Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel: An investigation of several outbreaks

E. van Duijkeren [a,*], M. Moleman [b], M.M. Sloet van Oldruitenborgh-Oosterbaan [b], J. Multem [a], A. Troelstra [c], A.C. Fluit [c], W.J.B. van Wamel [d], D.J. Houwers [a], A.J. de Neeling [e], J.A. Wagenaar [a,f]

[a] Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, PO Box 80165, 3508 TD Utrecht, Utrecht University, The Netherlands
[b] Department of Equine Medicine, Faculty of Veterinary Medicine, Utrecht University, The Netherlands
[c] Department of Medical Microbiology, University Medical Center Utrecht, Utrecht University, The Netherlands
[d] Department of Medical Microbiology and Infectious Diseases, Erasmus Medical Center, Rotterdam, The Netherlands
[e] National Institute of Public Health and the Environment, Laboratory for Infectious Diseases and Screening, Bilthoven, The Netherlands
[f] Central Veterinary Institute of Wageningen UR, Lelystad, The Netherlands

ARTICLE INFO

ABSTRACT

At the Veterinary Microbiological Diagnostic Center, the Netherlands, the percentage of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates found in equine clinical samples increased from 0% in 2002 to 37% in 2008. MRSA of *spa*-type t064, belonging to MLST ST8 and *spa*-types t011 and t2123, both belonging to the livestock-associated MLST ST398, predominated.

During an outbreak of post-surgical MRSA infections in horses at a veterinary teaching hospital in 2006/2007, MRSA isolates of *spa*-type t2123 were cultured from 7 horses and 4/61 personnel which indicated zoonotic transmission. After intervention the outbreak stopped. However, another outbreak occurred in 2008, where 17 equine MRSA isolates of *spa*-type t011 ($n = 12$), t2123 ($n = 4$), and t064 ($n = 1$) were found. This time, 16/170 personnel were positive for MRSA with *spa*-type t011 ($n = 11$) and t2123 ($n = 5$). Personnel in close contact with horses were more often MRSA-positive (15/106) than those without (1/64).

Screening of horses upon admission showed that 9.3% were MRSA-positive predominantly with *spa*-type t011. Weekly cross-sectional sampling of all hospitalized horses for 5 weeks showed that 42% of the horses were MRSA-positive at least once, again predominantly with *spa*-type t011, which suggests that nosocomial transmission took place. Fifty-three percent of the environmental samples were MRSA-positive, including samples from students' and staff members' rooms, and all were *spa*-type t011. This indicates that humans contribute to spreading the organism. Culturing of samples employing high-salt pre-enrichment performed better than a comparable method without pre-enrichment.

Our results show that nosocomial transmission occurs in equine clinics and suggests that personnel play a role in the transmission.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an emerging equine pathogen. Initial reports of MRSA infections in horses largely concerned sporadic infections associated with veterinary hospitals (Hartmann et al., 1997; Seguin et al., 1999). However, the number of reports on MRSA colonization and infections in horses is increasing and they are now also associated with private practices and the community (Weese and van Duijkeren, 2009). The prevalence of MRSA colonization published so far varied between 0% and 4.7% on horse farms in Europe, Canada and

---

North America (Busscher et al., 2006; Baptiste et al., 2005; Vengust et al., 2006; Weese et al., 2005), between 2.9% and 10.9% in horses admitted to equine clinics (Weese et al., 2006; Van den Eede et al., 2009) and 16% in horses in an equine hospital (Baptiste et al., 2005). In hospitalized horses, surgical site infections predominate, whereas joint, incision and skin/soft tissue infections are most common in community-onset infections (Weese and van Duijkeren, 2009). In Canada and North America most equine MRSA infections are caused by a human epidemic clone of Multilocus Sequence Type (MLST) ST8 (Canadian MRSA-5 or USA500) (Weese and van Duijkeren, 2009). It has been suggested that this MRSA is horse-adapted because of its predominance in horses and horse personnel (Weese and van Duijkeren, 2009). Recently, there have been reports on colonization and infection of horses with MRSA ST398 (Van den Eede et al., 2009; Witte et al., 2007), a MRSA clone associated with livestock (de Neeling et al., 2007; Gravel-and et al., 2008). The emergence of two clusters of MRSA infections at an equine veterinary teaching hospital in 2006/2007 and 2008, respectively, and the subsequent demand for outbreak management and MRSA control measures, prompted us to perform a study on colonization rates of horses and horse personnel, the possible occurrence of nosocomial transmission and the degree of contamination of the hospital's interior.

2. Materials and methods

2.1. First outbreak investigation

2.1.1. Horses
In December 2006 and early 2007, 7 horses originating from different provinces in the Netherlands developed MRSA infections shortly after colic surgery at the veterinary teaching hospital. Six patients had surgical wound infections and one had thrombophlebitis. All isolates were genotyped by Pulsed-Field Gel Electrophoresis (PFGE), spa-typing, SCCmec-typing and Multiple Locus Variable-number tandem repeat Analysis (MLVA) as described below. One isolate was typed by MLST.

