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Case Report Open Access

Polymicrobial Vaginal Infection and Chronic Infertility in a 3-year-old Bitch: Isolation of *Escherichia coli*, *Morganella morganii*, and *Staphylococcus* spp

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ABSTRACT

This case report describes the isolation of Escherichia coli, Morganella morganii, and Staphylococcus spp from the vagina of a 3-year-old bitch presenting with chronic infertility and abnormal vaginal discharge. The bitch, which had whelped only once at 1 year and 5 months, experienced multiple failed breeding attempts despite an up-to-date vaccination status and treatment with amoxicillin before the last breeding. Two weeks post-breeding, a blackish vaginal discharge was observed, prompting further investigation. Microbial culture from a vaginal swab revealed three distinct organisms: E. coli as a lactose fermenter producing a greenish metallic sheen on EMB agar, Staphylococcus spp as gram-positive cocci in clusters, and M. morganii as a non-lactose fermenter. Biochemical tests confirmed the identities of these isolates, and antimicrobial sensitivity testing indicated the susceptibility of E. coli and M. morganii to gentamicin and streptomycin. At the same time, Staphylococcus spp was most susceptible to ciprofloxacin. Resistance to amoxicillin and other commonly used antibiotics was observed. Treatment was instituted using gentamicin and ciprofloxacin. Four weeks after treatment, a follow-up swab was collected from the bitch for culture, which yielded no growth after 48 hours suggesting that the infection had been cleared. The bitch was bred during the subsequent estrus and she successfully conceived and whelped 7 healthy puppies. This case highlights the significance of polymicrobial infections in canine reproductive health and emphasizes the role of targeted antimicrobial therapy guided by culture and sensitivity results, especially following previous antibiotic exposure.

Keywords: Canine infertility, vaginal discharge, antimicrobial resistance, reproductive health, antibiotic susceptibility

INTRODUCTION

Reproductive health issues in bitches especially chronic infertility and abnormal vaginal discharge are common concerns in veterinary practice and can arise from a variety of causes, such as: hormonal imbalances, anatomical abnormalities, and infections (Zubair et al., 2014). Vaginal infections, especially those involving opportunistic pathogens, are recognized as a significant contributing factor to infertility in dogs, as they can disrupt the normal reproductive process and lead to infertility or pregnancy loss. Identifying and managing such infections is crucial to restoring fertility and maintaining reproductive health (Zubair et al., 2014).

Infections by bacteria such as Escherichia coli, Morganella morganii, and Staphylococcus spp are notable as these organisms, although often commensal or opportunistic, can become pathogenic under favorable conditions, especially in cases of altered immunity or disrupted vaginal microbiota. E. coli and Staphylococcus spp are commonly implicated in reproductive infections (Jagodka et al., 2023; Leps et al., 2024), whereas M. morganii, though less frequently isolated from the reproductive tract, has been documented as a potential opportunistic pathogen (Liu et al., 2016). Antibiotic treatments, while necessary to manage infections, can disrupt the microbial balance and may select for antibiotic-resistant strains, complicating subsequent treatment efforts (Muteeb et al., 2023).

CASE DESCRIPTION

A vaginal swab from a three (3) year old Caucasian shepherd bitch was submitted to the Bacteriology Laboratory, Department of Veterinary Microbiology, Ahmadu Bello University Zaria, Nigeria. History revealed that the bitch had whelped only once at the age of 1 year 5 months after which all breeding attempts had failed. The bitch had an up-to-date vaccination history and had been treated with amoxicillin before the last breeding. The client reported the observation of a blackish discharge from the vagina of the bitch two weeks after the last failed breeding.

Microbiological examinations

Culture and isolation

the vaginal swab was first subjected to pre-enrichment in buffered peptone water (Oxoid, UK) at 37°C for 24 hours, after which a swab of the turbid peptone water was cultured on blood agar at 37°C for 24 hours and observed for growth. Each of the distinct colonies observed was sub-cultured on blood agar (Oxoid, UK) and MacConkey agar (Oxoid, UK) with the presumptive colonies of *Escherichia coli* sub-cultured on Eosin methylene blue (EMB) agar (Oxoid, UK) at 37°C for 24 hours.

Biochemical tests

the presumptive *Escherichia coli* and *Morganella morganii* isolates were inoculated into Triple Sugar Iron (TSI), Indole, Urea, Citrate, Methyl Red, Voges Proskauer and SIM medium for motility, and incubated at 37°C for 24 hours.

