promoting infection are present. Other, better defined influencing factors are for instance:

**Sow- and birth-related factors**

Chapters three and four paid attention to sow characteristics influencing the actual outbreak of CM. It was found that the odds of CM in swine increased with a higher number of piglets born alive and stillborn piglets, with the application of manual birth intervention and for gilts. Moreover, previous CM increased the chance of suffering another CM disease. Only older investigations exist with which to compare these results. However, the identified risk factors are physiologically consequent and explicitly described in chapter three. Consideration of these findings in practice could prove useful in disease and herd management, and may be implemented in knowledge-based management information systems in pig production. Optimal herd management is the key element in reducing the prevalence of diseases in herds. This also includes the detection of weak points. In chapter four, a possibility of analysing critical points and decisions in management on an individual farm basis is suggested by the application of decision trees. Graphical trees are very demonstrative for the farmer or consultant, and enable a comparison between different herd data. In the context of multifactorial diseases, the utilisation of such a technique is shown feasible when certain conditions are fulfilled. The more observations are available, the better the quality of the decision rules. Only reasonable parameters should be included. The minimum number of instances per branch has to be adjusted to the total number of instances, i.e. a small number of instances in total requires a small minimum number of instances per branch.
The time during and soon after farrowing is a very sensitive period in pig production demanding great attention by the farmers. The sows are exposed to a physiologically extreme situation; their health is strongly influenced by their environment and immune defence. At the current state of knowledge, it still remains unknown as to why only some sows develop clinical signs of CM-infection. The outbreak of disease is most likely due to the interaction of invading ubiquitous pathogens and the host’s immune system. All known facts about this multifactorial pathogenesis are summarised in chapter one of this thesis. Immune competence, including resistance to diseases, is genetically determined (Mallard et al., 1992; Magnusson and Greko, 1998). Heritabilities for CM of approximately 10 % indicate the opportunity to use this trait for selection (Lingaas and Ronningen, 1991; Berg et al., 2001). In pigs, resistant genotypes have been identified for postweaning *E. coli* diarrhea and oedema disease (Vogeli et al., 1999; Frydendahl et al., 2003; Reiner, 2008). ‘Genetic disease resistance’ as a breeding tool is already applied in the United States, Canada, Denmark and Switzerland. The identification of factors leading to individual resistance is one main objective of future studies on infectious diseases. A holistic view, considering the multifactorial nature of CM, is the only way to reduce the diseases’ prevalence in problem herds.

**A note on the study design and phenotype**

Sampling and data collection took place in different rearing and nucleus herds in Northern Germany, supervised by PIC Germany GmbH Schleswig. Definition of diseased sows, selection of control sows and collection of milk samples were carried out by one person (farm manager or veterinarian), who was briefed in detail and monitored the herds routinely. The pig breeding company PIC governs an excellent database with pedigree information and performance data of all animals housed on the farms that was also the basis for the statistical analyses of this thesis. The study design was chosen due to the microbiological investigation (see above) and further studies on the genetic background of CM via genotyping (Preißler et al., unpublished data): healthy half- or full-sib sows from the same herd served as controls for CM-diseased sows. The option of the statistical analysis was therefore a case-control study performed by conditional logistic regression with parameters potentially predisposing for the occurrence of CM.
The new genotyping technologies required an accurate phenotyping of the respective animals. The diagnosis and definition of diseased sows is not that simple because the symptoms of CM differ from sow to sow and are not always apparent. In this thesis, sows were identified as CM-infected when their rectal temperature was above 39.5°C 24 h post-partum and the mammary glands showed defined signs of infection. Moreover, the appearance and the performance of the piglets were evaluated with regard to their behaviour and body condition. Measuring the rectal temperature after farrowing is the most common practice used to diagnose CM in sows. The critical temperature values range from 39.3°C to 40.5°C (Waldmann and Wendt, 2001) and are in practice often difficult to interpret due to possible physiological hyperthermia in postparturient sows. Therefore, the definition of the trait CM has to include further parameters such as clinical mammary gland changes, decreased milk secretion, reduced appetite and changes in piglets’ behaviour. Piglets of CM-affected sows are normally characterised by restlessness due to starvation (Klopfenstein et al., 2006). After exhaustion of their energy reserves, they commonly retreat to the warmest parts of the farrowing crate and reduce their attempts to nurse.

