BRIEF COMMUNICATION PEER REVIEWED

Bacterial flora on the mammary gland skin of sows and in their colostrum

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Summary

Mammary-gland skin swabs and milk samples were analysed bacteriologically. All skin samples were positive, with 5.2 isolates on average, Staphylococcaceae being the dominant organisms. In 20.8% of milk samples, no bacteria were detected. Two isolates on average, mainly Staphylococcaceae and Streptococcaceae, were isolated from the positive milk samples.

Keywords: swine, bacteria, colostrum, mammary gland, skin

Received: April 7, 2010
Accepted: July 1, 2010

Resumen - La flora bacteriana en la piel de la glándula mamaria de las hembras y en su calostro

Se analizaron bacteriológicamente hisopos de la piel de la glándula mamaria y muestras de leche. Todas las muestras de piel resultaron positivas, con 5.2 aislados en promedio, siendo los Staphylococcaceae los organismos dominantes. En 20.8% de las muestras de leche, no se detectaron bacterias. De las muestras de leche positivas, se aislaron dos aislados en promedio, principalmente Staphylococcaceae y los Streptococcaceae.

Résumé - Flore bactérienne cutanée de la glande mammaire de truies et de leur lait

Des écouvillons de la peau de la glande mammaire ainsi que des échantillons de lait ont été soumis à une analyse bactériologique. Tous les échantillons provenant de la peau étaient positifs, avec en moyenne 5.2 isolats bactériens, les Staphylococcaceae étant de loin les micro-organismes dominants. Aucune bactérie ne fut détectée dans 20.8% des échantillons de lait. En moyenne, on trouvait deux isolats bactériens par échantillon de lait positif, et ceux-ci étaient principalement des Staphylococcaceae et des Streptococcaceae.

Coliform mastitis in sows, as part of the postpartum dysgalactia syndrome, represents an economically very important disease complex.[1] In other species, for instance in cows, a higher susceptibility of the mammary gland to new infections prior to and during parturition was proposed by Oliver and Sordillo.[2] Though coliform mastitis is a multifactorial disease, bacteria play an important role in the pathogenesis of infection. Possibly, pathogenic bacteria are not ubiquitous only in the environment of the animals, but are also present on the skin. The skin flora of pigs, especially on sows' mammary glands, has not yet been the object of scientific examination. This is, to the authors' knowledge, the first published study to determine the bacterial flora in skin and colostral milk samples of a limited number of sows with clinically healthy mammary glands.

Materials and methods

All animal care and handling procedures used in this study followed the farms' written guidelines in accordance with guidelines set forth by the Federation of Animal Science Societies.[3]

The eight sampled crossbred sows were housed at the research farm of the Institute of Animal Breeding and Husbandry of the Christian-Albrechts-University Kiel, with 120 sows in total (average parity 2.5). A vaccination program against porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopneumoniae* was implemented on the farm. The sows were managed with a 28-day lactation period. One week prior to expected parturition, they were washed and confined in the cleaned and disinfected farrowing house, with eight pens per compartment. The floor in the single pens (2.47 m × 1.75 m) was partially slatted and no straw was provided. Farrowing-crate dimensions were individually adjusted to the size of the sow.

All samples were collected during the period between the beginning of parturition and the birth of the last piglet. All sampled sows showed neither fever nor clinical signs of mastitis during the sampling period, and none developed mastitis subsequently. Skin sampling from all available glands was performed in six sows. In two sows, skin sampling was restricted because of the animal's behavior, and only single glands were swabbed. Swab samples were taken from defined skin areas of mammary glands with the help of a circular stencil (10 cm in diameter), with the teat as the central point. One sterile cotton swab was moistened with sterile isotonic saline, rubbed evenly in the defined area, and transported in Amies medium (Transwab; Medical Wire and Equipment, Corsham, Wiltshire, England). In addition, colostrum samples from all sows were collected before the piglets started to suckle. All samples were obtained by hand-milking without oxytocin injection. For 19 single glands, milk sampling according to the sample scheme was not possible due to aggressive behavior of the sow or blocked access to the teat. Colostrum samples were obtained after cleaning each mammary gland with soap solution and disinfection with a local antiseptic (Kodan Tinktur forte; Schülke and Mayr, Norderstedt, Germany)

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This article is available online at http://www.aasv.org/shap.html.

