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MICROBIOLOGICAL AND HISTOPATHOLOGICAL ASPECTS OF CANINE PYOMETRA

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ABSTRACT

As pyometra is recognized as one of the main causes of disease and death in the bitch the purposes of this study were to evaluate microbiological and histopathological aspects of canine pyometra and to research the virulence factors of the *E. coli* isolates identifying possible risks to human health. The microbiological isolation from the intrauterine contents of 100 dogs with pyometra was carried out and the virulence factors in the *E. coli* strains were identified using PCR method. This study also consisted of the counting of microorganisms colonies forming units in samples of intrauterine content, tests of antimicrobial susceptibility of the *E. coli* isolates and the histological examination of the uterus. *E. coli* was the most prevalent microorganism isolated (76.6%) and 120 strains (79.5%) were positive for *sfa*, 86 (56.9%) were positive for *cnf*, 87 (57.6%) were positive for *pap*, 52 (34.4%) were positive for *hly*, 51 (33.8%) were positive for *iuc* and 5 (3.3%) were positive for *afa* genes. One observed more sensitivity of *E. coli* to norfloxacin, polimixin B, sulphazotrin, chloranfenicol and enrofloxacin. In 42% of the samples of uterine walls where microorganisms were isolated, the sizes of the areas of the inflammatory responses corresponded to 39-56%. Virulence factors were identified in 98.0% of the strains evaluated, demonstrating a high frequency of potentially pathogenic *E. coli*. It must be considered that dogs are animals that are living in close proximity to man for thousands of years and have an important role in the transmission of *E. coli* to other animals and to man.

Key-words: bitches, *Escherichia coli*, histopathological, microbiological, pyometra.

INTRODUCTION

Canine pyometra, also known as cystic endometrial hyperplasia complex, is a disease of the adult dog with inflammation of the uterus and accumulation of pus, and normally occurs in the luteal phase of the oestrous cycle (11). It is associated with hormonal alterations and bacterial infections (10,11). The incidence of pyometra in the bitch is high, and is recognized as one of the main causes of disease and death in this specie (10). The endometrial hyperplasia is induced by progesterone and is normally observed before the occurrence of pyometra (10,11).

The bacterial infection is a secondary condition (11). Bacteria ascend through the cervix and into the uterus during oestrous (10). Bitches with cystic endometrial hyperplasia, seem to be incapable of eliminating bacteria that can survive in the cystic fluid. One microorganism normally associated to this disease is *Escherichia coli* (2). Some strains of *E. coli* are pathogenic to man and animals. In humans it has been associated with severe gastrointestinal disorders, extra-intestinal infections and urinary infections (8).

As pyometra is considered one of the main diseases in the bitch, and this pathology represents a potential risk to public health because of the fact that the vaginal secretion can be a

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source of infection to man, the purposes of this study were to evaluate microbiological and histopathological aspects of canine pyometra and to research the virulence factors genes of the *E. coli* isolates identifying possible risks to human health.

MATERIALS AND METHODS

Source of the material

A total of 100 bitches were examined with confirmed diagnose of pyometra at the Obstetrics Sector of the Veterinary Hospital of the Faculty of Veterinary Medicine and Zootechny, University of São Paulo.

The bitches were between 2 and 16 years old and 91% of them were more than 6 years old. Eleven bitches had a history of having had birth control injections with progesterone substances in them. Sixty four per cent of the bitches were nuliparous, 21% were primiparous and 14% pluriparous.

Microbiological examination

Using aseptic techniques and the use of sterile discardable syringes and needles, 5 mL samples were taken from each uterine horn, after the bitches had undergone ovariohysterectomy surgery. The samples were transported to the laboratory in refrigerated conditions.

The obtained samples were cultured aerobically on sheep blood agar (Oxoid) and MacConkey's agar (Oxoid) at 37ºC for 24-96 hours. The samples were also cultured aerobically in BHI broth (Brain and Heart Infusion broth, Difco) at 37ºC for 24 hours. The samples cultured in BHI broth were also plated on blood agar and MacConkey's agar. The isolated microorganisms were identified according to Lennette *et al.* (15) and classified according to Krieg and Holt (14) and Murray *et al.* (17).

The samples were also cultivated on Sabouraud dextrose Agar (Oxoid) and incubated aerobically at room temperature for a minimum period of 7 days.

