

Staphylococcus pseudintermedius causing presumptive ascending canine abortion

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Abstract

Despite growing literature regarding evidence of commensal and sometimes pathogenic bacteria in the uterine and vaginal microbiome of dogs, no direct association with reproductive disease has been linked to *Staphylococcus pseudintermedius*, an opportunistic vaginal commensal (commonly cultured). A multiparous dog had an infectious abortion during late pregnancy over the course of a week. The infectious agent identified on fetal autopsy via culture of the stomach contents and spleen was *Staphylococcus pseudintermedius*.

Keywords: Dog, *Staphylococcus pseudintermedius*, abortion, ascending infection

Background

During the past few decades, more attention is given to the vaginal and uterine microbiome across domestic species and in human medicine. The dogma that the genital tract is a sterile environment is being replaced as evidence for commensal and pathogenic bacteria cultured from the genital tract is catalogued. Based on several studies, some of the most common bacterial isolates from the vagina are *Escherichia coli*, *Staphylococcus pseudintermedius* (*S. pseudintermedius*), *Streptococcus canis*, and *Mycoplasma* in ~ 60–80% of clinically healthy dogs.^{1–4} Most of these commensals have the potential of becoming opportunistic pathogens resulting in reproductive disease aside from *S. pseudintermedius*² that has been suggested to serve more of a protective function along with *Streptococcus* species against pathogenic strains via competition.¹ As a result of further research, we are recognizing that there is a urogenital microbiome in veterinary species that differs from women. In spayed dogs, lactobacillus is not a common isolate as in humans to protect the vaginal microbiome from pathologic infection.^{5,6} Study on the effects of sterilization on the urogenital microbiome suggested a blunting of microbiome diversity with *Pasteurella canis* and *Proteus* as the most common organisms cultured from healthy spayed females.⁷ Stage of the estrous cycle in dogs has also been documented to cause a shift in the vaginal microbiome with *S. pseudintermedius* and *Mycoplasma* as more common in estrus and *E. coli* in anestrus.¹ Top 3 bacterial isolates from the canine endometrium were *Pseudomonas*, *Staphylococcus*, and *Corynebacterium*.^{8–12}

This case report serves as a direct association between *S. pseudintermedius* and a presumptive ascending abortion in a dog

where fetal sepsis with *S. pseudintermedius* was confirmed on fetal autopsy. Possible theories on how the ascending infection occurred, the clinical presentation, and diagnostic approach to the case are discussed.

Case presentation

A 3-year, multiparous Australian labradoodle, was presented on 29 October 2021, on day 55 postLH for diagnostic work-up and treatment for premature labor. Although coowned and housed in a 'guardian home', the dog was kept throughout her reproductive cycle and pregnancy by a single experienced breeder. Dog was current on vaccination, flea/tick/heartworm prevention, and maintained on a dry kibble diet with no raw additives. She had been bred once previously in 2020 and had 8 healthy pups. On the most recent cycle, breeding management (consisting of repeated examinations, *Brucella canis* testing via rapid card agglutination test [RCAT; Zoetis, Parsippany, NJ]) progesterone assay, and vaginal cytology) was provided by the referral practice. Progesterone concentrations were: 0.74 on 31 August 2021, 0.95 on 2 September 2021, 7.76 on 6 September 2021, and 11.8 ng/ml on 7 September 2021. Dog was bred via a single TCI (transcervical insemination; Karl Storz, El Saegundo, CA) to a proven 3-year stud on 7 September 2021 with good-quality fresh semen per analysis via NucleoCounter® NC-100™ (ChemoMetec, Allerød, Denmark), computer-assisted sperm analysis (CASA; Sperm Vision® Minitube, Delevan, WI, USA), and manual wet-mount morphology (652.96 × 10⁶ progressively motile sperm [82.6 progressive motility, 85.73 viable, and 70% normal morphology]). On the same day, she was mated (unintentional) by a 7-year male (also proven, possibility of dual-sire litter) at the

breeder's home where she was housed during breeding management. She was confirmed pregnant via transabdominal ultrasonography on 11 October 2021 and was isolated from other dogs.