The presumptive *Staphylococcus* spp isolate was subjected to catalase and coagulase tests. The catalase test was performed by using a sterilized loop to pick from the colony on blood agar and placing it on a clean glass slide. This was followed by adding a drop of hydrogen peroxide to the colony and observation for bubble formation, which indicates a positive reaction. The coagulase test was carried out using the slide agglutination method, in which 2 drops of rabbit plasma were placed on a sterile glass slide. A sterilized loop was used to pick a colony from blood agar and mixed with the rabbit plasma to form a solution. The formation of cloths was indicative of a positive result.

Antibiotic susceptibility test

The disc diffusion method was used for antibiotic sensitivity testing of the isolates on Mueller-Hinton agar (Oxoid, UK). All three isolates were subjected to 10 antibiotics each namely Augmentin (amoxicillin and clavulanic acid), Ofloxacin, Chloramphenicol, Sparfloxacin, Gentamycin, Perfloxacin, Septrin (sulfamethoxazole and trimethoprim), Streptomycin, Ciprofloxacin, and Amoxicillin, for *Escherichia coli* and *Morganella morganii* isolates while Gentamycin, Perfloxacin, Septrin (sulfamethoxazole and trimethoprim), Streptomycin, Ciprofloxacin, Amoxicillin, Ampiclox (ampicillin and cloxacillin), Erythromycin, Rocephin (Ceftriaxone) and

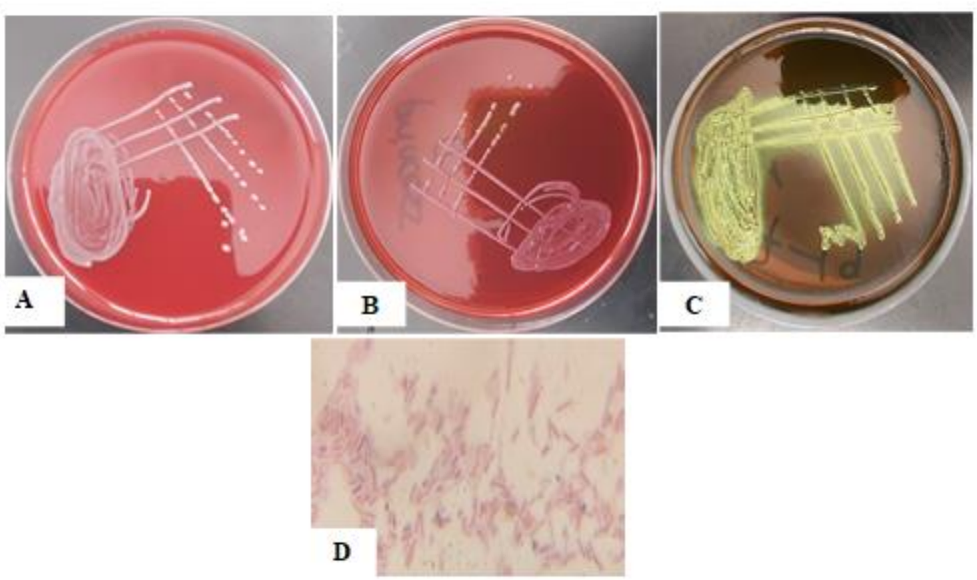

Figure 1: medium sized non-hemolytic colonies on blood agar (A); pinkish colonies on MacConkey (B); colonies with greenish metallic sheen on eosin methylene blue (C) and gram-negative rods (D), presumptive of Escherichia coli

Table 1: Outcome of biochemical test on isolates presumptive of Escherischia coli and Morganella morganii.

S/N	Biochemical tests	Lactose fermenter	Non-lactose fermenter
1	Triple sugar iron (TSI) agar	Acid/acid + gas	Alkaline/acid + gas
2	Indole	Positive	Positive
3	Urease	Negative	Positive
4	Citrate	Negative	Negative
5	Methyl red (MR)	Positive	Positive
6	Voges proskauer (VP)	Negative	Negative
7	Motility	Motile	Motile
		Escherichia coli	*Morganella morganii*

Zinnacef (cefuroxime) for *Staphylococcus* spp. isolate.

