



Original Articles

Culture of intrauterine secretion of female dogs during ovary hysterectomy for empirical choice of antimicrobials

Cultura da secreção intrauterina de cadelas durante a ovariohisterectomia para escolha empírica de antimicrobianos

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ABSTRACT

Pyometra occurs mainly in older animals; however, the use of progestogens contributes for the younger females to be affected as well. Its treatment consists of fluid therapy, antibiotic therapy and ovariohysterectomy, and the choice of antimicrobial, generally, is based on the knowledge of the agents most commonly isolated in this type of infection and to which active principles they are sensitive. Nevertheless, it is known that over time bacteria acquire natural resistance to antibiotics and that this resistance is usually accelerated by their indiscriminate use. In that sense, studies that update which antimicrobials are efficient to fight bacterial infections are important and necessary, especially, because when diagnosing an animal with pyometra, the antibiotic therapy should be initiated immediately and the choice of the active principle, at least, in the first moment, is empirical. Therefore, the objective of this work was to determine the microbiological profile, through culture and antibiogram, of the intrauterine content of bitches submitted to elective ovary hysterectomy, thus contributing to a more conscious and effective empirical choice in the treatment of these infections.

RESUMO

A piometra ocorre principalmente em animais mais velhos; entretanto, o uso de progestágenos contribui para que as fêmeas mais jovens também sejam afetadas. Seu tratamento consiste em fluidoterapia, antibioticoterapia e ovariohisterectomia, sendo a escolha do antimicrobiano, geralmente, baseada no conhecimento dos agentes mais comumente isolados nesse tipo de infecção e a quais princípios ativos são sensíveis. No entanto, sabe-se que com o tempo as bactérias adquirem resistência natural aos antibióticos e que essa resistência costuma ser acelerada pelo uso indiscriminado. Nesse sentido, estudos que atualizem quais antimicrobianos são eficazes no combate às infecções bacterianas são importantes e necessários, principalmente, porque ao diagnosticar um animal com piometra, a antibioticoterapia deve ser iniciada imediatamente e a escolha do princípio ativo, pelo menos, no primeiro momento, é empírico. Portanto, o objetivo deste trabalho foi determinar o perfil microbiológico, por meio da cultura e antibiograma, do conteúdo intrauterino de cadelas submetidas à ovariohisterectomia eletiva, contribuindo assim para uma escolha empírica mais consciente e eficaz no tratamento dessas infecções.

INTRODUCTION

Cystic endometrial hyperplasia (CEH) is a uterine inflammation with accumulation of fluid. CEH usually occurs in the diestrus, due to the high production of

progesterone which stimulates the secretion of the endometrial glands and reduces uterine contraction, thus accumulating sterile fluid inside the uterus. These factors make the environment favorable for the proliferation of bacteria and consequently the

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development of pyometra (BARNI, ALBUQUERQUE, CONTESINI, 2013).

Pyometra is characterized by an intrauterine bacterial infection, usually affecting adult or older animals, but young animals can also develop this disease due to the administration of exogenous hormones, progestogens, mainly (CHEN; ADDEO; SASAKI, 2007; SILVEIRA et al., 2013). This disease can occur with the open cervix, where vaginal secretion is present, or with the closed cervix, when there is abdominal distension without vaginal secretion (CHEN, ADDEO, SASAKI, 2007).

The main bacterium isolated in animals with pyometra is *Escherichia coli* (FELDMAN, 2008; NELSON; COUTO, 2015), however, *Klebsiellas*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Pasteurella* and *Serratia* have also been isolated (FELDMAN, 2008; FIENI, 2006). Thus, the collection of intrauterine secretion from female dogs with pyometra is an important source of studies on bacterial resistance to antibiotics (ISHII, FREITAS, ARIAS, 2011).

In order to minimize errors in clinical conduct and consequently determine the correct choice of antimicrobial to be used, it is indicated to perform microbiological tests to identify the bacterial species. When it is not possible to perform the identification, the target site of the action, the physiological bacterial flora present and the microorganisms that are more likely to ascend to these regions are considered (OLIVEIRA, MUNARETTO, 2010).

Among animal and human medicine professionals there is a concern in relation to the resistance of bacteria to antibiotics. Because it involves different physiological mechanisms in different genetic constitutions of bacteria, it becomes a very complex problem. Moreover, the constant mutation and recombination of bacteria is an even greater aggravating factor for the emergence of resistance to antimicrobials (ISHII, FREITAS, ARIAS, 2011).