2.1.2. Personnel
In February 2007, 61 persons who had been in contact with the above patients at the hospital were screened on a voluntary basis. Two persons refused to participate. All human samples were taken from both nares and from the throat and were cultured using the culturing method 2 described below.

This outbreak initially stopped after thorough cleaning of the hospital and strict isolation of the patients.

2.2. Second outbreak investigation

Almost a year later a second outbreak occurred from January to October 2008.

2.2.1. Horses
A total of 12 horses (hospitalized and outpatients) developed MRSA infections and 5 horses housed in the same stables were found to be MRSA-positive in nasal samples. Isolates were further indentified by spa-typing and 7 of these were analyzed by MLVA.

2.2.2. Personnel
From May to July 2008, a second screening of the personnel at the clinic was performed and 106 persons in close contact with the horses (veterinarians, animal attendants and students) were sampled. Persons who did not want to participate were asked to note all animal contacts on a daily basis. This time, no one refused to participate. Samples were taken from both nares and from the throat. In addition, samples were taken from 64 personnel without close contact with the horses but working at the radiology department, in technical maintenance and for the cleaning company. Samples were taken and cultured as described.

2.3. Screening of horses at admission to the hospital

During November and December 2008, samples from the nares of all horses (n = 259) just before entering the hospital were taken in duplicate and cultured for MRSA using two culture methods (below). All isolates were spa-typed.

2.4. Screening of the hospitalized horses

During the same period, cross-sectional sampling of all hospitalized horses was performed 5 times with a weekly interval leading to a total of 319 samples from 149 horses. On Mondays, all horses present at the clinic were sampled. The number of patients sampled varied between 79 on the first sampling day to 31 on the last. On average, a total of 2.1 samples were taken per horse. The first 79 samples were cultured by the two methods; the other 240 samples were cultured using pre-enrichment with Muller Hinton broth and 6.5% NaCl (method 1) only. All isolates were spa-typed.

2.4.1. Sampling of the horses
Per horse, one cotton-tipped swab was inserted into both nares (±10 cm) and retracted while rotating the swab in contact with the nasal mucosa. For screening of horses at admission and on the first sampling of the hospitalized horses, this was done twice in order to obtain duplicate samples. Samples were processed within 24 h.

2.4.2. Environmental sampling
To assess the environmental contamination in December 2008, surface wipes of the environment (n = 36) were taken using sterile gauzes (Cutisoft®, BSN Medical BV, Almere, the Netherlands). A surface of approximately 10 cm × 30 cm was gently rubbed using both sides of the gauze. Wipes were taken in duplicate at the adjacent sites. Each wipe was taken wearing new sterile gloves to prevent cross-contamination, put into sterile containers and immediately transported to the laboratory. The duplicate samples were assigned to both culture techniques at convenience. The sampling sites were seven stable units (A, B, D, F, H, O and R), six isolation units, two surgical theatres, two preparation rooms, two examination rooms, the equine reproduction clinic, the farriery, the intensive care supply room, the anaesthesia unit, the surgical

sterilizing unit (both sending side and receiving side), three locker rooms of the staff, animal attendants and students, two students' rooms, two staff rooms, animal attendants canteen, students canteen, pantry in the staff building and lunch room of the reproduction clinic. All MRSA isolates were *spa*-typed.

2.5. Culturing technique

Two culture techniques using a broth with aztreonam and ceftizoxime were compared, one with (method 1) and another without (method 2) pre-enrichment in Mueller Hinton with 6.5% NaCl. Method 1 had been used successfully to detect MRSA, including ST398, in meat samples (van Loo et al., 2007) and method 2 is the standard procedure at the University Medical Center Utrecht for clinical as well as screening cultures of humans. From a previous study we knew that method 1 performed better for culturing MRSA of ST398 from samples from pigs and calves (Graveland et al., 2009), but we did not know if this was also true for samples from horses.

2.5.1. Method 1

Swabs were put into tubes containing 5 ml Mueller Hinton Broth (MHB), containing 6.5% NaCl. Containers with the wipes were filled with 50 ml of this broth. After overnight incubation at 37 °C one ml of the pre-enrichment broth was transferred to 9 ml phenolred mannitol broth (PHMB) (BioMérieux, Marcy l'Etoile, France) with 5 μg/ml ceftizoxime and 75 μg/ml aztreonam. This enrichment broth was also incubated overnight at 37 °C and 10 μl of the PHMB broth was subsequently plated onto sheep blood agar (Biotrading, Mijdrecht, The Netherlands) and brilliance MRSA agar (Oxoid, Basingstoke, United Kingdom).

