

CASE REPORT

Management of a mixed enterococcal and streptococcal infection in a three-year old bull mastiff bitch: A case report

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ABSTRACT

Infertility in bitches poses a great concern to dog owners and breeders who intend to uplift their economic gain from sale of puppies. This case report represents a successful management of a mixed infection of *Enterococcus* and *Streptococcus* in a bull mastiff bitch, presented following repeated still birth in two consecutive pregnancies. Vital parameters were normal, but haematological parameters such as packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC) and total leukocyte count (WBC) were below the reference range on the day of presentation, indicative of anaemia and leukopenia. Also, vaginal cytology indicated estrus. Bacterial culture obtained from the vaginal swab revealed *Enterococcus* and *Streptococcus* species, which were highly susceptible to erythromycin and resistant to amoxicillin and ciprofloxacin. Since serum agglutination test returned negative for brucellosis, a confirmatory diagnosis of a mixed infection of *Enterococcus* and *Streptococcus* was made. The bitch was successfully treated with erythromycin. Post-treatment culture was negative, which allowed breeding at the next estrus post treatment; the hematological parameters demonstrated improvement, indicating a positive therapeutic response. This case report underscored the importance of laboratory diagnostics and management of systemic infections to sustain fertility.

Keywords: Bitch, *Enterococcus*, *Streptococcus*, vaginal cytology

INTRODUCTION

There is a wide range of causes of reproductive disorders in bitches. The most frequent are abnormal embryonic development due to genetic defects or competition between placental spaces, uterine abnormalities (uterine torsion, cystic endometrial hyperplasia, inflammation, etc), nutritional deficiencies, environmental stressors, hormonal imbalances (including hypoluteodism and hypothyroidism) and infections (Mantziaras and Zakosek, 2025). Given the complexity of these cases, accurate diagnosis often requires a comprehensive, multi-faceted approach to identify the underlying causes. Bacterial infections are the most common clinical presentations, which are associated with reproductive disorders in bitches. Infections can be caused by single bacterial line or in combination of several bacteria. *Enterococci* are Gram- positive facultative anaerobic cocci in short and medium chains. They were recognized as a separate genus from streptococci by DNA hybridization in 1984 (Said et al., 2024). *Enterococci* infection is commonly implicated in stillbirth and inflammation of the placenta, foetal membrane or umbilical cord (Gabrielli et

al., 2025). Also, they can be residents of intestinal flora, occasionally causing genital tract inflammation and urinary tract infections (Stepien-Pysniak et al., 2021).

Streptococci are Gram-positive, non-motile, non-spore-forming, catalase-negative cocci occurring in pairs or chains. Most streptococci are facultative anaerobes, while some are obligates anaerobes (Patterson, 1996). Streptococci invade tissues opportunistically, when there is a bridge in normal host barriers, resulting in disease manifestations, such as pneumonia, endocarditis and foetal death (Tsutsumi, 2025).

In a 10-year study of the trends in reproductive disorders in animals presented at a veterinary hospital in Enugu state by Ibe et al. (2025), the highest prevalence of reproductive disorders was in the canine species. This may be attributed to the fact that dogs are the most important companion domestic species to man, thus, the increased demand for their maintenance appropriate for specific purposes, such as company, sport, hunting and/or guarding (Costa et al., 2019). In another retrospective survey on 203 bitches with genital disorders, such as infertility, vaginitis, pyometra and puppy death by Bjurstrom (1993), majority of the infertile bitches gave rise to mixed cultures involving streptococci. Mixed infection has also been reported as a common cause of infertility in bitches (Antunes et al., 2016).

This case report reveals infertility in a bitch caused by mixed infection by the rare combination of the *Enterococci* and *Streptococci*. The systematic diagnosis, management and good prognosis of the case instigated its report in the literature.

CASE PRESENTATION

A 3-year-old Bull Mastiff bitch weighing 46.0 kg was presented to the Small Animal Clinics Unit of the Veterinary Teaching Hospital, University of Nigeria, Nsukka, on the 20th of May, 2024, with the chief complaint of ascertaining if the bitch was in estrus. It was revealed that the bitch previously had aborted in two consecutive pregnancies, after mating with two different dogs, and that no diagnostic testing was conducted to ascertain the cause of the abortions. There was no other dog in the compound. The vaccination history was up to date.

