Bugs and drugs: appropriate use of antibiotics in canine reproduction
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Introduction
While the use of antibiotics is necessary and rational in many areas of canine reproduction (pyometra, postpartum metritis, Brucella canis treatment, mastitis), their common empirical or unproven use in other canine reproductive situations may cause more harm than good, both to the individual being treated and to human and animal health as a whole. Understanding the health and function of the normal microbiome and the potential adverse effects of unnecessary use of antibiotics can help us form a reasoned approach to their rational use without causing unintended harm. Many questions remain about appropriate antibiotic choice and course of treatment.

Keywords: Reproduction, fertility, normal flora, antibiotic, microbiome

Normal flora in the bitch

Vaginal flora
Multiple authors have documented a range of normal vaginal flora in the bitch, including aerobic, anaerobic, and mycoplasma species. The variety of organisms and differences between studies may be related to sampling procedures, laboratory procedures (including choice of media), regional, management, or breed differences. Organisms isolated from the bitch’s vaginal vault include mycoplasma spp., Pasteurella spp., Bacteriodes, Streptococcus spp., Escherichia coli, Staphylococcus spp., Enterococcus spp., Klebsiella spp., Proteus spp., Bacillus, Pseudomonas spp., Arcanobacterium spp., Acenitobacter spp., and ureaplasma.1-7 Few studies looked at anaerobic flora in the vagina, but they were found in approximately equal numbers to aerobic flora when cultured.7 The types of organisms also vary between cycle stages, potentially due to the medium presented by normal fluid secretions of blood and mucus concordant with cycle stage. A study comparing presence and variety of vaginal flora between owned and stray dogs found no difference in the makeup of the population of organisms.8 Bacteria originating from skin and the gastrointestinal tract appear to make up the population of vaginal flora. Lactic acid producing bacteria (LAB), specifically Lactobacillus and enterococcus were found in equal numbers in a study of healthy and ill bitches and were not correlated with vaginal pH, age, or body temperature.9 Spayed bitches with recurrent urinary tract infections had no significant difference in vaginal microbiome than did unaffected spayed bitches.10

In most, but not all studies, the highest number of organisms was found during estrus and the lowest in diestrus.11,12 Increased numbers of organisms may also be found postpartum, but were not associated with neonatal morbidity.13

Uterine flora
While some studies documented no flora in the uterus of normal bitches, several others did find low numbers of flora in the uterus of bitches that had normal uterine histology and/or were clinically normal. The largest number of organisms was found in estrus and lowest in diestrus in most, but not all, studies.11,12 The use of pre-enrichment broth in culturing samples from the uterus was recommended to increase growth from samples from normal bitches.12 Organisms isolated from the bitch’s uterus include Staphylococcus spp., mycoplasma spp., Escherichia coli, Haemophilus, Streptococcus spp., Corynebacteria spp., Alcaligene faecalis, Bacterioides spp., Pasteurella spp., Clostridium perfringens, and Bacillus.1,11,14 Culture of postmortem samples routinely grew more organisms and a greater variety of organisms in the uterus than samples obtained from living bitches indicating the microbiome may be rapidly altered postmortem. Preliminary work using extracted DNA and taxonomic evaluation indicated multiple organisms present in the bitch’s vagina and uterus.15 This method of research holds great promise in further delineating the normal microbiome of the canine reproductive tract.
Some organisms appeared more likely to be involved in cases with uterine pathology, particularly *E. coli* although they were also often found in bitches with no pathology.5,11 *E. coli* is recognized as the most common organism found in canine pyometra and does recur with successive infections. This may be related to its ability to form biofilms that protect it from treatment.16 The presence of *Streptococcus* spp. in proestrus was negatively associated with the presence of uterine infections, indicating the possibility of a protective effect.5 Conversely, in one small study the presence of *Streptococcus canis* or *Streptococcus dysgalactiae* in parturum vaginal sampling was associated with increased neonatal deaths.17 In another study, the presence of organisms in the anterior vagina was increased in the early postpartum period but had no apparent adverse effect on neonatal morbidity.13

Ovarian bursa

Bacterial growth in the ovarian bursa has also been found, not solely in bitches with pyometra, but also in control bitches (*Enterococcus* sp, *Bacillus* sp. and an unidentified gram positive sp. There are no currently published data on the microbiome status of canine uterine tubes.

