Preventive Veterinary Medicine 117 (2014) 180–188

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Risk factors for MRSA in fattening pig herds – A meta-analysis using pooled data

Sabine Fromm[a,b], Elena Beißwanger[a], Annemarie Käsbohrer[a], Bernd-Alois Tenhagen[a,*]

[a] Federal Institute for Risk Assessment, Department for Biological Safety, Unit Epidemiology and Zoonoses, Diedersdorfer Weg 1, D-12277 Berlin, Germany

[b] University of Potsdam, Faculty of Science, Institute of Nutritional Sciences, Arthur-Scheunert-Allee 114-116, D-14558 Nuthetal, Germany

ARTICLE INFO

Article history:
Received 14 March 2014
Received in revised form 16 July 2014
Accepted 25 August 2014

Keywords:
Fattening pigs
Swine
MRSA
Methicillin resistant *Staphylococcus aureus*
Risk factor
Meta-analysis
Pooling analysis
Herd size
Herd type
Antimicrobial drugs
Slatted floor
Other livestock
Zoonoses

ABSTRACT

The importance of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) as an infectious agent for humans has increased in recent years in Germany. Although it is well known that the prevalence of MRSA in pig farms is high, risk factors for the presence of MRSA in herds of fattening pigs are still poorly understood. The aim of this study was to evaluate available data from previous studies on MRSA in fattening pigs in a meta-analysis to answer the question: What are the factors associated with the occurrence of MRSA in fattening pig herds?

The studies on MRSA in pigs that were identified by literature research were heterogeneous with respect to the risk factors investigated and the type of herds focused on. Therefore we decided to carry out a pooling analysis on herd level rather than a typical meta-analysis. Eligible herd data were identified based on the published literature and communication with the authors. The final data set covered 400 fattening pig herds from 10 different studies and 12 risk factors. The prevalence of MRSA in the 400 fattening pig herds was 53.5%.

Data were analyzed using generalized estimating equations (GEE). The resulting multivariate model confirmed previously identified risk factors for MRSA in pig herds (herd size and herd type). It also identified further risk factors: group treatment of fattening pigs with antimicrobial drugs (OR = 1.79) and housing fattening pig herds on at least partially slatted floors (OR = 2.39) compared to plain floor. In contrast, according to the model, fattening pig herds on farms keeping other livestock along with pigs were less likely to harbor MRSA (OR = 0.54).

The results underline the benefits from a pooling analysis and cooperative re-evaluation of published data.

1. Introduction

The importance of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) as an infectious agent for humans has increased in recent years in Germany (Cuny et al., 2013; Köck et al., 2013b) although the zoonotic pathogen mostly occurs as a colonizer of skin and

* Corresponding author at: Federal Institute for Risk Assessment, Department for Biological Safety, Unit Epidemiology, Zoonoses and Antimicrobial Resistance, Diedersdorfer Weg 1, D-12277 Berlin, Germany. Tel.: +49 30184122211; fax: +49 30184122952.
E-mail address: Bernd-Alois.Tenhagen@bfr.bund.de (B.-A. Tenhagen).

http://dx.doi.org/10.1016/j.prevetmed.2014.08.014

mucosa without clinical symptoms in humans. Farmers and veterinarians in contact with livestock are the main risk groups for colonization with LA-MRSA (Denis et al., 2009; Graveland et al., 2011; Witte et al., 2007). In 2011, 39% of all newly isolated MRSA from patients in hospitals in the Netherlands were of the livestock-associated type (Hetem et al., 2013). Also in the German province North Rhine-Westphalia located next to the Netherlands, from 2008 to 2011 a trend toward an increasing proportion of LA-MRSA in screening specimens in hospitals and by practitioners from 14% to 28% was found (Köck et al., 2013b). Across Germany, the proportion of LA-MRSA increased from 0.3% in the period of 2004–2005 to 5.4% (2010–2011), with significantly higher proportions in Lower Saxony and North Rhine-Westphalia (Schaumburg et al., 2012). By exchanging genetic material with more virulent bacteria LA-MRSA could become a serious health risk (Cuny et al., 2013).

LA-MRSA were first identified in swine herds and humans around 2004 in Europe (Meemken et al., 2010; Voss et al., 2005). They have meanwhile been observed in animal production in many parts of the world, with very heterogeneous prevalence. In Europe, in a survey using standardized sampling and testing methods the prevalence per country in holdings with breeding pigs ranged from 0% to 51.2% in 2008 (EFSA, 2009). Farm level studies showed a MRSA-prevalence of 52% of German fattening pig farms (Alt et al., 2011) and of 56% in pig holding companies in the Netherlands (Broens et al., 2011b).

Known risk factors for MRSA in pig farms are herd size, production type and purchase of pigs (Alt et al., 2011; Broens et al., 2011a,b; Crombe et al., 2012; EFSA, 2010). Introduction of MRSA in a herd could also be possible via MRSA colonized staff or other vectors such as rodents, flies or exhaust air from neighboring herds (Friese et al., 2012; Graham et al., 2009; Pletinckx et al., 2011; Schulz et al., 2012; van de Giessen et al., 2009; Van den Broek et al., 2009).

Within a herd, MRSA is spread predominantly by direct contact between animals. Dust and contaminated surfaces may serve as reservoirs for MRSA. Once present in the herd, MRSA have a selective advantage and may spread and persist if group treatments with antimicrobial drugs are carried out (Broens et al., 2011b; van Duijkeren et al., 2008). Some studies showed the highest MRSA prevalence in growing pigs. After the growing period the prevalence of MRSA in fattening pigs decreases with age (Broens et al., 2011a, 2012; Crombe et al., 2012; Dewaele et al., 2011; Smith et al., 2009; Weese et al., 2011).