Because of this diversity of symptoms, an unerring eye of the farmer is inevitable not only to maintain animal health and productivity, but also to provide the phenotype necessary for genetic investigations and breeding programs.

References


General Summary

The **overall aim** of this thesis is described by its title: *Coliform mastitis in sows: Analysis of potential influencing factors and bacterial pathogens with special emphasis on Escherichia coli*. This thesis is divided into four chapters.

In **chapter one**, current knowledge about the economically very important mastitis in sows is summarised. Instead of body temperature as single indicator for CM diagnosis and treatment, a combination of appropriate criteria should be applied to achieve a proper diagnosis and to minimise the use of antibiotics. ‘Genetic disease resistance’, as a new approach to disease reduction, offers promising potentialities for prevention. In pathogenesis, there have been several hints of a predominant influence of *Escherichia coli* since it is the pathogen most often isolated in previous studies.

To support these findings, a study was carried out and described in **chapter two**. The objective was to analyse milk samples of sows with and without CM for the presence of *E. coli* and, subsequently, to investigate virulence-associated genes because strains isolated from sows with CM have not yet been further investigated via molecular biological methods. *Escherichia coli* were most often found, but the prevalence and the virulence gene spectrum in both samples from CM-infected and healthy sows did not differ significantly. Particular virulence gene profiles or specific mammary pathogenic *E. coli* were not detected. These results support the hypothesis that other causative factors seem to have greater influence on the pathogenesis of porcine CM.

The emphasis of **chapter three** was, therefore, the investigation of potential influencing factors, in particular sow characteristics. The odds of CM in sows increased with a higher number of piglets born alive and stillborn piglets, with the application of birth intervention and for gilts.

These conclusions indicate the need for an holistic approach, considering management, especially documentation and selection of disease cases, as well as environmental and animal factors, to deal with Coliform mastitis in sows.
Chapter four presents an instrument enabling the farmer to make sow herd data visually accessible and to detect critical points in management. With the assistance of decision trees, the same influencing parameters for the disease and their relationships were illustrated, which were also identified by the case-control study of chapter three. Such an approach distinguishing diseased from healthy sows and predicting outcome could prove beneficial in disease and herd management and support the establishment of optimal and individual strategies.
Zusammenfassung


Um die Rolle von *E. coli* genauer zu analysieren, wurde eine Untersuchung durchgeführt, die in Kapitel zwei beschrieben ist. Zielsetzung dieser Untersuchung war die Analyse von Milchproben gesunder und an CM erkrankter Sauen in Hinblick auf das Vorkommen von *E. coli*. Weiterhin wurden die isolierten *E. coli*-Stämme auf das Vorhandensein bestimmter Virulenzgene untersucht. *Escherichia coli*-Stämme von Sauen mit CM sind bisher molekularbiologisch nicht näher differenziert worden. Auch diese Studie ergab, dass *E. coli* das vorherrschende Bakterium in den Milchproben erkrankter Sauen ist, jedoch mit ähnlicher Prävalenz auch in den Milchproben gesunder Sauen gefunden wurde. Gleiches gilt für das Virulenzgenspektrum, das sich nicht signifikant zwischen *E. coli*-Isolaten von CM-erkrankten und gesunden Tieren unterscheidet. Spezielle Virulenzgen-Profile oder sogar Mammaspzifisch pathogene *E. coli* wurden nicht detektiert. Diese Ergebnisse stützen die Hypothese, dass andere Faktoren einen größeren Einfluss auf die Pathogenese von porciner CM haben.

Im vierten Kapitel wird ein Instrument für die Praxis vorgestellt, das Betriebsdaten anschaulich und vergleichbar darstellen und Schwachstellen im Management aufdecken kann. Mit der Hilfe von sogenannten Entscheidungsbäumen konnten die gleichen Einflussfaktoren für CM, wie in Kapitel drei bereits identifiziert, und ihre Zusammenhänge visualisiert werden. Eine solche Technik, die erkrankte von gesunden Tieren unterscheidet und ein mögliches Auftreten von Krankheiten ankündigt, ist von großem Nutzen für die Betriebsführung und unterstützt die Etablierung von optimalen und individuellen Managementstrategien.

Mit den vorliegenden Untersuchungen wird ein wesentlicher Beitrag zur aktuellen MMA-Forschung geliefert.
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