Vaginal microbiome: what not to do

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Abstract

Little advancement has been made over the years in defining normal canine reproductive tract microbiota. Existing survey data were obtained using the practical, but flawed, culture-based technique. Many of our clinical decisions pertaining to canine vagina are impacted by vaginal culture swab results; however, based on literature, culture results significantly underestimate bacterial diversity and concurrently overestimate role of isolated bacteria as they are more "visible." As clinicians practicing in "Omics Era" we must be prepared to interpret data from 16S rDNA gene sequencing for bacterial identification. With recently published information paving the path for characterizing a resident microbiome in the canine reproductive tract, we can begin to integrate this information into clinical practices, particularly those that pertain to ever-frustrating cases of canine vaginitis. With this information, our clinical practices should aim to identify an accurate cause and to do no (further) harm to the canine vaginal microbiome.

Keywords: Microbiome, 16S rDNA, vaginitis, vaginal culture

Introduction

Based on various veterinary resources, it is clear that the vagina of the canine is far from sterile. However, general consensus, with a few outliers, tend to accept the paradigm that the uterus is a sterile environment and needs to remain as such in order to establish and sustain a successful pregnancy. Owners of breeding bitches and stud dogs frequently take this even one step further in presuming that both the uterus and vagina of a fertile bitch is sterile and, in efforts to assess prospective fertility of a breeding bitch, will request that vaginal cultures be obtained by the veterinarian providing cycle management services for the bitch. This then subjects the veterinarian to the responsibility of interpreting the results from vaginal culture swabs. What bacterial species are "acceptable" to the practitioner when assessing relevance to a positive culture result? Where is the literature to support "lack of pathology" in the presence of specific species of bacteria? How is the practitioner to determine if culture results are indicative of dysbiosis present in the bitch's reproductive tract or not?

The "sterile uterine environment" concept was recently challenged by the observation that human placenta harbors a diverse microbiome of viruses, bacteria and fungi.[1-2] Therefore, if a placenta containing such organisms can result in delivery of a healthy infant, then should the presence of these organisms not be considered normal to the uterus? This is not a new area to be investigated in theriogenology - studies reporting bacterial species identified in the canine uterus and vagina were published 40 years ago.[3] Still, little advancement has been made and minimal information exists that attempts to define "normal canine reproductive tract microbiota" because nearly all published data were obtained using practical, but flawed, culture-based techniques. Unfortunately, these techniques fail to detect > 90% of microflora present in the sampled environment.[4] In essence, culture vastly underestimates bacterial diversity present in any area of the body, while at the same time likely overestimates the role of the bacteria cultured simply because they are more "visible." Following content is intended to be useful in assisting clinicians as they navigate through the changing world of microbiomics as it relates to the canine reproductive tract, and more specifically, the ever-accessible canine vagina.

Avoid subscribing to "sterile vagina" or "sterile uterus" concepts

Is there a normal microflora within a bitch's reproductive tract? Our group at Oklahoma State University has proposed that results contained within our 2019 publication "Canine endometrial and vaginal microbiomes reveal distinct and complex ecosystems" is a first step in describing normal flora within canine female reproductive tract. Results obtained from vaginal swabs and endometrial tissue samples are reported by phyla and genera, as the SILVA database does not allow taxonomic classification at species level. Top 5 genera identified in vagina included Hydrotalea, Ralstonia, Mycoplasma,

Fusobacterium and Streptococcus. Top 5 genera identified in endometrium included Pseudomonas, Staphylococcus, Corynebacterium, Anaplasma and Dermacoccus. When comparing 2 different regions of the reproductive tract, vagina is higher in "richness" (number of bacteria present in a given area), but endometrium is higher in "diversity" (variety of organisms identified in a given area).

What phyla or genera do bacteria commonly cultured from the vagina and endometrium represent? As reported in various literature sources, common bacteria including, but not limited to, *Enterococcus* spp*., Streptococcus* spp*., Escherichia* spp*.,* and *Staphylococcus* spp. would all belong to one of the top 5 phyla represented in the canine reproductive tract.[3,5-8] One large theme was apparent while comparing the 25 samples compared in our project and that theme was "individual diversity." It seems that whereas there were phyla and genera most heavily represented within our population of bitches evaluated in our study, none of them closely resembled one another when comparing bacterial richness and diversity for bacteria present. Obviously, commitment to further defining the canine reproductive microbiome needs to be maintained if information obtained thus far will have any chance of impacting clinical practice with development of effective probiotics or infusion treatment regimens.

