Case Report

Pregnancy and spontaneous partial abortion associated with salmonella in a dog

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Abstract

A 2-year mixed breed dog was presented on day 50 after breeding for an emergency condition (greenish brown vulvar discharge and abortion). The patient was underweight (lower body condition score), had nonfetid vulvar discharge, and was negative for Ferguson reflux. Patient had 10 premature fetuses (determined via transabdominal ultrasonography and radiography). Serum progesterone concentration was 1.25 ng/ml. Overnight hospitalization therapies included intravenous fluids, terbutaline, fenbendazole, altrenogest, progesterone, and antibiotics. Based on vaginal discharge culture results (salmonella), antibiotics treatment was altered. Three days later, neutrophilia with left shift and a fetal death were observed. Because cesarean surgery with ovariohysterectomy was declined by the owner, altrenogest and terbutaline treatments were discontinued to permit whelping. Patient was in dystocia after delivering 2 stillborn and 2 live pups. One pup was delivered medically and 1 necrotic pup via cesarean surgery. Intrauterine culture (collected during surgery) was negative. On day 7 postpartum, the patient and 3 remaining pups were healthy. This case demonstrated the difficulties of managing fetal-placental infections.

Keywords: Dog, pregnancy, fetal death, infection, dystocia

Background

Abortion is defined as the expulsion of a dead or a living fetus that is incapable of having independent life.[1] Abortions typically occur during the second half of pregnancy. Other categories of pregnancy loss include early embryonic death up to 35 days (prior to fetal organogenesis) of pregnancy or stillbirth of mature dead fetus at term.[1,2] Causes of abortion are infectious (bacterial, viral, protozoal, or fungal) and noninfectious categories.[3] Duration of canine pregnancy can be defined in a number of ways, with the least accurate based on the number of days after a single mating (ranged from 57 to 72 days).[2] This wide range is due to breeding early in estrus or late at the start of diestrus. Final fetal maturation only occurs in the last 1 – 2 days of pregnancy. Delivery of premature fetuses generally results in decline and/or neonatal death.[2] Challenges due to salmonella infection with the management of fetal-placental infections are described.

Case presentation

A 2-year-old Bernese Mountain/standard poodle mixed breed (bernedoodle) pregnant dog was presented at a veterinary clinic on day 50 after a single natural breeding, for a brownish green vulvar discharge noticed earlier in the day. Serum progesterone concentrations were 2.1 ng/ml. The dog was discharged, presuming that labor was imminent. Later that evening, the dog aborted a premature fetus, 8 hours later, and was brought to the university emergency service. Prior history included a normal pregnancy and whelping, neither travels nor contact with wildlife, and the dog had no predation behaviors. Sire (bernedoodle) of this pregnancy was reportedly healthy with unknown brucellosis status.

Physical examination findings were underweight (body condition score of 3/9 [Royal Canin© scale])[4] and a moderate amount of nonfetid dark brown vulvar discharge. A large number of fetuses (fetal heart rates ranged between 200 and 250/beats per minute [bpm]) were observed via focal transabdominal ultrasonography. Serum progesterone concentration was 1.25 ng/ml. Bloodwork findings included mild normocytic normochromic anemia (categorized regenerative due to anisocytosis and polychromasia), mild monocytosis, mild hypocalcemia, and mild hyponatremia. Additional tests included leptospirosis witness test (WITNESS® Lepto, Zoetis, Parsippany, NJ), *Brucella canis* rapid slide agglutination test (D-TEC® CB, Zoetis), canine herpesvirus titer (Colorado State University, Fort Collins, CO), and SNAP 4Dx testing

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Citation: Clinical Theriogenology 2023, 15, 9379, http://dx.doi.org/10.58292/CT.v15.9379