Kemper N, Preissler R. Bacterial flora on the mammary gland skin of sows and in their colostrum. *J Swine Health Prod.* 2011;19(2):112–115.

and 70% isopropyl alcohol. The first streams of secretion from each teat were discarded in order to "wash out" bacteria in the distal end of the teat duct; the following streams were collected in sterile test tubes. In all, 96 skin samples (2, 8, 15, 16, 14, 14, 14, and 13 samples per sow, respectively) and 77 colostrum samples (2, 3, 12, 13, 14, 6, 14, and 13 samples per sow, respectively) were examined. Bacteriological analysis was performed by routine bacteriological diagnostics, including cultivation on Columbia sheep blood and Endo agar, Gram staining, and biochemical identification systems (ID32 STAPH, API20STREP, and API20E; bioMérieux, Nürtingen, Germany), as described previously.[4]

Statistical analyses included paired chi-squared tests for each sow and teat for the 77 corresponding skin and colostrum samples, with regard to the isolated bacteria families. The chi-squared test was performed using the procedure PROC FREQ in SAS statistical software (SAS Institute Inc, Cary, North Carolina). A statistical significance level of $P < .05$ was used.

Results

A broad but similar spectrum of bacterial genera was isolated from both skin and colostrum samples. All 96 skin samples were bacteriologically positive, with 496 isolates in total and 5.2 isolates per sample on average (Table 1). Only three skin samples, all from a single sow, were negative for Staphylococcaceae. Between one and five different species of Staphylococcaceae were isolated from the other 93 skin samples (96.9% of all skin samples), with 2.7 *Staphylococcus* species per sample on average. Staphylococcaceae were isolated most frequently from the skin (50.4% of all skin isolates), with *Staphylococcus simulans* the predominant species (Table 1). Streptococcaceae were isolated from 61 of 96 skin samples (63.5% of all skin samples, 14.1% of all skin isolates), with *Aerococcus viridans* the dominant species. Seven species of Enterobacteriaceae were isolated from 64 of the 96 skin samples (66.7% of all skin samples, 19.4% of all skin isolates), and *Escherichia coli* was the dominant species.

Among the colostrum samples (Table 2), 16 of 77 (20.8%) were culturally negative, while 61 of 77 (79.2%) were bacteriologically positive, with 122 isolates in total and two isolates on average per positive sample. Staphylococcaceae were isolated most frequently (54.1%) from both the colostrum (Table 2) and skin samples (Table 3), with *S simulans* the dominant species (Table 1).

Streptococcaceae, with *A viridans* as the dominant species, represented 30.3% of all isolates from colostrum samples. Enterobacteriaceae were isolated from only four colostrum samples (3.9% of all colostrum isolates), with *E coli* the dominant species. In one colostrum sample, two morphologically different *E coli* isolates were identified, but no *E coli* was found in the corresponding skin sample. Also, in two other sows, *E coli* was detected in only a single gland.

The difference between the total numbers of bacterial isolates in skin and in colostral milk samples was significant ($P < .001$), with a higher number in the skin samples. Regarding the agreement between colostrum and skin samples, in 35 of the 61 positive milk samples (57.4%), at least one bacterial species was isolated, which was also isolated from the skin sample of the corresponding teat. On isolate level, 54 of the 122 species isolated from colostrum (44.3%) were found on the corresponding teat skin as well. This correlation was especially distinct in *S stimulans* (13 of 54 species; 24.1%) and *Staphylococcus warneri* (12 of 54 species; 22.4%). However, paired analysis for each sow and teat revealed no significant relationship ($P > .05$) between the bacterial families in the skin flora and in colostrum flora.

Discussion

Because Staphylococcaceae were isolated from all sows, from 96.9% of all skin samples, and from 75.4% of all positive colostrum samples, we suggest that Staphylococcaceae represent an essential part of the resident flora on sows' mammary gland skin. Similar results were reported in a study by Nagase et al,[5] examining the distribution of staphylococci on pigs' back skin, isolating two to four *Staphylococcus* species per sample. While Staphylococcaceae are resident on sows' mammary gland skin, other bacterial families, such as Streptococcaceae species, seem to be transient, according to their infrequent distribution on the skin. This holds true especially for Enterobacteriaceae, belonging to the fecal flora and often isolated from skin after fecal contamination, as shown by Bertschinger et al.[6]

Comparison of these results to the results of other studies is complicated by the fact that in our study, colostrum was collected before the piglets started to suckle. However, the spectrum of isolated bacteria in the colostrum samples was in accordance with other studies on the milk flora of healthy sows and sows that developed clinical mastitis in a later period, for instance, Morkoc et al.[7] In that study, aseptic sampling by percutaneous aspiration from the gland cistern showed that bacteria were present in 41.6% of the samples from healthy sows examined and in 74.9% of the samples from dysgalactic sows examined. In dysgalactic sows, there was a higher prevalence of gram-negative bacteria.[7] *Escherichia coli* has been described as the major pathogen in coliform mastitis,[8] but neither systemic clinical signs nor local signs of mastitis were observed in the examined sows in our study.