2.5.2. Method 2

Swabs were put into a tube with 5 ml tryptone soya broth (TSB) containing 4% NaCl, 1% mannitol, 16 μg/ml phenol red, 50 μg/ml aztreonam and 5 μg/ml ceftizoxime. The containers with wipes were filled with 50 ml of this broth. After incubation for 48 h at 37 °C, 10 μl was plated onto sheep blood agar (Biotrading, Mijdrecht, The Netherlands) and MRSA brilliance agar (Oxoid, Basingstoke, United Kingdom).

2.6. Identification of bacteria

Suspect colonies were identified as *Staphylococcus aureus* (*S. aureus*) using standard techniques: colony morphology, Gram stain, catalase and coagulase and by a latex agglutination test (Pasteurex Staph Plus, Bio-Rad Laboratories, Hercules, USA). Colonies suspect of being MRSA were tested by PCR for the *S. aureus* specific DNA fragment (Martineau et al., 1998), the *mecA* gene (De Neeling et al., 1998), and the Panton-Valentine leucocidin toxin genes (Lina et al., 1999).

2.7. Genotyping

All isolates from the first outbreak and a selection of isolates from different *spa*-types were genotyped by PFGE using SmaI as restriction enzyme according to the Harmony protocol (Murchan et al., 2003), MLVA (Schouls et al., 2009) and SCC*mec*-typing (Ito et al., 2001; Ito et al., 2004; Okuma et al., 2002). At least one isolate of each *spa*-type was analyzed by MLST (Enright et al., 2000).

All human, equine and environmental MRSA isolates were *spa*-typed by sequencing of the repetitive region of the protein A gene *spa* (Harmsen et al., 2003). Data were analyzed using the Ridom Staphtype software version 1.4 (www.ridom.de/staphtype).

2.8. Retrospective study of S. aureus isolates from clinical samples from horses

VMDC's database was searched for all equine *S. aureus* isolates from 2002 to 2008. As from January 1st, 2002, all *S. aureus* isolates with unusual antimicrobial resistance patterns had routinely been tested for the presence of *mecA* by PCR and *mecA*-positive isolates were subjected to further genotyping and stored as part of VMDC's strain collection. During this period the culture techniques for *S. aureus* did not change and all isolates were identified as described above. Only first isolates from a patient and only isolates from clinical infections were included in the set of data. Four isolates of *spa*-type t064 and one isolate of *spa*-type t011 were chosen at random for SCC*mec*-typing and MLST as described above. In addition, eight isolates of *spa*-type t64 and one isolate of *spa*-type t011 were analyzed by MLVA.

3. Results

3.1. First outbreak investigation

3.1.1. Horses

The 7 clinical isolates from 2006 and 2007 were all non-typeable by PFGE, MLVA-type MT0568, SCC*mec*-type IVa and *spa*-type t2123 and were PVL-negative. The isolate analyzed by MLST was ST398.

3.1.2. Personnel

4/61 personnel were found positive, all with the same MRSA strain with MLVA-type MT0568 and *spa*-type t2123.

3.2. Second outbreak investigation

3.2.1. Horses

The clinical MRSA isolates (*n* = 12) in 2008 were *spa*-type t2123 (*n* = 2), t011 (*n* = 9) and t064 (*n* = 1), respectively. Seven isolates were analyzed by MLVA: the isolates of *spa*-type t2123 were both MLVA-type MT0568 and 5 isolates of *spa*-type t011 were MLVA MT0398 (Tables 1 and 2). The horse which had a surgical wound infection with t064 also carried an MRSA of *spa*-type t2123 in its nose. Of the five positive contact horses one had a MRSA with *spa*-type t2123, three type t011, whereas one isolate was not available for typing (Table 1). All isolates were PVL-negative.

3.2.2. Personnel

16/170 personnel at the clinic were found to be MRSA-positive. From the 106 persons in close contact with horses, 15 (14.2%) were found to be MRSA-positive. From

Table 1
Spa-types and repeat succession of the equine MRSA isolates found in the present study.

Spa-type	Tandem repeat
t011	008-16-02-25-34-24-25
t2123	008- - - - - - - - - - - - - -25
t588	008-16-02- - - - - -24-25
t064	11-19-12-05-17-34-24-34-22-25
t451	11- - - -12-05-17-34-24-34-22-25

the remaining 64 persons without direct contact with horses, only one person (1.6%) working for the cleaning company in the stables was found MRSA-positive. Persons only cleaning offices and persons working at the radiology department or in technical maintenance were negative. The spa-types found were t011 ($n = 11$) and t2123 ($n = 5$). All isolates were PVL-negative.