RESULTS

The outcome of culture and isolation

Three organisms were isolated from the vagina of the bitch. The first isolate was medium-sized, smooth, moist, raised, whitish, non-hemolytic colonies on blood agar (Figure 1A), which appeared as small, pinkish colonies on MacConkey (Figure 1B) indicative of a lactose fermenter, produced a greenish metallic sheen on EMB (Figure 1C) and were gram-negative rods which were presumptive of *Escherichia coli* (Figure 1D).

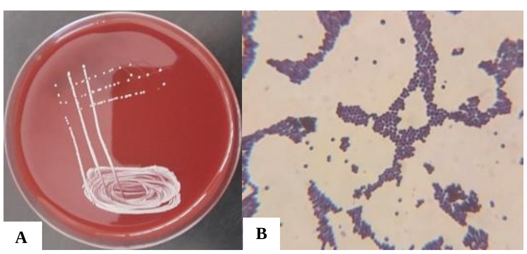

Figure 2: Small non-hemolytic colonies on blood agar (A) and gram-positive cocci in clusters (B), presumptive of Staphylococcus spp

The second isolate appeared as small, smooth, raised, moist, whitish, non-hemolytic colonies on blood agar (Figure 2A), grew poorly on MacConkey, and appeared as gram-positive cocci in clusters which were presumptive of *Staphylococcus* spp. (Figure 2B).

The third isolate appeared as medium size, smooth, moist, raised, whitish, non-hemolytic colonies on blood agar (Figure 3A), which appeared as small, colorless colonies on MacConkey (Figure 3B) indicative of a non-lactose fermenter and appeared as gram-negative rods (Figure 3C).

Biochemical test

The biochemical test identified the lactose fermenters as *Escherichia coli*, the non-lactose fermenter as *Morganella morganii* (Table 1, and Figure 4), and the gram-positive cocci as *Staphylococcus* spp.

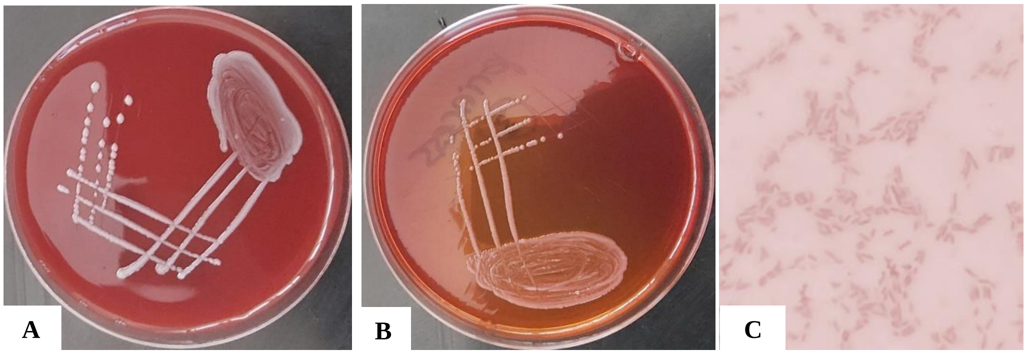

Figure 3: medium size whitish colonies on blood agar (A); colorless colonies on MacConkey (B) and gram-negative rods (C), presumptive of Morganella morganii

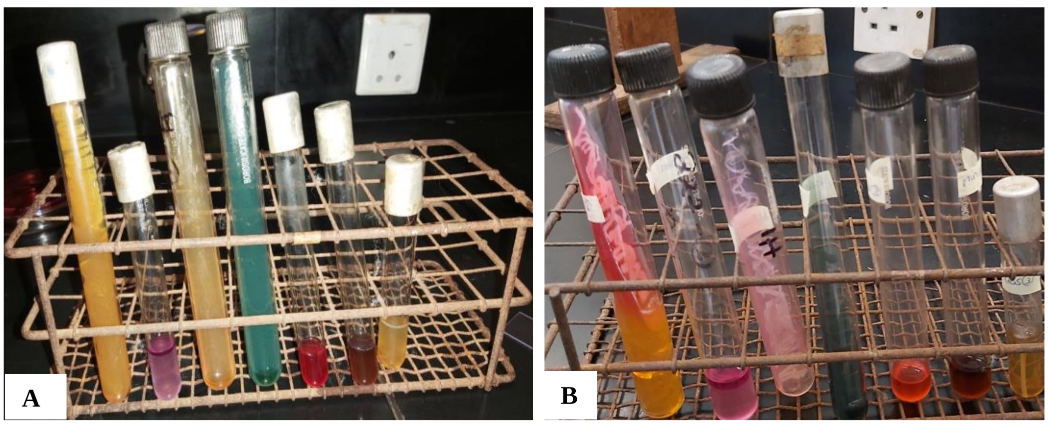

Figure 4: Biochemical test outcome indicative of Escherischia coli (A) and Morganella morganii (B). From left to right: Tripple Sugar Iron (TSI), Indole, Urease, Citrate, Methyl Red, Voges Proskeuar and motility tests.