Bacterial resistance is a natural process due to the bacteria's ability to adapt to the environment. However, the inadequate use of antibiotics (ISHII, FREITAS, ARIAS, 2011; NETO, ALMEIDA, 2017), such as early interruption of treatment and the use of this medication for diseases where there is no need, is accelerating this process (OLIVEIRA, MUNARETTO, 2010).

Such conduct may become a risk for both human and animal medicine, since bacterial resistance allows bacteria to multiply and persist at therapeutic levels of a given antimicrobial (ISHII, FREITAS, ARIAS, 2011; NETO, ALMEIDA, 2017). If measures are not taken, by 2050 super bacteria could kill up to 10 million people per year (FRANCO et al., 2015; LIMA, BENJAMIM, SANTOS, 2017). In view of this, bacterial resistance has become a serious public health problem, because when a micro-organism acquires resistance to a certain antibiotic, it will not be

effective, allowing the disease to progress and increasing the risk of death.

In view of the above, the objective of this work is to establish the microbiological profile, through culture and susceptibility test to antimicrobials, of the intrauterine content of female dogs submitted to elective ovary hysterectomy, thus contributing to a more conscious and effective choice in the treatment of these infections.

MATERIAL AND METHODS

The study was performed in female dogs submitted to elective ovary hysterectomy during the practical classes of the surgical disciplines of the Veterinary Medicine course of UNIJUÍ. These classes have CEUA approval under the protocols: 002/2016, 003/2016, 001/2017, 002/2017, 002/2018, 003/2018.

The animals selected were those who, when performing the ovariohysterectomy, presented increased uterine volume, in which swabs were performed (Figure 1) and 5 mLs of secretion were collected for culture and antibiogram until a total of 30 samples were obtained. The weight of the bitches, race, and age were varied (Table 1).

Figure 1. Collection of intrauterine secretion through swab.



To isolate the bacterial agent, samples were sown using the depletion method on nutrient agar and MacKonkey agar and were incubated at 37°C for 48 hours. The isolated colonies were intended for morphotintorial and biochemical analysis for gender and species characterization according to Macfaddin (2000).

The isolates were then subjected to susceptibility testing to the following antimicrobials: vancomycin, amoxicillin,

amoxicillin with clavulanate, azithromycin, ceftriaxone, enrofloxacin, gentamicin, ampicillin, cephalixin, cephalothin, tetracycline, and penicillin G. The sensitivity or resistance analysis protocol was in accordance with the CLSI (2013).

After culture and reading of the results, all material was submitted to sterilization and later discarded as biological material.

Table 1. Relationship of race, weight and age of the female dogs submitted to intrauterine secretion sampling.

ANIMALS	RACE	WEIGHT (kg)	AGE (years)
Dog 1	SRD	5.8	3
Dog 2	Dachshund	7.5	2
Dog 3	Fila	23	8
Dog 4	SRD	14.3	7
Dog 5	SRD	11.4	3
Dog 6	SRD	5.9	Uninformed
Dog 7	SRD	13	Uninformed
Dog 8	SRD	13.9	4
Dog 9	Poodle	5.6	4
Dog 10	SRD	15	Uninformed
Dog 11	SRD	13.8	4
Dog 12	SRD	4.8	7
Dog 13	SRD	8	4
Dog 14	SRD	15.8	1
Dog 15	Yorkshire terrier	5	10
Dog 16	SRD	5.4	5
Dog 17	SRD	8.3	12
Dog 18	SRD	15	2
Dog 19	SRD	5.8	11
Dog 20	SRD	8.6	11
Dog 21	SRD	7.6	Uninformed
Dog 22	SRD	16.9	Uninformed
Dog 23	SRD	7.6	2
Dog 24	SRD	18.5	9
Dog 25	SRD	11.4	Uninformed
Dog 26	SRD	4.7	10
Dog 27	Doberman	26.5	1
Dog 28	SRD	23	8
Dog 29	SRD	8.6	Uninformed
Dog 30	SRD	17.5	Uninformed

RESULTS AND DISCUSSION

From the 30 samples collected, in 20, there was no bacterial growth; from the 10 remaining samples, *Staphylococcus aureus*, *Klebsiella rhinoscleromati*, *Streptococcus dysgalactiae*, *Staphylococcus coagulase negative*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Streptococcus sp.* and *Escherichia coli* were isolated twice (Table 2).