Research of virulence factors in *E. coli* isolates

For the research of virulence factors genes present in *E. coli* strains isolated from intrauterine contents of bitches with pyometra the polymerase chain reaction (PCR) was used. Different sets of primers (Invitrogen, California) were used. The primer sequences were used to detect genes encoding pili associated with pyelonephritis (*pap*), haemolysin (*hly*), aerobactin (*iuc*), cytotoxic necrotizing factor 1 (*cnf*1), S fimbriae (*sfa*), afimbrial adhesin I (*afa*), heat labile (*LT*) and heat stable (*STa* and *STb*) enterotoxins and verotoxins (VT). Amplicon sizes and the relevant literature are shown in Table 1.

Table 1. The primers used for detection of the various genes by PCR, the amplicon size and relevant references.

Gene	Primer	Oligonucleotide pairs (5′→3′)	Amplicon (*bp*)	Reference
LT	LTA-1	GGCGACAGATTATACCGTGC	696	Schultsz
	LTA-2	CCGAATTCTGTTATATATGTC		*et al.*, 1994
STa	STI-1	TTAATAGCACCCGGTACAAGCAGG	147	Olsvik
	STI-2	CTTGACTCTTCAAAAGAGAAAATTAC		*et al.*, 1993
STb	STb-1	ATCGCATTTCTTCTTGCATC	172	Blanco
	STb-2	GGGCGCCAAAGCATGCTCC		*et al.*, 1997
VT1	VT1-A	GAAGAGTCCGTGGGATTACG	130	Pollard
	VT1-B	AGCGATGCAGCTATTAATAA		*et al.*, 1990
VT2	VT2-3	CCGTCAGGACTGTCTGAAAC	726	Woodward
	VT2-5	GAGTCTGACAGGCAACTGTC		*et al.*, 1992
pap	pap-*1*	GCAACAGCAACGCTGGTTGCATCAT	336	Yamamoto
	pap-2	AGAGAGAGCCACTCTTATACGGACA		*et al.*, 1995
hly	hly-*1*	AACAAGGATAAGCACTGTTCTGGCT	1177	Yamamoto
	hly-2	ACCATATAAGCGGTCATTCCCGTCA		*et al.* 1995
iuc	iuc-*1*	TACCGGATTGTCATATGCAGACCGT	602	Yamamoto
	iuc-2	AATATCTTCCTCCAGTCCGGAGAAG		*et al.*,1995
cnf	cnf1	AAGATGGAGTTTCCTATGCAGGAG	498	Yamamoto
	cnf2	CATTCAGAGTCCTGCCCTCATTATT		*et al.,* 1995
sfa	sfa-*1*	CTCCGGAGAACTGGGTGCATCTTAC	410	Yamamoto
	sfa-2	CGGAGGAGTAATTACAAACCTGGCA		*et al.* 1995
afa	afa-*1*	GCTGGGCAGCAAACTGATAACCTC	750	Yamamoto
	afa-2	CATCAAGCTGTTTGTTCGTCCGCCG		*et al.* 1995

E. coli strains were cultured in brain and heart infusion broth (BHI, Difco), at 37ºC for 18-24 h. Two hundred µl of culture were submitted to DNA extraction using the guanidium thiocyanate method described by Boom *et al.* (1990). DNA samples were kept at -20ºC until PCR assays. Amplification was conducted on a DNA thermal cycler (PT-200, MJ Research Watertown, Massachusetts, USA). The standard PCR amplification mixture consisted of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin, 200 µM of each of the four deoxinucleosides triphosphates, 20 pico moles of each primer being tested, 0.2 µL of Taq DNA polymerase, 0.5 µL of DNA template and ultra pure water to a final volume of 50 µL. The mixture was submitted to an initial denaturing step of 95ºC for 5 minutes, followed by 35 cycles of 95ºC for 1 minute, 55ºC for 1 minute, 72ºC for 1 minute and a final cycle of 72ºC for 5 minutes. The amplified products were separated on a 2% agarose gel and examined eletrophoretically after staining with ethidium bromide. A 100 bp DNA ladder (Invitrogen, California) was used as a molecular size marker.

Antimicrobial susceptibility of the E. coli isolates

The most frequent microorganism isolated from the samples of intrauterine content was submitted to tests of susceptibility to antimicrobials. Commercially prepared antimicrobial sensitivity discs (Laborclin®, Paraná, Brazil) having the following antimicrobial agents and concentrations were used: chloramphenicol (30 µg), tetracycline (30 µg), oxacillin (1 µg), penicillin (10 U.I.), cephalothin (30 µg), lincomicyn (2 µg), gentamicin (10 µg), vancomycin (30 µg), erythromycin (15 µg), sulphazotrin (25 µg), ampicillin (10 µg), cephacetril (30 µg), enrofloxacin (5 µg), norfloxacin (30 µg), cefalexin (30 µg), polimixin B (300 µg), tobramicin (10 µg), amoxicilin (10 µg), cefoxitin (30 µg), clindamicin (2 µg), neomicin (30 µg) and amicacin (30 µg).