Factors associated with MRSA contamination of piglet production holdings have been extensively studied in the EFSA baseline study (EFSA, 2010). Risk factors for herds of fattening pigs have less intensively been studied so far, and the results of the studies dealing with them are largely heterogeneous with respect to the risk factors investigated and the sampling and testing methodologies. Most of the studies are based on small numbers of herds. MRSA in fattening pigs can lead to introduction of MRSA in the food chain and the number of fattening pigs and fattening pig herds in a country is commonly much higher than the number of breeding pig units.

Only if the risk factors for MRSA are known we will be able to implement measures to successfully reduce the occurrence of MRSA in the farms. Our aim was to evaluate available data from previous studies in a meta-analysis, in order to increase statistical power. To establish a relationship between potential risk factors of the various studies, a pooling analysis was performed. The question for the analysis was: What are the factors associated with the occurrence of MRSA in fattening pig herds?

2. Methods

2.1. Study identification and data collection

To identify studies on MRSA in pigs and potential associated risk factors (in the following referred to as 'risk factors' or simply 'factors') a literature research was performed in the online databases PUBMED, WEB OF SCIENCE and SCOPUS on February 20, 2013. The search terms "pig/sow/swine" were combined with "methicillin/meticillin/MRSA". Only German and English language full text articles were considered. Exactly 600 scientific publications matched the search terms. After manually screening the studies for content, we identified 21 studies that had determined the prevalence of MRSA and examined various risk factors for MRSA in pigs. Additionally, four dissertations were found searching the online databases PROQUEST and DIMDI. Although all 25 studies dealt with MRSA prevalence in pigs, the specific research questions varied. They investigated a variable set of risk factors for MRSA and categorized these factors differently. Moreover, several studies included other types of pig herds, besides fattening pigs.

For this reason, it was necessary to analyze data on herd level instead of study level. The risk factors analyzed in the individual studies were compiled, regardless whether their role as a risk factor had been confirmed in the respective study. Based on this compilation, an EXCEL spreadsheet was developed for collecting data on herd level from the individual studies.

2.2. Interaction with authors

The authors of the studies were contacted via email and supplied with the EXCEL spreadsheet and instructions for completion. They were requested to either complete the sheet or to send the appropriate information in their own data format. Subsequently, an intensive process of communication with the authors or contact persons took place, to avoid misinterpretation when using their data. In the next step, the data provided by the authors were matched against strict inclusion criteria. Only data were accepted that concerned fattening pig herds and included information on the MRSA status of herds (positive or negative), as well as a substantial number of risk factors.

As a result of this procedure, 16 studies dropped out from the analysis, for at least one of the following reasons:

- raw data were no longer available for a study;
- raw data did no longer refer to specific herds;

– the data on risk factors on herd level was insufficient;
– only herd types other than fattening pigs were analyzed; and
– the authors did not respond, even after repeated request.

On the other hand, data from one additional, previously unpublished study and some additional data on herds from published studies were obtained and included in the analysis. Table 1 shows the number of analyzed herds per study and the sampling methods used for detecting MRSA. One herd per farm was included.

2.3. Analysis

2.3.1. Definition of outcome variable

For this analysis, a herd was considered as MRSA positive, if at least one of the used MRSA-sampling methods yielded a positive result for at least one sample. Herds were considered as MRSA negative if no MRSA were detected in any of the samples taken. The MRSA status of herds was taken as the binary dependent variable.

2.3.2. Selection of risk factors

From the set of risk factors that had originally been analyzed in the ten studies, 12 factors were selected for the meta-analysis based on their availability for the herds of the different studies: herd size (HERD SIZE), herd type (HERD TYPE), purchase pigs from other origin (PURCHASE), group treatment with antimicrobial drugs in the fattening period prior to sampling (AM DRUG), animal flow system all in/all out (ALL IN/OUT), regular clean up (CLEAN UP), regular disinfection (DISINFECTION), at least partially slatted floor compared to plain floor (SLATTED), organic farm (ORGANIC), indoor housing without outdoor access (INDOOR), other livestock herds than pigs on the farm (OTHER LIVESTOCK) and companion animals on the farm (COMPANION). Factors were included if the respective information was available for more than 80% of the 400 herds that had finally been selected. Table 2 shows the categorization of the 12 risk factors in the original study and our re-categorization for the analysis.

2.3.3. Model building

Logistic regression was used to investigate the effect of the12 factors on the MRSA status of herds of fattening pigs. Since our data originated from different studies, it was reasonable to assume that data from the same study is more similar than data across studies. To account for this fact, generalized estimating equations (GEE) were used as regression technique with an exchangeable working correlation structure and p-values based on model-based variance estimates. GEEs extend ordinary logistic regression in a way that allows modeling correlated data. To enable the joint evaluation of the risk factors across the 400 data sets, in a preliminary step, missing values were imputed using a simple hot deck procedure. Subsequently, the effect of single factors on the MRSA status of herds was analyzed by carrying out univariate regression analyses. Factors that were not significantly associated with the outcome in these analyses (at a significance level of 0.05) were excluded from further analysis. The factors that remained were used as candidates for inclusion in a multivariate regression model. To avoid multicollinearity in this model, the correlation matrix for the candidate factors was analyzed. Pairs of factors with a strong correlation (matrix values $\geq$0.5) were considered as being potentially redundant. For each such pair, an expert judgment was rendered, deciding whether one of the factors should be dropped. The remaining factors were entered in the multivariate model. In an additional step, the potential effects of second-order interaction terms on the MRSA status of herds were explored. For this purpose, all second-order interaction terms (i.e., interaction terms involving two risk factors) were created and added individually, one by one, to the multivariate regression model. Those terms that showed a significant effect were then considered for final inclusion in the model.

