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Kısa Bildiri ***Short Communication***

Examination of Aerobic Bacteria from Milk Samples of Bitches with Clinical Mastitis

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Abstract

Canine mastitis occurs primarily during the postpartum period and may also occur during pseudopregnancy, as well as after early weaning of puppies. Clinical and bacteriological examinations of mammary secretion were performed in 17 bitches and results of the bacteriological examination of milk samples were evaluated. *Staphylococcus intermedius* (n=11) was the predominant isolate from the canine milk while the other microorganisms were *Escherichia coli, Pseudomonas aeruginosa*, *S. aureus, Citrobacter freundii, S. epidermidis* and *S. hyicus*. According to the antimicrobial susceptibility test results, isolates were found mostly to be sensitive to gentamycin, while cefixime was detected as the least effective antimicrobial agent. As we had limited number of dogs in our study, further studies on this subject will be helpful for the veterinarians working with pet animals. Because dogs and humans live very closely in urban life style zoonotic transmissibility of *S. intermedius* shall be of interest to examine further in the future.

Özet

Klinik Mastitli Köpeklerden Alınan Süt Örneklerinde Aerobik Bakteri Muayenesi

Köpek mastitisi genellikle postpartum dönemde veya bazen de hayali gebelik süreci geçiren köpeklerde oluşabilmektedir. On yedi köpeğin klinik olarak ve sütlerininde bakteriyolojik olarak muayenesi yapılmış ve sonuçlar değerlendirilmiştir. Örneklerin muayenesi sonucunda köpek sütünden en fazla *Staphylococcus intermedius* (n=11) izole edilmiş, bunu *Escherichia coli, Pseudomonas aeruginosa*, *S. aureus, Citrobacter freundii, S. epidermidis* ve *S. hyicus* izlemiştir. Yapılan antibiyogram testine göre ise izolatlar en fazla gentamisin'e, en az ise cefiksim'e duyarlı bulunmuştur. Çalışmamızda nispeten az sayıda hayvan olduğu için bu konuyla ilgili daha sonra yapılabilecek çalışmalar küçük hayvanla uğraşan veteriner hekimler için yararlı olacaktır. Yine şehir hayatında insanlar köpeklerle çok yakın bir şekilde yaşadıklarından *S. intermedius*'un zoonotik geçişi ile ilgili çalışmalar yapılabilir.

Introduction

Mastitis is an inflammation of the mammary gland often associated with an infection and not a very common problem in the lactating bitches (Feldman and Nelson, 2004). It can be septic or non septic and may involve one or more mammary glands (Aiello, 1998). Canine mastitis occurs primarily during the postpartum period and may also occur during pseudopregnancy, as well as after early weaning of puppies. Etiology of canine mastitis has not been studied in detail before, but previous studies emphasizes that the disease is important for the post-parturient bitch (Ververidis et al., 2007).

A bitch with mastitis may or may not be ill and systemic signs (e.g. depression, fever) may also be evident. The mammary gland (s) involved is usually warm and painful with red-to purple-coloured skin, having areas within the tissue that feel firm and hard. Milk from affected glands may be hemorrhagic or purulent, may have alkaline pH, and often is more viscous than normal milk (Aiello, 1998). Diagnosis is based upon clinical signs, haematology and cytological

examination of the milk, which may reveal bacteria, white blood cells and erythrocytes (Argyle, 1998). Usually the source of the mammary infection is not found. Potential causes including haematogenous spread from the other infection sites and ascending infection via like the nipples, penetrating wounds and sometimes uterus is also possible. Death of the bitch or puppies ('toxic milk syndrome') is possible as a result of the acute mastitis (Aiello, 1998; Marti and Fernandez, 2010; Ververidis et al., 2007).

Neonatal septicemia is thought to be possible cause of puppy deaths and it might be because of the non-hygienic environment, high animal concentrations at the kennels, the capability and experience of kennel staff and whelping facilities, and some diseases of the bitch like puerperal mastitis and metritis (Somi et al., 2003).

