Brief Communication Communication brève

Staphylococcus aureus colonization in healthy horses in Atlantic Canada

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Abstract — Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization was not identified in any of 497 horses from Atlantic Canada. Methicillin-susceptible *S. aureus* (MSSA) was isolated from a subsample of 19/242 (7.9%) horses. Colonization with MSSA is relatively common in healthy horses in Atlantic Canada, but MRSA is currently rare or absent.

Résumé — Colonisation par *Staphylococus aureus* de chevaux en santé sur la côte atlantique du Canada. La colonisation par *Staphylococus aureus* résistant à la méthicilline (SARM) n'a été identifiée sur aucun des 497 chevaux testés provenant de la côte atlantique du Canada. Le *Staphylococus aureus* sensible à la méthicilline (SASM) a été isolé à partir d'un sous échantillonnage de 19/242 chevaux (7,9 %). La colonisation par le SASM est relativement fréquente chez les chevaux de la côte atlantique du Canada mais le SARM est actuellement rare ou absent.

(Traduit par Docteur André Blouin)

Can Vet J 2008;49:797–799

Staphylococcus aureus, a gram-positive bacterium, is a member of the commensal microflora and a common opportunistic pathogen in many animal species, including humans and horses. While *S. aureus* has traditionally been an important pathogen, its role in disease has increased since the emergence of methicillin-resistant strains. Methicillin-resistant strains of *S. aureus* (MRSA) are resistant to all beta-lactam antimicrobials (penicillins and cephalosporins) because of the production of an altered penicillin-binding protein encoded by the *mecA* gene (1). The MRSA strains are often resistant to many other antimicrobials, limiting treatment options.

Methicillin-resistant *S. aureus* is an increasingly serious problem in human medicine, where it is a leading cause of hospital-associated infections worldwide and has now emerged as an important pathogen in the general population, otherwise referred to as community-associated MRSA (2).

Concerns about MRSA are also spreading to equine veterinary medicine, as MRSA seems to be emerging as an important equine pathogen. Sporadic cases and outbreaks have been reported in Canada and internationally (1,3–5). Clinical MRSA infections in horses can be highly variable, ranging from mild to rapidly fatal. Soft tissue and joint infections are most common in horses with community-associated MRSA infections, while postoperative and invasive device (IV catheter) infections predominate in equine hospitals (3,4). A variety of other infections can occur, including skin infections, pneumonia, sinusitis, septicemia, metritis, and omphalophlebitis (3,4).

Colonization, wherein *S. aureus* resides at a body site without producing clinical disease, is more common than clinical infection in humans and other species. In humans, methicillin-susceptible *S. aureus* (MSSA) can be found in the nasal passages of 29% to 38% of healthy adults (6,7). Colonization with MRSA is less common and rates vary tremendously among different populations, depending on factors such as exposure to the healthcare system, and the geographic region. A recent study involving science teachers in Ontario provides a good example of the prevalence of colonization in a presumptive low risk population in Canada; colonization was identified in fewer than 3% of healthy individuals. Studies in horses have reported colonization rates of 0–16% in horses (3–5,8,9), 13% in people that work with horses (5), and 9.7% in veterinary hospital personnel working at a large animal clinic (10). Several studies have provided evidence of MRSA transmission from humans to horses and vice versa (4,5). Isolates of MRSA from horses and horse personnel in previous studies have typically been identified as Canadian epidemic MRSA-5 (CMRSA-5), equivalent to USA500, a putatively horse-adapted strain (4,5,11). Little is known, however, about the basic ecology of MSSA in horses, hampering the understanding of MRSA.

The objective of this study was to determine the prevalence of MRSA and MSSA colonization in horses in Atlantic Canada, and to compare the results with those of similar studies in other parts of Canada and in other countries.

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This study was funded by the Public Health Agency of Canada.

A mixture of draft, race, pleasure, breeding, school, and show horses from New Brunswick, Nova Scotia, Prince Edward Island, and Newfoundland were enrolled in the study from May 26 to August 31, 2006. Convenience sampling was used. This study was approved by the University of Guelph Animal Care Committee.

Single nasal swab specimens were collected from each horse. The cotton-tipped culture swabs were inserted approximately 10 cm into 1 nasal passage and withdrawn while contacting the nasal mucosa with the swab. Swabs were inserted into liquid Stuart's medium and stored at between 5°C and 25°C until processing. Demographic information (age, breed, gender, and use) was recorded for each horse at the time of sample collection.

Selective culture for MRSA was performed: nasal swabs were inoculated into 2 mL of MRSA enrichment broth, consisting of 10 g tryptone T/L, 75 g sodium chloride/L, 10 g mannitol/L, and 2.5 g of yeast extract/L, and incubated aerobically for 24 h at 35°C. Ten microliters of broth was then streaked onto mannitol-salt agar containing 10 μg of cefoxitin/mL.