**Alteration of genital microbiome in bitches**

Antibiotics may be used for unrelated infections, for diagnosed reproductive tract infections such as pyometra or postpartum metritis, or given empirically as a preventative or presumptive treatment without clinical indication. Effects on the vaginal and uterine microbiome are likely and the results may allow overgrowth of organisms with increased pathogenic potential by removing competing organisms and allowing resistant organisms to multiply more readily.19 Treatment of normal bitches with ampicillin or trimethoprim-sulfamethoxazole (TMS) for ten days increased growth of mycoplasma (both) and *E. coli* (TMS).19 The use of antibiotics in estrus bitches may adversely affect the attraction of the male dog and his interest in and pursuit of mating.20 Antibiotic resistance has not been sufficiently evaluated in the bitch’s reproductive tract; however one study found a low number of B-lactamase producing and fluoroquinolone resistant organisms in *Enterobacteriaceae* spp. in the urogenital microbiome of dogs.21 The use of LAB in positively improving or maintaining a healthy microbiome in the vaginal vault is intriguing, however in one study the oral administration of a probiotic with LAB did not increase the number of bitches from which they were isolated after 14 or 28 days of treatment.22 Further study of different probiotic compounds, duration of treatment, and a larger treatment group is warranted due to the success of such treatment in other species. Canine vaginal LAB isolates *Enterococcus caninestiniti* and *Weisella* spp. showed increased bacterial adhesion to canine vaginal epithelial cells as well as inhibition against *E. coli* and *P. vulgaris* (*Weisella*) and *Klebsiella* and *E. faecalis, E. faecium* (*E. caninestiniti*). This indicates a potential therapeutic use for canine vaginally sourced LAB in promoting a healthy urogenital microbiome.23

**Fetal and neonatal concerns**

It is not unusual for pregnant and lactating bitches to be treated with antibiotics, either for a documented medical issue that requires treatment (mastitis, metritis) or on an empirical basis by the owner or veterinarian in hopes of preventing a future problem. We do not have canine data evaluating any short or long-term effects of such treatment on the puppies in question, but if they are like humans and rodents then there are reasons to think carefully about antibiotic use without a diagnosed infection. In human infants where the mothers were treated with antibiotics during labor to prevent vertical transmission of group B *Streptococcus* the infants were at greater risk of developing late-onset serious bacterial infections and those infections were more likely to be resistant to ampicillin than infants whose mothers were not treated intrapartum with antibiotics.24 Several other studies have reported an increase in early-onset antibiotic-resistant neonatal infections in preterm infants when mothers were treated with antibiotics.25 27 Therefore in humans there can be an increase in both early and late-onset neonatal sepsis, potentially fatal, when the mothers are treated with antibiotics prepartum or intrapartum.
Antibiotic exposure during gestation or parturition can affect development of other morbidities in children. Maternal use of chloramphenicol or penicillin increased the risk of asthma in children by the age of seven years, particularly when used in the first trimester.28

Obesity is a serious concern in both the canine and human populations. Prenatal, or early postnatal, exposure to antibiotics increases the risk of obesity in children. In one study children exposed to antibiotics during the second or third trimester had an 84% higher risk of obesity by seven years of age than children who were not so exposed.29 Interestingly, cesarean section, evaluated independently, also increased risk of childhood obesity by 46%. The connection between perinatal exposure to antibiotics and obesity has also been documented in rodents-this occurred regardless of the later healthy state of the intestinal microbiome in the exposed mouse pups.30 Not surprisingly, use of antibiotics in the peripartum period also affects the development of the microbiome in infants. Both direct treatment of the infants, as well as treatment of the mother with antibiotics, alter the infant’s microbiome, with a lesser effect of the latter.31 *Bifidobacterium* presence was reduced, enterococci overgrew and continued to increase over the month of monitored time in the study.31

Areas for further study

Additional research to understand the canine reproductive microbiome is clearly needed. Evaluation of what constitutes the normal microbiome in the entirety of the bitch’s reproductive tract is primary. Current studies have largely ignored anaerobic flora yet studies that looked for it found it in significant numbers. Speciation of organisms such as *Streptococcus* spp. and *Mycoplasma* spp. is important in understanding how differences may impact the pathogenicity of the organism. Studies should be performed in living bitches, as postmortem microbiome changes appear to occur quite rapidly. Careful thought must be given to sampling techniques, media used, cycle stage, breed, population, housing, age, parity, reproductive history, and precise location of the sampling so that reproduction of results is feasible and variables controlled. Terminology must be used with precision as colonization is not synonymous with infection. Research on promising LAB that can be used to help prevent overgrowth and sustained infection with more pathogenic organisms is an important and promising area of work, and potentially an urgent one as antibiotic resistance is increasingly a concern. Effects of gestational and peripartum antibiotics on the microbiome in neonatal dogs is another area of needed research.

Knowing when to treat is a serious concern. Failure to treat when needed has serious consequences; however, we often do not consider the significant consequences of treating when inappropriate. Defining those situations is important and could be a goal for the Society for Theriogenology and American College of Theriogenology similar to the ACVIM Consensus Statement on Therapeutic Antimicrobial Use in Animals and Antimicrobial Resistance or the Antimicrobial Use Guidelines for Treatment of Urinary Tract Disease in Dogs and Cats by the International Society for Companion Animal Infectious Diseases.32,33

Conclusion

The reproductive tract of the bitch is richly endowed with a diverse microbiome. Understanding it, respecting it, and supporting it are goals we, as a profession, need to support. When treatment is necessary, we must consider the totality of potential consequences of treatment. This is an area with great research potential for the dog, but in the meantime we should make every effort to use antibiotics only when there is a true need, use the antibiotic least likely to affect unintended targets, and to do so for only the necessary time frame. Understanding what the adverse consequences can be may allow us to least anticipate them and possibly ameliorate them as more data become available.

References