Resist your startle reflex when viewing culture results or 16S rDNA data

By comparing previously compiled lists of "commonly isolated bacteria in culture" to our "top 5 phyla/genera reported via 16S rDNA" from vaginal and endometrial samples, we realized that today's emerging information failed to confirm culture-based results obtained from previous years; in addition, formerly obtained culture-based results are failing to support today's discoveries.[3,5-8] Previously published results revealed that bacteria isolated with highest frequency from vaginal swabs from a group of bitches included Enterococcus, Streptococcus, Pasteurella, and Escherichia genera.[8] However, if comparing those culture results to our 16S rDNA data, none of those organisms were represented in top 5 genera most represented in canine vagina.[9]

So then, how do we interpret the significance, or even reliability, of either result? Are bacteria isolated on petri dishes responsible for subfertility or infertility in the bitch or not? Are bacteria truly representative of the environment from which the sample was taken, or were the presence of these bacterial species exaggerated after media enrichment? In order to fully answer these questions in a way that will impact clinical practice, we will need to be on the lookout for progress updates coming our way that includes data of samples from infertile or infected bitches as analyzed via metagenomic methods. However, in spite of the incongruency in the results obtained from 2 distinct diagnostic methods, it would appear that we do have enough information at our fingertips to troubleshoot some commonly encountered clinical scenarios. For example, if a bitch owner expresses anxiety over a *Mycoplasma* spp. isolated from a routine vaginal swab, we can subdue their concerns in an evidence-based fashion rather than solely from a practice-based approach. Alas, this would be a prime opportunity to communicate progress of information being shared at continuing education events today!

Resist utilizing antibiotics in the absence of careful case (i.e. vagina) selection

Vaginitis can be frustrating; key to accurately troubleshooting this feminine issue is to carefully define the cause for owner concern and pet presentation. The entirety of canine vagina (reaching up to cervix) is difficult to characterize in terms of appearance and characteristics in most clinical settings, due to equipment or cost limitations. We may become frustrated and, therefore, tempted to reach for anything that would be effective for treating a supposed infection; in other words, we feel at a loss and partake in a response to treatment approach.

Nevertheless, impact of antibiotic consumption reaches much farther than most of us consider with each prescription dispensed. Many of us have worked with cases, not to mention owners, that have tried to force use to prescribe an antibiotic despite little justification based on diagnostic or clinical evidence. What is even more perplexing is when this reported approach appears to result in pregnancy when previous infertility was unexplained. What should a veterinarian do? There are significant incentives to exercise the sagacious use of antibiotics in our patients including, but not limited to, an antibiotic's ability to disturb host metabolism and absorption of vitamins, alteration of susceptibility to

host infections, and overgrowth of yeast and/or *Clostridium difficile*.[10] Broad-spectrum antibiotics that primarily target anaerobic bacteria, for example clindamycin, have a known negative impact on intestinal microbiota which results in reduced host protection against gut colonization by bacterial pathogens. Even use of amoxicillin can result in increased resistant enterobacteria.[10] Whereas some antibiotic regimens will only alter gut microbiota from baseline for a short interval (e.g. days or weeks), other regimens have been documented to alter gut microbiota in humans for up to 4 years.[10,11] A prospective pilot study reported that of 54 women administered a short course of oral antibiotics for nonreproductive issues, treated patients had an increased prevalence of asymptomatic vaginal colonization of *Candida* species compared to nontreated and age-matched controls (10 of 27 versus 3 of 27, respectively). Similarly, an increase in incidence of symptomatic vulvovaginal candidiasis was also documented when treated patients were compared to nontreated patients (6 of 27 versus 0 of 27, respectively).[12]

Although microbiomes of human and canine reproductive tracts cannot be described as similar, and therefore a predicted response to commonly utilized antibiotics should not be extrapolated from one species to the other, veterinarians and owners likely underestimate the impact of antibiotics on systemic microbiome; therefore, this impact should be consciously discussed, weighed, and clinical decisions managed accordingly. To improve case selection for antibiotic use in our daily practices, we can be cognizant of other causes of subfertility or infertility in the bitch and perform appropriate case work-ups following client education. Other than a presumed bacterial vaginitis or endometritis, other causes of subfertility or infertility such as inappropriate timing, suboptimal semen quality, or even intrauterine fluid accumulation around the time of ovulation, should be evaluated.[13,14] With increased accessibility of ultrasound equipment, perhaps more of us should begin performing more detailed examinations of the bitch's reproductive tract as is done by our more equine-focused colleagues during estrous cycle management prior to breeding.

Avoid unwise douching practices

Once again, vaginitis can be frustrating. While it may be tempting to lavage (i.e. douche) vagina or vestibule with fluids spiked with small amounts of antiseptic, it will behoove clinicians to avoid this practice. However, in the case of a primary noninfectious vaginitis, with the intent of clearing tenacious mucoid secretions, physiologic saline solution alone may be safely utilized sparingly.[15] In women, *Lactobacillus* bacteria that inhabit vagina are thought to maintain an appropriate pH environment that makes it inhospitable for other bacterial species that can induce a symptomatic bacterial vaginitis to survive; in contrast, utilizing antiseptics or a high-frequency douching schedule will inarguably impact normal vaginal flora and make the patient *more* susceptible to a bacterial vaginosis by upsetting vaginal flora. Although a step by step description regarding how to work up a canine vaginitis case is outside the scope of these proceedings, this should be a priority when presented with a case of suspected vaginitis. Appropriate diagnostic procedures will prevent inappropriate treatment from being implemented and avert secondary problems from arising.

Conclusion

More information pertaining to vaginal (and uterine) dysbiosis is forthcoming in veterinary species. Be proactive in understanding and seeking information that, while currently in its infancy, will impact the way we approach cases in the clinic. In the meantime, resist practices that can weaken, or even obliterate, normal microbiome of the canine reproductive tract. When possible, rededicate yourself to pursuit of a diagnosis and be accurate in interpretation of results from diagnostic tests.

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