The presence of bacteria in colostrum or milk is not an indicator of mastitis, as stated by Zhu et al.[9] Bacterial invasion without signs of inflammation occurs mainly before parturition and lactation, at the earliest on gestation day 108, as shown by repeated sampling by Bertschinger et al.[6] In our study, mastitis without clinical signs could not be excluded, because no other parameters, for instance somatic cell count or quantitative bacteriology, were assessed.

Whether the high percentage of skin flora, especially Staphylococcaceae, in sows' colostral milk is a consequence of contamination via the teat duct or the mammary epithelia from the teat cistern during the milking process remains unknown. Colonization of the teat duct has been described in cows[10] and ewes,[11,12] and can therefore be assumed for sows. Even though the number of sows sampled was low, the total sample number provided a good first overview of the bacteria present in skin and colostrum samples. However, the situation might differ for sows in other housing conditions, and further studies are needed to provide deeper insights.

This study clearly showed that several bacterial species are present both on the skin and in the colostral milk of clinically healthy sows, with Staphylococcaceae as the predominant family. Furthermore, the results of culturing the milk samples indicated that the bacterial flora differ between individual teats. To clarify a possible relationship between skin and milk flora and their potential influence on the occurrence of clinical mastitis in sows, further studies, for instance, quantitative bacteriology in healthy and diseased animals, are recommended.

Implications

- The bacterial flora on the mammary gland skin of sows consists mainly of Staphylococcaceae.

Table 1: Species distribution, classified in families, of bacterial isolates cultured from skin samples (n = 96) and colostrum samples (n = 77) of eight healthy sows*

Bacterial family	Species	Skin		Colostrum	
		No. of isolates	Distribution of all isolated family species (%)	No. of isolates	Distribution of all isolated family species (%)
Staphylococcaceae	All species	250	100	66	100
	Staphylococcus simulans	59	23.6	15	22.7
	Staphylococcus aureus	44	17.2	3	4.5
	Staphylococcus warneri	25	10.0	13	19.7
	Staphylococcus hyicus	22	8.8	3	4.5
	Staphylococcus xylosus	22	8.8	8	12.1
	Staphylococcus chromogenes	21	8.4	5	7.6
	Staphylococcus haemolyticus	17	6.8	4	6.1
	Staphylococcus species	17	6.8	15	22.7
	Staphylococcus sciuri	15	6.0	0	0
	Gemella morbillorum	7	2.8	0	0
	Gemella haemolysans	1	0.4	0	0
Enterobacteriaceae	All species	96	100	5	100
	Escherichia coli	74	77.1	4	80
	Enterobacter species	10	10.4	0	0
	Klebsiella pneumoniae	4	4.2	0	0
	Escherichia species	3	3.1	0	0
	Citrobacter species	2	2.1	0	0
	Proteus species	2	2.1	0	0
	Others	1	1.0	0	0
	Serratia species	0	0	1	20
Streptococcaceae	All species	70	100	37	100
	Aerococcus viridans	35	50.0	23	62.2
	Lactococcus lactis	13	18.6	4	10.8
	Streptococcus dysgalactiae	11	15.7	2	5.4
	Streptococcus species	8	11.4	7	18.9
	Aerococcus urinae	3	4.3	1	2.7
Enterococcaceae	All species	41	100	7	100
	Enterococcus faecium	18	43.9	3	42.9
	Enterococcus faecalis	16	39.0	1	14.3
	Enterococcus durans	6	14.6	3	42.9
	Enterococcus avium	1	2.4	0	0
Leuconostocaceae	All species	18	100	4	100
	Leuconostoc species	18	100	4	100
Others	All species	5	100	2	100
Spore formers	All species	16	100	1	100

* Sows were housed in single pens (2.47 m × 1.75 m) with partially slatted floors and no bedding. Farrowing-crate dimensions were individually adjusted to the size of the sow. Skin swabs and colostrum samples were collected during the period between the beginning of parturition and the birth of the last piglet. Skin samples were taken by a sterile cotton swab, moistened with sterile isotonic saline, from defined areas with help of a 10-cm diameter stencil with the teat as the central point; colostrum samples were taken without oxytocin injection by hand milking from each teat. The first stream from each teat was discarded and the following streams were collected in sterile glass test tubes.