The cultures were tested for antimicrobial susceptibility by the Kirby and Bauer standardized disk diffusion method (1). Cultures were classified as sensitive, intermediate and resistant considering the diameter of the growth zone of inhibition. The concentrations as well as the criteria used for interpretation were the ones recommended by the "Clinical and Laboratory Standards Institute- CLSI" (5).

Counting of microorganisms colonies forming units in samples of intrauterine content of bitches with pyometra

The samples of intrauterine content from which microorganisms were isolated were diluted in sterile physiological solution (0.85%). From each of the dilutions, 0.1 mLs were cultured (using the *pour plate* technique) in duplicate in Plate-Count agar (Oxoid). The plates were then incubated at 37ºC for 48 hours in order to evaluate the quantity of colonies forming units (C.F.U.) of the microorganisms.

Histopathological examination

Two hundred samples of fragments of uterine walls from bitches with pyometra were collected for histopathological examination and placed in vials containing 10% formaldehyde.

The fragments were fixed in 10% formaldehyde, embedded in paraffin and cut in 5 mm sections. The sections were stained with haematoxylin and eosin (H & E), placed on a glass slide and covered with a cover slip and examined under the light microscope for histological changes.

In order to evaluate the intensity of the inflammatory response, a calibrated reticulum was acoplated to the microscope's ocular. Ten microscopical fields with cellular inflammatory infiltrates were examined in each fragment.

Using the 400X magnification, the presence or absence of inflammatory response was evaluated. A score ranging from 0 to 4 was established according to the area occupied by the inflammatory response, as can be seen in Table 2.

Table 2. Correlation between the scores and the sizes of the areas occupied by inflammatory responses.

Score	Size of the area occupied by the cellular infiltrate which characterizes the inflammatory response (in percentage)
0	<20
1	20 - 38
2	39 - 56
3	57 - 75
4	>75

RESULTS

Microbiological examination

From a total of 200 samples analyzed, bacterial growth was observed in 197 samples (98.5%). The 3 samples without bacterial growth corresponded to one uterine horn from three bitches, where the other uterine horn presented bacterial growth. So, the 100 bitches analyzed presented bacterial growth in at least one uterine horn.

Considering the 197 samples in which the presence of microorganims was verified, the following bacteria were isolated: *E.coli* (74.1%) from 146 samples, *Klebsiella pneumoniae* subsp. *pneumoniae* (3%) from 6 samples, *Citrobacter diversus* (3%) from 6 samples, *Pseudomonas aeruginosa* (2%) from 4 samples, *Staphylococcus kloosii* (2%) from 4 samples, *Salmonella* spp. (2%) from 4 samples, *Proteus mirabilis* (2%) from 4 samples, *Streptococcus* sp. (1%) from 4 samples, *Morganella morgani* (1%) from 2 samples, *Klebsiella pneumoniae* subsp. *azanae* (1%) from 2 samples, *Staphylococcus schleiferi* subsp.

coagulans (1%) from 2 samples, *Staphylococcus intermedius* (1%) from 2 samples, *Staphylococcus epidermidis* (1%) from 2 samples, *Streptococcus canis* (1%) from 2 samples, and *Corynebacterium jeikeium* (1%) from 2 samples. The occurrence of associations of microorganisms was observed in five (2.5%) samples and the following microorganisms were isolated: *E. coli* and *Staphylococcus kloosii* from two uterine horns, *E. coli* and *Enterococcus faecium* from two uterine horns, *E. coli* and *Streptococcus* sp. from one uterine horn and *E. coli* from the other uterine horn. The frequency of isolations of *E. coli* (76.6%) was statistically higher (P<0.05) when compared with the other microorganisms, considering the samples that *E. coli* was isolated alone and in association with other bacteria. The occurrence of moulds and yeasts was not observed in any of the samples.