3.3. Screening of horses at admission to the veterinary teaching hospital

Screening of the horses before entering the hospital showed that 24/259 horses (9.3%) tested MRSA-positive. Twenty-one of these MRSA (87.5%) were spa-type t011, one t064, one t2123 and one was lost before typing. All typed isolates were PVL-negative.

3.4. Screening of the hospitalized horses

Screening of the hospitalized horses revealed that 88 of 324 (27%) samples were MRSA-positive and 62 of 149 (42%) horses. Four of these horses were already MRSA-positive at admission while 21 were negative and 37 were not sampled at admission, because they were already hospitalized when the investigation started. Most horses were MRSA-positive only once, but one horse was MRSA-positive 5 times (Table 3). All MRSA isolates except one were spa-type t011. One horse had spa-type t2123, but this horse was already positive with the same spa-type at admission and had been hospitalized at this clinic before in 2004 and 2007. All MRSA were negative for the PVL toxin genes.

3.5. Screening of the environment

From the environmental wipes, 19/36 (53%) tested positive for MRSA: 4/7 stable units, 3/6 isolation units, 2/2

Table 3
Cross-sectional sampling of the hospitalized horses.

Number of samples/horse	Number of positive samples/horse	Number of horses with this result
1	0	47
1	1	25
2	0	13
2	1	7
2	2	6
3	0	3
3	1	2
3	3	3
4	0	22
4	1	12
4	2	2
4	3	1
4	4	2
5	0	2
5	1	1
5	5	1

preparation rooms, 2/2 examination rooms, the farriery, the anaesthesia unit, the intensive care supply room, 2/2 staff rooms, the animal attendants'canteen, the students' room and the students' canteen. All environmental isolates had spa-type t011 and were PVL-negative.

3.6. Comparing two culture methods

Culturing method 1 using MHB with 6.5% NaCl as pre-enrichment yielded more MRSA-positive samples than method 2 using TSB broth with 4% NaCl and aztreonam and ceftizoxime as enrichment. Of the 338 equine samples tested by both methods, 53 (15.7%) were MRSA-positive using pre-enrichment with MHB and NaCl (method 1), whereas only 12 (3.5%) samples were MRSA-positive using the TSB broth (method 2). All MRSA-positive samples found by method 2 were also detected using method 1. Of the 36 environmental wipes, 18 (50%) samples were found positive by method 1 whereas only 6 (16.7%) samples were positive by method 2. One sample was found positive by method 2 and not by method 1.

3.7. Retrospective study of clinical MRSA isolates at the VMDC

The percentage of clinical MRSA isolates on the total number of clinical equine S. aureus isolates found at the VMDC increased from 0% in 2002 to 37% in 2008 (Fig. 1).

Table 2
Distribution of spa-types of clinical equine MRSA isolates among the equine clinics.

| Clinic	Spa-types found at this clinic	Number of isolates with this spa-type	Clinical condition
Veterinary teaching hospital	t011, t064, t2123	$n = 9$, $n = 1$, $n = 9$	Post-operative wound infections, wound infections, thrombophlebitis
Referral clinic A	t064, t011	$n = 8$, $n = 4$	Arthritis, endometritis, conjunctivitis, peritonitis, post-operative wound infections
Referral clinic B	t064, t011, t588	$n = 6$, $n = 3$, $n = 1$	Post-operative wound infections, abscess, pneumonia
Clinic C	t064	$n = 1$	Arthritis
Clinic D	t011	$n = 2$	Abscess, endometritis
Clinic E	t451	$n = 1$	Endometritis
Clinic F	t064	$n = 1$	Post-operative wound infection
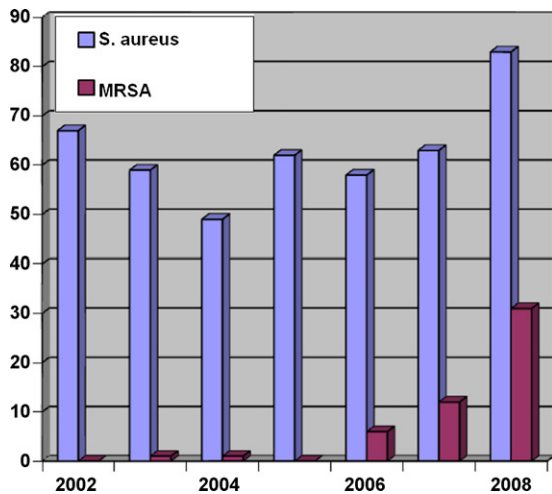
Fig. 1. The number of clinical equine S. aureus and MRSA isolates found at the VMDC from 2002 to 2008.