(anaplasma, ehrlichia, Lyme, and heartworm) (SNAP® 4Dx Plus Test, Idexx, Westbrook, ME); all were negative. Ten premature fetuses were counted (abdominal radiography), and their immaturity was determined based on the level of calcification, without evidence of fetal death (Figure 1). An immediate cesarean surgery with ovariohysterectomy was recommended but was declined by the owner. As the patient appeared stable, outpatient therapy was discussed initially; however, another fetus aborted necessitating overnight hospitalization. Therapies included intravenous lactated Ringer's solution (90 ml/kg/day [Excel®, B. Braun Medical Inc., Bethlehem, PA]), subcutaneous terbutaline (0.03 mg/kg three times a day [Brethine®, Hikma Pharmaceuticals PLC, London, United Kingdom]), oral altrenogest (0.088 mg/kg once a day [Regu-Mate®, Merck Animal Health, Rahway, NJ]), oral amoxicillin and clavulanate potassium (14 mg/kg twice a day [Clavamox®, Zoetis, Parsippany, NJ]), oral clindamycin (11.8 mg/kg twice a day [Cleocin®, Lannett Company, Inc., Philadelphia, PA]), and oral fenbendazole (50 mg/kg once a day for 3 days [Panacur®, Merck Animal Health, Rahway, NJ]). Both aborted fetuses were submitted for necropsy, and were premature (lack of hair growth).

Next day physical examination findings remained unchanged. Reexamination with targeted transabdominal ultrasonography confirmed many fetuses with heart rates above 200/bpm and poor development of fetal kidney parenchyma and gastrointestinal tract that indicated prematurity. Manual digital examination of the vagina did not elicit either a visible or palpable Ferguson reflex, and neither fetuses nor associated fetal membranes were present. Moderate amount of brown mucoid discharge in the vagina prevented viewing of the cervix during vaginoscopy. There was neutrophilic inflammation without intracellular bacteria (Figure 2) in the vaginal discharge cytology. A sample of the discharge obtained from the inside of the cranial portion of the vaginoscope (to decrease the risk for contamination) was submitted for aerobic culture. Intramuscular progesterone in oil (9.8 mg/kg [Progesterone injection USP, Hikma Pharmaceuticals PLC, London, UK]) was given once. It was recommended to increase the caloric intake. Due to financial constraints, the patient was discharged from the emergency service for continued outpatient care.

Outpatient reexamination was performed 2 days later (on day 53 after breeding). Nonfetid brown vulvar discharge continued (the rest of the examination was unremarkable). Upon further inquiry, the dog, reportedly, had been hyporexic throughout pregnancy, and the diet was supplemented with boiled chicken breast and fried eggs. Patient also had an increase in thirst. A free-catch urine specific gravity was 1.008 (> 1.030).[5] Due to borderline hyposthenuria that can be identified in pyometra, a renal panel was performed, which revealed that the dog was not azotemic (Table). Progesterone concentrations were 6.24 ng/ml. Multiple viable fetuses and 1 fetus without a heartbeat were observed via targeted transabdominal ultrasonography. Air shadowing surrounding the fetus (Figure 3) indicated fetal demise. Final aerobic culture of the vaginal discharge grew Salmonella sp., with subspeciation and serotype pending. Based on culture and sensitivity results, clindamycin (Cleocin®, Lannett Company, Inc.) was discontinued, and oral ciprofloxacin (30 mg/kg once a day [Cipro®, Bayer HealthCare Pharmaceuticals Inc., Wayne, NJ]) was prescribed. In spite of fetal demise, as the patient was clinically stable, further outpatient management continued.

