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Short communication

Longitudinal study of horses for carriage of methicillin-resistant *Staphylococcus aureus* following wound infections

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ABSTRACT

An outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in horses in Sweden raised questions concerning the risk posed by horses to their surroundings following MRSA infections. This initiated a longitudinal study to investigate how long MRSA-infected horses remained positive and to test the sensitivity of different anatomical sampling sites for detection of MRSA.

Between October 2008 and June 2010, 9 of 15 horses notified as having MRSA-infected wounds fitted the case criteria for the study. The cases were sampled at five anatomical sites (nostrils, corner of mouth, pastern, perineum, and previous infection site) on six to seven occasions or more during approximately 12–18 months.

MRSA-specific broth and agar were used for culture. Verified MRSA isolates were *spa*-typed. The sensitivity of sampling sites was calculated.

The most sensitive sampling site was the nostrils, with a sensitivity of 0.91 (95% CI: 0.59–1.00). The other test sites had a sensitivity of 0–0.09. Individual cases tested positive, but with time all tested negative. The observed carriage time ranged from 55 to 711 days (median = 143, IQR: 111–172 days), but these data should be interpreted with caution since only a small number of cases were studied.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an emerging infective agent in horses. Carriage of MRSA on admittance to hospital is a risk for developing infection (Weese et al., 2006) and types found in horse nostrils on admittance are also identified in infections (van Duijkeren et al., 2010).

Human screening for MRSA includes the nares, but sampling of multiple locations is customary (Bitterman et al., 2010; Senn et al., 2012). The practice to sample horse nostrils for MRSA is probably based on studies of *S. aureus* in horses (Devriese et al., 1985; Shimizu and Kato, 1979) and extrapolation from human medicine. However, a recent study compared nine anatomical sites in clinically normal, hospitalised horses for MRSA detection and the nostrils were the most sensitive site (Van den Eede et al., 2012). Internationally, sampling of horse in hospitals and/or farms report 0–12% MRSA prevalence (Axon et al., 2011; Bagcigil et al., 2007; SVARM, 2010; Tokateloff et al., 2009; Van den Eede et al., 2009). According to those sources and van Duijkeren et al. (2010), the clonal complex (CC) 398 and CC8 (ST8, ST254, ST612 and Canadian MRSA-5/USA500) dominate in horses. MRSA in animals are notifiable in Sweden (regulations SJVFS 2007:90 and SFS 2004:168) and the two MRSA types found in horses belong to the common complexes (SVARM, 2010). According to notification data, MRSA is rare in animals (SVARM, 2010). To date, 17 infected horses have been notified.

Decolonisation of MRSA without use of antimicrobials has been achieved in horses using interventions to prevent re-colonisation (Weese and Rousseau, 2005). However, to

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our knowledge decolonisation of MRSA in horses post-infection has not been studied. When a Swedish outbreak of MRSA infection in horses (Bergstrom et al., 2012) raised questions concerning the risk posed by post-infected horses to their surroundings, a study was initiated to investigate whether and for how long MRSA can be detected post-infection in horses. In addition, the hypothesis that the nostrils are sensitive site for detection of MRSA carriage in horses was tested.

2. Materials and methods

2.1. Study design

In this longitudinal study, horses notified as having an MRSA infection were continuously enrolled. Cases were sampled post-infection at five predetermined anatomical sites on seven occasions for detection of MRSA. The study was approved by the Ethical Committee on Animal Experiments, Uppsala, Sweden (C 309/8).

2.2. Sample collection

Inclusion criteria were horses (hereafter named cases) with clinical symptoms of infection and a positive culture of MRSA that led to notification. In addition, the owner's informed consent was required. Eleven of 15 notified horses were available and nine fitted the inclusion criteria. The cases originated from two equine hospitals in Sweden. The MRSA status of the cases prior to notification was unknown, as screening is not customary. The infective MRSA *spa*-types were t011 ($n=7$) and t064 ($n=2$), described earlier (SVARM, 2010). Eight cases had surgical site infection and one, Case 4, had an infected pressure wound. No case was treated with antimicrobials after diagnosis of infection. Cases 3 and 4 were four and six months old, respectively, and the other seven cases were >3 years on the first sampling occasion.

Bacteriological sampling of cases started in October 2008. No definitive stop point for enrolling cases was decided a priori, but when no case was notified for over a year, the study was closed in October 2011 after the last sampling.

Day 0 of the study was set to the date MRSA infection was detected, as the most natural day 0, the date of wound healing, could not be determined for all cases (Table 1). Only the infected site was sampled on day 0. Five anatomical sites were selected for sampling on seven occasions, the first six occasions at roughly monthly intervals and the seventh 6–12 months after the sixth. More sampling occasions were added if required. One swab was used for each site. The sites were: (1) both nostrils, approximately on the border of skin and mucosa; (2) both corner of the mouth; (3) the skin of the perineum; (4) the skin on the right front pastern; (5) the area of the previous infection site. In addition, new wounds, skin lesions, etc. were included if present on any sampling. The cases were sampled in home stables with the exception of the first sampling of Case 4, which was performed in hospital. On the first sampling occasion, the wounds had healed (skin closure without inflammatory symptoms but scar tissue) in eight of nine cases. Case 5 still had an open wound, but without signs of infection.

The first sampling, and subsequent samplings if convenient, was conducted by the first author and otherwise carried out by veterinary practitioners. One exception was an extra sampling of Case 4 performed by the owner and a human nurse. The instruction was: disinfect hands, wear disposable gloves during sampling and without prior disinfection of the area, gently rub a

Table 1
Longitudinal testing of nine horses at five different anatomical sites following MRSA wound infection.

Case	1	2	3	4	5	6	7	8	9	Total[d]	Pos[e]
Sampling occasion				Time (days)[a]							
1	116	107	102	17 (N)	46	44 (N)	39 (N,P,W)	37	28	9/45	3/5
2	143	172	129	58 (N)	77 (N)	76	75	64 (N)	55	9/45	3/3
3	179	219	165	91 (N)	112	111	103	98 (Pa)	84	9/45	2/2
4	213	240	200	122 (N)[g]	141 (N)	137	129	128	119	9/44	2/2
5	243	288	229	170	169	167	166	163	155	9/45	0/0
6	277	392	263	205 (N)	210	202	195	200	182	9/45	1/1
7	467	567	453	660	400	–	405	425	363	8/40	0/0
8 E				711					396[f]	2/13	0/0
9 E				744[h]						1/1	0/0
Total[b]	7/35	7/35	7/35	9/40	7/35	6/30	7/35	7/35	8/43	65/323	
Pos[c]	0/0	0/0	0/0	5/5	2/2	1/1	1/3	2/2	0/0		11 (17%)/13 (4.0%)

Sampled sites: N: nostrils, M: corner of mouth, P: perineum, W: site of previous infection, Pa: pastern.; Red: positive testing, in brackets positive site/s; E: extra sampling occasions outside original plan; –: deceased, not available.

[a] Days between the diagnosis of MRSA infection (day 0) and the sampling occasions.
[b] No. of sampling occasions/no. of samples per case.
[c] No. of positive occasions/positive samples per case.
[d] No. of sampled cases/no. of total samples per occasion.
[e] No. of positive cases/positive sites per occasion.
[f] Eight samples collected; from the five predetermined sites plus three from a newly injured area.
[g] M not sampled.
[h] Only N sampled.