Diagnostic Plan

Physical Examination

Physical examination revealed a normal mucous membrane and uninflamed mandibular and prescapular lymph nodes. There was a mild tick infestation. The rectal temperature, respiratory rate, pulse rate and heart beat were all within the normal range (Table 1).

Table 1 Vital parameters of the patient on the day of presentation

Vital Parameter	Patient's Value	Normal Range in Dogs
Rectal temperature	39.3°C	37.9°C - 39.9°C
Respiratory rate	36 cycles/minute	15-40 cycles/minute
Pulse rate	82 beats/minute	70 - 120 beats/minute
Heart rate	90 beats/minute	70 - 120 beats/minute
Capillary refill time	<2 seconds	

Laboratory Screening

Laboratory analyses included vaginal cytology, *Brucella* screening, haematology and microbial culture and sensitivity tests. Vaginal cytology was conducted using a sterile vaginal mucosal swab pre-soaked in normal saline and inserted from an angle of 45° on the vaginal mucous membrane. A smear of the swab on an albuminized slide preceded air-drying and methanol fixation for about 20 minutes. The fixed slides were

rinsed with running water and stained with Giemsa. Stained slides were examined and photomicrographs were obtained at different magnifications, using a digital eyepiece (Scopetek®, DCM500, Hangzhou, Zhejiang Province, China; Resolution: 5M pixels) attached to a light microscope (OLYMPUS®, Model XSZ107BN, Hamburg, Germany).

Brucella screening was conducted using 3 ml of cephalic blood into a plain sample bottle. Serum was

obtained from the sample after keeping the sample in a slanting position for 12 hours at room temperature to allow for blood clotting and separation of the serum. Serum was aspirated into labeled 2 ml serum vials, using a Pasteur pipette and maintained on ice. The sera samples were thawed and used for *Brucella* screening, using the serum agglutination method as described by Brown et al. (1981). For haematological analysis, cephalic blood sample was aseptically collected using a 2 ml sterile syringe and 23-gauge needle into a sterile ethylenediaminetetraacetic acid (EDTA) sample bottle. Red blood cell (RBC) and total leukocyte count (WBC) were determined by haemocytometer method, packed cell volume (PCV) was determined by microhaematocrit centrifugation method, while haemoglobin concentration (HC) was determined by cyanomethaemoglobin method (Kachmar, 1970).

Vaginal swab, collected as already described, was used for microbial culture and sensitivity test. The swab sample was enriched by inoculating on an enrichment media containing nutrient broth and peptone water at 42°C for 48 hours. A colony was taken from the enrichment media, using a sterile metal loop, and sub-cultured onto a blood agar, Eosin Methylene Blue, Mannitol Salt Agar, *Salmonella* *Shigella* Agar, McConkey Agar, by streaking. These were incubated at 37°C for 24 hours aerobically. Growing colonies were

examined with Gram-staining techniques. Suspicious colonies were identified using biochemical tests. The antibiotic sensitivity test for the identified bacteria was applied with multi-discs containing ceftriaxone (10 µg), azithromycin (20 µg), amoxicillin (30 µg), ampicillin (30 µg), levofloxacin (10 µg), chloramphenicol (20 µg), ciprofloxacin (10 µg), gentamycin (10 µg) and streptomycin (30 µg). The antibiotics sensitivity test was performed according to the Kirby Bauer disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines (2020).

Management

Based on the tick infestation, management commenced on the day of presentation, prior to laboratory results. Injection of ivermectin at dosage of 0.4 mg/kg was given subcutaneously and repeated after 2 weeks.

Tentative Diagnosis

These included Streptococcal infection and Brucellosis.

Laboratory Findings

Vaginal cytology revealed very scarce parabasal cells and many anucleated superficial cells. This was indicative for the estrus stage of estrous cycle.