The dog was presented for an outpatient reexamination once more (on day 56 after breeding). The dog had 2 episodes of vomiting since prior visit and was trembling in the car for an hour prior to the appointment. The dog had lost 0.5 kg since prior visit, was tachycardic (160/bpm), had mild erythema of the skin and ear pinnae, and was mildly hyperthermic (39.4°C). There was continued vulvar discharge and initial mammary secretions from a few teats, consistent with colostrum. Due to the concern for systemic inflammatory response syndrome (SIRS)[6] on clinical examination (elevated temperature and tachycardia), bloodwork was performed. Patient had moderate normocytic normochromic anemia of pregnancy, with a marked neutrophilia and a left shift, and mild monocytosis. A mild hypoglycemia and mild hypocalcemia were also noted (Table). Progesterone concentrations were lower (1.10 ng/ml). Targeted transabdominal ultrasonography revealed similar findings as before (multiple normal viable fetuses and 1 dead fetus [Figure 4]). Further maturation of the live fetuses was evident by slight distinction of the cortical and medullary parenchyma of fetal kidneys (Figure 5) and a more thickening of gastrointestinal tract layers in two of the fetuses[7] (Figure 6). Given the patient's deterioration and concerning laboratory findings, immediate cesarean surgery with ovariohysterectomy

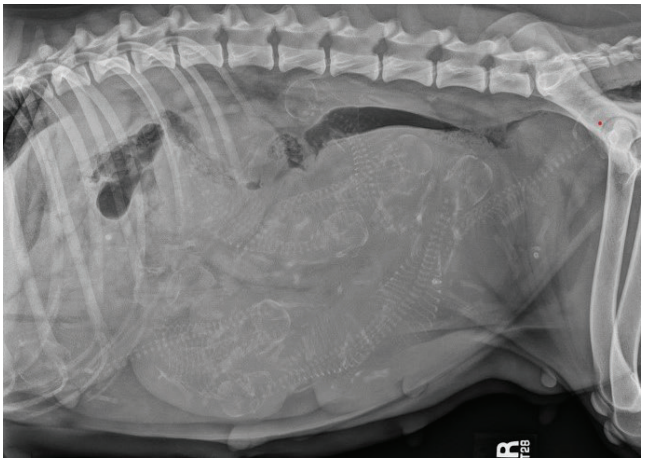

Figure 1. Right lateral abdominal radiograph (50 days after breeding), note the 10 fetuses and poor calcification (immaturity).

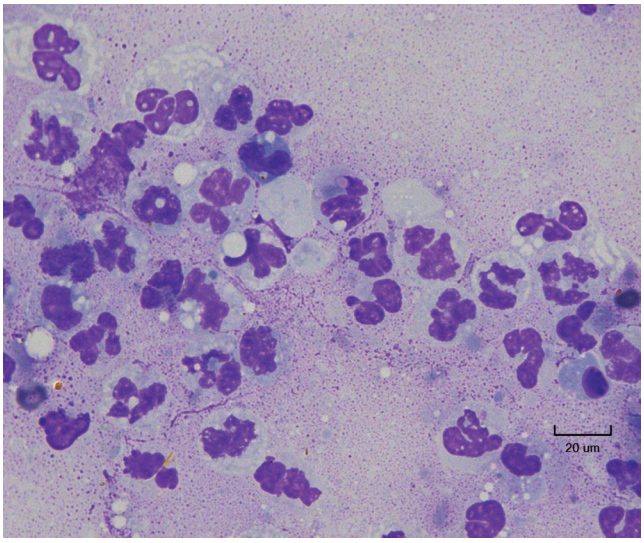

Figure 2. Vaginal discharge cytology (note degenerate neutrophils), 1000× total magnification.

Citation line: Clinical Theriogenology 2023, 15, 9379, http://dx.doi.org/10.58292/CT.v15.9379

Table. Complete blood count, serum chemistry, venous blood gas panel, and progesterone concentrations on days 50, 53, 56, and 59 after mating

Test	Day 50 after mating	Day 53 after mating	Day 56 after mating	Day 59 after mating	Reference range
Hematocrit (calculated)	34	N/A	26.4	30.6	40.0–56.0%
Neutrophils	N/A	N/A	19.64	N/A	2.7–8.9 K/µl
Band neutrophils	N/A	N/A	5.86	N/A	0 K/µl
Monocytes	N/A	N/A	2.9	N/A	0.1–0.8 K/µl
Glucose	87	73	73	N/A	78–124 mg/dl
Calcium (total)	N/A	N/A	8.2	N/A	8.7–10.4 mg/dl
Calcium (ionized)	1.17	N/A	N/A	1.37	1.18–1.35 mmol/l
Blood urea nitrogen	N/A	11	N/A	N/A	7–27 mg/dl
Creatinine	N/A	0.58	N/A	N/A	0.6–1.5 mg/dl
Progesterone	1.25	6.24	1.10	N/A	0.0–0.4 ng/ml