In most samples there was no growth of bacteria, which was already predicted, because they were bitches undergoing elective ovary hysterectomy, which when they passed the clinical examination and blood count did not indicate that they had pyometra. Thus, the thickening of the uterus with accumulation of secretion was considered an unexpected finding. This change is called cystic endometrial hyperplasia (CEH), which consists of increased size, number and chronic and repeated exposure to progesterone and endometrial hyperplasia,

with accumulation of fluid inside the uterine lumen and endometrial glands (FELDMAN, 2008; NELSON, COUTO, 2015).

Nevertheless, the accumulation of fluid in CEH makes the uterus a favorable environment for bacterial growth, originating from bacteria of the vaginal microbiota, which can result in the development of pyometra (FELDMAN, 2008). Thus, it is stressed that samples with a negative result could contaminate themselves at any time.

Animals previously diagnosed with pyometra were excluded from the study, because in these animals, the support treatment with antimicrobials and fluid therapy had already been started until the moment of the ovarihiysterectomy, and thus, by the use of the antimicrobial, there could be interference in the culture result.

Table 2. Results of antimicrobial susceptibility testing of bacteria *Staphylococcus aureus*, *Klebsiella rhinoscleromatis*, *Streptococcus dysgalactiae*, *Staphylococcus coagulase negative*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Streptococcus sp.* and *Escherichia coli* isolated from intrauterine chain secretion.

Antibiotics	<i>S. aureus</i>	<i>K. rhinoscleromatis</i>	<i>S. dysgalactiae</i>	<i>Streptococcus sp.</i>	<i>E. coli</i>	<i>E. coli</i>	<i>Streptococcus sp.</i>	<i>Staphylococcus coagulase negativa</i>	<i>Pseudomonas aeruginosa</i>	<i>Enterobacter aerogenes</i>
Ampicillin	R	R	S	S	R	R	R	R	R	R
Amoxicillin	S	S	S	S	S	R	S	R	R	R
Amoxicillin+Clavulonate	S	S	S	S	S	R	S	R	R	R
Azithromycin	S	R	R	S	R	S	R	S	R	R
Cephalotin	S	S	R	S	R	R	S	R	R	S
Cephalexin	I	R	R	S	I	I	R	S	R	S
Ceftriaxone	S	S	R	S	S	S	I	R	R	S
Enrofloxacin	S	S	S	S	S	S	S	S	I	S
Gentamicin	S	R	S	R	S	R	R	S	S	S
Penicillin G	R	R	R	S	R	R	R	R	R	R
Tetracycline	R	S	R	R	R	R	R	S	R	S
Vancomycin	S	S	S	S	R	R	S	R	R	R

"R" refers to antibiotic resistant bacteria, "S" sensitive bacteria and "I" intermediate resistance.

With the results obtained in the present study, it should be noted that the simple presence of intrauterine content does not mean that there is bacterial infection, since only in 33.33% of the cases with content there was the presence of bacteria. The relevance of these results is the importance of the clinical examination (which includes assessment of body temperature) and performance of the blood count, because even in animals in which there was bacterial isolation, if they did not present fever or deviation to the left, antibiotic therapy was not instituted. That is, when the surgeon encounters a thickened uterus with secretion in its lumen during an elective ovary hysterectomy, this may indicate both a CEH and a pyometra. Therefore, the immediate decision for the use or not of the antibiotic should be anchored in the temperature and blood count of the animal, because in this way the indiscriminate use of antimicrobials will be avoided.

It was observed that in at least 17 female dogs synthetic progestogens had already been applied, which favor the development of CEH and pyometra. In the study by Silveira et al. (2013), animals that used these drugs also developed CEH and pyometra. The use of exogenous hormones increases the incidence of these disorders, as the exogenous estrogen increases the number of receptors for progesterone in the uterus (NELSO, COUTO, 2015).