Serum agglutination test for Brucellosis was negative. Haematology test revealed low values of PCV, Hb, RBC, WBC and neutrophils (Table 2)

Table 2 Haematological parameters of the patient on the day of presentation

Haematological value	SI Unit	Patient's Value	Normal Range in Dogs (Khan et al., 2011)
PCV	%	30.0	35.0-57.0
Hb	g/µl	9.89	11.9 - 18.9
RBC	x10 ⁶	3.90	4.95- 7.87
WBC	x10 ³ µ/L	3.2	5.0 – 14.0
Lymphocyte	%	23.6%	8.0-38.0
Neutrophils	%	76.0	51.0-84.0
Monocyte	%	0.4	1.0-9.0
Eosinophils	%	0.0	0.0-9.0
Basophils	%	0.0	0.0-1.0

Haemo-analyzer used was ABAXIS Zoetis Vetscan (VS2 HM5) blood analyzer

The microbial culture result showed heavy growth of *Enterococci* and *Streptococci* (Figure 1).

The microbes were very sensitive (++) to streptomycin, azithromycin and erythromycin, mildly sensitive to levofloxacin (++) and resistant to ceftazidime,

rifampicin, ceftriaxone, amoxicillin and ciprofloxacin (Figure 2).

Confirmatory Diagnosis

Mixed infection of *Enterococci* and *Streptococci*.

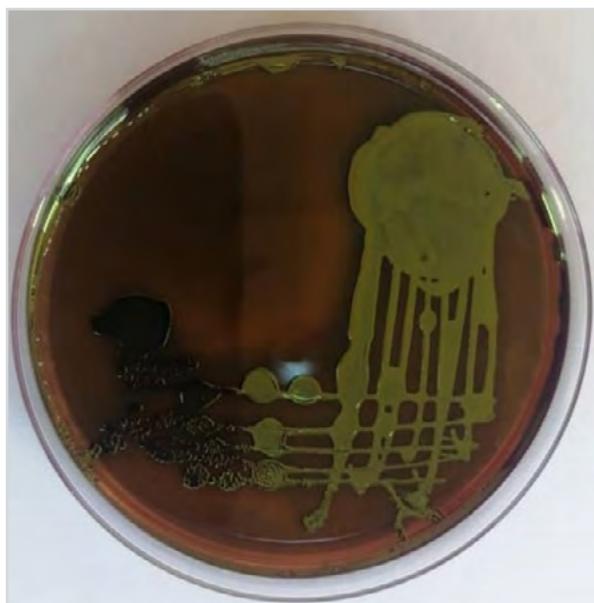


Figure 1 Result of bacterial culture, showing heavy growth of *Enterococci* and *Streptococci*

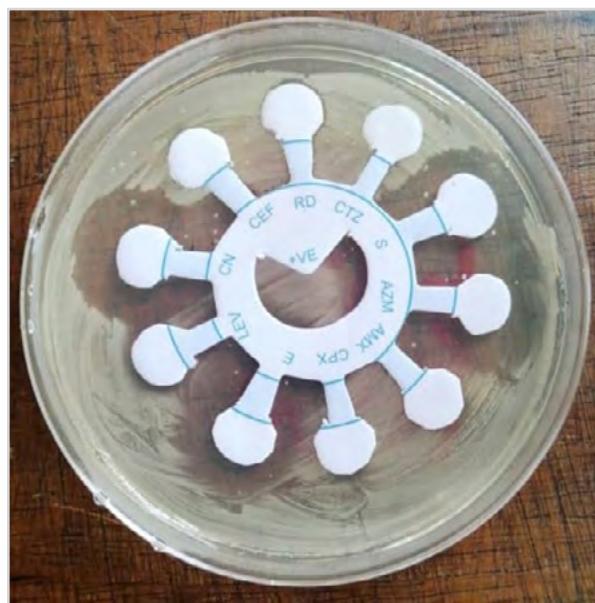


Figure 2 Result of bacterial sensitivity test

Continuation of Management

Following confirmation and antimicrobial sensitivity tests, treatment continued with administration of azithromycin at dosage of 10 mg/kg I.M. for seven days; vitamin B complex was also administered at dose of 2 ml I.M., x 5/7.