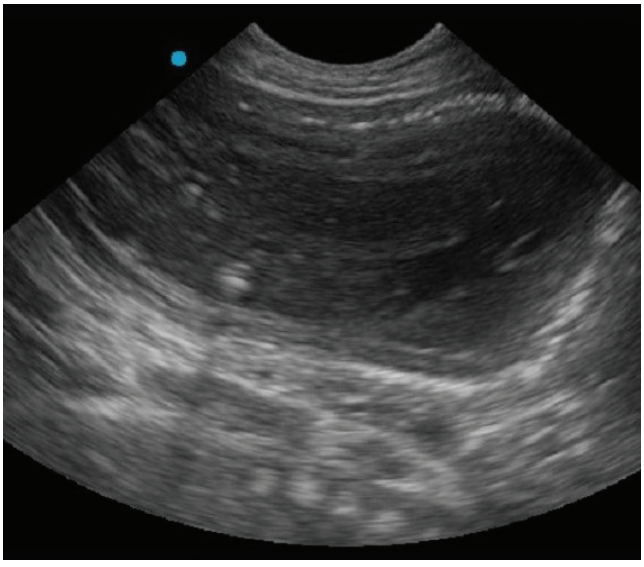

Figure 3. Transabdominal ultrasonographic image of fetal demise, note the hyperechoic foci around fetus (air shadowing), and fetus is poorly defined due to autolysis.

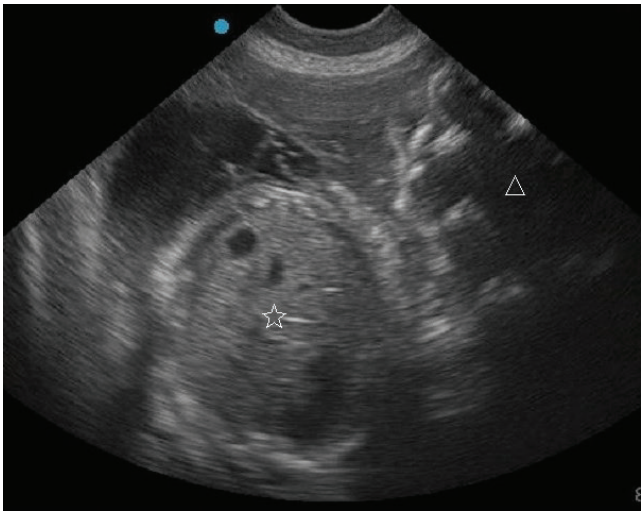

Figure 4. Transabdominal ultrasonographic image of live (star) and autolyzed (triangle) fetuses.

was again recommended; however, surgery was declined. Recommendation was made to discontinue terbutaline (Brethine®, Hikma Pharmaceuticals PLC) and altrenogest (Regu-Mate®, Merck Animal Health), to allow the dog to whelp at home. Amoxicillin and clavulanate potassium (Clavamox®, Zoetis) and ciprofloxacin (Cipro®, Bayer HealthCare Pharmaceuticals Inc.) were continued.