On 28 October 2021 (day 54 postLH), the dog went into premature labor and aborted 4 fetuses in a span of 2.5 hours (between 4:00 and 6:30 PM) with none surviving. She was taken to a local emergency facility, where radiography and transabdominal ultrasonography were performed. Five remaining fetuses had normal heartrates (200–250 beats per minute [bpm]), and the dog was clinically stable with no abnormalities on physical examination without signs of distress aside from panting. Bloodwork was not performed and the dog was discharged after subcutaneous fluid (Plasmolyte, 100 ml) treatment. Breeder was advised to monitor overnight and to have a follow-up examination the next morning.

On presentation on 29 October 2021 (55 days postLH), another transabdominal ultrasonography was performed (S9 US-diagnostic system; C611, 13–3.9 MHz Micro-convex transducer; Sonoscape Medical Corp, Shenzhen, China) that revealed 5 viable fetuses with heartrates >190 bpm, some intestinal layering, and no free fluid in the uterus or overt fetal abnormalities. Based on the partial abortion of her litter, blood was drawn for progesterone, complete blood count, and serum chemistry. A vaginal swab was obtained for cytology and a swab from a day-54 aborted fetus that was kept in the refrigerator after delivery for autopsy. Her serum progesterone concentrations were lower (1.37 ng/ml) (TOSOH Bioscience AIA 360, San Francisco, CA), and other bloodwork had a moderate leukocytosis (16,670 white blood cells [reference range 4,360–11,900]) characterized by a mature neutrophilia (13,669 cells [reference range 2,841–9,112]) and a reticulocytosis (129,000). Her serum chemistry had minor protein and electrolyte imbalances that were attributed to aborting part of the litter and uterine contractions (calcium, albumin, sodium, and chloride). Vaginal cytology revealed multiple neutrophils, lymphocytes, and plasma cells with intracellular cocci bacteria. Based on these diagnostic results, an infectious cause of abortion causing a secondary hypoluteoidism was suspected. Differential diagnoses considered included ascending infection from opportunistic vaginal microflora (*Staphylococcus*, *Streptococcus*, or *Escherichia coli*), primary hypoluteoidism, and *Brucella canis*. Brucellosis was lower on the differential list as the dog and stud dogs had a repeated history of negative RCATs, including ones obtained at the time of breeding. A brucella RCAT was not repeated for the dog at abortion.

Treatment

Oral Augmentin® (amoxicillin/clavulanic acid, at a dose of 17 mg/kg twice daily for 10 days) and Regumate® (0.088 mg/kg altrenogest once daily until 2 days before her estimated whelping window of 7 November 2021 ± 1 day^{13,14}) treatment were initiated on day 55 postLH, and the dog was scheduled for reevaluation on the following Monday (1 November 2021, day 59 postLH) after the weekend.

Altrenogest, a synthetic progestin, was selected to combat the effects of low progesterone. The goal for progestin therapy was to increase cervical tone and myometrial relaxation to prevent further abortion of remaining fetuses in hopes the remaining litter would survive to maturity. Progestogens inhibit gap junction formation in the uterus by preventing the expression

of contraction associated proteins via estrogen receptor binding on myometrial cells.^{15–18} As altrenogest is a synthetic progestin, it has some affinity to progesterone receptors in the endometrium allowing it to have a similar effect. A recent review on altrenogest¹⁹ reported that altrenogest has a molecular structure similar to androgen, allowing it to also bind to androgen receptors. This would explain reports of masculinization of female fetuses and hypospadias of male fetuses in dogs if exposed before day 45 of pregnancy with only clitoral hypertrophy noted after day 45 of pregnancy exposure in female fetuses.¹³

Amoxicillin and clavulanic acid were selected as broad-spectrum therapy due to the history and the identification of numerous intracellular cocci on vaginal cytologic examination in order to limit the spread of an ascending or hematogenous infection to the other fetuses.²⁰ Empirical antibiotic therapy without culture and sensitivity results was considered vital for fetal outcome due to the potential for rapid spread of infection and fetal compromise. Augmentin® was selected due to its broad-spectrum nature, as well as its high margin of safety for pregnancy as a Class B FDA labelled drug.^{21,22} It is a hydrophilic penicillin with low protein binding (~ 25%) allowing it to have a high volume of distribution into extracellular fluid such as amniotic fluid. It also freely crosses the placenta via passive diffusion making it an ideal antibiotic to treat uterine and fetal infections.^{22,23} In women, maternal serum, placental tissue, and umbilical tissue had concentrations of amoxicillin above the minimum inhibitory concentration (MIC) for *Streptococcus agalactiae* 2 hours after oral treatment.²⁴ The only compartment tested that was barely below MIC concentrations in between doses was the amniotic fluid at 72 compared to > 75%.²⁴ Unfortunately, the autopsy report for the fetus was not available (including culture and sensitivity results) until 21 days after the last pup whelping.