The patient was re-examined 7 days post-treatment. Physical examination revealed that the dog continued to weigh 46 kg and maintained a normal mucous membrane. The mandibular and prescapular lymph nodes were normal. There was no tick infestation. The rectal temperature, respiratory rate, pulse rate and heart beat were all within the normal range in dogs (Table 2).

Table 3 Vital parameters of the patient on the day 7 post-treatment

Vital Parameter	Patient's Value	Normal Range in Dogs
Rectal temperature	38.9°C	37.9°C - 39.9°C
Respiratory rate	34 cycles/minute	15-40 cycles/minute
Pulse rate	84 beats/minute	70 - 120 beats/minute
Heart rate	89 beats/minute	70 - 120 beats/minute
Capillary refill time	<2 seconds	

Analysis of blood sample collected 7 days post-treatment also revealed normal values of PCV, Hb concentration, RBC, WBC and neutrophils (Table 4)

Table 4 Haematological values of the patient on the day 7 post-treatment

Haematological value	SI Unit	Patient's Value	Normal Range in Dogs (Khan et al., 2011)
PCV	%	38.0	35.0-57.0
Hb	g/µl	12.3	11.9 - 18.9
RBC	$\times 10^6$	5.10	4.95- 7.87

Haematological value	SI Unit	Patient's Value	Normal Range in Dogs (Khan et al., 2011)
WBC	$\times 10^3$ u/L	3.5	5.0 – 14.0
Lymphocyte	%	30.5	8.0-38.0
Neutrophils	%	68.8	51.0-84.0
Monocyte	%	0.7	1.0-9.0
Eosinophils	%	0.0	0.0-9.0
Basophils	%	0.0	0.0-1.0

Haemo-analyzer used was ABAXIS Zoetis Vetscan (VS2 HM5) blood analyzer

Repeated bacterial culture result did not yield any bacterial growth.

Recommendations to the owner of the bull mastiff bitch

The owner was advised not to breed the bitch, until the next estrus/heat. This was to enable completion of management of the Enterococcal and Streptococcal infections. The client was also advised to present the dog intended for breeding to the hospital prior to breeding. This was to screen the dog for any sexually transmitted diseases before breeding.

Prognosis

The bitch was bred on the next heat after treatment (6 months post-treatment). She whelped successfully.

DISCUSSION AND CONCLUSION

Laboratory confirmation of *Streptococcus* species in the present study is similar to other reports (DeWinter et al., 1999; Okpara et al., 2018), in which abortions and stillbirths in dogs were linked to streptococcal infections. *Streptococcus canis* is an opportunistic pathogen of dogs. However, the Lancefield group G of *Streptococcus canis* has been incriminated in infertility in bitches. Although Lamm et al. (2010) opined that confirmation of the diagnosis requires histopathologic examination and routine aerobic cultures from aborted material, the present case had no access to the aborted placenta or fetus. However, isolation of streptococci from maternal vaginal swabs was also indicative of a likely cause of the abortions; similar findings have been reported by other authors mentioned above.

This case underscores the importance of holistic laboratory examination in the management of potential infective causes of infertility in bitches. It is a known fact that *Brucella canis* is the most common cause of abortion and stillbirths in bitches (Buhmann et al.,

2019). However, negative isolation of *Brucella* species should not rule out other bacterial causes of abortion, as the present case has revealed. Viral, bacterial, fungal and protozoal infections have been implicated in canine abortions. The progression of foetal development may be affected by the direct action of microorganisms such as Streptococci, which either degenerates the placenta or causes the release of placental toxins by inflammatory processes leading to hyperthermia, hypoxia and endotoxemia, leading to abortion (Antunes et al., 2016). In a study conducted by Shambulingappa et al. (2010), Streptococci ranked the second highest frequently occurring causes of canine abortions after *Escherichia coli*. Haematology revealed anaemia and leukopenia on the day of presentation (PCV, Hb, RBC and WBC were below their reference range). Studies have reported an association between severe anaemia and bacteraemia in dogs (Miller et al., 2004), although more often anaemia is due to inflammatory disease and cancer-related anaemia (Chervieret al. 2012; Num-Adom and Amali, 2024). Bacteraemia can cause anaemia in dogs through the immune response, which leads to the destruction of red blood cells. This immune-mediated damage, driven by pro-inflammatory cytokines, is the primary cause, not a direct effect of the bacteria themselves (Miller et al., 2004). These haematological values returned to normal post-treatment, underscoring the importance of early therapeutic intervention of infections. Leukopenia was recorded in the present study, similar to what was reported in a pit bull terrier cross breed with bacterial infection (Hanekom et al., 2020). In the present study, there was an improvement in the total white blood cell count on day 7 compared to day 1 (although the result was still below the normal range), likely due to the therapeutic control of the bacterial infection.