Research of virulence factors in *E. coli* isolates

Of the 151 *E.coli* isolates, 120 (79.5%) were positive for *sfa*, 87 (57.6%) were positive for *pap*, 86 (56.9%) were positive for *cnf*, 52 (34.4%) were positive for *hly*, 51 (33.8%) were positive for *iuc* and 5 (3.3%) were positive for *afa*. None of the samples analyzed were positive for LT1, LT2, Sta, STb, VT1 and VT2. Of the 151 samples, 3 (2.0%) did not present positivity for any of the virulence factors studied in this research.

Antimicrobial susceptibility of the isolates

Considering the 151 strains of *E. coli*, 86.1% were resistant to cephalothin, 68.9% to ampicillin, 46.4% to cefoxitin, 34.4% to tobramicin, 32.5% to tetracycline, 29.8% to amicacin, 27.8% to cefalexin, 15.2% to gentamicin, 13.9% to cefotaxim, 13.2% to sulphazotrin, 12.6% to enrofloxacin, 10.6% to aztreonam, 7.9% to chloramphenicol, 6% to neomycin, 2% to norfloxacin and 0.7% to polimixin B.

The highest sensitivity was to norfloxacin (94%), polimixin B (82.8%), sulphazotrin (76.8%), enrofloxacin (75.5%) and chloramphenicol (75.5%).

Counting of microorganisms colonies forming units in samples of intrauterine content of bitches with pyometra

The counting of microorganism colonies forming units in samples of intra-uterine content of bitches with pyometra is shown in Table 3.

Histopathological examination

The results of the histopathological examinations of samples of uterine walls of bitches with pyometra are showed in Table 4.

In 42% of the samples on which microorganisms were isolated, the sizes of the areas of the inflammatory responses corresponded to 39-56% (score 2).

The size of the inflammatory response in samples of uterine fragments where *E. coli* was isolated was not larger when compared to the ones with other isolated microorganisms.

It was observed that the larger the number of microorganisms colonies forming units/mL, the greater the intensity of the inflammatory response (P<0.0004) and the correlation coefficient (r) was 0.2338.

Table 3. Results of the counting of microorganisms colonies forming units in samples of intrauterine content of bitches with pyometra.

Microorganism (MO) isolated	Number of samples from which the MO was isolated	Median of C.F.U./mL	Minimum-Maximun
Escherichia coli	151	7,600,000	300 - 13,700,000,000
Klebsiella pneumoniae subsp. *pneumoniae*	6	13,450,000	695,000 - 15,600,000
Citrobacter diversus	6	11,950,000	500,000 - 99,000,000
Staphylococcus kloosii	6	1,140,500	225,000 - 11,200,000
Streptococcus spp.	5	18,500,000	16,200,000 - 104,000,000
Pseudomonas aeruginosa	4	907,500	80,000 - 1,440,000
Salmonella spp.	4	12,100,000	10,000,000 - 18,900,000
Proteus mirabilis	4	850,350	600 - 1,790,000
Morganella morgani	2	1,450	1,300 - 1,600
Klebsiella pneumoniae subsp. *azanae*	2	862,500	685,000 - 1,040,000
Staphylococcus schleiferi subsp. *coagulans*	2	36,100,000	9,700,000 - 62,500,000
Staphylococcus intermedius	2	1,308,000,000	266,000,000 - 2,350,000,000
Staphylococcus epidermidis	2	1,185,000	1,060,000 - 1,310,000
Streptococcus canis	2	624,000	138,000 - 1,110,000
Corynebacterium jeikeium	2	10,000	5,000 - 15,000
Enterococcus faecium	2	440,000	405,000 - 475,000

Table 4. Results of the histopathological examination of samples of the uterine walls of bitches with pyometra considering the microorganism and the size of the inflammatory response (in score).

Microorganism	Number of samples from which the MO was isolated	Score 0 (%)	Score 1 (%)	Score 2 (%)	Score 3 (%)	Score 4 (%)
E.coli	146	6.2	21.2	41.1	24.7	6.8
Gram negative bacteria (other than *E. coli*)	28	0	21.4	50	25	3.6
Gram positive bacteria	18	0	44.4	44.4	5.6	5.6
Association between *E. coli* and Gram positive bacteria	5	0	40	40	20	0
Absence of microorganism	3	33.3	0	33.3	0	33.3

DISCUSSION

Fransson *et al.* (9) isolated a 90% rate of *E.coli* in bitches with pyometra. In this study, *E.coli* was isolated in 76.6% of the samples with bacterial growth, with occurrence statistically larger ($P<0.05$) than other agents isolated, data that was also observed by and Fransson *et al.* (9).