Most MRSA originated from the veterinary teaching hospital described in the first part of this study and two large equine referral clinics, but these 3 clinics together account for about 50% of all equine samples submitted to the VMDC. Only 5 incidental cases were found at 4 other equine clinics (Table 2). The most prevalent MRSA at both referral clinics had spa-type t064, which is typeable by PFGE using SmaI (RIVM-type 6b), MLST ST 8, MLVA-type MT0265 and SCCmec-type IVa. MRSA with spa-type t011 (non-typeable by PFGE, MLVA-type MT0398, SCCmec-type IVa and known to be ST398) was the second most common MRSA in those two referral clinics (Table 2, Fig. 2). Spa-type t588 was only found once. Spa-type t451, which differs from t064 by only one repeat, was also found once in one of the 4 clinics with only incidental cases of MRSA. Notably, spa-type t2123 was not found in horses at any equine clinic except at the veterinary teaching hospital (Table 2) nor in any other animal species (data not shown).
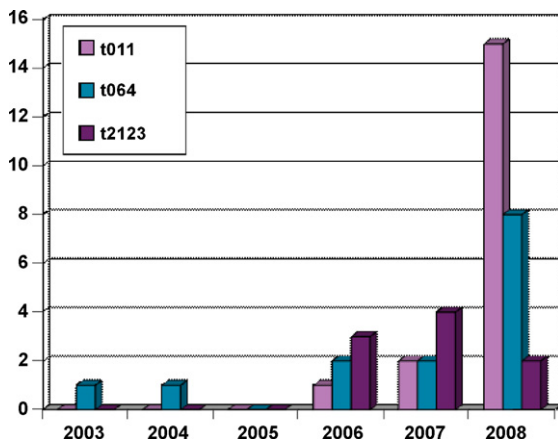

Fig. 2. The distribution of the 3 predominant spa-types found at the VMDC in clinical equine samples from 2002 to 2008.

4. Discussion

Earlier studies among equine clinical isolates in the Netherlands (van Duijkeren et al., 2004) and a survey among 200 healthy horses (Busscher et al., 2006) did not reveal MRSA. The reason for the sharp increase in the number of equine MRSA infections since 2006 is currently unknown. The high prevalence of ST398 in pigs and veal calves in the Netherlands (de Neeling et al., 2007; Graveland et al., 2008) and the fact that horses are often kept on farms with other livestock and are sometimes treated by veterinarians who are also involved in the treatment of livestock are likely explanations for the increase of ST 398 in horses. MRSA ST8 of spa-type t064 have also been found in horses and staff at a veterinary hospital in Ireland (Moodley et al., 2006) and MRSA ST398 spa-type t011 have been found in horses and staff in a teaching hospital in Vienna (Cuny et al., 2008). The international travel and trade of horses may favour the dissemination of the MRSA. Whether changes in the use of certain antimicrobials are responsible for the increase remains to be determined.

All equine and human MRSA isolates from the first outbreak at the hospital were of the rare spa-type t2123. As the horses had had no direct contact with each other it is likely that nosocomial transmission took place, which is supported by the fact that MRSA with this spa-type had and has not been found in animals outside this clinic with one exception: a horse which was positive with spa-type t2123 during our pre-admission study in 2008. This horse, however, had been admitted to this hospital in November 2004 and in October 2007. Unfortunately, no cultures were performed at that time, but it is most likely that this horse became infected at an earlier hospitalization. Spa-type t2123 is also very rare in humans in the Netherlands: in 2007 and 2008 only 4 isolates had this spa-type, while it was not found in 2006, except for the isolates related to this outbreak (https://mrsa.rivm.nl/flash/flash.aspx). In this view there is a strong case that zoonotic transmission did occur at the hospital.

The index case of this outbreak was not identified, but we postulate that spa-type t2123 (repeat succession 08-25) has emerged from the more common and closely related spa-type t011 (08-16-02-25-34-24-25) by the loss of five repeats. Notably, the isolates of spa-type t011 and t2123 found at the hospital shared the same resistance pattern (data not shown) and had the same SCCmec-type. A similar observation was made during an MRSA outbreak in a permanent care facility for people with visual and intellectual disabilities, where two different spa-types were discovered: the rare spa-type t2383 (08–16) and spa-type t011 (Fanoy et al., 2009). Another indication that new spa-types emerge through the loss of repeats is that spa-type t451 (11-12-05-17-34-24-34-22-25) was found once and this spa-type seems to be related to the more common spa-type t064 (11-19-12-05-17-34-24-34-22-25) from which it differs only by the absence of repeat 19. spa-type t064 is also closely related to spa-type t008 (11-19-12-21-17-34-24-34-22-25), which has been isolated from horse personnel in Vienna (Cuny et al., 2008).