Whelping, dystocia, and cesarean surgery

A last dose of altrenogest (Regu-Mate®, Merck Animal Health) was given at home the following morning (day 57 after breeding). Later that night, the dog delivered a stillborn fetus. The dog then went into spontaneous labor ~ 60 hours after the last dose of altrenogest (Regu-Mate®, Merck Animal Health) and delivered 1 live pup. Subsequently, the dog was given an unknown dose of oral calcium (Oral Cal Plus™ Gel, Breeder's Edge®, Orange City, IA) at home by the owners. Three hours passed prior to delivery of another live pup. Forty-five minutes later, a stillborn fetus was delivered. Contractions failed to progress. The dog was presented for emergency management of dystocia (day 59 after breeding). The dog had lost 2.5 kg, moderate amount of nonfetid brown vulvar discharge, and colostrum secretions in all teats. A mild Ferguson reflex was present on digital vaginal examination, and fetal membranes were visualized at the cervix via vaginoscopy. Targeted transabdominal ultrasonography was performed; there was 1 dead fetus, 1 with a normal heart rate (240/bpm), and one with a critically lower heart rate (120/bpm). Blood gas analysis revealed mild anemia and normal ionized calcium concentrations (Table). Cesarean surgery was recommended due to evidence for severe fetal distress. Owner, however, declined surgery due to purported concern of the current welfare of the patient than of the fetuses and requested medical therapy for dystocia.

Monitoring fetal heart rates (via ultrasonography) and ensuring no obstruction (via vaginal examination prior to each injection), 3 serial doses (starting at 2 IU, then

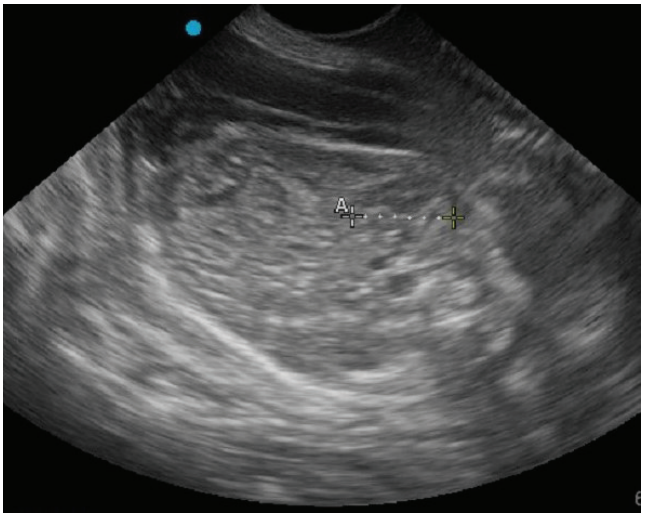

Figure 5. Transabdominal ultrasonographic image of a canine fetal kidney (outlined with caliper), note the degree of architectural detail indicates progression of fetal maturity.

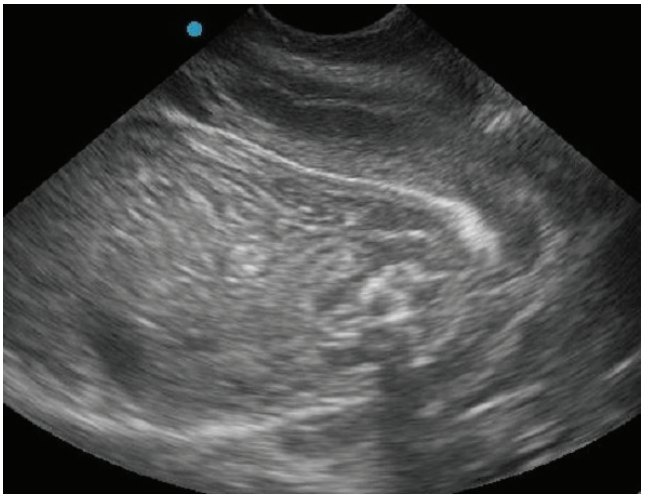

Figure 6. Transabdominal ultrasound image of a fetal gastrointestinal tract, note the architectural detail indicating progression of fetal maturity.

increasing to 3 IU and 5 IU with each subsequent dose) of intramuscular oxytocin (oxytocin, VetOne, Ontario, Canada) were given every 20 – 30 minutes. Doses were increased with each injection until a notable clinical response of contractions was observed. Subcutaneous 2 ml calcium gluconate 10% (Fresenius Kabi, Lake Zurich, IL) diluted with sterile saline was given every 2 hours to aid uterine contractions. One live pup with meconium staining was delivered. A second round of medical management with oxytocin (oxytocin, VetOne) injections every 20 – 30 minutes was performed starting at a 5 IU dose, but contractions failed to continue. As a result, cesarean surgery was approved by the owner. One live pup and one necrotic fetus were delivered via hysterotomy. Uterus contained a large amount of thick black fluid, and a sample was submitted for aerobic culture. Uterus was copiously lavaged prior to closure.

