

SHORT COMMUNICATION

Detection of *Simonsiella* spp. in the Vagina of Lions and Leopard in Oestrus

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Summary:

Reports of the vaginal flora of wild cats such as lions or leopards are scarce. The microorganisms most commonly found in the vagina of clinically healthy cats are aerobic bacteria such as coagulase-negative *Staphylococcus*, *Streptococcus canis*, and *E. coli*. *Simonsiella* spp are large Gram-negative bacteria belonging to the Neisseriaceae family, typically found in the oral cavity and upper respiratory tract of many species. To date, there are no reports of the detection of *Simonsiella* spp in the vaginal flora of any felid.

For a period of six months, daily behaviour monitoring was performed on six captive lionesses at a South African conservation centre, in parallel with the collection of vaginal swabs and interpretation of the resultant vaginal cytologies every other day. Oestrus was identified by typical female reproductive behaviours, as well as by enlarged and separated vulvar lips, and a predominant proportion of superficial cornified cells, clearing of the background, and high bacterial presence in the vaginal smear.

Simonsiella spp were identified by their characteristic morphology in 58% (60 of 103) of the vaginal samples collected during oestrus. They were also found in oral swabs of 3 out of 3 lions tested.

Additionally, *Simonsiella* spp were opportunistically found in a vaginal smear from a zoo housed female Sri Lankan leopard in oestrus, during a routine reproduction assessment.

The finding of *Simonsiella* spp may be more common than previously suspected, transitory, and without detectable clinical relevance. A connection between occurrence of these bacteria and oestrus was apparent.

Keywords:

African lion; oestrus; *Simonsiella* spp; Sri Lankan leopard; vaginal cytology; vaginal flora.

Main text:

In the domestic cat, the vaginal bacterial flora is mainly constituted by aerobic bacteria of the genus *Acinetobacter*, *Actinomyces*, *Corynebacterium*, *Escherichia*, *Haemophilus*, *Klebsiella*, *Lactobacillus*, *Pasteurella*, *Staphylococcus*, and *Streptococcus* (Clemetson & Ward, 1990; Holst *et al.*, 2003). Anaerobic bacteria such as *Bacteroides* and *Peptococcus* have also been isolated in a lesser extent (Clemetson & Ward, 1990; Holst *et al.*, 2003). The most commonly found organisms in the vagina of clinically healthy domestic cats are coagulase-negative *Staphylococcus*, *Streptococcus canis*, and *E. coli*. (Clemetson & Ward, 1990; Holst *et al.*, 2003). These bacteria originate from the skin and the bowel, and their presence, even in high concentration, is not considered to be an indication of reproductive disorder (Clemetson & Ward, 1990; Holst *et al.*, 2003, Johnston *et al.*, 2001). While mating does not seem to have a direct effect, the stage of the oestrous cycle does influence the bacterial populations present on the vagina of the cat (Holst *et al.*, 2003). To date, however, information about the vaginal flora of non-domestic felids, such as African lions or leopards remains scarce.

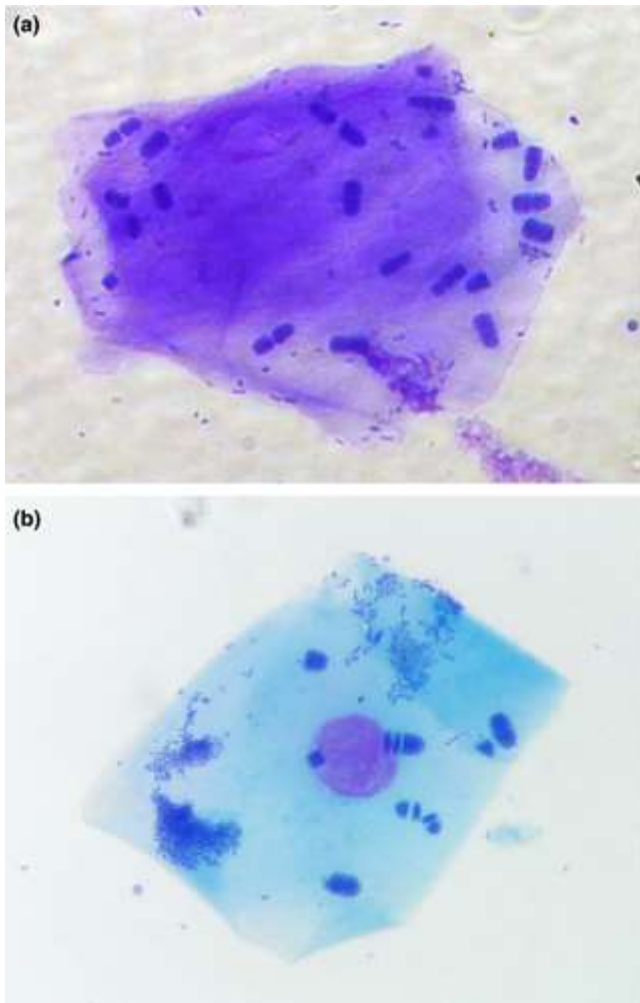


Fig1: Vaginal cytology of two lionesses in oestrus. Simonsiella spp can be observed attached to a superficial epithelial cell, forming typical clusters. Multiple bacteria of smaller size can be observed as well. Diff-Quik stain. x1000.