Oluoch *et al.* (18) studied 674 strains of *E. coli* isolated from different types of infections in dogs including the genitourinary tract and observed 90% of sensitivity of these microorganisms to norfloxacin, 87.5% to enrofloxacin, 90.7% to gentamicin and 85.9% to amicacin. Considering the 151 strains of *E. coli* isolated in the present study, a higher resistance was observed to cephalothin (86.1%) and ampicillin (68.9%) and the highest sensitivity was to norfloxacin (94%), and considering enrofloxacin, gentamicin and amicacin, the percentages of sensitivity were of, respectively, 75.5%, 70.2% and 55.6%, inferior percentages when compared to those observed by Oluoch *et al.* (18).

Dhaliwal *et al.* (7) tried to correlate sorotypes of isolated *E. coli* with histological changes on the uterine wall. The study was carried out with 34 samples of uterine tissue, which can be considered as a small number of samples to make an adequate correlation between clinical signs, strains of *Escherichia coli* and the severity of the lesions, and taking into account the diversity of the sorotype characteristics of this microorganism. The study was carried out in different veterinary clinics with the participation of different surgeons, each one adopting their own procedure for the surgical removal of the uterus and the fragments used in the study. The researchers concluded that a much higher number of samples would be necessary to make correlations, and preferably all samples should come from one unique place, and with the participation of only one surgeon, to allow the research and sampling to be fair. In the present study, differently as done by Dhaliwal *et al.* (7), 100 samples of uterus from bitches with pyometra were collected for histopathological examinations, being one sample from each uterine horn, totalizing 200 samples. Only one surgeon performed all the surgeries, and the uterine wall samples were always collected by the same person using the same procedure every time.

Considering the 146 strains of *E. coli* which were isolated, 46 (31.5%) were associated to an inflammatory response that occupied an area larger than 57%. The sizes of the inflammatory responses present in the uterine fragments where the isolation of *E. coli* was observed, were not larger than the ones verified in samples in which other microorganisms were isolated ($P<0.05$). However, regarding all the microorganisms which were isolated including *E. coli*, it was observed that the larger the quantity of microorganism colonies forming units/mL the larger the size of the inflammatory response ($P<0.0004$). Although this correlation was statistically significant, it was low ($r = 0.2338$), pointing out that factors other than the quantity of microorganisms colonies forming units/mL may exist, influencing the occurrence and intensity of the inflammatory response.

The uropathogenic *E. coli* (UPEC) causes urinary tract diseases in humans and in animals. The bacteria move from the gastrointestinal tract to the urinary tract. Most of the urinary tract infections start by the colonization of the colon by a *E. coli* strain capable of causing urinary tract infections (22).

The most important adhesin in strains that cause kidney infections is the P pili. The gene involved is called *pap* (pyelonephriti associated pili) (22). Johnson *et al.* (13) analyzed 63 samples of feces of dogs and cultured *E. coli* in 30% and observed the *pap* gene in 56%. With this data they concluded that ExPEC (extra-intestinal pathogenic *E. coli*) is present in canine feces and could be a reservoir of ExPEC to humans. In the present study, 57.6% of the *E.coli* isolated were positive for the *pap* gene, data very similar to what Johnson *et al.* (13) found in feces of healthy dogs. This suggests that *E. coli* cultured from the uterus of dogs with pyometra can be originally from the intestinal flora of the same dog.

The S fimbria (*sfa*) is also an important adhesin associated with urinary tract diseases in humans and animals (22). In the current study, 79.5% of the *E. coli* isolated were positive for *sfa*, showing that this adhesin must have an important role in the colonization of the uterus.

The uropathogenic strains also have afimbrial adhesions (*afa*I and *afa*III) (22). In this study only 3.3% of the samples of *E. coli* isolated were positive for the *afa* gene. This adhesin probably has a very small role in the colonization of the uterus.

Some uropathogenic *E.coli* produce an extra-cellular toxin originally called hemolysin (*hly* gene) because it destroys erythrocytes and other cells with the stimulation of an inflammatory response (17,22). Studies have already proven that the production of hemolysin is associated with strains of *Escherichia coli* that causes extra-intestinal infections in humans (12). Strains of *E. coli* isolated from dogs and cats with urinary tract infections have a high prevalence of *hly* (25). Low *et al.* (16) compared *pap* and *hly* genes of dogs and humans with urinary tract infections and observed that all the isolates had a similar DNA. These results suggest that some *E.coli* strains can be capable of infecting dogs and humans.