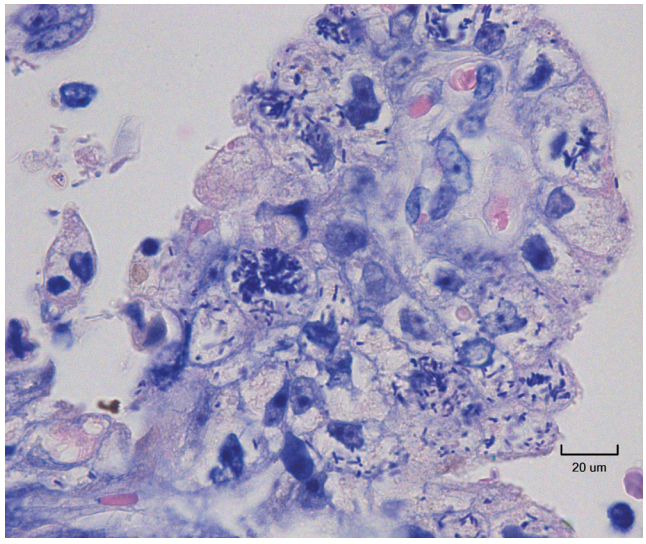

Figure 7. Histopathology of fetal chorionic membrane obtained at abortion, note the rod-shaped (bacilli), 1000× total magnification.

Nine fetuses were accounted for (2 aborted, 4 whelped at home, one delivered vaginally at hospital, and two delivered via cesarean surgery) out of an initial total of 11 (10 observed via abdominal radiology and one aborted prior to presentation). There was a large amount of gas in the abdomen (introduced intraoperatively) with no evidence of fetal calcification in the reproductive tract or the gastrointestinal tract (radiography) that would have suggested fetal ingestion by the dog at home. Pups nursed, urinated, and defecated prior to discharge. Amoxicillin and clavulanate potassium (Clavamox®, Zoetis) and ciprofloxacin (Cipro®, Bayer HealthCare Pharmaceuticals Inc.) were continued for 2 weeks. Additionally, the dog was prescribed medications as needed: oral tramadol (4.4 mg/kg twice a day for 5 days [Amneal Pharmaceuticals LLC, Glasgow, KY]), subcutaneous oxytocin (three times a day for 10 days [VetOne]), and oral metoclopramide (0.22 mg/kg three times a day for 5 days [Teva Pharmaceuticals, Parsippany, NJ]). Due to risk of fetal infection, the pups were prescribed oral trimethoprim sulfa (10 mg/kg twice a day for 2 weeks [Aurobindo Pharma, Dayton, NJ]), subcutaneous ceftiofur sodium (2.5 mg/kg twice a day for 2 weeks [Naxcel®, Zoetis, Parsippany, NJ]), and subcutaneous 0.9% NaCl (0.9% sodium chloride injection USP, B. Braun Medical Inc., Bethlehem, PA) 3 – 5 ml per pup as needed.

Outcome

Dam and litter were presented 1 week postpartum. One neonate died 2 days postpartum. The dam was eating well, there were no concerns with incision site, and pups were nursing well and were gaining weight. Physical examination was unremarkable. Intraoperative aerobic culture (uterine fluid) had no growth. Cornell abortion panel had lower titer (1:50) suggesting exposure to *Toxoplasma gondii*. Second titer was recommended to confirm active infection but was declined. A free-catch urine was concentrated (specific gravity >1.050). Fetal necropsies and placental histopathology were consistent with neutrophilic chorioplacentitis, associated with gram negative rods (Figure 7). Partial abortion was attributed to *Salmonella enterica* subsp. *houtenae* serotype IV 50:z4,z23:- (Flint). Three live pups were recovered from a total of 11 fetuses. The dam was healthy and cleared of the infection based on clinical signs and negative uterine culture.