Table 2: Bacterial families isolated from colostrum samples of healthy sows in total and in proportion to the total number of sampled sows, samples, and isolates*

Bacterial family	Sows (n = 8) No. positive (%)	Samples (n = 77) No. positive (%)	Isolates (n =122) No. positive (%)
Streptococcaceae	8 (100.0)	35 (45.5)	37 (30.3)
Staphylococcaceae	7 (87.5)	46 (59.7)	66 (54.1)
Enterobacteriaceae	4 (50.0)	4 (5.2)	5 (4.1)
Enterococcaceae	4 (50.0)	7 (9.1)	7 (5.7)
Leuconostocaceae	2 (25.0)	4 (5.2)	4 (3.3)
Others	2 (25.0)	2 (2.6)	2 (1.6)
Spore formers	1 (12.5)	1 (1.3)	1 (0.8)

* Sows and sampling methods described in Table 1.

Table 3: Bacterial families isolated from skin samples of healthy sows in total and in proportion to the total number of sampled sows, samples, and isolates*

Bacterial family	Sows (n = 8) No. positive (%)	Samples (n = 96) No. positive (%)	Isolates (n = 496) No. positive (%)
Enterobacteriaceae	8 (100.0)	64 (66.7)	96 (19.4)
Staphylococcaceae	8 (100.0)	93 (96.9)	250 (50.4)
Streptococcaceae	8 (100.0)	61 (63.5)	70 (14.1)
Enterococcaceae	7 (87.5)	40 (41.7)	41 (8.3)
Leuconostocaceae	6 (75.0)	18 (18.8)	18 (3.6)
Spore formers	4 (50.0)	16 (3.2)	16 (3.2)
Others	3 (37.5)	5 (5.2)	5 (1.0)

* Sows and sampling methods described in Table 1.

- Streptococcaceae and Enterobacteriaceae, including *E coli*, may be present transiently on the mammary gland skin of sows.
- Under the conditions of this study, the colostrum of many healthy sows is not free of bacteria; therefore, isolation of bacterial species from the milk of sows with clinical coliform mastitis for diagnostic purposes should be regarded against this background.

Acknowledgement

This work was partially financially supported 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

1. Gerjets I, Kemper N. Coliform mastitis in sows: a review. *J Swine Health Prod*. 2009;17:97–105.

2. Oliver SP, Sordillo LM. Udder health in the periparturient period. *J Dairy Sci.* 1988;71:2584–2606.

3. Federation of Animal Science Societies. *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching*. 3rd rev ed. Champaign, Illinois: Federation of Animal Science Societies; 2010. Available at: www.fass.org/docs/agguide3rd/Ag_guide_3rd_ed.pdf. Accessed 14 October 2010.

4. Kemper N, Gerjets I. Bacteria in milk from anterior and posterior mammary glands in sows affected and unaffected by postpartum dysgalactia syndrome (PPDS). *Acta Veterinaria Scandinavica*. 2009;51:26–32.

5. Nagase N, Sasaki A, Yamashita K, Shimizu A, Wakita Y, Kitai S, Kawano J. Isolation and species distribution of *Staphylococci* from animal and human skin. *J Vet Med Sci.* 2002;64:245–250.

6. Bertschinger HU, Burgi E, Eng V, Wegmann P. Reduction of the incidence of puerperal mastitis in the sow by protection of the mammary gland against fecal contamination. *Schweizer Archiv für Tierheilkunde*. 1990;132:557–566.

7. Morkoc A, Bäckström L, Lund L, Smith AR. Bacterial endotoxin in blood of dysgalactic sows in relation to microbial status of uterus, milk and intestine. *JAVMA*. 1983;7:786–789.

8. Awad-Masalmeh M, Baumgartner W, Passering A, Silber R, Hinterdorfer F. Bacteriological studies in sows with puerperal mastitis in different herds in Austria. *Tierärztliche Umschau*. 1990;45:526–535.

9. Zhu Y, Fossum C, Berg M, Magnusson U. Morphometric analysis of proinflammatory cytokines in mammary glands of sows suggests an association between clinical mastitis and local production of IL-1beta, IL-6 and TNF-alpha. *Vet Res*. 2007;38:871–882.