The statistical analyses were carried out using the statistical software programs PASW Statistics (Version 18.02, IBM Deutschland, Ehningen, Germany) and R, version 3.0.1. GEEs in R were computed using the gee package, version 4.13-18.

3. Results

3.1. Study identification and data collection

Data on herd-level was retrieved from ten different studies (Alt et al., 2011; Brockers, 2011; Fischer, 2011;

Table 1
Number of analyzed herds per study and their sampling methods for detecting MRSA.

Study	Analyzed herds (n)	Sampling method				
		Nasal swabs (n)	Dust (n)	Boot swabs (n)	Air samples (n)	Manure samples (n)
van Duijkeren et al. (2008)	14	10				
Frick (2010)	29	10				
Alt et al. (2011)	291		5 (pooled)			
Brockers (2011)	12	12	5 (pooled)	1		
Fischer (2011)	4	12	5 (pooled)	1		
Heine (2011)	19	10–60 (a 5 pooled)	5			
Friese et al. (2012)	8	12	5 (pooled)	1	6	
Schulz et al., 2012	7	12	1–3	1–2	6	
Köck et al. (2013a)	10		12			12
Merialdi et al. (2013)	6		10			
Overall	400					

n = number of.

Table 2
Factors inclosed in meta-analysis: their categorization in the original study and resulting categories for the meta-analysis.

Factors: in the original study	Herd size (*n*)	Age (weeks)	Herd type ff/wf/gf	Purchase pigs from other origin (*n*)	Other livestock herds than pigs	Companion animals	Indoor housing
van Duijkeren et al. (2008)	n.a.	10–26	ff, finishing unspecified	Y/N	n.a.	n.a.	n.a.
Frick (2010)	Exact number	10–26	ff, gf	Y/N	No, cattle, horse, poultry	Contact with dog, cat	Y/N
Alt et al. (2011)	Categories: <100, 100–499, 500–999, 1000–4999, ≥5000	10–25	ff, wf, gf	0, 1–2, ≥3 or retailer	Cattle, horse, sheep, goat	Dog, cat	Indoor, indoor with outdoor access, pure outdoor
Brockers (2011)	Exact number	26	ff, gf	0, 1, 2, >2	Cattle, poultry, sheep, goat	Dog, cat, horse, rabbit	Y/N
Fischer (2011)	Exact number	26	ff, gf	0, 1, 2, >2	Cattle, poultry, sheep, goat	Dog, cat, horse, rabbit	Y/N
Heine (2011)	Exact number	10–25	ff, gf	Exact number of origins	No, cattle, poultry, several	Y/N	Y/N
Friese et al. (2012)	Exact number	10–25	ff, gf	0, 1, 2, >2	No, cattle, broiler, turkey, other	Y/N	Y/N
Schulz et al., 2012	Exact number	10–25	gf	0, 1, 2, >2	No, cattle, broiler, turkey, other	Y/N	Y/N
Köck et al. (2013a,b)	Exact number	10–26	ff, gf	Y/N	No, cattle, broiler, turkey, other	n.a.	n.a.
Merialdi et al. (2013)	Sows (*n*) → calculated	17	ff	0, 1, 2, >2	Y/N	Y/N	Y/N
Factors in meta-analysis	HERD SIZE <500, 500–999, 1000–4999, >5000		HERD TYPE ff, wf, gf	PURCHASE Y/N	OTHER LIVESTOCK Y/N	COMPANION Y/N	INDOOR Y/N

Factors in the original study (cont.)	Organic farm	All in/all out	Clean up	Disinfection	Slatted floor	Group treatment with AM DRUGs, fattening period
van Duijkeren et al. (2008)	Conventional only	n.a.	n.a.	n.a.	n.a.	AM DRUGs, age
Frick (2010)	Conventional or organic	Continuous, all in/all out	Y/N	Y/N	Totally, partially, straw	Against which disease, AM DRUGs, duration
Alt et al. (2011)	Conventional or organic	Y, Y+ clean up, Y+ disinfection	See all in/all out	See all in/all out	Totally, partially, concrete with bedding	In the last 4 month: Y/N, Date last administration, duration, AM DRUGs
Brockers (2011)	Conventional only	Per whole barn, compartment, stock or continuous	No, regularly, occasionally	No, regularly, occasionally	Totally, partially, concrete with bedding	Regular group treatments: Y/N, AM DRUGs, duration
Fischer (2011)	Conventional only	Per whole barn, compartment, stock or continuous	No, regularly, occasionally	No, regularly, occasionally	Totally, partially, concrete with bedding	Regular group treatments: Y/N, AM DRUGs, duration
Heine (2011)	Organic farms only	Per whole barn, compartment or continuous	No, regularly, occasionally	No, regularly, occasionally	Straw bedding mandated	Individual treatment: Y/N, group treatment not allowed
Friese et al. (2012)	Conventional only	Per whole barn, compartment or continuous	Y/N	Y/N	Totally, partially, concrete with bedding	AM DRUGs, age, duration
Schulz et al., 2012	Conventional only	Per whole barn, compartment or continuous	Y/N	Y/N	Totally, partially, concrete with bedding	AM DRUGs
Köck et al. (2013a)	Conventional only	Per whole barn, compartment or continuous	Y/N	Y/N	Totally, partially, concrete with bedding	n.a.
Merialdi et al. (2013)	Conventional only	All in/all out, continuous	Y/N	Y/N	Totally, partially, concrete with bedding	AM DRUGs, age, duration
Factors in meta-alalysis (cont.)	ORGANIC Y/N	ALL IN/OUT Y/N	CLEAN UP Y/N	DISINFECTION Y/N	SLATTED Y/N	AM DRUG Y/N

n.a., not available; Y, yes; N, no; *n*, number of; ff, farrow-to-finish; wf, wean-to-finish; gf, grow-to-finish; AM DRUG, antimicrobial drug.

