Article

Prevalence of methicillin-resistant *Staphylococcus aureus* colonization in horses in Saskatchewan, Alberta, and British Columbia

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Abstract — This study estimated the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in nasal swabs of 458 horses in western Canada. The rate of colonization was 1.3% ± 5.84% [95% confidence interval (CI)], a rate similar to those reported elsewhere. Colonization tended to be transient and seemed unrelated to stress or administration of antimicrobials. Five of the 6 isolates were Canadian epidemic MRSA-5, a human clone that appears to predominate in horses in North America. The other isolate was spa type 539 (t034), a sequence type 398 strain, and this is the first report of this clone in horses in North America. Surveillance is warranted because of the potential of MRSA to cause disease in horses and humans.

Résumé — Prévalence de la colonisation par *Staphylococcus aureus* résistant à la méthicilline chez des chevaux en Saskatchewan, en Alberta et en Colombie-Britannique. Cette étude a estimé la prévalence de *Staphylococcus aureus* résistant à la méthicilline (SARM) dans des prélèvements nasaux chez 458 chevaux de l'Ouest canadien. Le taux de colonisation était de 1,3 % ± 5,84 % [intervalle de confiance (IC) de 95 %], un taux semblable à celui déclaré ailleurs. La colonisation avait une tendance transitoire et semblait non liée au stress ou à l'administration d'antimicrobiens. Cinq des 6 isolats étaient le SARM-5 épidémique canadien, un clone humain qui semble prédominer chez les chevaux en Amérique du Nord. L'autre isolat était de type spa 539 (t034), une souche à la séquence de type 398, et cela est le premier rapport de ce clone chez les chevaux en Amérique du Nord. La surveillance est justifiée en raison du potentiel du SARM pour causer la maladie chez les chevaux et les humains.

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Introduction

Methicillin was first developed in the 1960s to combat the influenza-like pandemic of penicillin-resistant *Staphylococcus aureus* which caused high mortality rates in Australia, the United States, and the United Kingdom in the late 1950s (1). Within 6 months of methicillin's appearance on the market, methicillin resistance was found in strains of *S. aureus* (1). Since then, methicillin-resistant *S. aureus* (MRSA) has been reported worldwide in humans and has become a significant cause of hospital- and community-associated infections (1).

Domestic animals are becoming increasingly recognized as potential sources for human infection with MRSA (2–6). In the last 10 years, MRSA has emerged as a veterinary pathogen and potential zoonosis of particular clinical importance in dogs and horses (2,3,6–10). There have been multiple cases of horses and other domestic animals with or without clinical signs of infection as the source for infection or re-infection of humans in contact with them. This has led to a high prevalence of MRSA colonization in populations in contact with animals, such as horse personnel (3,11,12), veterinarians (4), and pig farmers (13), as well as clinical cases of MRSA infection in humans in contact with infected animals (2). As MRSA and other multi-resistant bacteria emerge as an increasing problem in human and veterinary medicine, surveillance of

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their presence in normal populations is becoming increasingly crucial to better understand and prevent the diseases they cause.

There have been various studies reporting MRSA infection or colonization in horses. Reported prevalence rates vary. A community-based study in Ontario and New York state reported a prevalence of 4.7% (12). However, this included a prevalence of 12% on farms with a history of MRSA on the farm but 0% on farms without known exposure to MRSA. A study of horses admitted to an equine teaching hospital in Ontario from 2002 to 2003, reported a 2.7% prevalence of MRSA colonization (14). There have been multiple other prevalence studies in horses which yielded reports as low as 0% in Denmark (15), Slovenia (16), and the Netherlands (17), while in others, absolute prevalence could not be determined due to the nature of the population sampled or the nature of the study (4,7,9). There has been minimal investigation of the prevalence of MRSA in horses in North America outside of Ontario, and it is unclear how widely dispersed MRSA is in the horse population. The purpose of this study was to estimate the prevalence of nasal colonization of horses with MRSA in western Canada through nasal swabbing of a convenience sample of horses of various breeds and disciplines in Saskatchewan, Alberta, and British Columbia.