Catalase and coagulase test reactions:

The presumptive isolate of *Staphylococcus* spp tested positive for both catalase and coagulase tests.

Antibiotic susceptibility test

Escherichia coli and *Morganella morganii* isolates were most susceptible to gentamycin and streptomycin, showed intermediate susceptibility to ciprofloxacin, and were resistant to the other seven antibiotics (augmentin, ofloxacin, chloramphenicol, sparfloxacin, perfloxacin, septrin, and amoxicillin).

Staphylococcus spp isolate was most susceptible to ciprofloxacin, followed by erythromycin, streptomycin, perfloxacin, gentamycin, septrin, and zinnacef, showed intermediate susceptibility to rocephin, and was resistant to amoxicillin and ampiclox.

DISCUSSION

The isolation of *Escherichia coli*, *Morganella morganii*, and *Staphylococcus* spp from the vagina of a bitch with chronic infertility and abnormal discharge has significant implications for the management, treatment, and understanding of reproductive health in canines. The presence of these multiple bacterial species underscores the complexity of polymicrobial infections in reproductive health. Each organism contributes unique pathogenic factors that may worsen the infectivity and impede fertility. For instance, *E. coli* produces toxins that damage epithelial cells, some *Staphylococcus* spp release virulence factors that can initiate local inflammation, and *M. morganii*, though less frequently associated with reproductive infections, can also disrupt the local microbiota balance (Jones-Dias *et al*., 2016; Vasiu *et al*., 2021). Together, these pathogens create an environment hostile to conception, suggesting that chronic infertility in this case is likely linked to bacterial presence and associated inflammation.

Additionally, the resistance of *E. coli* and *M. morganii* to multiple antibiotics, including amoxicillin, points to the risks associated with empirical antibiotic treatment. The prior use of amoxicillin may have disrupted the normal microbiota, reduced beneficial organisms, and selected resistant strains, which limits treatment options and highlights the necessity of culture and sensitivity testing (Jagodka *et al*., 2023). This testing provided a clear basis for selecting effective antibiotics; gentamicin and streptomycin for *E. coli* and *M. morganii*, and ciprofloxacin for *Staphylococcus* spp, for increasing the likelihood of treatment success. Without such testing, treatment failure and recurrence of infection would be likely, further exacerbating resistance and potentially contributing to long-term infertility (Oguejiofor, 2018).

The identification of these pathogenic bacteria also indicates a disruption in the normal vaginal microbiota, which plays a critical role in maintaining a healthy reproductive environment. Resistant infections can hinder the restoration of a balanced microbiome, resulting in chronic inflammation, scarring, and other complications that reduce fertility (Vasiu *et al*., 2021). Pre-breeding health assessments with microbial screening may also benefit bitches with a history of infertility or recurrent infections, as early detection and management of infections could improve the chances of successful breeding and reduce the likelihood of chronic reproductive issues in future cycles (Jones-Dias *et al*., 2016; Jagodka *et al*., 2023).

Treatment was instituted using gentamicin (14 mg/kg body weight, once daily) and ciprofloxacin (25 mg/kg body weight, once daily) combination for 5 days. The vaginal swab was collected for culture one month after the completion of treatment, which yielded no growth after 48 hours of culture, which indicated that the treatment was successful. Subsequently, the bitch was bred and she successfully conceived and whelped 7 healthy puppies.

CONCLUSION

This case demonstrates that polymicrobial infections with antibiotic-resistant organisms can significantly impact reproductive health, particularly in cases of infertility with a history of antibiotic use. Comprehensive diagnostic workups, including microbial culture and sensitivity testing, are crucial for identifying causative pathogens and guiding effective treatment. Judicious use of antibiotics and holistic management strategies can play an essential role in managing and preventing complex reproductive infections, thereby improving fertility outcomes in canine patients.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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