Outcome

When the dog was presented for her reevaluation on the following Monday (1 November 2021 day 59 postLH), the breeder reported that she had aborted 2 more fetuses over the weekend (fetus # 5 and # 6) leaving only 3 fetuses left in the pregnancy. On ultrasonography, fetuses appeared viable with heartrates ranging from 195 to 243 bpm with intestinal layering and renal corticomedullary distinction noted in all fetuses. At this point, all prescribed medications were continued to try to have at least 1 viable fetus make it to her expected whelping window. She was sent home to be monitored with the understanding that if she aborts anymore fetuses, they would be premature and would not survive. Dog aborted another fetus (fetus # 7) 3 days after her first reevaluation (day 62 postLH) and was examined again on Friday 5 November 2021 (day 63 postLH). At this reexamination (day 63 post LH), there was only 1 viable fetus (heart rate 230 bpm) out of 2. At this time, progestin therapy was discontinued and her antimicrobials were continued until completed on 8 November 2021. The next evening (day 64 postLH), the breeder reported that the dog had whelped the remaining 2 pups with only the viable pup surviving. Despite partially aborting her pregnancy over the course of a week, the dog remained bright, active, and had a normal appetite throughout.

A few weeks after the dog aborted her litter, the final autopsy report had been uploaded to her medical record. Based on the autopsy report, the submitted fetus had moderate acute sepsis and mild pulmonary hemorrhage with presence of multiple

gram-positive cocci on the lung and renal surfaces. Stomach contents and spleen were cultured that had growth of *S. pseudintermedius* confirming sepsis to be the cause of abortion. Based on sensitivity test, *S. pseudintermedius* was susceptible to all antimicrobials on the panel including amoxicillin/clavulanic acid. A secondary isolate of *Pseudomonas aeruginosa* was also cultured from the spleen with growth only in thiogluco-late; on sensitivity, this was resistant to amoxicillin/clavulanic acid. Antibiotics that the *Pseudomonas* were susceptible to were aminoglycosides and ceftazidime. Pathology results were discussed with the breeder on 30 November 2021 (24 days after the dog whelped her last pup), breeder reported that the sole surviving pup was doing well and thriving.

Discussion

To the author's knowledge, this is the first published report of a highly suspected ascending cause of abortion from *S. pseudintermedius* based on fetal autopsy. There have been previous reports on canine abortions resulting from ascending *Streptococcal*, *Salmonella*, and *E. coli*^{4,8,10,12} infections and others regarding vaginal microbiome altering between proestrus, estrus, and diestrus.¹⁻³ Due to the high number of gram-positive cocci on the pleural surface, renal surface, and cultured from both the stomach and spleen of the fetus submitted for autopsy, the author and the pathologist believe that the cause of the fetal sepsis was *S. pseudintermedius* instead of the *P. aeruginosa*. The autopsy report was prepared by a board-certified pathologist who also did not mention any gram-negative rods noted on any surface via cytology, and *P. aeruginosa* was only discovered on splenic culture only in thiogluco-late.