Materials and methods

Study population

During the summers of 2006 and 2007, nasal swabs were taken from 458 horses of various breeds and occupations in Saskatchewan, Alberta, and British Columbia, Canada. The study population included 50 horses admitted to the Veterinary Teaching Hospital (VTH) of the Western College of Veterinary Medicine (WCVM) at the University of Saskatchewan in Saskatoon for various complaints. Convenience samples (n = 123) were taken from horses attended by the clinicians of the WCVM field service practice, and 285 samples were taken from a convenience sample of a cross-section of horses at ranches, local rodeos, and agricultural fairs throughout Saskatchewan, Alberta, and British Columbia. Follow-up samples were taken from positive horses found in Saskatchewan and as many other horses as possible on their farm of origin as soon after original sampling as practicality allowed (3 wk to 3 mo). This was only possible for horses in Saskatchewan due to practicality and cost.

Sampling method

One sterile, individually packaged cotton-tipped swab (BBL Culture Swab, Becton Dickinson and Company, Sparks, Maryland, USA) was inserted approximately 10 cm into the anterior nares of each horse by a trained individual wearing new disposable latex gloves for each animal. A short questionnaire was completed with the owner's assistance and included questions regarding the horse's breed, age, sex, use, or occupation, recent travel, and history of antimicrobial use in the 30 d prior to swabbing. The swabs were stored at 4°C until processing, which occurred within 48 h of collection.

Laboratory methods

Enrichment culture was performed as previously described (13). The swabs were inoculated into 2 mL of enrichment broth consisting of 10 g/L Tryptone T, 75 g/L sodium chloride, 10 g/L mannitol, and 2.5 g/L yeast extract, and incubated at 35°C for 24 h. A 100-μL volume of broth culture was inoculated onto MRSA Chromogenic agar (BBL CHROMagar, Becton Dickinson and Company) and incubated aerobically at 35°C for 48 h.

Colonies were identified as *S. aureus* based on colony morphology, Gram stain appearance, an ability to ferment maltose, positive tube coagulase test, and *S. aureus* latex agglutination test (Pastorex Staph Plus; Bio-Rad Laboratories, Missisauga, Ontario). Methicillin-resistance was identified by demonstration of growth on Mueller-Hinton agar with 4% NaCl and 6 μg/mL oxacillin, and detection of penicillin-binding protein 2a by latex agglutination test (MRSA SCREEN antibody kit; Denka Seiken Company, Tokyo, Japan). Isolates were typed by *Sma*I pulsed-field gel electrophoresis (PFGE) (18) and by sequence analysis of the X region of the protein A gene (*spa* typing) (19). Sequences were analyzed using the eGenomics software (http://tools.egenomics.com). Ridom database equivalents were identified using the Ridom Spaserver Web site (20). eGenomics *spa* types are reported using a numerical system (*spa* type 539) while Ridom *spa* types are reported using a numerical system preceded by a 't' (*spa* t034). Isolates were also tested for the presence of genes encoding Panton Valentine leukocidin (PVL) production by real-time polymerase chain reaction (RT-PCR) (21).

Results

A total of 458 nasal swabs were taken from 458 horses in western Canada: 119 from Alberta, 75 from British Columbia, and 264 from Saskatchewan. Methicillin-resistant *Staphylococcus aureus* was cultured and identified from 6 of the 458 nasal swabs, resulting in a prevalence of 1.31% ± 5.84% [95% confidence interval (CI)]. The bacterium (MRSA) was isolated from 3/264 (1.1%) horses from Saskatchewan, 1/119 (0.8%) from Alberta, and 2/75 (2.7%) from British Columbia. All positive samples were from horses originating from different farms, although all 3 positive samples in Saskatchewan were from horses swabbed upon arrival at the WCVM for unrelated conditions.

In Saskatchewan, 123 (26.9%) of the total of 458 swabs were taken in and around Saskatoon by the field service clinicians, including 41 (9.0%) swabs taken at Marquis Downs racetrack in Saskatoon. Ninety (19.7%) horses were sampled in other areas of Saskatchewan at rodeos and horse shows. No colonized horses were identified amongst these populations. Fifty (10.9%) swabs were taken from horses at the time of admission to the VTH, and 3 (6.0%) colonized horses were identified from 3 different farms.

