Journal of Equine Veterinary Science 35 (2015) 170–173

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Short Communication

Variations of Potentially Pathogenic Bacteria Found on the External Genitalia of Stallions During the Breeding Season

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ARTICLE INFO

Article history:
Received 21 October 2014
Received in revised form 26 November 2014
Accepted 3 December 2014
Available online 9 December 2014

Keywords:
Stallion
Culture
Pathogenic
Bacteria
Infertility

ABSTRACT

Potentially pathogenic bacteria such as, beta-hemolytic *Streptococcus equi* subspecies *zooepidemicus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*, are commonly isolated from cultures of the external genitalia of healthy stallions and can pose a risk to the fertility of stallions and mares. We hypothesized that there would be no difference in the frequency of cultures testing positive for potentially pathogenic bacteria throughout the breeding season and that when individual stallions cultured positive, they were not more likely to continue to culture positive. Nine Thoroughbred stallions, bred exclusively by live cover, were used. Samples for bacteriologic evaluation were taken from the prepuce and postejaculate urethra at a sampling interval of every 10th mare bred. All stallions were considered to be healthy and of sound breeding status as well as breeding a minimum of 20 mares during the season. The breeding season was divided into three different time periods, with February and March being early breeding season, April being midseason, and May and June being late breeding season. There was no difference in the frequency of positive cultures between the three separate time periods ($P = .1677$). However, a post hoc pairwise comparison revealed a tendency ($P = .07$) for more cultures to be positive at the end of the breeding season compared with the beginning of the season. Furthermore, the presence of a positive culture did not increase the incidence of positive cultures from the same stallion on subsequent collections ($P = .1025$). In conclusion, these results suggest a transient colonization of potentially pathogenic bacteria of the external genitalia of stallions.

1. Introduction

Previous studies have found that various bacterial isolates can be cultured from the external genitalia of healthy stallions. A majority of these bacteria are nonpathogenic and considered normal microflora with no effect on fertility [1–3]. However, potentially pathogenic bacteria such as, beta-hemolytic *Streptococcus equi* subspecies *zooepidemicus* (β-hemolytic streptococci), *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*, are also commonly isolated from cultures of the external genitalia of healthy stallions and have been suggested to pose a risk to the fertility of both stallions and mares [1,2,4].

The presence of potentially pathogenic bacteria on the external genitalia of stallions can cause fertility issues due to the potential transfer of bacteria from stallion to mare at the time of breeding by live cover. Most healthy mares have

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http://dx.doi.org/10.1016/j.jevs.2014.12.007

natural defense mechanisms that protect against the development of endometritis and subsequent infertility [2,4]. However, mares that may have compromised defense mechanisms may develop an infection due to the transfer of potentially pathogenic bacteria from the stallion [2,3,5]. Additionally, semen can also become contaminated with bacteria when artificial insemination techniques are used [5,6] and affect fertility of the mare.

Previous studies have also investigated the presence of bacteria on different locations on the stallion's external genitalia and found that there is a difference in colonization between anatomic sites [7]. It is also known that environment and management play a role in the bacterial isolates found [1,7,8]. However, there is less known about how the presence of potentially pathogenic bacteria on the stallion's external genitalia varies over a breeding season using multiple stallions in one location and how repeatable a positive culture is from a given stallion.

Using stallions bred exclusively by live cover in central Kentucky, our hypothesis was that there would not be a difference in the frequency of cultures positive for potentially pathogenic bacteria throughout the breeding season. In addition, we hypothesized that when an individual stallion cultured positive for potentially pathogenic bacteria, they were not more likely to continue to have positive cultures throughout the season. To address these hypotheses, our objectives were to (1) determine if there is a difference in the frequency of cultures positive for potentially pathogenic bacteria (specifically, β-hemolytic streptococci, *E. coli*, *P. aeruginosa*, and *K. pneumonia*) as the breeding season progressed based on cultures from the external genitalia of stallions and (2) determine if there is an association with an increase of cultures positive for potentially pathogenic bacteria when individual stallions have already been confirmed positive on the previous culture.

2. Materials and Methods

2.1. Stallions and Management

Similar to a previous study completed in our laboratory, nine stallions were used during one breeding season [9]. Briefly, stallions were housed at one central Kentucky farm with a veterinarian and a stallion manager handling all bacteriologic samples. Stallions were housed in box stalls with concrete floors and straw bedding. When not in stalls, stallions were turned out in individual grass paddocks. Fibar (The Fibar Group, LLC, Armonk, NY) was used as the footing in the breeding shed, and new Fibar was added to the breeding shed as needed during the breeding season. As previously described [9], stallions bred a minimum of 20 mares, exclusively by live cover during the breeding season. Before the breeding season, all stallions were considered to be healthy and of sound breeding status according to a breeding soundness examination performed by a veterinarian.

2.2. Collection of Swabs and Bacteriologic Evaluation

Swab sample collection and bacteriologic evaluation were done as previously described [9]. Briefly, before breeding, stallions were teased to stimulate an erection, and a swab sample was collected for bacteriologic evaluation from the prepuce using a sterile transport swab with Amies media and with charcoal (Fisherfinest, Houston, TX). After the sample was collected, the stallion was allowed to mount and breed the mare. After ejaculation was confirmed, a second swab sample was collected from the postejaculate urethra. The stallion's penis was washed with water only after breeding. Occasionally, the stallion's penis was rinsed with water before breeding only if debris such as mud or large amounts of smegma was noticed. Swab samples were obtained from stallions at an interval of every 10 mares.

After sample collection, swabs were sealed in transport tubes, labeled, and refrigerated. Samples were transported refrigerated to the Rood and Riddle Equine Hospital laboratory in Lexington, Kentucky, within 24 hours for bacteriologic evaluation as previously described [9]. Individual stallions were considered positive if one or both sampling sites were found to have a growth of any of the four potentially pathogenic bacteria of interest in this study. Potentially pathogenic bacterium was considered as a growth of one or more colony forming units of *E. coli*, *P. aeruginosa*, and *K. pneumonia*. At least eight colony forming units of β-hemolytic streptococci were needed to be considered positive. The number of colony forming units to be considered as a positive growth was determined previously at the Rood and Riddle Equine Hospital laboratory in Lexington, Kentucky [9]. Cultures were considered negative if both sampling sites yielded no growth or growth of nonpathogenic bacteria. To determine whether there was a difference in the frequency of positive cultures as the breeding season progressed, we divided the 6-month breeding season into three time periods. The time periods were determined based on the number of mares bred, with February and March categorized as early breeding season, April being midseason, and May and June categorized as late breeding season. Organizing the culture results in this manner allowed for fairly even numbers of cultures in each period as well as to address whether there was a specific time period within the breeding season where cultures tested positive more frequently.

2.3. Statistical Analysis

Statistical analysis was performed using SAS 9.3 software from SAS Institute, Inc, Cary, NC. Because of the nature of the data, the generalized linear model procedure was used for complete randomized block design to determine the frequency of positive cultures throughout the breeding season. Significance was set at $P < .05$. The generalized McNemar test was used to account for the independent nature of the data to determine association of positive cultures within individual stallions. Significance was set at $P < .05$.

3. Results

Table 1 gives an overview of the total sampling occasions performed, frequency of positive cultures occurring within those sampling occasions, and percentage of positive cultures on all stallions during the different time periods investigated. Overall, there was no difference in the