Of the 10 positive samples, *Escherichia coli* were isolated in two. Although it is not a significant number due to the few isolated samples, this result matches data from the literature where it is cited as the most present agent in intrauterine secretions (NELSON, COUTO, 2015). In a similar study prepared by Oliveira et al. (2016) at the Federal University of Bahia, approximately 40% of *E. coli* samples were isolated, followed by *Enterobacter* spp., *Staphylococcus* spp., *Klebsiella* spp., *Pseudomonas aeruginosa* and *Streptococcus* spp.

Other bacteria that are normally found in the genital tract of females are also isolated in the uterus with pyometra (EGENVALL et al., 2000), notably *Streptococcus*, *Klebsiella* and *Staphylococcus* (FIENI, 2006), which were also identified in this study.

It was observed that 80% of positive samples were resistant to ampicillin and 90% to penicillin. Tetracycline and azithromycin also did not obtain good results, with 70% and 60% of the samples being resistant to these active principles, respectively. Oliveira et al. (2016) also found resistance to antimicrobials azithromycin (80%) and ampicillin (40%). For Sfaciotte, Vignoto, e Wosiacki (2014), who analyzed samples of 25 animals from the Veterinary Hospital of the State University of Maringá, it was found that amoxicillin, ampicillin and penicillin showed resistance to 92% of the samples tested. This

bacterial resistance is directly related to the indiscriminate use of these drugs (LARA et al., 2008).

Another antibiotic widely used in skin and orthopedic infections, cephalexin (FOSSUM, 2019), has been shown to be developing bacterial resistance, as 40% of the samples were resistant to this active principle and 30% had intermediate sensitivity. Due to the frequent routine use, there was an increase in the resistance of this antimicrobial (ISHII, FREITAS, ARIAS, 2011).

Rofloxacin was one of the most prescribed antimicrobials in the late 1990s and early 2000s in pets and its efficiency is currently being questioned by several professionals due to possible resistance. However, in the present study, enrofloxacin stood out as the most efficient antibiotic, with 90% of samples being sensitive to it and 10% being intermediate. It was also observed that this antimicrobial was effective in both gram-positive and gram-negative strains, which is in accordance with the expectations of this antibiotic due to its broad spectrum of action. According to the results obtained in this study, if in fact some strains of bacteria have already been resistant to enrofloxacin, perhaps not using it for a certain period of time may have eliminated the resistant strains, since other antimicrobials were used instead. Therefore, most of the strains in circulation today are susceptible.

Staphylococcus aureus showed resistance to two aminoglycoside antibiotics, sensitivity to two antibiotics belonging to the same class and intermediate cephalixin resistance. This was also found in the studies of Ishii, Freitas, and Arias (2011), in which four samples isolating *Staphylococcus* spp. from ear disorders were shown to be resistant to cephalixin, two of which showed resistance to one class of antibiotics, aminoglycosides. It should be noted that aminoglycosides are not antimicrobials of choice for the treatment of Gram-positive infections, such as *S. aureus*, but for Gram-negative infections.

E. coli was isolated twice in separate samples. It was observed that in one of them this bacterium was resistant to amoxicillin and amoxicillin associated with clavulanic acid and in the other sample it was sensitive to both antibiotics. One reason for resistance may be the mechanism of action of amoxicillin, which inhibits the deposition of peptide glycan in the composition of the bacterial cell wall, more important for Gram-positive that has multiple layers and less important for Gram-negative that has a single layer (SPINOSA, 2017).

In the study of Ishii, Freitas, and Arias (2011), *Pseudomonas*, *E. coli*, and *Klebsiella* were isolated from animals seen at the Veterinary Hospital of the State University of Londrina that had peritonitis. These bacteria demonstrated resistance to amoxicillin associated with clavulanic acid, ampicillin, cephalothin, cephalexin, enrofloxacin, gentamicin, and penicillin. Although the specific history of each patient is not known, the conception that not only regional variations but also individual variations can be

determinant for the success of an antibiotic is reinforced with these results.

CONCLUSIONS

The results obtained showed that 50% of the samples isolated from the intrauterine content were gram-positive and 50% were gram-negative. It is noted that several antibiotics, often used routinely, are losing their efficiency against bacterial strains, to which they should be sensitive. Among them, cephalexin stands out, as it obtained 30% of bacteria sensitive to it. In contrast, enrofloxacin has been almost 100% sensitive in both gram-positive and gram-negative samples isolated. The importance of this study is emphasized as a way of updating on the sensitivity x resistance to the most commonly used antibiotics against bacterial agents normally observed in infections.

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