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, polymixin 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
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 nulliparous, 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 37°C for 24-96 hours. The samples were also cultured aerobically in BHI broth (Brain and Heart Infusion broth, Difco) at 37°C 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*), *S* fimbiae (*sfa*), afimbrial adhesin 1 (*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.

| Gene | Primer | Oligonucleotide pairs (5´→3´) | Amplicon (bp) | Reference |
|------|--------|-------------------------------|--------------|-----------|
| *LT* | LTA-1  | GCCGACAGATTATACGC              | 696          | SCHULTSZ et al., 1994 |
|      | LTA-2  | CCGAATTCTGTTATATGTC            |              |           |
| STa  | STI-1  | TTAATAGACCGGTACAAGCGG          | 147          | OLSVIK et al., 1993 |
|      | STI-2  | CGTACTCTTCTAAAAGATAAAATTAC     |              |           |
| STb  | STb-1  | ATCGCATTCTTCTGGCATC            | 172          | BLANCO et al., 1997 |
|      | STb-2  | GGGCAGCAGCAAGCATGCTCC          |              |           |
| VT1  | VT1-A  | GAAGAGTCGTTGGAATTCG            | 130          | POLLARD et al., 1990 |
|      | VT1-B  | AGCGATGCAAGTATAAA             |              |           |
| VT2  | VT2-3  | CCGTCAGAAGCTGCTGAAC           | 726          | WOODWARD et al., 1992 |
|      | VT2-5  | GAGTCGAGAGGCAACTTCTC          |              |           |
| pap  | pap-1  | GCAACAGCAAGCCTGTTGACTCAT      | 336          | YAMAMOTO et al., 1995 |
|      | pap-2  | AGAGAGCCACTTCTTACGGA          |              |           |
| hly  | hly-1  | AACAAGGAATAAGCCTGTTGCT        | 1177         | YAMAMOTO et al., 1995 |
|      | hly-2  | ACCATATAAGCGGCTATTCCGCT       |              |           |
| iuc  | iuc-1  | TACCGAGTGTCATATTGCAAGAAG      | 602          | YAMAMOTO et al., 1995 |
|      | iuc-2  | ACTATCTCTCTCAGGCTGGAAGA       |              |           |
| cnf  | cnf1   | AAGATGAGATTCTTTCTATGCAAGAG    | 498          | YAMAMOTO et al., 1995 |
|      | cnf2   | CATTCAAGTCTCTGCCCATTATT       |              |           |
| sfa  | sfa-1  | CTCGGAGAAGCTGGTGCACTTACTC     | 410          | YAMAMOTO et al., 1995 |
|      | sfa-2  | CGAGAGAGTAAATACAACCTGGCA      |              |           |
| afa  | afa-1  | GCTGGGCGAGCAAAGCTGATAACCTC    | 750          | YAMAMOTO et al., 1995 |
|      | afa-2  | CATCAAGCTTGGTTCTGCAGGCC       |              |           |
E. coli strains were cultured in brain and heart infusion broth (BHI, Difco), at 37°C 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 -20°C 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 MgCl₂, 0.001% (w/v) gelatin, 200 μM of each of the four deoxynucleosides 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 95°C for 5 minutes, followed by 35 cycles of 95°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute and a final cycle of 72°C for 5 minutes. The amplified products were separated on a 2% agarose gel and examined electrophoretically 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), lincomycin (2 μg), gentamicin (10 μg), vancomycin (30 μg), erythromycin (15 μg), sulphadiazine (25 μg), ampicillin (10 μg), cephalaxin (30 μg), enrofloxacin (5 μg), norfloxacin (30 μg), cefalexin (30 μg), sulphamethoxazole/trimethoprim (15 μg), amoxicillin (10 μg), clindamycin (2 μg), neomycin (30 μg) and amoxicillin (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 37°C 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 microorganisms 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.
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colans (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 tobramycin, 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 polymixin B. The highest sensitivity was to norfloxacin (94%), polymixin B (82.8%), sulphazotrin (76.8%), enrofloxacin (75.5%) and chloramphenicol (75.5%).

**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                                         | 1,030,000,000      | 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           | 1.4         | 5.0         | 25          | 3.6         |
| Gram positive bacteria               | 18                                               | 0           | 44.4        | 44.4        | 5.6         | 5.6         |
| Association between E. coli and      | 5                                                | 0           | 40          | 40          | 20          | 0           |
| Gram positive bacteria               |                                                  |             |             |             |             |             |
| 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, totaling 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 pilu) (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 (afaI and afaII) (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, cnf1 and cnf2, have been already described. The production of cnf1 is associated to the production of α-hemolysin, and E. coli producing cnf1 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 (iap 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 iap 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 these 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 paredes uterinas 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 patogênicas. Deve-se considerar que os cães são animais que vivem em proximidade aos homens há milhares de anos; se embora não infectem diretamente, podemos considerá-los como fonte de transmissão de microrganismos patogênicos.
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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