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Discussion

This case demonstrated the challenges associated with the management of severe canine pregnancy infections. *Escherichia coli* is the most common bacterial organism to cause uterine infection in dogs.[3] Though uncommon, Salmonella sp. can also cause uterine infections leading to abortions. Salmonella is a mobile, nonspore forming gram negative bacteria of the family enterobacteriaceae. There are multiple subspecies and serovars present worldwide: *S. panama* was isolated from aborted pups[8] and *S. typhimurium* from newborn kittens.[8] Mode of transmission is most commonly by fecal-oral route, as the bacteria are shed through feces and through contact with fomites or vertical transmission in avian and reptile species.[8] This bacteria is of public health importance, as it is a zoonotic pathogen.[8,9] Dogs are typically asymptomatic when infected or have gastrointestinal signs of diarrhea, vomiting, and/or anorexia. Rare, but severe, in-utero infections in dogs result in fever, abortion, stillbirth, weak neonates, or death.[8,9] Historically, antibiotics used successfully for therapy for salmonellosis included amoxicillin, enrofloxacin, trimethoprim-sulfonamide, and chloramphenicol. It is a good practice to decide antibiotic choice based on culture and sensitivity.[9]

Salmonella enterica was recovered in 11.9% of environmental isolates, 5% of raw meat feeds, 14% of raw meat-fed dog fecal samples, and 10.5% and 4.5% from vacuum cleaner waste samples from households where dogs were and were not fed raw meat, respectively.[9] *Salmonella enterica* subsp. *houtenae* (*Salmonella subspecies IV*) was initially isolated from cockatiels and now is prevalent in many reptile species, raw poultry, and racoons.[10] Infection in children can lead to meningitis.[10] In another recent case (University of Florida) with the same outcome of a dog abortion, an organism from *Salmonella subspecies IV* was isolated in a dog that consumed raw beef and lizards.[11] The bacteria was not further speciated in that case. Dog in that prior study did not have gastrointestinal signs as in this case, but instead was febrile and hyporexic. To our knowledge, this is only the second reported canine abortion caused specifically by Salmonella subspecies IV. In this case, the owner reported that the dog frequently drank water from a well in the backyard, inhabited with lizards and other reptiles, that could have served as a potential salmonella reservoir. Another possible source of infection was from improperly cooked chicken and eggs that were fed during pregnancy. Poultry kept at temperatures between 7 and 47°C allowed growth of Salmonella sp.[9] Although a final etiology of salmonella was determined in this case, the definitive source of the organism remains unclear.

Diagnostics and early treatment for infections in pregnant dogs are crucial in efforts to provide a positive case outcome. Aerobic culture revealed Salmonella sp., and paired with sensitivity results, allowed for appropriate antibiotic choice. During pregnancy, certain antibiotics are harmful to the dog and/or fetuses. Fluoroquinolone class caused cartilage damage in canine fetuses;[12,13] however, cartilage lesions in equine fetuses were not identified after fluoroquinolone treatment between 46 and 60 days of pregnancy.[12] In this case, ciprofloxacin was prescribed as the bacteria was susceptible to fluoroquinolones. Although ovariectomy was indicated (welfare of the dog), owner declined. Amoxicillin and clavulanate potassium (a beta lactam) and clindamycin (a lincosamide) used were safe during pregnancy.[13]