Canine mastitis is frequently seen from 6th to 10th day after whelping and mortality in puppies during the first weeks of life ranges from 10 to 34% (Somi et al., 2003; Ververidis et al., 2007). In the subclinical mastitis the appearance of milk is normal and there are no obvious clinical signs. Subclinical mastitis has also been recognized as a predisposing factor to septicemia in puppies and nursing from their mothers with mastitis may be the cause of fatal gastroenteritis (Milani et al., 2012; Somi et al., 2003). Undergrowth of puppies is another important aspect to be considered in bitches with subclinical mastitis (Araújo et al., 2011).

The bacteriological assessment of the vaginal tract thought to be the origin of pathogenic bacteria responsible for mastitis and due to the neonatal infections. *Escherichia coli*, *Staphylococcus pseudointermedius*, *Pasteurella spp*., *Klebsiella spp*. and *Streptococcus canis* are the most commonly isolates from the vagina of healthy bitches (Kustritz, 2006). Also, an enterococcal infection by *Enterococcus faecalis* from a dog with mastitis has been reported (Manson *et al.,* 2003). Coagulase-positive hemolytic Staphylococci are important veterinary pathogens that cause various diseases in animals. Among them, *Staphylococcus intermedius* mostly associated with canine pyoderma, otitis and mastitis. Hajek first differentiated this microorganism in 1976 from *S. aureus* based on a limited number of biochemical phenotypes (Yoon et al*.,* 2010).

Because there is limited data in the literature, the aim of this retrospective study was to assess the incidence of bacterial growth in milk from bitches with clinical signs of mammary gland inflammation, antimicrobial susceptibility properties of these bacterial infections and clinical outcome of the patients.

Materials and Methods

Animals

A total of 17 bitches of different breed, age and parity with suspected mastitis were included into the study. All of the bitches had clinical signs of mastitis. Ten bitches were at the post-partum period (group I), whereas seven bitches (group II) were only pseudopregnant. Clinical signs of mastitis were warm, painful, edematous and pink-to purple-colored mammary glands and a pinky or yellowish-green discoloration of the milk.

Experimental procedures

Milk Sampling

Firstly, teats were disinfected with 70% alcohol for the sampling and first drops of milk were discarded. Milk samples from each mammary gland were taken from the bitches with sterile swabs (Figure 1). The swabs were placed into the swab transport medium and transported to the laboratory within 30 min after sampling.

Figure 1. Sampling of milk with sterile swabs.
Şekil 1. Steril pamuklu çubukla süt örneği alınışı.

Bacteriological Examinations

Bacteriological examinations were performed from milk samples and identified by using conventional techniques. Swabs were inoculated into Nutrient broths supplemented with 2% horse sera and incubated at 37°C for 24 to 48 hrs. The cultures were passaged onto Nutrient agar plates with 7% sheep blood and MacConkey agar plates and incubated under microaerobic and aerobic conditions. The colonies were observed macroscopically and microscopically following after Gram staining. Pure cultures were identified by conventional biochemical tests (Quinn et al., 1994).

Antimicrobial Susceptibility Test

The antimicrobial susceptibility properties were determined with the disc diffusion method according to the guidelines of the Clinical Laboratory Standards Institute (CLSI). The isolates were tested with the

commercially available antimicrobial discs containing ampicillin (10 μg), amoxicillin (25 μg), amoxicillin-clavulanic acid (10 and 20 μg), cefixime (5 μg), cefoperazone (75 μg), cloxacillin (5 μg), enrofloxacin (5 μg), erythromycin (15 μg), gentamycin (10 μg), kanamycin (30 μg), tetracycline (30 μg), streptomycin (10 μg), trimethoprim-sulfamethoxazole (1.25 and 23.75 μg). The criteria for interpreting zone size limits and quality control of material and reagents were those of the CLSI (CLSI, 2002; CLSI, 2006).

Results

In the present study, 11 dogs (10 were in group I, and one was in group II) were showing a few clinical signs such as depression and fever, but no bitches showed anorexia. Six dogs did not show any clinical signs in group II. None of the dogs in both groups died because of the severity of mastitis.

