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Transmission of methicillin resistant *Staphylococcus aureus* among pigs during transportation from farm to abattoir

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ABSTRACT

The prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in pigs at abattoirs is higher than in pigs sampled on farms. This study investigated whether MRSA negative pigs can become MRSA positive during transportation from the farm to the abattoir after exposure to other pigs and environmental sources of MRSA. Nasal swabs were collected from four batches of pigs during loading at the farm, on arrival at the abattoir and after stunning. Environmental wipes were taken from lorries after transporting pigs and from lairages after holding pigs. All pigs ($n = 117$) tested MRSA negative before transportation. On arrival at the abattoir, 12/117 (10.3%) pigs in two batches tested MRSA positive. In lorries that tested positive after transportation, the prevalence of MRSA positive pigs was 21.1%, whereas no MRSA was detected in pigs that had been transported in lorries that tested negative after transportation. At stunning, all batches and 70/117 (59.8%) pigs tested MRSA positive. Pigs can become MRSA positive in the short period of time during transportation from the farm to stunning at the abattoir.

Introduction

In 2005 in The Netherlands, a distinct clone of methicillin resistant *Staphylococcus aureus* (MRSA CC398) was found in pigs and in humans who had been in contact with pigs (Voss et al., 2005). Since then, this clone has been identified in several countries in both pigs and other livestock (Khanna et al., 2008; EFSA, 2009; Smith et al., 2009; Van Den Broek et al., 2009; Wagenaar and Van De Giessen, 2009; Mulders et al., 2010). In a study performed in Dutch abattoirs, 209/540 (39%) pigs and 44/54 (81%) slaughter batches were MRSA positive in 2005–2006 (De Neeling et al., 2007). In another Dutch study on pig farms using similar diagnostic methods, 7/31 (23%) farms and 35/310 (11%) pigs were MRSA positive in 2006 (Van Duijkeren et al., 2008).

The higher prevalence of MRSA in pigs in abattoirs compared to the prevalence on farms might be due to MRSA transmission at abattoirs (De Neeling et al., 2007) or during transportation to the abattoir. For *Salmonella enterica* serovar Typhimurium, a short exposure to contaminated environments (such as lorries and lairages in abattoirs) is sufficient to result in positive pigs (Hurd et al., 2001; Boughton et al., 2007). The objective of the present study was to determine whether pigs become positive for MRSA CC398 during transportation from the farm to the abattoir and while being held in lairages at the abattoir.

Materials and methods

Study design

From July 2008 to April 2009, four MRSA negative farrow-to-finish farms (farms A–D) were selected out of a national prevalence and risk factor survey on MRSA (Wagenaar and Van De Giessen, 2009). In this survey, 60 nasal swabs from pigs (in 10 pools of six swabs each) and five environmental wipes were tested for the presence of MRSA. A farm was classified as MRSA negative if all samples tested negative. Information on the selected farms is presented in Table 1.

After transportation, lorries were cleaned using high pressure water, followed by disinfection, according to Dutch guidelines for animal transportation (Anonymous, 2005). The time of transportation ranged from 2 to 5 h. On the way to the abattoir, pigs from other farms were picked up by the same lorry for batches A, B and C. Pigs from other farms were located in separate lorry sections, but contact between pigs from different farms was possible, either directly by nose-to-nose contact between pigs in different lorry sections or indirectly through contact with excreta. Slaughter pigs from the four farms were transported to three different commercial abattoirs (abattoirs I–III).

The floors of all lairages were constructed of concrete with rough surfaces, as were the separation walls between lairage sections in abattoir II. In the other two abattoirs, open metal fences separated the lairage sections. Production units in all abattoirs, including the lairages, were cleaned at the end of every working day using high pressure water, followed by alkaline or acid disinfectants. Lairages were disinfected twice weekly. The killing method in all abattoirs was electrical stunning, followed by bleeding.

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Table 1
Information on farms, abattoirs, transportation and positive samples at loading, on arrival and at stunning.

	Batch A	Batch B	Batch C	Batch D
Farm size (number of sows)	160	160	320	380
Abattoir	I	II	III	III
Pick up of other pigs during transportation	Yes	Yes	Yes	No
Transport time in lorry (h)	5	5	4	2
Holding time in lairage (h)	9	1.75	11.5	2
Total time (h)	14	6.75	15.5	4
Number of pigs present in lairage	~1000	~500	~800	~800
Type of fencing in lairage	Open	Closed	Open	Open
Number of pigs in batch	60	65	27	63
Sex of pigs (M, male; F, female)	M, F	M	M	F
Number of pigs tested	30	30	27	30
Number of positive pigs at loading	0	0	0	0
Number (%) positive pigs on arrival	0 (0.0)	0 (0.0)	7 (25.9)	5 (16.7)
Number (%) positive pigs at stunning	2 (6.7)	13 (43.3)	27 (100.0)	28 (93.3)
Positive/total wipes lorry[a]	0/3	0/5	1/5	1/5
Positive/total wipes lairage[a]	0/3	4/5	1/5	1/5

[a] Environmental wipes were taken after transportation in lorries and holding in lairages.