Frick, 2010; Friese et al., 2012; Heine, 2011; Köck et al., 2013a; Merialdi et al., 2013; Schulz et al., 2012; van Duijkeren et al., 2008). During intensive communication with the authors additional data were made available by the authors of these studies. Overall, data on 400 fattening pig herds were obtained. Sampling dates ranged from 2006 to 2013. Most of the data originated from cross-sectional studies, focusing on pigs of ages from 10 to 26 weeks

Table 3
Risk factors analyzed in the meta-analysis, their categorization, herds observed per category and proportion of MRSA-positive herds per category.

Risk factor	Categories	Number of herds	MRSA-positive herds in %
HERD SIZE	0–499	110	29.1
	500–999	112	58.9
	1000–4999	140	68.6
	≥5000	21	71.4
HERD TYPE	Farrow-to-finish	107	40.2
	Wean-to-finish	38	63.2
	Grow-to-finish	242	59.1
PURCHASE	No	107	43.9
	Yes	279	59.5
AM DRUG	No	180	38.9
	Yes	199	65.8
ALL IN/OUT	No	121	38.0
	Yes	260	61.9
CLEAN UP	No	99	39.4
	Yes	281	59.8
DISINFECTION	No	129	39.5
	Yes	251	62.2
SLATTED	No	42	19.1
	Yes	331	58.0
ORGANIC	No	373	55.2
	Yes	23	21.7
INDOOR	No	18	27.8
	Yes	354	56.2
OTHER LIVESTOCK	No	281	58.7
	Yes	103	42.7
COMPANION	No	154	52.6
	Yes	217	55.3

(Table 2). However, data on 23 herds originated from longitudinal studies, i.e., studies in which the same herds were tested repeatedly at different ages. In these cases, the data collected at the oldest tested age were selected (ages of 17 and 26 weeks, Table 2).

3.2. Descriptive analysis of prevalence of MRSA

A descriptive analysis of the 400 fattening pig herds revealed that 214 (53.5%) were MRSA positive. MRSA prevalence differed between the herds with respect to the different categories of the 12 considered risk factors.

Table 3 shows the 12 risk factors, the derived categories, the number of herds per category and the percentage of MRSA positive herds amongst them.

3.3. Model building

Ten of the 12 tested risk factors were significantly associated with the MRSA status of herds in univariate logistic regression analyses, using generalized estimating equations (GEE) (Table 4). COMPANION and INDOOR were the only two factors which were not significantly associated and therefore excluded from multivariate modeling.

Strong correlations were observed between the factors HERD TYPE and PURCHASE, furthermore between ALL IN/OUT, CLEAN UP and DISINFECTION (pairwise), and between SLATTED and ORGANIC. Based on expert judgment, HERD TYPE, ALL IN/OUT, SLATTED and ORGANIC were considered as the more relevant factors that should be kept for multivariate modeling. As a result the factors PURCHASE, CLEAN UP and DISINFECTION were excluded from further analyses, in order to reduce redundancy.

Adding individual second-order interaction terms to the multivariate model, a significant effect was only observed for the term HERD TYPE*OTHER LIVESTOCK. However, since for the analysis of nearly one third of all second-order interaction terms the available data was too sparse, we decided not to include interaction terms in the model at all.

The final multivariate logistic regression model was established based on data from 400 fattening pig herds. It covered seven risk factors for MRSA in fattening pigs. These factors had been significantly associated with the MRSA status of herds in the univariate analysis and were mostly not strongly correlated with each other.

3.4. Risk factors associated with occurrence of MRSA

Our multivariate model suggests that five of the factors involved influence the risk for MRSA in fattening pig herds (Table 4).

According to this model, the risk for MRSA increases with the HERD SIZE in a farm. More precisely, it suggests that herds with 500–999 fattening pigs are more likely to harbor MRSA than herds with less than 500 fattening pigs. The same applies to herds with 1000–4999 fattening pigs. However, no significant effect could be shown for herds with more than 5000 animals. Furthermore, wean-to-finish herds and grow-to-finish herds have a greater risk for occurrence of MRSA compared to farrow-to-finish herds. HERD TYPE wean-to-finish has the greatest risk. The model associated also herds with groupwise antimicrobial treatment during the fattening period (AM DRUG) with a higher likelihood of a MRSA detection in the herd compared to herds without such group treatments. Herds kept on at least partly SLATTED floor are associated with MRSA compared to herds that were kept on plain floor.

Herds on farms that also housed OTHER LIVESTOCK than pigs on the farm were less likely found positive for MRSA compared to pure pig production farms.

The factors ALL IN/OUT and ORGANIC were not significantly associated with the likelihood of MRSA detection.

4. Discussion

The goal of the present work was to analyze risk factors for MRSA in fattening pigs across existing studies. We aimed to increase the statistical power of the analysis by increasing the number of analyzed herds, compared to the individual studies.

This study confirms previously known risk factors for MRSA in fattening pigs, such as HERD SIZE and HERD TYPE. Furthermore, it reveals a strong correlation between HERD TYPE and PURCHASE. PURCHASE is another known risk factor for MRSA in fattening pigs but was removed from analysis on account of its strong correlation with HERD TYPE (Broens et al., 2011b; Espinosa-Gongora et al., 2012).