Apart from antimicrobial therapy, additional medications were given to the dog during pregnancy. Fenbendazole was given as an anthelmintic. Terbutaline was given in an effort to prevent uterine contractions during pregnancy. As there was systemic infection present, it was assumed that the fetuses would have congenital neonatal sepsis, and thus, treatment was initiated at birth. Ceftiofur sodium is a beta-lactam antibiotic that is labeled for dogs with a wide margin of safety in neonates.[14] This Salmonella sp. was also susceptible to trimethoprim based on the antibiogram; however, a lower dose was used for the neonates than what is labeled for dogs due to underestimated pup birth weights. Additionally, risk with use of this antibiotic included transient hypothermia and bone marrow suppression.[14–16]

Maintenance of pregnancy is dependent on progesterone production and secretion from the corpora lutea (CL). Systemic inflammation caused CL lysis and decreased progesterone concentrations.[2] Progesterone or progestogens can be given in these cases to maintain pregnancy and prevent abortion. Altrenogest is an oral, short-acting, synthetic progestogen given daily.[17] This medication can be given at home by owners. Altrenogest maintained pregnancy in ovariectomized dogs.[18] Progesterone in oil is an injectable, more rapid, and longer acting formulation that is typically given every 72 hours in hospital when there is an imminent risk for pregnancy loss due to hypoluteoidism.[17] Altrenogest does not cross-react with progesterone assays, whereas progesterone in oil is readily measured by the assay.[18] On day 5 prepartum, progesterone concentrations (determined via radioimmunoassay) in seven normal dogs ranged between 2.6 and 7.8 ng/ml.[19] Progesterone concentrations in the morning diestrual dogs' blood samples were typically double compared to afternoon samples.[20] Progesterone concentrations are measured either via radioimmunoassay or chemiluminescence; the latter concentrations were ~ 85% of those of the radioimmunoassay.[21] In the present case, progesterone concentrations were measured in the early afternoon via chemiluminescence; both may account for the lower concentrations identified around 1 week prepartum. Additionally, the dog was receiving altrenogest supplementation, and it is unknown if a diurnal pattern occurs in dogs given exogenous progesterone or progestogen. Differences between pre and postprandial progesterone concentrations in dogs may also affect these results, as a substantial decrease in concentrations was observed 1 hour after feeding in pregnant dairy cows.[22] Progesterone in oil treatment provided quick hormone replacement initially and then was maintained with oral altrenogest.

Risk factors for salmonellosis include a weakened immune system, improper diet including raw foods, and poor hygienic conditions.[8] Canine pregnancy is maintained by elevated progesterone concentrations.[2] Progesterone in women created an immunosuppressed state that made pregnant women more susceptible to infections.[23] Similarly, this is speculated to occur in pregnant dogs, with steroid hormones suppressing neutrophilic activity.[24] Client education is important to implement hygiene protocols in the household and prevent zoonosis in pets with known infections. Best practices include wearing gloves, frequent hand washing, and isolation from those in the household. Having a designated area in the yard for the infected animal to urinate and defecate is recommended. People, especially pregnant women, should avoid contact with feces from infected animals at home to prevent zoonotic spread.[25]

Learning points

- Salmonella is not a common organism identified in utero-placental infections in dogs.
- Salmonella is a gram-negative, zoonotic bacteria prevalent worldwide that typically causes asymptomatic

infections in dogs; however, treatment is required in severe cases.
- Culture and sensitivity testing is important in guiding appropriate antibiotic therapy; however, in the case of salmonellosis, fluoroquinolones use during pregnancy may be associated with neonatal arthropathy.
- Progesterone or a synthetic progestogen (e.g. altrenogest) treatment in cases of hypoluteoidism can maintain pregnancy.
- In pregnancy infections, establishing a priority to manage either dam or fetal health is necessary, if a positive outcome is not expected for both.

Acknowledgment

Authors thank Dr. Randall Walker (Department of Veterinary Pathology) for gross necropsy and histopathological examinations.

Conflict of interest and funding

Anum Ahmed's residency program was funded by the American Kennel Club/Canine Health Foundation/ Theriogenology Foundation grant. Authors report no conflict of interest.

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