The 1st MRSA-positive horse in Saskatchewan was a 9-year-old quarter horse (QH) gelding (Case 1) from an extensive cattle ranch 300 km east of Saskatoon. He was admitted on July 10, 2007 for evaluation of a chronic low-grade lameness. The 2nd was a 2-week-old Paint filly (Case 2) from a small private breeding facility 100 km east of Saskatoon, admitted on July 18, 2007 for evaluation of mastitis caused by *Streptococcus*

dysgalactiae. The 3rd was a 10-year-old Paint gelding (Case 3) housed in the Saskatoon area during the summer months and taken to Arizona for cattle work every winter. He was admitted on July 18, 2007 for an acute lameness. All 3 horses were housed on pasture with many other horses of similar age, breed, and use and none of the horses had had antimicrobials administered in the month prior to admission. Case 3 had changed pastures and pasture mates often during the year prior to admission, but the other 2 had not left their properties in the past year.

In a follow-up investigation, all 3 MRSA-positive horses were re-sampled along with some of their pasture mates on their home farms. On the ranch of origin of Case 1, a cross-section of 30 (38.0%) of the 79 other horses on the property were swabbed on August 8, 2007 (5 wk after original swab), as was the original case. All samples were negative for MRSA, including the gelding that had tested positive 5 wk previously.

At the smaller Paint breeding facility, Case 2 and all 7 horses that had contact with her were swabbed on August 9, 2007 (3 wk after original swab). Case 2 was still positive for MRSA nasal colonization despite antimicrobial therapy for her mastitis (oral sulfamethoxazole-trimethoprim tablets (Nu-Cotrimox DS; Nu Pharm, Richmond Hill, Ontario) at 30 mg/kg q24h for 3 wk, as was her dam, and a yearling Paint gelding, for an overall farm prevalence of 38%. This gelding had a history of antimicrobial therapy (penicillin) at the time of his castration approximately 1 mo prior to swabbing. This farm was visited again on October 22, 2007, and the same 8 horses as well as an additional 8 that had been out to pasture (stallions and mares with foals at foot) were swabbed again (10 wk after 2nd swab); only the swab from the original filly remained positive for MRSA.

Case 3 was kept in a pasture with 2 other horses that had also travelled with him to Arizona and back in the past year. The gelding and his companions were re-sampled on October 18, 2007 (3 mo after swab). Case 3 and the 2 other horses were negative for MRSA.

In Alberta, 119 (26.0%) horses were sampled on 9 farms. Only 1 horse was positive for MRSA carriage, so the community prevalence was 0.84% ± 6.59%. The positive horse (Case 4) was an 8-year-old Canadian horse from a large show barn of 120 horses in Alberta. He had no history of antimicrobial use and had not left the property in the past year.

The 75 (16.4%) horses sampled in British Columbia were sampled on 9 farms, and 2 colonized horses were identified. The prevalence from this small sample was 2.67% ± 47.79%. The first positive horse (Case 5) was a 30-year-old quarter horse gelding with no history of antimicrobial use. It had no history of leaving the property but had had contact with another horse that did leave the property once a week to a location where it had contact with other horses. The final horse (Case 6) was a 7-year-old quarter horse gelding. He had no history of antimicrobial use, and little is known about his travel history as the owner was unwilling to complete the questionnaire. There was no known contact or link between these 2 horses.

All but 1 of the isolates were identified as Canadian epidemic MRSA-5 (CMRSA-5) by PFGE and *spa* type 7 (t064). None contained genes encoding for production of PVL. The remaining isolate, from Alberta, was non-typeable by *smaI* PFGE and was classified as *spa* type 539 (t034). It was also PVL negative.

Discussion

This is the first study of MRSA colonization in horses in western Canada, and a small but detectable prevalence of colonization was identified. The 1.31% ± 5.84% (95% CI) colonization rate reported here is similar to that reported elsewhere, including Ontario (12).