Table 4
Results of univariate and multivariate logistic regression analyses using GEEs.

Factor	Category	Univariate			Multivariate		
		p	OR	95% CI	*p*	OR	95% CI
HERD SIZE	0–499		Ref			Ref	
	500–999	0.000	3.59	(2.08, 6.21)	0.005	2.35	(1.30, 4.26)
	1000–4999	0.000	4.95	(2.91, 8.41)	0.002	2.63	(1.43, 4.85)
	≥5000	0.001	5.41	(2.03, 14.43)	0.167	2.18	(0.72, 6.58)
HERD TYPE	Farrow-to-finish		Ref			Ref	
	Wean-to-finish	0.016	2.53	(1.19, 5.40)	0.012	3.14	(1.29, 7.63)
	Grow-to-finish	0.001	2.19	(1.38, 3.45)	0.006	2.08	(1.24, 3.49)
PURCHASE	No		Ref				
	Yes	0.006	1.87	(1.20, 2.91)			
AM DRUG	No		Ref			Ref	
(fattening period)	Yes	0.000	3.00	(2.00, 4.51)	0.015	1.79	(1.12, 2.85)
ALL IN/OUT	No		Ref			Ref	
	Yes	0.000	2.69	(1.74, 4.16)	0.698	1.11	(0.66, 1.88)
CLEAN UP	No		Ref				
	Yes	0.000	2.28	(1.45, 3.61)			
DISINFECTION	No		Ref				
	Yes	0.000	2.45	(1.61, 3.74)			
SLATTED	No		Ref			Ref	
	Yes	0.000	5.65	(2.64, 12.12)	0.048	2.39	(1.01, 5.69)
ORGANIC	No		Ref			Ref	
	Yes	0.004	0.15	(0.04, 0.55)	0.477	0.56	(0.11, 2.79)
INDOOR	No		Ref				
	Yes	0.058	2.60	(0.97, 6.94)			
OTHER	No		Ref			Ref	
LIVESTOCK	Yes	0.006	0.53	(0.34, 0.83)	0.015	0.54	(0.33, 0.89)
COMPANION	No		Ref				
	Yes	0.568	1.12	(0.75, 1.67)			

Within this study new risk factors for MRSA in fattening pig herds could be identified. AM DRUG denotes the group treatment of pigs with antimicrobial drugs during the fattening period. Its role as a risk factor for MRSA in pigs has previously been shown in univariate analyses, but not in multivariate analyses (Alt et al., 2011; Broens et al., 2011a). For another zoonotic agent, *Salmonella*, group treatment of fattening pigs with antimicrobial drugs has been reported as risk factor even in multivariate analyses (Meyer et al., 2005). Furthermore antibiotic group treatment has been identified as a risk factor for MRSA carriage in veal calves (Graveland et al., 2010). Once present in a herd, MRSA have favorable conditions for multiplication if group treatments with antimicrobial drugs are carried out (van Duijkeren et al., 2008).

The second identified previously unreported risk factor is SLATTED. It denotes keeping herds on partially or fully slatted floor. Slatted floors are widely used in commercial pig production. A detailed epidemiological study of factors associated with bacterial enteric diseases in England identified slatted floors as a hygienic risk for finisher-pigs (Pearce, 1999). Pearce's result confirmed a suggestion (McOrist, 1997) that the inadequate cleaning of slatted floors may be associated with dissemination of microbes. Besides, studies showed that MRSA can survive on inanimate dry hospital surfaces over six months (Otter et al., 2013; Wagenvoort et al., 2000). On the other hand, antimicrobial drugs like tetracyclines in pig slurry achieve concentrations around 200 mg/kg dry matter (Gans et al., 2010). The increased risk of MRSA occurrence on slatted floors could be a result of inadequate cleaning and disinfection with insufficient elimination of MRSA, which allow the bacteria to survive and accumulate on and under slatted floors. This may increase the likelihood of MRSA detection in subsequent fattening batches kept in the same barn. Pigs are curious animals exploring their environment intensively using their nose, the typical place for MRSA colonization.

On the other hand the use of disinfectants can also coselect for resistance in bacteria, which could provide a selective advantage for MRSA (Argudin et al., 2013). In our bivariate analyses regularly disinfected barns were slightly more strongly associated with MRSA detection (OR = 2.45) than barns that were only regularly cleaned (OR = 2.28). The issue of optimal cleaning and disinfection procedures (Madec et al., 1999; Riedl, 2013) for slatted floors needs to be reconsidered also with respect to potential transmission of other pathogens than MRSA.

A rather surprising result from our study is the effect of OTHER LIVESTOCK. The multivariate model suggests that fattening pig herds kept in farms that also house other livestock have a reduced risk for MRSA-detection. There is little reason to assume a direct protective effect of OTHER LIVESTOCK against MRSA in fattening pig herds. Rather, there might be other factors behind OTHER LIVESTOCK, which cause this effect. Running chi-square tests on our data revealed significant associations between OTHER LIVESTOCK and the factors SLATTED and INDOOR, at a significance level of 0.05 (data not shown). This finding suggests that traditional family farms could to be one such factor, which keep several species of livestock, allow pigs some outdoor access and keep them less strictly on slatted floor.

Factors ALL IN/OUT and ORGANIC were not significantly associated with the outcome in the multivariate analysis. A previous study suggested that organic herds

were less likely positive for MRSA (Heine, 2011). A potential explanation of the discrepancy could be that in our study the proportion of organic herds was low and other factors may have masked a potential association.