Bacteriological examinations of milk samples from 17 bitches were evaluated. Bacterial growth was observed in 14 samples and three samples were bacteriologically negative. Out of a broad spectrum of bacteria isolated from the milk of clinically diseased bitches (n=14), only one species of bacteria was isolated from nine of them while two different bacteria species were isolated from the other five bitches. *Staphylococcus intermedius* (n=11) was the most common isolate from the canine milk while the other microorganisms were *Escherichia coli, Pseudomonas aeruginosa*, *S. aureus, Citrobacter freundii, S. epidermidis* and *S. hyicus* (Table 1 and Figure 2).

According to the antimicrobial susceptibility test results, isolates were found mostly to be sensitive to gentamycin, while cefixime was detected as the least effective antimicrobial agent (Table 2).

Table 1. Distribution of the bacterial species isolated from the bitches.

Tablo 1. Köpeklerden izole edilen bakteri türlerinin dağılımı.

Number of Dogs	Bacteria Species
7	*S. intermedius*
1	*S. intermedius, E. coli*
1	*S. epidermidis*
1	*S. intermedius, S. aureus*
1	*S. intermedius, C. freundii*
1	*S. aureus*
1	*S. intermedius, P. aeruginosa*
1	*S. hyicus, E. coli*

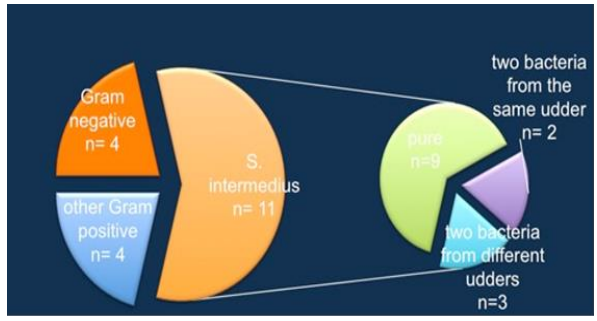

Figure 2. Distribution of bacterial species isolated from mammary glands.

Şekil 2. Meme bezlerinden izole edilen bakteri türlerinin dağılımı.

Table 2. Antimicrobial susceptibility test results.

Tablo 2. Antibiyogram test sonuçları.

Bacteria Species	AMP	AML	AMC	CFM	CFP	OB	ENR	E	GM	K	T	S	SXT
***S. intermedius* (n=11)**	6	7	9	0	7	8	9	3	10	6	4	3	5
***E.coli* (n=2)**	0	0	1	1	2	0	2	0	1	1	1	1	2
***S. aureus* (n=2)**	1	0	0	0	0	2	2	0	2	2	2	0	1
***S. epidermidis* (n=1)**	0	0	0	0	0	0	0	1	1	0	0	0	0
***P. aeruginosa* (n=1)**	0	0	0	0	0	0	0	0	0	0	0	0	0
***C. freundii* (n=1)**	0	0	0	0	0	0	1	0	1	1	1	1	0
***S. hyicus* (n=1)**	0	0	0	0	0	1	1	0	0	0	0	0	0
Total	7	7	10	1	9	11	15	4	15	10	8	5	8

AMP: ampicillin (10 μg); **AML:** amoxicillin (25 μg); **AMC:** amoxicillin/clavulanic acid (20/10 μg); **CFM:** cefixime (5 μg); **CFP:** cefoperazone (75 μg); **OB:** cloxacillin (5 μg); **ENR:** enrofloxacin (5 μg); **E:** erythromycin (15 μg); **GM:** gentamycin (10 μg); **K:** kanamycin (30 μg); **T:** tetracycline (30 μg); **S:** streptomycin (10 μg); **SXT:** trimethoprim- sulfamethoxazole (1.25/23.75 μg). The numbers indicate the susceptible isolates. The remaining isolates are intermediate susceptible or resistant to that antimicrobial agent.

Discussion

Mastitis in dogs generally occurs in postpartum period and can be classified as subclinical and clinical. Systemic signs like depression, high fever and anorexia may be apparent in some cases of acute mastitis.