In this study, 34.4% of the samples of *E. coli* isolated were positive for *hly*. This number is below what is observed in humans with urinary tract infections, suggesting that this virulence factor has a smaller role in canine pyometra than in urinary tract infections.

The cytotoxic necrotizing factor (*cnf*) has been described in recent years as being associated to a large variety of infections in man and animals, being considered an important virulence mechanism of *E. coli* (27). Two types of cytotoxic necrotizing factors, *cnf*1 and *cnf*2, have been already described. The production of *cnf*1 is associated to the production of *a*-hemolysin, and *E. coli* producing *cnf*1 have been isolated from intestinal and extra-intestinal infections of dogs and from feces of healthy dogs (20). In this study *cnf* was detected at a high rate (56.9%), data also observed by Pohl *et al.* (20), Wray and Woodward (27) and Beutin (2).

Other factors also contribute to the virulence of uropathogenic *E. coli*, such as the acquisition of iron. These *E. coli* have many mechanisms of uptake of iron (22). The aerobactin (*iuc* gene) is the most effective system of chelate of iron used by enteric bacteria for the acquisition of iron (6).

In this study, 33.8% of the samples were positive for the *iuc* gene, data also observed by De Lorenzo and Martinez (6) in feces of healthy dogs. This again suggests that *E. coli* present in pyometra in dogs is originally from the fecal flora of the very same dog.

Von Sydow *et al.* (24) verified the presence of 89.21% of *E. coli* in feces of healthy dogs and observed that 76% of the strains were positive for virulence factors. The virulence factors detected with higher rates were *iuc* (48%), *sfa* (40%) and *pap* (24%), and 57.14% of the strains were positive to more than one virulence factor. In this study virulence factors were identified in 98.0% of the *Escherichia coli* strains evaluated, showing a high rate of potentially pathogenic *E. coli* ($P<0.05$), and the factors with higher rates were *sfa* (79.5%), *pap* (57.6%) and *cnf* (56.9%) and 80.4% of the strains were positive to more than one virulence factor. These rates are different to those observed by Von Sydow *et al.* (24) in feces of healthy dogs, suggesting that these virulence factors have an important role in the colonization of the uterus.

In this study none of the *E. coli* strains were positive for the LT, Sta, STb, VT1 and VT2 toxins, suggesting that theses virulence factors don't have an important role in canine pyometra.

Virulence factors were identified in 98.0% of the strains evaluated, demonstrating a high frequency of potentially pathogenic *E. coli*. It must be considered that dogs are animals that are living in close proximity to man for thousands of years and have an important role in the transmission of *E. coli* to other animals and to man.

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RESUMO

Aspectos microbiológicos e histopatológicos da piometria canina

A piometra é uma enfermidade da cadela adulta, sendo a doença reconhecida como uma das causas mais comuns de morte desta espécie animal. Os objetivos deste trabalho foram a avaliação de aspectos microbiológicos e histopatológicos da piometra canina e pesquisa de fatores de virulência de *E. coli,* identificando possíveis riscos para a saúde humana. Foi realizado o exame microbiológico de conteúdo intra-uterino de 100 cadelas com piometra bem como a contagem de unidades formadoras de colônias de microrganismos nestas amostras, testes de susceptibilidade "in vitro" aos antimicrobianos de *E. coli* e pesquisa de fatores de virulência nestas estirpes, e exame histopatológico de amostras de útero. *Escherichia coli* foi o microrganismo mais freqüentemente isolado (76,6%), sendo que 120 estirpes (79,5%) foram positivas para os genes *sfa*, 86 (56,9%) foram positivas para *cnf*, 87 (57,6%) foram positivas para *pap*, 52 (34,4%) foram positivas para *hly*, 51 (33,8%) foram positivas para *iuc* e 5 (3,3%) foram positivas para *afa*. Observou-se maior sensibilidade de *E.coli* à norfloxacina, polimixina B, sulfazotrim, cloranfenicol e enrofloxacina. Em 42% das amostras de parede uterina nas quais foram isolados microrganismos, os tamanhos das áreas de processo inflamatório corresponderam a 39-56%. Foram identificados fatores de virulência em 98,0% das estirpes de *Escherichia coli* avaliadas, demonstrando uma alta freqüência de *E. coli* potencialmente patogênica. Deve-se considerar que os cães são animais que vivem em proximidade aos homens há milhares

de anos e possuem um papel importante na transmissão de *E. coli* aos outros animais e também ao homem.

Palavras-chave: cadelas, *Escherichia coli*, histopatológico, microbiológico, piometra

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