Although LA-MRSA are a major issue in health and consumer protection, we found only few studies on risk factors for MRSA in fattening pigs. These studies on MRSA in pigs were heterogeneous with respect to the risk factors investigated and the types of herds focused on. On account of this heterogeneity we could not carry out a classical meta-analysis, in combining the *results* of individual studies and exploring using appropriate statistical techniques. Instead, we carried out a pooling analysis on herd level. In a pooling analysis, the data from different studies are combined and analyzed, as if they were a single dataset. However, by using GEEs for modeling, instead of ordinary logistic regression, we still considered potential correlations between data from the same study. GEEs with an exchangeable working correlation structure were used, because we assumed that each pair of data originating from the same study has the same correlation. Model-based (instead of empirical) variance estimates were used for computing *p*-values and confidence intervals for odds ratios, because the number of studies, from which our data originates, was only 10 and empirical estimates in that case tend to underestimate variance.

In the studies that were considered in our analyses different sampling and testing methods were used to detect MRSA. Due to differences in sensitivity, these methods may lead to different MRSA prevalence rates of herds. However, the estimation of prevalences was not the target of our analysis. Instead, a herd was considered as MRSA positive, if at least one sample of a herd was detected positive with at least one used sampling method, reducing the risk for false MRSA negative herds. The absolute values of the prevalences may differ between studies, however the difference between positive and negative herds is less likely influenced by differences in sampling methods.

A simple hot deck procedure was used to replace missing values proportionally with existing ones. This imputation step enabled the analysis of 12 potential risk factors across ten different studies on MRSA in pig herds. For each of the 12 factors, between one and seven percent of the values were imputed. A common criticism against imputation is that it may introduce bias and make data too uniform. However, the imputation step was carried out after assuring that we can assume that the herds are 'comparable' across studies. In fact, they were all kept in Western Europe and housed under similar conditions, except for the organic herds from one study (Heine, 2011). Since only two values were missing across all 12 factors for the herds of that study, this fact was considered as of little consequence for the imputation. By deciding for imputation of missing values we could include the 67 of herds with at least one missing value. To avoid major bias we decided not to include variables that had more than 20% missing values. An alternative strategy to deal with missing values would have been to exclude all herds from the analysis for which at least one value was missing ('listwise deletion' or 'complete-case analysis'). Deletion of these herds might likewise have introduced some kind of bias. We also carried out the analysis using listwise deletion and found the odds ratios largely unchanged (data not shown). However, confidence intervals were wider and therefore significance levels differed.

Our final multivariate model contains no interaction terms. Second-order interaction terms were considered for inclusion in the model, but only one significant interaction was identified. However, the analysis of their potential effects proved difficult because some combinations of categories of risk factors occurred only rarely in our data, resulting in numerical problems. As also biological correlations did not suggest respective interactions we decided not to include them in the model.

A limitation of our approach might be that it might miss risk factors for MRSA in pig holdings that were surveyed in no or too few studies. These factors include the MRSA status of herds from which pigs are purchased, the MRSA status of herds in the neighborhood, the MRSA status of the staff in stables and the excessive occurrence of rodents or flies in stables that are known carriers for MRSA. Such factors should therefore be included in further studies on the issue.

5. Conclusion

This study analyses risk factors for MRSA in fattening pigs across various existing studies. While the process of data collection from these studies was time-consuming and tedious, it enabled the analysis of 12 potential risk factors for MRSA based on 400 fattening pig herds. We were able to confirm known risk factors (herd size, herd type), but also to identify factors that had not been identified previously (group treatment of fattening pigs with antimicrobial drugs and housing fattening pig herds on at least partially slatted floor compared to plain floor). Furthermore, we identified factors associated with a reduced risk for MRSA in fattening pig herds (farms keeping other livestock along with pigs).

The results of this work underline the usefulness of cooperation and comprehensive analysis of published data in order to provide a broader basis for discussion and possible solutions to problems of health and consumer protection.

Acknowledgements

The authors gratefully acknowledge all study authors and contact persons for their support in compiling the data, the work they have done and the always friendly and constructive cooperation: Katja Alt, Maja von Borries, Katja Brase, Birgit Brockers, Patrick Butaye, Bénédicte Callens, Susanne Kathrin Fischer, Johannes Evangelist Frick, Anika Friese, Carmen Espinosa-Gongora, Jürgen Harlizius, Ulrike Heine, Maho Imanishi, Robin Köck, Jesper Larsen, Diana Meemken, Giuseppe Merialdi, Lynda Osadebe, Larissa J. Pletinckx, Josef Schulte-Wülwer, Jochen Schulz, Engeline van Duijkeren, Arje van Nes, Marijke Verhegghe, Birgit Vossenkuhl, Jaap A. Wagenaar, Scott Weese. We also would like to thank Antje Dörendahl for additional research of

dissertations and Matthias Eckardt and Daniela Schlichting for support related to the data analysis. This study was conducted in the framework of the EMIDA ERA-NET project "LA-MRSA" (support code 015868A), which was funded by the Federal Ministry of Education and Research.

References

Alt, K., Fetsch, A., Schroeter, A., Guerra, B., Hammerl, J., Hertwig, S., Senkov, N., Geinets, A., Mueller-Graf, C., Bräunig, J., Käsbohrer, A., Appel, B., Hensel, A., Tenhagen, B.-A., 2011. Factors associated with the occurrence of MRSA CC398 in herds of fattening pigs in Germany. BMC Vet. Med. 7, 69.

Argudin, M.A., Lauzat, B., Kraushaar, B., Kelner-Burgos, I., Fetsch, A., Tenhagen, B.-A., Guerra, B., 2013. Metal and disinfectant resistance genes among animal *Staphylococcus aureus* isolates from Germany. In: 5th Symposium on Antimicrobial Resistance in Animals and the Environment, Gent, Belgium, 2013-06-30 to 2013-07-03.

