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Presence and distribution of fungi and bacteria in the reproductive tract of healthy stallions

A. Rota[a,*], E. Calicchio[a,1], S. Nardoni[b], F. Fratini[b], V.V. Ebani[b], M. Sgorbini[a], D. Panzani[a], F. Camillo[a], F. Mancianti[b]

[a] *Dipartimento di Clinica Veterinaria, Università di Pisa, San Piero a Grado (PI), Italy*
[b] *Dipartimento di Patologia Animale, Profilassi e Igiene degli Alimenti, Università di Pisa, Pisa, Italy*

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Abstract

A saprophytic bacterial flora is present on the penis and the distal part of the urethra of stallions. Little is known about the fungal flora of their reproductive tract. As micro organisms play an important role in mares fertility, the aim of the study was to describe the distribution of fungi and bacteria in the normal genital apparatus of stallions. The microbic flora of the reproductive tract of 11 healthy, fertile stallions was evaluated, collecting samples from 5 different locations: urethral fossa, penis/internal lamina of the prepuce, urethra pre- and post-ejaculation, and semen. For fungal examination samples were taken on 3 different occasions (N = 165), while for bacteriologic examination samples were taken on one occasion only (N = 55). There was a statistical difference in the presence of filamentous fungi between urethral fossa or penis/prepuce (45.4%) and urethra pre- or postejaculation or semen (15.1%, 6.0%, and 0.0%, respectively). Yeasts were isolated in 9.1% of the samples, never in semen. The most represented mycelial fungi were *Penicillium spp*., *Aspergillus spp*., *Scopulariopsis spp*., *Trichosporon spp*. and Mucoracee. The proportion of samples showing a total bacterial count ≥10 000 colony forming units (CFU) was higher for urethral fossa than for urethra pre- or postejaculation or for semen. Some bacterial growth was always observed in all locations, including the ejaculate. Differences between sampling locations were observed also for Staphylococci, both coagulase positive and negative. *Salmonella enterica* Abortus equi and sulphite reducing clostridia and other pathogens (including *Klebsiella* spp. and *Pseudomonas* spp.) were never isolated. *Escherichia coli* and coliforms always showed a low or absent flora. These data add information to the literature.

Keywords: Stallion; Reproductive tract; Fungal flora; Bacterial flora

1. Introduction

Physiologically, the penis and the distal urethra of stallions are colonized by a nonpathogenic bacterial population, representing the normal flora of these organs. The presence of bacteria in other areas of the male reproductive tract (testes, epididymes, accessory sex glands) probably represents a pathologic condition [1]. During natural mating, bacteria of the stallion's genital organs can be transferred to the female [2–4]. Artificial insemination may reduce the amount of transferred bacteria, even more if semen is preserved in an extender containing antibiotics [1,2]. When the normal stallion's bacterial flora reaches the genital tract of a healthy mare it is rapidly eliminated, within 24–72 h

[1] Present address: Private Practitioner, Cagliari, Italy.

* Corresponding author: Tel.: +39 050 2210163; fax +39 050 2210182.
E-mail address: alerota@vet.unipi.it (A. Rota).

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[5–7]. However, when the penile flora or the normal vaginal and/or uterine defense mechanisms of the mare are altered, transfer of bacteria in the female may lead to endometritis. Moreover, microbial flora in the ejaculate may affect the ability of spermatozoa to survive preservation processes [8]. Factors that can modify the normal bacterial flora of the stallion's penis are frequent washing with detergent or disinfectants and use of topical antibiotics, or mucosal lesions, neoplasia, or parasitism [9,10].

Several studies have shown the presence of different bacterial species in stallion semen, most of which are not pathogenic for healthy mares and can be considered the normal flora of the male genital tract [1,4,11–20]. The bacterial species present in all studies were *Streptococcus* spp. and *Staphylococcus* spp.; *Escherichia coli* was isolated by most of the authors in semen samples. Less information is available in the literature on samples collected from different locations of the stallion's penis. Malmgren et al. [18] observed that the presence of bacteria was lower in urethral samples collected postejaculation than in semen, while Vaissaire et al. [12] collected samples from prepuce, urethral fossa, and urethra, concluding that these sites were often heavily contaminated and the prepuce was the most colonized site. Mycoplasmas, on the contrary, occurred in similar proportions in urethra, fossa glandis, and pre-ejaculatory fluids [19].

Knowledge regarding the presence of fungi in semen or of the stallion's genital tract is scarce, even though mycotic agents are considered responsible for 2%–9% of the endometritis [21–23] and of up to 10% of abortions in mares [24–26]. The term fungus includes yeasts and molds: yeasts are single-celled budding fungi, while molds are multicellular filamentous fungi [27].

In fresh stallion semen, Malmgren et al. [18] observed only one positive culture for filamentous fungi on 115 samplings, while in another study 5% of the 118 samples were positive [16]. In both cases fungi belonged to the Mucoraceae family, known as potentially responsible for placentitis and abortions in horses [24,26,28]. In cooled-preserved and frozen semen samples, filamentous fungi and/or yeasts were found in 6% and in 17.5%–33% of samples, respectively, and the more frequently represented species were *Candida* spp., *Aspergillus* spp., and *Cryptococcus* spp. [16,18,29]. To our knowledge, the only available information on the mycotic flora of the stallion's penis and/or urethra is that one of 54 samples collected from the urethra postejaculation was positive [17], and that some yeasts were present on the stallion's penis and prepuce [12]. Moreover, Spergser et al. [19] collected samples from the fossa glandis, penis shaft, urethra, and semen of 116 Noric stallions, and found yeasts or filamentous fungi in $<1\%$ of the samples.

The aim of this study was to describe the bacterial and fungal flora in samples taken from urethral fossa, penis/prepuce, urethra pre- and postejaculation and semen of healthy, fertile stallions.

2. Materials and methods

2.1. Animals

Samples were collected from 11 stallions of different breeds (7 Maremmano, 2 Standardbred, 1 Italian Draught Horse, 1 Monterufolino) between June 2008 and March 2009. The stallions, ranging from 4 to 23 years of age, were kept in individual boxes (4 m $\times$ 4 m), with doors opening in a corridor, a concrete floor, and wood shavings bedding, at the Tuscany Region Institute for Equine Development (Istituto Incremento Ippico, Regione Toscana). All animals passed the sanitary tests required by the Italian law for their use as breeding stallions, which include culture for *Taylorella equigenitalis* and serology for equine herpes virus-1 and equine herpes virus-4 (if not vaccinated), equine infectious anemia, equine viral arteritis, dourine, and glanders. Moreover, during the sampling period the animals showed no evidence of reproductive tract pathology (orchitis, epididymitis, lesions of the penis) and were not allowed to mate mares naturally. All stallions were fertile (i.e., giving at least one confirmed pregnancy) in breeding seasons 2008 and/or 2009.

2.2. Samples collection for microbial examination

Sterile swabs with Amies and without charcoal as transport media (Nuova Aptaca, Canelli AT, Italy) were used to collect samples for bacteriological examination. Samples for mycological examination were collected by sterile swabs without transport media (LP Italiana S.p.A., Milano, Italy). In both cases, samples were transported refrigerated to the laboratory within 24 h after collection.

Samples were collected from 5 different sites: (1) urethral fossa, and (2) penis/prepuce (comprising free portion of the penile body, internal and external laminae of the internal prepuce, preputial ring), before penis was washed; (3) urethra pre-ejaculation, after washing; (4) urethra postejaculation, immediately after semen collection; and (5) semen.