Simonsiella spp are Gram-negative bacteria belonging to the Neisseriaceae family (Bruckner & Fahey, 1969). These bacteria are short (0.5-1.3 μm) and wide (1.9-6.4 μm), and usually stay together forming characteristic single-series groups of 8-12 cells that make them easily recognizable under the microscope (Fig.1) (Hedlund & Staley, 2002). They may be found attached to epithelial cells of the oral cavity and upper respiratory tract of many species (Bruckner & Fahey, 1969; Hedlund & Staley, 2002; Nyby *et al.*, 1977; Kuhn *et al.*, 1978). To the authors' knowledge, *Simonsiella*

spp have only been opportunistically found once in the vagina of a bitch in oestrus (Valle *et al.*, 2006).

In this short communication, we report the detection of *Simonsiella* spp in the vagina of six African lionesses and one female Sri Lankan leopard.

All lionesses, housed at a wildlife conservation center in South Africa, were trained by means of positive reinforcement conditioning to voluntarily allow collection of vaginal swabs as part of a research project concerning wild felid reproductive physiology, approved by the Animal Ethics Committee of the University of Pretoria.

Vaginal samples were collected every other day, at least three times per week, for a period of six months in 6 animals. In parallel, behaviour monitoring was performed two hours a day, five days per week, throughout the same period of time, to record and describe any sexual activity and oestrous signs present on these females. In total, 22 oestrous cycles and 3 pregnancies were recorded, and 400 vaginal swabs were collected from the lionesses throughout the research period. Smears were prepared from the vaginal swabs, and these were stained with the Diff-Quik method (*i.e.* modified Wright-Giemsa) and evaluated according to the technique previously described by Johnston *et al.* (Johnston *et al.*, 2001).

Oestrus was identified by the presence of specific behavioural signs such as: purring, flirting, lordosis, rolling, and increased ano-genital grooming, as well as by enlarged and separated vulvar lips. The presence of a predominant proportion of superficial cornified cells, the clearing of the background, and a moderate to high number of

bacteria associated to the epithelial cells in the vaginal smear (Fig.2a, b) was additionally considered as an indicator of oestrus as previously described for other carnivore species (Johnston *et al.*, 2001).

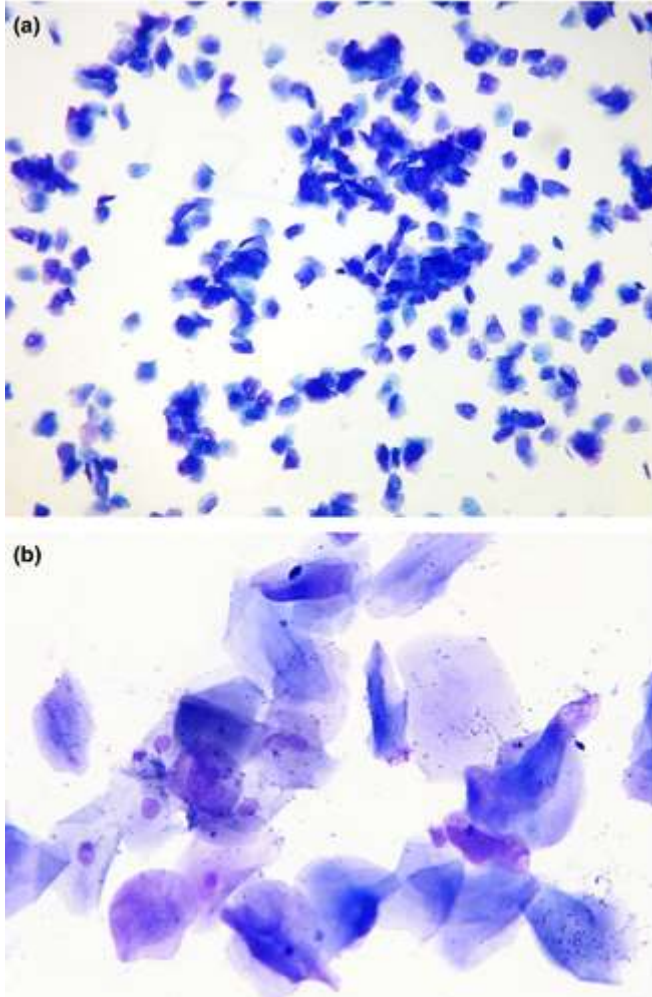


Fig2a,b: Vaginal cytology of one lioness in oestrus. A predominant proportion of superficial cornified cells, clearing of the background, and a high number of bacteria may be observed. Diff-Quik stain. x40 (left) and x200 (right).