Brockers, B., (Thesis) 2011. Untersuchung zum Vorkommen und zur Kolonisationsdynamik von Methicillinresistenten *Staphylococcus aureus* (MRSA) bei Schweinen in Mastbeständen in Nordwestdeutschland und Ostdeutschland. Stiftung Tierärztliche Hochschule, Hannover, Germany.

Broens, E.M., Espinosa-Gongora, C., Graat, E.A.M., Vendrig, N., van der Wolf, P.J., Guardabassi, L., Butaye, P., Nielsen, J.P., de Jong, M.C.M., van de Giessen, A.W., 2012. Longitudinal study on transmission of MRSA CC398 within pig herds. BMC Vet. Res., 8.

Broens, E.M., Graat, E.A., van der Wolf, P.J., van de Giessen, A.W., de Jong, M.C., 2011a. Prevalence and risk factor analysis of livestock associated MRSA-positive pig herds in The Netherlands. Prev. Vet. Med. 102, 41–49.

Broens, E.M., Graat, E.A., van der Wolf, P.J., van de Giessen, A.W., van, D.E., Wagenaar, J.A., van, N.A., Mevius, D.J., De Jong, M.C., 2011b. MRSA CC398 in the pig production chain. Prev. Vet. Med. 98, 182–189.

Crombe, F., Willems, G., Dispas, M., Hallin, M., Denis, O., Suetens, C., Gordts, B., Struelens, M., Butaye, P., 2012. Prevalence and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* among pigs in Belgium. Microb. Drug Resist. 18, 125–131.

Cuny, C., Kock, R., Witte, W., 2013. Livestock associated MRSA (LA-MRSA) and its relevance for humans in Germany. Int. J. Med. Microbiol. 303, 331–337.

Denis, O., Suetens, C., Hallin, M., Catry, B., Ramboer, I., Dispas, M., Willems, G., Gordts, B., Butaye, P., Struelens, M., 2009. Methicillin-resistant *Staphylococcus aureus* ST398 in swine farm personnel, Belgium. Emerg. Infect. Dis. 15, 1098–1101.

Dewaele, I., Messens, W., De Man, I., Delputte, P., Herman, L., Butaye, P., Heyndrickx, M., Rasschaert, G., 2011. Sampling, prevalence and characterization of methicillin-resistant *Staphylococcus aureus* on two Belgian pig farms. Vet. Sci. Dev. 1, 1–6.

EFSA, 2009. Analysis of the baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU, 2008: Part A. MRSA prevalence estimates; on request from the European Commission. EFSA J., 82pp.

EFSA, 2010. Analysis of the baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU, 2008: Part B. Factors associated with MRSA contamination of holdings. EFSA J., 1597.

Espinosa-Gongora, C., Broens, E.M., Moodley, A., Nielsen, J.P., Guardabassi, L., 2012. Transmission of MRSA CC398 strains between pig farms related by trade of animals. Vet. Rec. 170, 564.

Fischer, S.K., (Thesis) 2011. Untersuchungen zur Intraherdenprävalenz von methicillin-resistenten *Staphylococcus aureus* (MRSA) in Schweinebeständen in Süddeutschland. Stiftung Tierärztliche Hochschule, Hannover, Germany.

Frick, J., (Thesis) 2010. Prävalenz von Methicillin-resistenten Staphylokokken (MRSA) in bayerischen Schweinebeständen. Ludwigs-Maximilians-Universität, Munich, Germany.

Friese, A., Schulz, J., Hoehle, L., Fetsch, A., Tenhagen, B.A., Hartung, J., Roesler, U., 2012. Occurrence of MRSA in air and housing environment of pig barns. Vet. Microbiol. 158, 129–135.

Gans, O., Pfundtner, E., Winckler, C., Bauer, A., 2010. Antibiotika in Biogasanlagen: Abbauverhalten und Einfluss auf die Biogasproduktion. Umweltbundesamt, Dessau.

Graham, J.P., Price, L.B., Evans, S.L., Graczyk, T.K., Silbergeld, E.K., 2009. Antibiotic resistant enterococci and staphylococci isolated from flies collected near confined poultry feeding operations. Sci. Total Environ. 407, 2701–2710.

Graveland, H., Wagenaar, J.A., Bergs, K., Heesterbeek, H., Heederik, D., 2011. Persistence of livestock associated MRSA CC398 in humans is dependent on intensity of animal contact. PLoS ONE 6, e16830.

Graveland, H., Wagenaar, J.A., Heesterbeek, H., Mevius, D., van Duijkeren, E., Heederik, D., 2010. Methicillin resistant *Staphylococcus aureus* ST398 in veal calf farming: human MRSA carriage related with animal antimicrobial usage and farm hygiene. PLoS ONE 5, e10990.

Heine, U., (Thesis) 2011. Epidemiologische Studie zum Vorkommen von MRSA (Methicillin-resistente *Staphylococcus aureus*) in ökologisch wirtschaftenden Schweinebeständen. Stiftung Tierärztliche Hochschule, Hannover, Germany.

Hetem, D.J., Bootsma, M.C., Troelstra, A., Bonten, M.J., 2013. Transmissibility of livestock-associated methicillin-resistant *Staphylococcus aureus*. Emerg. Infect. Dis. 19, 1797–1802.

Köck, R., Brase, K., Harlizius, J., Köksal, M., Nienhoff, H., Schulte-Wülver, J., Sicken, S., Friedrich, A.W., 2013a. MRSA and ESBL-producing enterobacteria in German pig holdings. In: 65 Jahrestagung der Deutschen Gesellschaft für Hygiene und Mikrobiologie, Rostock, 22–25.9.2013.