Pigs were held in lairages for 1.75–11.5 h. During this time period, there were frequent movements of pigs in and out of the lairages, due to delivery of pigs from other farms, while others were removed for slaughter. Pigs from other farms were located in separate lairage sections, but contact between pigs from different farms was possible, directly or indirectly, particularly in the abattoirs with open fencing.

Sampling from pigs, lorries and lairages

Nasal swabs (MW102, Medical Wire and Equipment) were taken from 27 to 30 slaughter pigs at the following time points: (1) at the farm just before loading; (2) on arrival at the abattoir; and (3) just after stunning; pigs were not restrained for sampling and the same pigs were sampled at each of the three time points. Environmental wipes (S1 Kit Ringer Solution, Sodibox) were taken from lorries after transportation and from lairages after holding pigs.

Isolation and typing of MRSA

All samples were sent to the Animal Health Service, Deventer, The Netherlands, for analysis within 10 days of sampling. Samples were first enriched using Mueller Hinton Broth with 6.5% NaCl (MHB+). Nasal swabs were put into 10 mL MHB+ and environmental wipes into 100 mL MHB+. After 18 h incubation at 37 °C, 1 mL MHB+ was transferred into 9 mL phenol red mannitol broth containing 75 mg/L aztreonam and 4 mg/L ceftizoxime (PMB+; NL020, BioMérieux), which was incubated for a further 18 h at 37 °C. A loopful of PMB+ was spread onto sheep blood agar (PB5008A, Oxoid) and a chromogenic MRSA screen agar (PO5196A, Oxoid), then incubated for a further 18 h at 37 °C. One suspected colony per sample was confirmed as MRSA by multiplex PCR (De Neeling et al., 1998; Martineau et al., 1998) and *spa* typed (Harmsen et al., 2003).

Statistical analysis

Data were analysed using SAS version 9.1. Confidence intervals (CIs) for prevalences were calculated based on the binomial probability function (PROC FREQ). Because of the limited number of batches and therefore potential entanglement between batch and explanatory variables (MRSA status of environment, fencing type, lorry, abattoir and holding time), and since environmental samples were taken after pigs had been removed from lorries and lairages, a Generalised Estimating Equations model was performed, with batch as a random effect to solely estimate the clustering effect using an exchangeable covariance structure (PROC GENMOD).

Results

Detection of MRSA in samples from pigs, lorries and lairages

All pigs ($n = 117$) tested MRSA negative before transportation (Table 1). On arrival at the abattoir, 12/117 (10.3%) pigs tested positive for MRSA; 7/27 (25.9%) pigs were positive from batch C and 5/30 (16.7%) pigs were positive from batch D, whereas all pigs from batches A and B were negative. In the lorries transporting batches C and D, MRSA was isolated from 1/5 environmental wipes after transportation of pigs, whereas no MRSA was detected in lorries after transporting batches A and B.

At stunning, 70/117 (59.8%) pigs were MRSA positive; positive pigs were found in all batches and the MRSA prevalence within each batch ranged from 2/30 (6.7%) for batch A to 27/27 (100.0%) for batch C (Table 1). MRSA positive environmental wipes were found in three lairages after holding pigs, ranging from 1/5 positive wipes for batches C and D to 4/5 positive wipes for batch B, whereas the lairage holding batch A tested negative (0/3).

The MRSA prevalence in pigs transported in lorries that tested positive after transportation was 21.1%, whereas MRSA was not detected in pigs transported in lorries that tested negative after transportation (Table 2). Mixing batches of pigs with pigs from other farms during transportation did not appear to increase MRSA transmission; the MRSA prevalence was 8.0% (95% CI 3.3–15.9) in pigs transported together with pigs from other farms, compared to 16.7% (95% CI 5.6–34.7) in pigs transported without pigs from other farms. The MRSA prevalence did not appear to increase with increasing transport time, since both negative batches at arrival had the longest transport time (Table 1).

The MRSA prevalence in pigs from lairages that tested positive after holding pigs was 78.2% (95% CI 68.0–86.3) compared to 6.7% (95% CI 0.8–22.1) in pigs from lairages that tested negative after holding pigs (Table 2). All pigs that were MRSA positive on arrival were also MRSA positive at stunning, whereas 55.2% of pigs that were negative on arrival were MRSA positive at stunning. The MRSA prevalence in pigs at stunning that were transported in lorries that tested positive after transportation was 96.5% (95% CI 87.9–99.6) compared to 25.0% (95% CI 14.7–37.9) in pigs that were transported in lorries that tested negative after transportation.

The MRSA prevalence in pigs held in lairages with open fencing was 43.3% (95% CI 25.5–62.6) compared to 65.5% (95% CI 54.6–75.4) in pigs that were held in lairages with closed fencing. The MRSA prevalence in pigs did not appear to be related to holding time in lairages (Table 1). In MRSA negative batches after transport (batches A and B), the MRSA prevalence in pigs that were held in lairages that tested positive was 43.3% (95% CI 25.5–62.6) compared to 6.7% (95% CI 0.8–22.1) in pigs that were held in lairages that tested negative after holding pigs.

Spa typing of MRSA isolates

One MRSA isolate from every positive nasal and environmental sample was *spa* typed ($n = 90$). Five *spa* types belonging to CC398 were identified, all previously detected in pigs (Huijsdens et al., 2009; Table 3). The most frequent *spa* types were t011 (60.0%) and t108 (25.6%).