A subset of samples was also tested for the presence of MSSA by inoculation of the enrichment broth used for MRSA isolation onto mannitol salt agar not containting antimicrobials. The agar plates were incubated aerobically for 24 h at 35°C. Colonies were then identified as *S. aureus,* based on morphological features, Gram stain, fermentation of maltose, polymixin B susceptibility, and positive results to the catalase, tube coagulase, and *S. aureus* latex agglutination tests (Pastorex Staph Plus; Bio-Rad Laboratories, Mississauga, Ontario). Isolates were tested for methicillin-resistance by an agglutination test (PBP2a latex agglutination test; Denka Seinken, Tokyo, Japan) and inoculation onto Mueller-Hinton agar with 4% NaCl and 6 μg of oxacillin/mL.

The chi-squared test for independence was used to compare the prevalence of MSSA colonization between different provinces. A P-value of < 0.05 was considered significant.

Four hundred ninety-seven horses were enrolled: 134 horses from 15 farms in New Brunswick, 149 horses from 13 farms in Nova Scotia, 111 horses from 11 farms in Prince Edward Island, and 103 horses from 11 farms in Newfoundland. The average number of horses sampled on each farm was 9.9 ± 6.6 (range, 1–32). Breeds of horses included standardbreds [n = 112 (22.5%)], quarterhorses [n = 86 (17.3%)], Morgans [n = 26 (5.2%)], Appaloosas [n = 21 (4.2%)], Thoroughbreds [n = 19 (3.8%)], Hanoverians [n = 18 (3.6%)], warmbloods [n = 16 (3.2%)], Pintos [n = 15 (3.0%)], unspecified ponies [n = 13 (2.6%)], Canadians [n = 12 (2.4%)], Shetland ponies [n = 8 (1.6%)], Newfoundland ponies [n = 7 (1.4%)], Holsteiners [n = 6 (1.2%)], Belgians [n = 6 (1.2%)], Clydesdales [n = 6 (1.2%)], Arabians (n = 6 [1.2%]), and saddlebreds [n = 4 (0.8%)]. The remaining 116 horses consisted of many other breeds and mixed breeds.

Methicillin-resistant *S. aureus* was not isolated from any of the 497 horses sampled. Methicillin-susceptible *S. aureus* was isolated from 19 of 242 (7.9%) horses sampled. The prevalence of MSSA colonization by province was as follows: 9.0% (6/67) in Nova Scotia, 9.2% (10/109) in Prince Edward Island, and 4.5% (3/66) in Newfoundland (P = 0.50). No horses from New Brunswick were tested for MSSA.

The apparent lack of MRSA colonization in the study population is in agreement with some other community-based studies that did not identify MRSA colonization in healthy horses in the United Kingdom (3), the Netherlands (12), and Slovenia (9). This is in contrast with other North American studies that identified MRSA colonization in 4.7% of horses in Ontario and New York State (5) and in 2.7% of horses admitted to the Ontario Veterinary College (13).

A variety of factors could account for the low prevalence of MRSA in this study. For instance, horse farms in Atlantic Canada tend to be smaller in size, fewer in number, and have a reduced frequency of national and international movement. Additionally, it is possible that horse management, including use of antimicrobials, is different in this region; but this was not evaluated.

This is the first reported evaluation of the prevalence of MSSA colonization of the nasal passages of healthy horses. The 7.9% prevalence indicates that *S. aureus* colonization is common in horses. Overall, the ecology of *S. aureus* in horses is poorly understood; evaluation of the dynamics of MSSA colonization may be important for understanding *S. aureus* infections in horses, as well as for controlling MRSA colonization. Risk factors for MSSA colonization were not evaluated in this study.

This study does not necessarily indicate an absence of MRSA within the Atlantic Canadian horse population. For instance, a very low prevalence of colonization or clustering of MRSA in certain groups or areas could have been overlooked because of the convenience sampling. Moreover, the sensitivity of single nasal swabs for the detection of MRSA is unknown, and an objective evaluation of screening methods is currently lacking. In the authors' experience, however, collection and processing of nasal swabs in this manner is an effective means of testing. Furthermore, the use of the enrichment protocol described here has been demonstrated to be more sensitive than direct culture (5). Although a commercial real time PCR assay is available for rapid identification of MRSA colonization in humans, a recent study reported that it is not useful in horses (11).

This study has identified that MRSA colonization is likely rare in the horse population in Atlantic Canada at this time. Because of the apparent international dissemination of MRSA in horses and the evidence of inter-species transmission between humans and horses, surveillance for the emergence of this pathogen is warranted. Further evaluation of the epidemiology of MSSA colonization and transmission may be useful for developing prevention and control protocols for the spread of MSSA and MRSA in horse populations.

Authors' contributions

Ms. Burton was responsible for the field work and data analysis. Dr. Reid-Smith coordinated the project and contributed to the writing and editing of the manuscript. Dr. McClure coordinated the field work and contributed to the writing and editing of the manuscript. Dr. Weese assisted with the study design and data analysis, and supervised the laboratory work.

Acknowledgments

The authors thank Drs. Liane Nelson, Jeannine DeLuca, Tammy Muirhead, Kathleen MacMillan, Nicole Wanamaker, Robert Arnott, Carl Dingee, Beverly Dawe, Kevin Anderson, Rhonda Hamilton, and Nicky Schaefer for their tremendous assistance in locating the sample population. The authors also thank Nicol Janecko for coordinating sample shipments and Joyce Rousseau for conducting the laboratory testing. CVJ

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