Köck, R., Schaumburg, F., Mellmann, A., Koksal, M., Jurke, A., Becker, K., Friedrich, A.W., 2013b. Livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) as causes of human infection and colonization in Germany. PLOS ONE 8, e55040.

Madec, F., Humbert, F., Salvat, G., Maris, P., 1999. Measurement of the residual contamination of post-weaning facilities for pigs and related risk factors. Zentralbl. Veterinarmed. B 46, 37–45.

McOrist, S., 1997. Enteric diseases: porcine proliferative enteropathies. Pig J. 39, 74–76.

Meemken, D., Blaha, T., Tegeler, R., Tenhagen, B.A., Guerra, B., Hammerl, J.A., Hertwig, S., Kasbohrer, A., Appel, B., Fetsch, A., 2010. Livestock associated methicillin-resistant *Staphylococcus aureus* (LaMRSA) isolated from lesions of pigs at necropsy in northwest Germany between 2004 and 2007. Zoonoses Public Health 57, e143–e148.

Merialdi, G., Galletti, E., Guazzetti, S., Rosignoli, C., Alborali, G., Battisti, A., Franco, A., Bonilauri, P., Rugna, G., Martelli, P., 2013. Environmental methicillin-resistant *Staphylococcus aureus* contamination in pig herds in relation to the productive phase and application of cleaning and disinfection. Res. Vet. Sci. 94, 425–427.

Meyer, C., große Beilage, E., Krieter, J., 2005. Untersuchungen zur Salmonella-Seroprävalenz in unterschiedlichen Produktionssystemen beim Schwein. Tierärztl. Prax. 33, 104–112.

Otter, J.A., Yezli, S., Salkeld, J.A., French, G.L., 2013. Evidence that contaminated surfaces contribute to the transmission of hospital pathogens and an overview of strategies to address contaminated surfaces in hospital settings. Am. J. Infect. Control 41, S6–S11.

Pearce, G.P., 1999. Epidemiology of enteric disease in grower-finisher pigs: a postal survey of pig producers in England. Vet. Rec. 144, 338–342.

Pletinckx, L.J., Verhegghe, M., Dewulf, J., Crombe, F., De, B.Y., Rasschaert, G., Goddeeris, B.M., De, M.I., 2011. Screening of poultry-pig farms for methicillin-resistant *Staphylococcus aureus*: sampling methodology and within herd prevalence in broiler flocks and pigs. Infect. Genet. Evol. 11, 2133–2137.

Riedl, A.M., (Thesis) 2013. Nachhaltige Stärkung der Wertschöpfungskette von Schweinefleisch. Verlag Dr. Kovac, Hamburg, Germany.

Schaumburg, F., Köck, R., Mellmann, A., Richter, L., Hasenberg, F., Kriegeskorte, A., Friedrich, A.W., Gatermann, S., Peters, G., von Eiff, C., Becker, K., Study Group, 2012. Population dynamics among methicillin-resistant *Staphylococcus aureus* Isolates in Germany during a 6-year period. J. Clin. Microbiol. 50, 3186–3192.

Schulz, J., Friese, A., Klees, S., Tenhagen, B.A., Fetsch, A., Rosler, U., Hartung, J., 2012. Longitudinal study of the contamination of air and of soil surfaces in the vicinity of pig barns by livestock-associated methicillin-resistant *Staphylococcus aureus*. Appl. Environ. Microbiol. 78, 5666–5671.

Smith, T.C., Male, M.J., Harper, A.L., Kroeger, J.S., Tinkler, G.P., Moritz, E.D., Capuano, A.W., Herwaldt, L.A., Diekema, D.J., 2009. Methicillin-resistant *Staphylococcus aureus* (MRSA) strain ST398 is present in midwestern U.S. swine and swine workers. PLoS ONE 4, e4258.

van de Giessen, A.W., van Santen-Verheuvel, M.G., Hengeveld, P.D., Bosch, T., Broens, E.M., Reusken, C.B., 2009. Occurrence of methicillin-resistant *Staphylococcus aureus* in rats living on pig farms. Prev. Vet. Med. 91, 270–273.

Van den Broek, I.V., van Cleef, B.A.G.L., Haenen, A., Broens, E.M., van der Wolf, P., van den Broek, M.J., Huijsdens, X.W., Kluytmans, J.A., van de Giessen, A.W., Tiemersma, E.W., 2009. Methicillin-resistant *Staphylococcus aureus* in people living and working in pig farms. Epidemiol. Infect. 137, 700–708.

van Duijkeren, E., Ikawaty, R., Broekhuizen-Stins, J.J., 2008. Transmission of methicillin-resistant *Staphylococcus aureus* between different kinds of pig farms. Vet. Microbiol. 126, 383–389.

Voss, A., Loeffen, F., Bakker, J., Klaassen, C., Wulf, M., 2005. Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg. Infect. Dis. 11, 1965–1966.

Wagenvoort, J.H., Sluijsmans, W., Penders, R.J., 2000. Better environmental survival of outbreak vs. sporadic MRSA isolates. J. Hosp. Infect. 45, 231–234.

Weese, J.S., Zwambag, A., Rosendal, T., Reid-Smith, R., Friendship, R., 2011. Longitudinal investigation of methicillin-resistant *Staphylococcus aureus* in piglets. Zoonoses Public Health 58, 238–243.

Witte, W., Strommenger, B., Stanek, C., Cuny, C., 2007. Methicillin-resistant *Staphylococcus aureus* ST398 in humans and animals, Central Europe. Emerg. Infect. Dis. 13, 255–258.