



ISOLATION AND ANTIBIOGRAM OF BACTERIA CAUSING REPRODUCTIVE DISORDERS IN BITCHES

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Received-23.07.2014

Accepted- 30.09.2014

Abstract

A study on the infectious etiology of reproductive disorders was carried out in bitches presented at University Veterinary hospitals Mannuthy and Kokkalai. Vaginal swabs and discharges were subjected to isolation trials which revealed the presence of Gram negative organisms, *Escherichia coli* (44 per cent), *Klebsiella* (12 per cent) and *Proteus* (1.2 per cent) and Gram positive organisms belonging to genus *Staphylococci*. The antibiogram revealed maximum sensitivity to Chloramphenicol followed by Gentamicin, Co-trimoxazole and Ciprofloxacin. The present study advocates early identification of the causative organisms, if any, and appropriate selection of antibiotics for prompt treatment and cure of various reproductive disorders in canines

Key words: Reproductive disorders in bitches, isolation, *E.coli*, antibiogram, chloramphenicol

The vaginal and cervical epithelium of healthy bitches in oestrus harbour microflora similar to those detected in the vagina of infertile bitches (Mshelia *et. al.*, 2001, Gunay *et. al.*, 2010). Such organisms may become opportunistic pathogens under conditions of stress and immunological incompetency, causing a multitude of reproductive disorders ranging from subclinical infections to vaginitis, pyometra, abortion and infertility. It is often difficult to distinguish pathogenic bacteria from normal vaginal flora. The most commonly isolated

bacteria, in bitches with genital disorders, are *Escherichia coli*, beta-hemolytic *Streptococci*, *Staphylococcus intermedius* and *Pasteurella multocida* (Bjurström, 1993). Bacteria like *Enterococcus*, *Klebsiella*, *Enterobacter* and *Proteus* has also been identified (John *et. al.*, 2011). Early identification of the causative agent can assist in appropriate choice of antibiotics and prevent casualties in life threatening conditions like pyometra. This can also reduce the huge financial losses borne by dog breeders, due to infertility problems in bitches.

Materials and Methods

Twenty seven female dogs of different breeds and ages (1 to 13 years) presented at the University Veterinary hospitals at Mannuthy and Kokkalai, with history of various genital disorders, from February 2013 to October 2013, became the object of study. Of these, 15 were suspected of pyometra, nine suspected of abortion, two vaginitis and two, fading puppy syndromes. Vaginal swabs and vaginal discharges, collected aseptically from these animals were inoculated on Brain Heart Infusion Agar (BHI) and incubated for 24 hours at 37°C. The isolates were characterized based on morphology, cultural characters and biochemical characters such as growth on selective media, IMViC, Nitrate reduction, Urease, Triple Sugar Iron (TSI), Coagulase and Sugar fermentation tests as per Quinn *et. al.* (2002). The antibiogram of the isolates were carried out on *Mueller Hinton Agar*, as per Bauer

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Table 1. Percentage Sensitivity and Resistance to the different antibiotics

Antibiotic Used	Total No. of tests	Percentage of Sensitivity (%)	Percentage of Resistance (%)
Gentamicin	23	78.26	21.7
Ceftriaxone	20	60	40
Ciprofloxacin	22	68.18	31.8
Co-trimoxazole	14	70	30
Chloramphenicol	10	92.85	7.1
Amoxycillin	23	21.73	78.2
Oxytetracyclin	8	25	75
Cefotaxim	9	11.11	88
Enrofloxacin	7	14.25	85

et. al. (1966), against nine antibiotics namely Gentamicin, Ciprofloxacin, Chloramphenicol, Ceftriaxone, Amoxycillin, Co-trimoxazole, Enrofloxacin, Cephataxime and Oxytetracyclin.

Results and Discussion

In the present study, out of the 27 samples subjected to bacteriological culture, 25 yielded bacterial growth. Gram's staining of the colonies revealed Gram negative short bacilli in 17 plates (68 per cent), Gram positive cocci in six (2.4 per cent) and mixed colonies of Gram negative short bacilli and Gram positive cocci in three plates (1.2 per cent). Two samples failed to yield any growth. Eleven of the Gram negative short bacilli were characterized as *E.coli* (44 per cent), three as *Klebsiella* (12 per cent) and three as *Proteus* (1.2 per cent). The Gram positive cocci isolated from samples were characterized as belonging to genus *Staphylococcus* (36 per cent).