Vaginal cytology has many practical applications in the evaluation of both the normal and abnormal estrous cycles in bitches. It was employed in the present case because it is a simple, quick, non-invasive and inexpensive

procedure used to identify the stage of the estrous cycle in dogs and to diagnose some pathologic conditions of the genital tract. There are different vaginal cell types viz. parabasal, intermediate, superficial and anuclear cells. It is generally accepted that vaginal cell types in the smear are related to the stage of the estrous cycle, which makes the vaginal exfoliative cytology a valuable add-on test in reproductive clinical diagnostics. Although the vaginal mucosal swab of a clinically healthy dog is not expected to be sterile, bacterial species isolated from bitches with reproductive disorders do not differ significantly from those found in healthy bitches (Groppetti et al., 2012). However, specific microorganisms within the vaginal mucosa, such as *Enterococci* and *Streptococci*, may cause infertility. The few parabasal and anucleated cells observed in the present case were indicative of the estrous stage of estrous cycle.

This case report is a re-emphasis on the need for a holistic laboratory examination, beyond the primary complaint of a client. It has demonstrated that mixed infection of *Enterococcus* and *Streptococcus* can cause reproductive disorders in dogs, and the use of the correct antibiotic therapy can cure the condition. It is hoped that this report will be useful to small animal clinicians.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – ICC, AKO, TEY; Design – ICC, TEY; Supervision – AKO; Fundings – ICC; Materials – AEA; Literature review - ICC; Analysis and interpretation of data - ICC; Writing – ICC, AKO, TEY, AEA; Critical review - AEA

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Liječenje miješane enterokokne i streptokokne infekcije kod trogodišnje kuje pasmine bull mastiff: Prikaz slučaja

SAŽETAK

Neplodnost kod kuja izaziva značajnu zabrinutost kod vlasnika pasa i uzgajivača koji nastoje ostvariti ekonomsku korist prodajom štenaca. Ovaj prikaz predstavlja uspješan slučaj liječenja miješane *Enterococcus* i *Streptococcus* infekcije kod kuje pasmine bulmastif, koja je dovedena nakon ponovljenih mrtvorodenih štenaca u dvije uzastopne trudnoće. Vitalni parametri su bili uredni, ali su hematološki parametri poput zapremine pakovanih ćelija (PCV), koncentracije hemoglobina (Hb), broja crvenih krvnih zrnaca (RBC) i ukupnog broja leukocita (WBC) bili ispod referentnih vrijednosti na dan pregleda, što ukazuje na anemiju i leukopeniju. Vaginalna citologija je pokazala da je kuja bila u estrusu. Bakterijska kultura uzeta iz vaginalnog brisa otkrila je prisustvo *Enterococcus* i *Streptococcus* vrsta koje su veoma osjetljive na eritromicin, a otporne na amoksicilin i ciprofloksacin. Pošto je serološki test aglutinacije bio negativan na brucelozu, postavljena je konačna dijagnoza miješane *Enterococcus* i *Streptococcus* infekcije. Kuja je uspješno liječena eritromicinom. Kultura posijana nakon provedenog tretmana je bila negativna, što je omogućilo parenje u narednom estrusu nakon liječenja; hematološki parametri su pokazali poboljšanje, što ukazuje na pozitivan terapijski odgovor. Ovaj prikaz slučaja naglašava važnost laboratorijske dijagnostike i liječenja sistemskih infekcija radi očuvanja plodnosti.

Ključne riječi: *Enterococcus*, kuja, *Streptococcus*, vaginalna citologija