Out of the 400 vaginal cytologies, 103 slides were classified as oestrus smears by means of the above described criteria. *Simonsiella* spp were identified by their characteristic morphology (*i.e.* large, clustered microorganisms associated to the exfoliated cells) in 60 of the oestrus smears (58%) belonging to all six females under

study. Vaginal swabs from a female in oestrus presenting the presumed bacteria were sent to the Laboratory of Bacteriology of the Faculty of Veterinary Sciences of the University of Pretoria (South Africa) to confirm the identity of the suspected microorganism by means of bacterial culture and Gram staining. Although, *Simonsiella* spp were not isolated in the culture, they were clearly identified in the Gram stain as well as microscopically by the criteria described earlier (Hedlund & Staley, 2002). A heavy growth of *Streptococcus canis* and *E. Coli* was isolated in this sample; nevertheless, this growth was not associated with disease. No microorganisms belonging to genus *Simonsiella* were detected in the vaginal cytologies prepared from samples collected during interestrous interval or diestrus (*i.e.* predominant proportion of parabasal and intermediate cells, with or without neutrophils) (Fig.3a, b). Exceptionally, *Simonsiella* spp were found in the vaginal cytology of one female two days after parturition.

In addition, oral swabs were taken and cytologies performed from three females during anaesthetic procedures for routine health checks. *Simonsiella* spp were again identified by their characteristic morphology in all of these samples.

Noteworthy, *Simonsiella* spp were also found opportunistically in a vaginal cytology from a captive female Sri Lankan leopard in oestrus, at a zoological institution in Singapore, during a routine reproduction assessment.

To date, there is no reports of the detection of *Simonsiella* spp in the vaginal flora of any felid, despite the large number of bacteria already identified to be normal in healthy cats (Clemetson & Ward, 1990; Holst *et al.*, 2003).

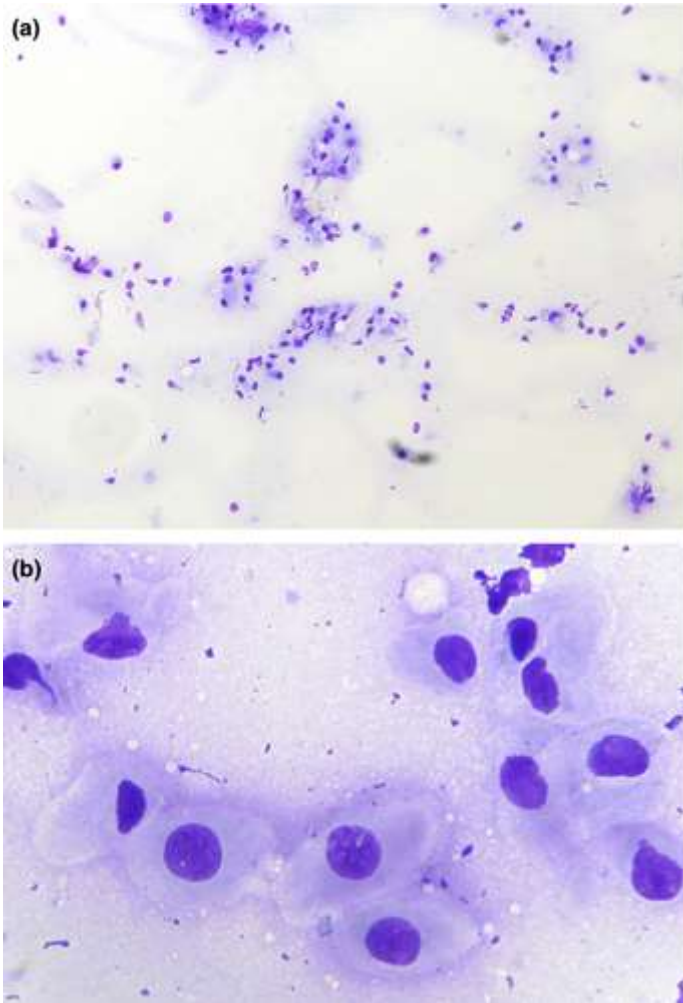


Fig3a,b: Vaginal cytology of one lioness in interestrous interval. A predominant proportion of parabasal cells and some neutrophils may be observed. Diff-Quik stain. x40 (left) and x400 (right).

The number of neutrophils present on the surface of the vaginal mucous membrane during oestrus is low, compared to any other stage of the oestrous cycle (Johnston *et al.*, 2001). This may create a favourable environment, and facilitate the growth of the resident bacteria and a transient colonization of the vaginal surface by other microorganisms such as *Simonsiella* spp. In this case, *Simonsiella* spp may have originated in the mouth, and migrated to the vagina as a result of increased anogenital grooming. This behaviour was recorded in all females under study during

oestrus stage and immediately postpartum.

The detection of *Simonsiella* spp in the vagina of six lionesses in oestrus, in addition to the casual finding of this microorganism in one Sri Lankan female leopard in oestrus may confirm this finding to be more common than previously suspected, transitory, and with no clinical relevance. As described previously in the bitch (Valle *et al.*, 2006), the occurrence of *Simonsiella* spp may be an additional cytology result helping to identify oestrus by vaginal smears.

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Conflict of Interest:

No economic interest nor conflict of interest existed during the performance of this study.

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