Two of the 3 horses that were initially colonized with MRSA in Saskatchewan were no longer identified as being colonized upon follow-up. This is consistent with a previous report in horses demonstrating that colonization tends to be transient and naturally eradicated over time (22), and gives further support to the notion that application of good general infection control practices may be critical for controlling the circulation of MRSA in the horse population. The 2 additional horses found to be positive on the first follow-up visit to the farm of origin of Case 2 had also cleared the infection by the time they were swabbed the 2nd time. Since they had contact with the filly, which remained colonized throughout the follow-up period, it is highly likely that they were transiently colonized after contact with her or colonized farm personnel. Colonized humans are a common finding on farms that have colonized horses (12).

We had hypothesized that there might be an increased prevalence in show and racing horses, due to their high levels of stress, their contact with multiple different premises and horses, and the frequent use of antimicrobials in these animals. Interestingly, 5 of the 6 cases were working or pleasure horses and the other was a young filly on pasture. None of the horses had a stressful level of work, nor had they been administered antimicrobials in the previous 30 d.

The predominance of CMRSA-5 was not surprising, as it has been the most common clone isolated from horses with colonization or infection in North America (3,8,12,14). It, or related clones, have also been found in horses in Europe (4,9), leading to the suspicion that this is a horse-adapted strain. Rarely found in hospital-associated or community-associated cases of MRSA in humans (23,24), CMRSA-5 is common in personnel who work with horses (2,3,11,12), supporting the hypothesis that CMRSA-5 can be transmitted between humans and horses. The MRSA isolates recovered from horses and equine veterinary personnel in a study in Ireland were indistinguishable, and were different from the strain isolated from small animals and human hospitals (4). Although the direction of transmission could not be confirmed, personnel working with horses most likely represent a vector for transmission from horse to horse either through skin contact, or through indirect contact via objects. The finding of the PFGE non-typeable MRSA spa type 539 was unexpected, as this is the first report of this strain in horses in North America. Spa type 539, or the more broadly classified sequence type 398 (ST398) MRSA have been associated with food animals (particularly pigs) in North America (13), Europe (24–27), and Asia (28), and animals have been implicated in the emergence of this clone in human infections in Europe (23,25,27). Infections caused by ST398, but of a different spa

type (t011) have been reported in Austria (7). Little is known about ST398 MRSA in North America, and continued surveillance of horses and other animals is warranted because of the significant concern regarding this clone internationally.

The risk factors and epidemiology for nasal colonization of horses with MRSA are poorly understood. A retrospective case-control study of horses admitted to a teaching hospital found previously known colonization of the horse or of other animals on the farm of origin, antimicrobial administration within 30 d of admission, admission to the neonatal intensive care unit, and admission to a service other than surgery to be risk factors for nasal colonization (29). The low prevalence of MRSA in horses in this study precluded evaluation of risk factors.

While colonized horses do not necessarily have clinical MRSA infections, there are still relevant implications of MRSA colonization. Weese et al (14) reported that clinical infection developed in 16% of hospitalized horses colonized with MRSA and that colonization was a significant risk factor for development of clinical MRSA infection during hospitalization. Further, contact with colonized horses is presumably a risk for MRSA colonization in humans, with the potential for subsequent infection. Zoonotic MRSA infections have been reported in people who work with horses (2,11).

It is interesting to note that all 3 positive swabs in Saskatchewan were obtained on admission to the VTH of the WCVM, indicating community-associated colonization as opposed to hospital acquisition. It also highlights the potential for silent introduction of MRSA into a veterinary hospital from areas where MRSA has not previously been diagnosed. The sampling policy was very strict about having horses swabbed upon admission, making it unlikely that the VTH environment was the source of the MRSA colonization in these horses, as has been previously reported in another hospital (8). Environmental sampling within the Large Animal Clinic of the VTH has not been performed to date, nor has human sampling of clinical and support staff.

Because of the potential implications for equine and public health, it is imperative that surveillance of MRSA-colonization and resistance patterns be continued to make possible the timely implementation of screening and infection control programs should the prevalence increase. CVJ

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