In a survey study, the data of 111 bitches with mastitis were evaluated and *Staphylococcus sp.* was the species isolated most frequently (86.4%) and other bacteria like *Esherichia coli* (13.5%) and *β-hemoliytic streptococci* (9.1%) were detected from the milk with mastitic mammary glands (Jung et al., 2002). In accordance with Jung et al. (2002), in our study *Staphylococcus sp.* was the species isolated most frequently (82.4%). *Staphylococcus intermedius* (64.7%) was the most common isolate within the samples while following most common microorganisms were *Escherichia coli* (11.8%) and *Staphylococcus aureus* (11.8%). Differentiation between *S. intermedius* and *S. pseudointermedius* was based on molecular techniques. At the time of sampling this differentiation has not been performed, due to the laboratory limitations. Along with, since this study is based on retrospective evaluation of the canine mastitis cases, the authors could not have the possibility to re-examine those isolates with molecular techniques. Therefore the isolates were reported as *S. intermedius* in this study.

Antimicrobial susceptibility tests showed that gentamycin (100%), enrofloxacin (100%), nitrofurantoin (100%), and amoxicilline/clavulanic acid (92.3%) have a good effectiveness on *Staphyloccocus spp*., in vitro (Jung et al., 2002). According to the antimicrobial suspectibility test results, in our study isolates were found mostly to be sensitive to gentamycin, while cefixime detected as the least effective antimicrobial agent. Effectiveness on *Staphyloccocus intermedius,* in vitro, was as follow: gentamycin (90.9%), enrofloxacin (81.8%), amoxicilline/clavulanic acid (81.8%), and cloxacillin (72.7%).

Canine mastitis has a good prognosis if it is early diagnosed and appropriately treated. The treatment of acute mastitis consists of systemic antibiotic therapy, hot water compresses and massage of mammary glands. Mastitis results to destruction of mammary parenchyma, drainage and surgical approach might be needed when there are abscesses and gangrenous mastitis (Araújo et al., 2011; Ververidis et al., 2007). No bitches in our study needed any mammary gland drainage or any surgical approach.

In our study, neonatal infection and health of the puppies were not evaluated. It is, however, mentioned by Somi et al. (2003), that bacteria isolated from the bitches with subclinical mastitis do not necessarily cause an infection in their puppies. It is more likely that bacterial toxins or bacteria itself from the milk, affecting the immune system of the neonates, might predispose the puppies for other bacterial and viral infections. As an opportunistic pathogen, it is known that the *S. intermedius* strains carry various virulence factors including like haemolysin, pyogenic toxins including staphylococcal enterotoxin and toxic shock syndrome toxin 1, and etc. (Yoon et al., 2010).

Mastitis studies are more common in dairy cattle because it is economically important in those species. Canine mastitis is not a very frequent condition and it does not draw attention and cause as much interest as in farm animals (Araújo et al., 2011). In 2010, the first case of human infection by *S. pseudointermedius* has been reported. Additionally, transmission of *S. intermedius* between dogs and their owners has been recently documented. Also, the emergence and methicillin-resistant population of *S. pseudointermedius* among the animals have been demonstrated. All of the mentioned reports show that *S. intermedius* family (SIG; *S. intermedius, S. pseudointermedius* and *S. delphini*) may be a serious problem not only in canine health also in public health (Yoon et al., 2010).

In conclusion, the most common bacterial species has been detected with this study in some population of canine patients with clinical mastitis. *Staphylococcus intermedius* was the most common isolate within the samples while following most common microorganisms was *Escherichia coli* and *Staphylococcus aureus.* Gentamycin was the most effective antibiotic choice and it was followed by enrofloxacin, amoxicilline/clavulanic acid, and cloxacillin. All of the bitches were treated properly as accordance with their clinical needs, and healthy at the end. Further studies on bacteriological studies on this subject will be helpful for the veterinarians working with pet animals. It shall be of interest to examine further about *S. intermedius group* and their zoonotic transmissibility from dogs to humans or vice versa.

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