The high prevalence of MRSA-positive horses entering the veterinary teaching hospital is in accordance with the prevalence of 10.9% found in a Belgian equine clinic (Van den Eede et al., 2009) and provides further evidence for the emergence of ST398 in Western European horses. The results of the screening study show that nosocomial transmission occurred as the prevalence of MRSA-positive samples was higher at the first sample during hospitalization than at the time of admission and individual horses which were negative upon admission turned MRSA-positive while hospitalized. Routine screening of all horses at admission in order to identify colonized or infected animals could help to prevent the spread of MRSA, but is impractical and expensive. In addition, given the high number of MRSA-positive horses entering the clinic, quarantine of positive patients is simply impossible. Therefore, the main goal of the preventive measures should be the containment of the MRSA with the patient thus limiting the risk of nosocomial infections. It is clear that preventing nosocomial MRSA infections presents a new challenge to equine clinics. Most hospitalized horses were MRSA-positive only for a short time. Interestingly, 87 horses were MRSA-negative although they were stabled in a contaminated environment. Twenty-seven of these were cultured 3–5 times and tested consistently negative. On the other hand, there were also horses that were found to be MRSA-positive repeatedly. One horse was even found positive five times. This suggests that some MRSA-positive horses are not colonized, but merely contaminated, probably through MRSA carrying dust particles. Colonization implies adherence and multiplication of the bacteria. Horses that remain positive for longer periods are more likely to be colonized. Longitudinal studies on *S. aureus* or MRSA colonization in horses are lacking, but are urgently needed. To date most reports on MRSA are one point prevalence studies which implies that they must be interpreted cautiously. In humans, longitudinal studies showed that about 20% of individuals are persistent *S. aureus* nasal carriers, approximately 30% are intermittently positive and about 50% non-carriers (van Belkum et al., 2009). A similar situation may also appear to be true for horses and suggest that host genetic factors may be involved.

The culturing technique using pre-enrichment with high salt concentrations was superior to the method without pre-enrichment. This is likely caused by the pre-enrichment step, but the composition of the enrichment broth was also slightly different, which might also have contributed to the different results. More studies on the use of different enrichment protocols are needed, because high salt may inhibit the growth of some MRSA strains (Brown et al., 2005).

Dissemination of MRSA in the environment occurred as MRSA of *spa*-type t011, the predominant *spa*-type found in the horses during the second outbreak, was also found in environmental wipes from the hospital's interior. Especially samples of rooms where horses were being treated and stabled were positive. Remarkably, samples from rooms where students and staff members gather, like the canteens, the staff room and the students' room were also MRSA-positive. This shows that staff members and students played a role in the dissemination of MRSA in the environment, either through MRSA on their hands (e.g. after contact with infected patients), by relocating contaminated dust (e.g. on their clothing or hair) or by spreading the organism from colonized sites (e.g. by sneezing). Obviously, strict implementation of hygienic measures is required to curb the spread of MRSA within a clinic. Isolation of horses diagnosed with or suspected of MRSA infections is important in order to minimize the risk of nosocomial and zoonotic transmission and environmental contamination. Personnel must avoid contaminating themselves and their environment. Transmission of the organism via hands is thought to be an important route of transmission within human and veterinary hospital settings (Leonard and Markey, 2008). Consequently, hand hygiene is a crucial part of any infection control programme. Refillable clip-on alcohol gel pouches can be used as a rapid and convenient method of hand sanitizing in places where water and soap is not available (Leonard and Markey, 2008). Proper cleaning and disinfection of contaminated environments is also crucial. Transmission of MRSA through dust is of greater importance in equine clinics than in human hospitals because horses produce more dust particles and are often stabled on beddings that constitute immense sources of – potentially contaminated – dust. In addition, it has been shown that MRSA survive very well in dust (Oie and Kamiya, 1996). It is noteworthy in this regard that personnel with direct contact with horses (e.g. veterinarians, students) were more often MRSA-positive (13%) than personnel without (1.6%), implying that direct contact is an important risk factor.

In conclusion, this study shows that the livestock-associated MRSA of ST398 (*spa*-type t011 and t2123) and ST8 (*spa*-type 64) recently emerged in horses in the Netherlands and that zoonotic and nosocomial transmission occurred at an equine veterinary teaching hospital where environmental contamination appeared to be widely distributed. Since the prevalence of non-clinical MRSA in horses before entering the clinic was already high, preventing nosocomial infections should focus on containment rather than identifying and isolating MRSA sources. Above all, this requires improving hygiene awareness among all personnel, beginning with strict hand sanitation.

Further studies on the risk factors associated with MRSA-positivity are needed to explain the high prevalence of MRSA in horses. Obviously, the immediate exposure of the horse to MRSA-positive horses, persons and environments will be a potential risk factor, but ultimately, the horse's genotypic sensitivity to colonization should also be considered. In addition, longitudinal studies should be performed in horses to elucidate to what extent and length true MRSA-carriership occurs in this species.

Acknowledgement

References

Baptiste, K., Williams, K., Willams, N., Wattret, A., Clegg, P., Dawson, S., Corkill, J., O'Neill, T., Hart, C., 2005. Methicillin-resistant staphylococci in companion animals. Emerg. Infect. Dis. 11, 1942–1944.