Infertility problems in bitches, varying from mild vaginitis to fatal pyometra, have always been a matter of concern for veterinarians and dog owners in Kerala. The causes of infertility are varied, like nutritional, infectious, immunological etc. Prompt diagnosis and early treatment of the condition are of utmost importance to ensure early recovery. Infectious causes of infertility have been implicated by several authors (Mshelia *et. al.*, 2001, John *et. al.*, 2011, Bassessar *et. al.*, 2013). The vestibule and vagina of healthy bitches is normally inhabited by a variety of aerobic and anaerobic bacteria. During periods of stress or immunological incompetence, these bacteria overcome the natural protective barriers of the body and cause infection. Hence it is often difficult to distinguish pathogenic bacteria from

the normal vaginal flora. Also it is difficult to associate disease with specific bacteria, as several organisms may be isolated at the same time from the vagina of bitches with reproductive diseases (Olson *et. al.*, 1978, Mshelia *et. al.*, 2001). However, a heavy growth of a uniform bacterium may be suggestive of a pathogen (Freshman, 1991).

Several authors have identified *E.coli* as the most prevalent bacteria in the vagina of bitches suspected of pyometra (Bjurström 1993, Coggan *et. al.*, 2008, Agostinho *et. al.*, 2014). Several studies have also been conducted on the various virulence genes present in *E.coli* isolated from canine vagina (Chen *et. al.*, 2003, Coggan *et. al.*, 2008, Agostinho *et. al.*, 2014). The presence of virulence genes, *sfa*, *cnf*, *pap*, *hly* and *afa* in *E.coli* isolated from canine vagina has been reported by Coggan *et. al.* (2008). *Staphylococcus spp.*, *Corynebacterium spp.*, *Klebsiella spp.* and *Streptococcus spp.* were the common bacteria isolated from vagina of healthy bitches during different stages of oestrus cycle by Mshelia *et. al.* (2001), and they opined that the mere isolation of bacterial agents in the presence of an on-going genital disease does not necessarily be incriminative of that organism.

Appropriate choice of antibiotics is decisive for the early cure of the disease. Hence the importance of invitro testing for antibiotic sensitivity is of prime importance. In the present study, the bacterial isolates showed maximum sensitivity to chloramphenicol (92.85 per cent) followed by gentamicin (78.26 per cent), co-trimoxazole (70 per cent) and ciprofloxacin (68.18 per cent). The maximum resistance was against

cefotaxim, followed by enrofloxacin, amoxicillin and oxytetracyclin (Table.1). *Escherichia coli*, Enterococcus, Klebsiella, Enterobacter, Proteus and β -haemolytic Streptococci isolated from vagina of infertile canines were reported to have sensitivity to gentamicin and resistance to amoxicillin (John *et. al.*, 2011). In this study, among the nine antibiotics used for antibiotic sensitivity testing, gentamicin and amoxicillin were the most frequently used ones, whereas, 6enrofloxacin, oxytetracyclin, cefotaxim and cotrimoxazole were incorporated the least (Table.1). Several strains of *E.coli* isolated from canine vagina have been reported to show low resistance to the antimicrobials used in canine practice (ampicillin 10 per cent, enrofloxacin four per cent, gentamicin zero per cent, streptomycin five per cent, sulfamethoxazole eight per cent, tetracycline four per cent and trimethoprim two per cent) (Hagman and Greko, 2005). In a microbiological study on intra uterine contents of dogs with pyometra, *E. coli* was isolated, which showed sensitivity to norfloxacin, polymixinB, sulphazotrin, chloramfenicol and enrofloxacin (Coggan *et. al.*, 2008). Antimicrobial susceptibility of bacteria isolated from canine pyometra cases showed lower proportions of resistance compared to those isolated from canine urinary tract infections (Hagman and Greko, 2005). In a study conducted by Coggan *et. al.* (2008), antimicrobial susceptibility of *E. coli* isolated from cases of canine pyometra, showed maximum resistance to cephalothin followed by ampicillin, cefoxitin, tobramycin, tetracycline, amikacin, cefalexin, gentamicin, cefotaxim, sulphazotrin, enrofloxacin, aztreonam, chloramphenicol, neomycin, norfloxacin and polymixin B. The highest sensitivity was to norfloxacin followed by polymixin B, sulphazotrin, enrofloxacin and chloramphenicol.

The present study showed a high prevalence of Gram negative organisms, especially *E.coli*, in vagina of canines manifesting various reproductive problems. This is in accordance with earlier reports of isolation of *E.coli* from vagina of canines, with and without reproductive disorders. Hence a possible link between canine infertility and *E.coli* infection cannot be ruled out. The antibiogram revealed chloramphenicol, co-trimoxazole and gentamicin as the most potent antibiotics against these organisms. Hence these antibiotics may

be included in the initial treatment regime for canine infertility. The present study advocates early identification of the causative organisms, if any, and appropriate selection of antibiotics for prompt treatment and cure of reproductive disorders in canines.

Acknowledgement

The authors are thankful to the Dean, College of Veterinary and Animal Sciences, Mannuthy, for providing all kind of facilities.

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