10. Linde K, Holmberg O, Aström G. The interference between coagulase negative staphylococci and Corynebacterium bovis and the common udder pathogens in the lactating cow. *Nord Vet Med*. 1980;32:552–558.

11. Fragkou IA, Mavrogianni VS, Cripps PJ, Gougoulis DA, Fthenakis GC. The bacterial flora in the teat duct of ewes can protect against and can cause mastitis. *Vet Res*. 2007;38:525–545.

12. Mavrogianni VS, Cripps PJ, Fthenakis GC. Bacterial flora and risk of infection of the ovine teat duct and mammary gland throughout lactation. *Prev Vet Med*. 2007;79:163–173.

Checkoff research highlighted at International PRRS Symposium

The Pork Checkoff once again demonstrated its commitment to finding solutions for porcine reproductive and respiratory syndrome (PRRS) by helping fund this year's International PRRS Symposium and present more that 26 research studies on the subject. The annual meeting, held in Chicago, Illinois, drew more than 275 researchers and pork-industry participants from 22 countries, making it the world's largest yearly gathering on PRRS.

Checkoff Director of Swine Health Information and Research Dr Lisa Becton was co-chair of the 2010 International PRRS Symposium along with Dr X. E. Meng of Virginia Tech University. Becton said, "The message we continue to hear is very clear – there are still many unanswered questions regarding the PRRS virus and how to effectively control and eliminate it from swine herds, but we are making progress. That's why this forum and all the participating researchers play such a critical role in helping to maintain the momentum that has built over the years."

For additional PRRS information, go to **pork.org** and **www.prrs.org**. For proceedings from the symposium, go to **www.prrssymposium.org**. Also, contact Dr Lisa Becton at **LBecton@pork.org** or 515-223-2791.

Litter-rearing environment may affect sow productivity

Can the litter size that a gilt was raised in affect her lifetime productivity as a sow? And does age at first boar exposure limit her potential parities? These were questions addressed in a Pork Checkoff-funded study led by Dr William Flowers at North Carolina State University.

At the end of six parities, significantly more sows raised in small litters (< 7 piglets) were still in production than were those raised in large litters (> 10 piglets), regardless of age of puberty induction. Similarly, regardless of the size of the litter in which they nursed, significantly more sows exposed to boars at 140 days of age remained in the herd after six parities than did their counterparts, given boar exposure at 170 days of age. Collectively, the total number of pigs produced through six parities per gilt bred in each management system was determined and these estimates are as follows: Small neonatal litter + boar exposure at 140 days = 43.2 pigs; small neonatal litter + boar exposure at 170 days = 29.8 pigs; large neonatal litter + boar exposure at 140 days = 29.7 pigs; and large neonatal litter + boar exposure at 170 days = 21.9 pigs.

"These findings represent changes commercial producers can make in their herds today that can increase profitability. By strategic cross-fostering, earlier puberty stimulation, or both, there is potential for increased sow-lifetime productivity," said Dr Mark Knauer, Pork Checkoff's director of animal science.

For more information contact Dr Mark Knauer, **MKnauer@pork.org** or 515-223-2606.

Comprehensive disease surveillance moves forward

In recent meetings organized by the Pork Checkoff staff, stakeholders representing pork producers, veterinarians, government, and industry have come together to provide perspectives and recommendations to create a comprehensive disease surveillance system for the nation's swine herd. Aside from the obvious benefits at the farm level, it is generally recognized that such a move would help protect the entire US pork industry from the threats of domestic and foreign animal diseases, which often have trade implications.

Iowa State University economist Dermot Hayes notes that the US pork industry currently exports 20% of annual production. He estimated that the 2009 H1N1 outbreak cost producers $27.29 per animal in lost revenue due to loss of exports.

"We hope to build upon the work that we've done with influenza surveillance in swine," said Dr Paul Sundberg, vice president of science and technology for the National Pork Board. "We're well on our way to prioritizing the top health issues for the swine industry and we're confident we can work together to create a comprehensive and integrated system to benefit the entire industry."

For more information, contact Dr Paul Sundberg, **PSundberg@pork.org** or 515-223-2764.

Swine premises identification surpasses 90%

As of the end of 2010, 92% of all US swine premises had nationally standardized premises identification numbers (PINs). This milestone figure, calculated by the Pork Checkoff using USDA data, represents 65,907 premises. Nearly half of these farms were registered over the last 3 years in conjunction with a cooperative agreement between the pork industry and USDA.

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