Brown, D.F., Edwards, D.I., Hawkey, P.M., Morrison, D., Ridgway, G.L., Towner, K.J., Wren, M.W., 2005. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). J. Antimicrob. Chemother. 56, 1000–1018.

Busscher, J., van Duijkeren, E., Sloet van Oldruitenborgh-Oosterbaan, M., 2006. The prevalence of methicillin-resistant staphylococci in healthy horses in the Netherlands. Vet. Microbiol. 113, 131–136.

Cuny, C., Strommenger, B., Witte, W., Stanek, C., 2008. Clusters of Infections in Horses with MRSA ST1, ST254, and ST398 in a Veterinary Hospital. Microb. Drug Resist. 14, 307–310.

De Neeling, A.J., van Leeuwen, W.J., Schouls, L.M., Schot, C.S., van Veen Rutgers, A., Beunders, A.J., Buiting, A.G., Hol, C., Ligtvoet, E.E., Petit, P.L., Sabbe, L.J., van Griethuysen, A.J., van Embden, J.D., 1998. Resistance of staphylococci in The Netherlands: surveillance by an electronic network during 1989–1995. J. Antimicrob. Chemother. 41, 93–101.

de Neeling, A., van den Broek, M., Spalburg, E., van Santen-Verheuvel, M., Dam-Deisz, W., Boshuizen, H., van de Giessen, A., van Duijkeren, E., Huijsdens, X., 2007. High prevalence of methicillin resistant Staphylococcus aureus in pigs. Vet. Microbiol. 122, 366–372.

Enright, M.C., Day, N.P., Davies, C.E., Peacock, S.J., Spratt, B.G., 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. J. Clin. Microbiol. 38, 1008–1015.

Fanoy, E., Helmhout, L.C., van der Vaart, W.L., Weijdema, K., van Santen-Verheuvel, M.G., Thijsen, S.F., de Neeling, A.J., van Wamel, W.J., Manaskova, S.H., Kingma-Thijssen, J.L., 2009. An outbreak of non-typeable MRSA within a residential care facility. Eur. Surveill. 14, 19080.

Graveland, H., Wagenaar, J.A., Broekhuizen-Stins, M., Oosting-Schothorst, I., Schoormans, A., van Duijkeren, E., Huijsdens, X., Mevius, D., Heedrik, D., 2008. Methicillin-resistant Staphylococcus aureus (MRSA) in veal calve farmers and veal calves in The Netherlands. In: ASM Conference on Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens, Copenhagen, Denmark, June 15–18, pp. 62–63.

Graveland, H., van Duijkeren, E., van Nes, A., Schoormans, A., Broekhuizen-Stins, M., Schothorst, I.O., Heederik, D., Wagenaar, J.A., 2009. Evaluation of isolation procedures and chromogenic agar media for the detection of MRSA in nasal swabs from pigs and veal calves. Vet. Microbiol., doi:10.1016/j.vetmic.2009.05.019 ePub ahead of print.

Harmsen, D., Claus, H., Witte, W., Rothganger, J., Claus, H., Turnwald, D., Vogel, U., 2003. Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. J. Clin. Microbiol. 41, 5442–5448.

Hartmann, F.A., Trostle, S.S., Klohnen, A.A., 1997. Isolation of methicillin-resistant Staphylococcus aureus from a postoperative wound infection in a horse. J. Am. Vet. Med. Assoc. 211, 590–592.

Ito, T., Katayama, Y., Asada, K., Mori, N., Tsutsumimoto, K., Tiensasitorn, C., Hiramatsu, K., 2001. Structural comparison of three types of staphylococcal cassette chromosome mec integrated in the chromosome in methicillin-resistant Staphylococcus aureus. Antimicrob. Agents Chemother. 45, 1323–1336.

Ito, T., Ma, X.X., Takeuchi, F., Okuma, K., Yuzawa, H., Hiramatsu, K., 2004. Novel type V staphylococcal cassette chromosome mec driven by a novel cassette chromosome recombinase, ccrC. Antimicrob Agents Chemother. 48, 2637–2651.

Leonard, F.C., Markey, B.K., 2008. Methicillin-resistant Staphylococcus aureus in animals: a review. Vet. J. 175, 27–36.

Lina, G., Piemont, Y., Godail-Gamot, F., Bes, M., Peter, M.O., Gauduchon, V., Vandenesch, F., Etienne, J., 1999. Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. Clin. Infect. Dis. 29, 1128–1132.

Martineau, F., Picard, F.J., Roy, P.H., Ouellette, M., Bergeron, M.G., 1998. Species-specific and ubiquitous-DNA-based assays for rapid identification of Staphylococcus aureus. J. Clin. Microbiol. 36, 618–623.