The inciting cause allowing *S. pseudintermedius* access to the uterus and fetuses determined, as is the outcome for most clinical abortions that present to the hospital. Primary hypoluteoidism allowing relaxation of the cervix and ascending infection is a possibility but unlikely since primary hypoluteoidism is uncommon in dogs. The most likely progression of disease was an ascending infection from poor cervical tone leading to secondary hypoluteoidism.²⁵ Another possible cause could be vaginal trauma from the natural mating that could have led to a break in the vaginal mucosa allowing ascension of infection during immune suppression in diestrus. A potential reason for why the dog aborted over the course of the week could be from a delay caused by progestin therapy or from the next fetus becoming nonviable closest to the cervix. The latter reason is more likely due to her aborting despite being on Regumate®. Serum progesterone concentrations were not reevaluated as the initial value was 1.37 ng/ml and the likelihood that it would not have increased from endogenous sources, the corpus luteum. Also, it is known in equine medicine, progestins do not cross react with progesterone on most analyzers, so her progesterone concentration would not artificially increase due to Regumate®.²⁶ Although this was not a sterile abortion, the dog was systemically and clinically stable having no signs of sepsis. This suggested that her leukocytosis was in response to the local inflammation and local infection of the abortion. Another area of discussion would have been monitoring fetal heart rates at home by the breeder between evaluations. This addition to the dog's treatment plan was not pursued, as the breeder did not have a doppler ultrasound unit nor the university (potential renting to breeders).

Regarding the treatment initiated in this case, there are many ways this could have been modified. Other methods of uterine quiescence could have been used in adjunction to

Regumate® or in place of, the most common being terbutaline that has a tocolytic effect on the canine uterus. This was not chosen due to ease of access to Regumate® in the hospital and the need to call terbutaline into a local human pharmacy, thereby delaying treatment. However, some may argue that a transition from Regumate® to terbutaline would have been advised to reduce the risk of genital abnormalities and possibly better control of premature uterine contractions.

Due to the late availability (27 November 2021) of autopsy report that included culture and sensitivity of both bacterial isolates, antibiotic adjustments were not possible prior to last pup whelping (6 November 2021). If this was known sooner, addition of gentamicin sulfate or transitioning to ceftazidime (a cephalosporin) could have been initiated to cover both the *S. pseudintermedius* and *P. aeruginosa*. Human literature has conflicting reports of cephalosporins potentially causing congenital malformations in a small percentage of newborns exposed in the first trimester are made.²² There are also reports regarding aminoglycosides causing nephrotoxicity in newborns exposed during pregnancy due to renal immaturity causing lowered clearance with gentamicin.²⁷ Both amikacin and streptomycin have been reported to cause congenital deafness from ototoxicity.²⁷ Despite these reports, though coverage of the *P. aeruginosa* would have been done with either ceftazidime or gentamicin sulfate. A second point of discussion in antimicrobials is extended coverage to any surviving pups with Clavamox® drops after delivery to treat neonatal sepsis. This was not done in this case but is a valid point that was overlooked during the time.

Brucellosis was not highly suspected as a cause of this abortion due to both studs had negative brucellosis results within 7 months from breeding, as did the dog. A repeat RCAT was not performed as she had no direct interaction with any other dog outside of 2 males at breeding. However, there are other ways that brucellosis can be transmitted aside from breeding such as urine contamination, mucosal-oral contact, vaginal discharge, and aborted materials.¹²

Lastly, human studies have reported success in 'rescuing fetuses' via cesarean surgery before term. This could have been attempted in this case, but conservative management was elected as not all fetuses were fully mature on ultrasonography with intestinal peristalsis on day 59 postLH. If surgical rescue was attempted on day 62 postLH, the last 3 remaining pups may have survived if a glucocorticosteroid (e.g. solumedrol) was given to hasten final maturation and surfactant production by type II pneumocytes. As it was reported by the breeder when fetus # 7 was aborted on day 62 postLH, the pup was unable to sustain respiration and passed away after a few minutes. This is most likely due to not having produced enough surfactant to maintain alveolar opening; in dogs, surfactant is produced in the last 12–24 hours before parturition.

Although it is commonly cultured from vaginal samples, ascending infection and abortion from *S. pseudintermedius* are uncommon,^{4,8,10} compared to *S. canis* or *E. coli*. We encourage practitioners to gain client consent to submit neonates aborted for diagnostic autopsy as this may be the most clinically relevant diagnostic and lead to a confirmatory or presumptive diagnosis as in this case. Open discussion on management of premature labor and abortion in dogs is necessary between clinicians.

Learning points

- *S. pseudintermedius* is a commensal bacterium of the skin that shares the same embryologic origin as the vestibule of the vulva¹²
- *S. pseudintermedius* is a commensal of the endometrium in healthy dogs
- Although *S. pseudintermedius* is a commensal caudal vaginal microflora, for the first time, its association with canine abortion is reported

Conflict of interest

None to report.

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