Moodley, A., Stegger, M., Bagcigil, A.F., Baptiste, K.E., Loeffler, A., Lloyd, D.H., Williams, N.J., Leonard, N., Abbott, Y., Skov, R., Guardabassi, L., 2006. spa typing of methicillin-resistant Staphylococcus aureus isolated from domestic animals and veterinary staff in the UK and Ireland. J. Antimicrob. Chemother. 58, 1118–1123.

Murchan, S., Kaufmann, M.E., Deplano, A., de Ryck, R., Struelens, M., Zinn, C.E., Fussing, C.E., Salmenlinna, S., Vuopio-Varkila, J., El Solh, N., Cuny, C., Witte, W., Tassios, P.T., Legakis, N., van Leeuwen, W., van Belkum, A., Vindel, I., Laconcha, J., Garaizar, S., Haeggman, S., Olsson-Liljequist, B., Ransjo, U., Coombes, G., Cookson, B., 2003. Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant Staphylococcus aureus: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. J. Clin. Microbiol. 41, 1574–1585.

Oie, S., Kamiya, A., 1996. Survival of methicillin-resistant Staphylococcus aureus (MRSA) on naturally contaminated dry mops. J. Hosp. Infect. 34, 145–149.

Okuma, K., Iwakawa, K., Turnidge, J.D., Grubb, W.B., Bell, J.M., O'Brien, F.G., Coombs, G.W., Pearman, J.W., Tenover, F.C., Kapi, M., Tiensasitorn, C., Ito, T., Hiramatsu, K., 2002. Dissemination of new methicillin-resistant Staphylococcus aureus clones in the community. J. Clin. Microbiol. 40, 4289–4294.

Schouls, L.M., Spalburg, E.C., van Luit, M., Huijsdens, X., Pluister, G.N., van Santen-Verheuvel, M.G., van der Heide, H.G.J., Grundmann, H., Heck, M.E.O.C., de Neeling, A.J., 2009. Multiple-Locus Variable number tandem repeat Analysis of Staphylococcus aureus: comparison with pulsed-field gel electrophoresis and spa-typing. Plos One 4, e5082.

Seguin, J.C., Walker, R.D., Caron, J.P., Kloos, W.E., George, C.G., Hollis, R.J., Jones, R.N., Pfaller, M.A., 1999. Methicillin-resistant Staphylococcus aureus outbreak in a veterinary teaching hospital: potential human-to-animal transmission. J. Clin. Microbiol. 37, 1459–1463.

Van den Eede, A., Martens, A., Lipinska, U., Struelens, M., Deplano, A., Denis, O., Haesebrouck, F., Gasthuys, F., Hermans, K., 2009. High occurrence of methicillin-resistant Staphylococcus aureus ST398 in equine nasal samples. Vet. Microbiol. 133, 138–144.

van Belkum, A., Melles, D.C., Nouwen, J., van Leeuwen, W.B., van Wamel, W., Vos, M.C., Wertheim, H.F., Verbrugh, H.A., 2009. Co-evolutionary aspects of human colonisation and infection by Staphylococcus aureus. Infect. Genet. Evol. 9, 32–47.

van Duijkeren, E., Box, A., Heck, M., Wannet, W., Fluit, A., 2004. Methicillin-resistant staphylococci isolated from animals. Vet. Microbiol. 103, 91–97.

van Loo, I.H.M., Diederen, B.M.W., Savelkoul, P.H.M., Woudenberg, J.H.C., Roosendaal, R., van Belkum, A., Lemmens-den Toom, N., Verhulst, C., van Keulen, P.H.J., Kluytmans, J.A.J.W., 2007. Methicillin-resistant Staphylococcus aureus in meat products, the Netherlands. Emerg. Infect. Dis. 13, 1753–1755.

Vengust, M., Anderson, M., Rousseau, J., Weese, J., 2006. Methicillin-resistant staphylococcal colonization in clinically normal dogs and horses in the community. Lett. Appl. Microbiol. 43, 602–606.

Weese, J.S., van Duijkeren, E., 2009. Methicillin-resistant Staphylococcus aureus and Staphylococcus pseudintermedius in veterinary medicine. Vet. Microbiol., doi:10.1016/j.vetmic.2009.01.039 ePub ahead of print.

Weese, J., Rousseau, J., Traub-Dargatz, J., Willey, B., McGeer, A., Low, D., 2005. Community-associated methicillin-resistant Staphylococcus aureus in horses and humans who work with horses. J. Am. Vet. Med. Assoc. 226, 580–583.

Weese, J., Rousseau, J., Willey, B., Archambault, M., McGeer, A., Low, D., 2006. Methicillin-resistant Staphylococcus aureus in horses at a veterinary teaching hospital: frequency, characterization, and association with clinical disease. J. Vet. Intern. Med. 20, 182–186.

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.