Journal of Animal and Veterinary Advances 10 (8): 965-971, 2011
ISSN: 1680-5593

Aerobes and Levels of Estradiol and Progesterone in Cystic Endometrial Hyperplasia-Pyometra Complex Bitches

Xian Gao, Lixia Zhou, Ganzhen Deng, Chang Liu, Chengye Li, Changwei Qiu and Xiuli Peng
Veterinary Medical College, Huazhong Agricultural University, 430070 Wuhan, China

Abstract: To determine which aerobic bacteria exert a marked effect on Cystic Endometrial Hyperplasia-Pyometra complex (CEH-P) and to determine from where these bacteria originate. About 48 privately owned bitches were diagnosed with CEH-P. Healthy vagina and uterine samples were obtained with cotton pledgets and cumulative uterine fluids from CEH-P cysts were collected with a syringe in utero to isolate and identify aerobic bacterial. A total of 99 isolates were identified and the primary aerobe in the CEH-P bitches was found to be *Escherichia coli* (33/99). The levels of estrode in bitches with endometritis and in bitches with pyometra and estrus were higher than the levels in diestrus bitches ($p<0.01$). When ranked according to the levels of progesterone in a descending order (i.e., from high to low levels), the ranking was bitches with pyometra, bitches with endometritis, bitches in estrus and bitches in diestrus. These results suggest that in addition to common pathogenic bacteria differential in every area, casual bacterial contamination invariably occurred in bitches with CEH-P and the primary infection ascended from the vagina when the cervix was open. The researchers also inferred that both estrode and progesterone levels in CEH-P bitches were increased relative to the levels in healthy bitches and were even higher than that of the levels observed in estrus bitches. Estrode and progesterone pretreatment and uterine mucosa damage were interpreted as the preconditions for bacterial infection. The hormone levels in CEH-P bitches were similar to the endocrinal characteristics of peripartum bitches.

Key words: CEH-P, aerobes, progesterone and estrode levels, bitch, preconditions, China

INTRODUCTION

Pyometra is an emergent disease in both dogs and cats that causes dysfunction in animal reproduction and results in death due to septicemia and toxemia (Pretzer, 2008). Dogs with pyometra exhibit symptoms such as fever, polydipsia, vomiting, polyuria, abdominal distention, purulent vaginal discharge, tachycardia, tachypnea, rapid deterioration and sepsis (Wiebe and Howard, 2009). Due to its pathological characteristics (endometrial hyperplasia, adenomyosis, hemorrhage, purulence and mucometra or hydrometra) and its pathogenic characteristics, the disease is referred to as Cystic Endometrial Hyperplasia-Pyometra complex (CEH-P) (Ajadi *et al*., 2008; Bigliardi *et al*., 2004; Martinez-Jimenez *et al*., 2009; Hagman *et al*., 2006; Iglesias-Nunez *et al*., 2008).

CEH-P is usually considered to be induced by pathological changes in the endometrium due to overexposure to progesterone by successive heat cycles and progesterone treatment (Niskanen and Thrusfield, 1998) and bacteria are considered to be the primary factors causing CEH-P (Pretzer, 2008; Smith, 2006).

However, very few studies of the relative estrode levels in dogs with CEH-P have been reported. The objective of this study was to determine which aerobic bacteria produce a marked effect on CEH-P pathology, the origin of these bacteria. It was also analyzed the relative progesterone and estrode concentrations in the blood of bitches with CEH-P.

MATERIALS AND METHODS

Cumulative fluids and serum from CEH-P bitches: This protocol was approved by Huazhong Agricultural University (Wuhan 430070, China). About 48 privately owned bitches were diagnosed with CEH-P including pyometra and endometritis. All bitches were treated by ovariohysterectomy. The diagnosis was based on case history and physical examinations and ultrasonography was applied to demonstrate an enlarged, fluid-filled uterus (Pretzer, 2008).

Further diagnosis of CEH-P was verified visually during the ovariohysterectomy and was validated by histopathological examination (Schlafer and Gifford, 2008). Cumulative fluids were collected with a syringe from

Corresponding Author: Ganzhen Deng, Veterinary Medical College, Huazhong Agricultural University, 430070 Wuhan, China

pyometra cysts *in vitro*. The samples were brought back to the laboratory for bacterial culture and identification.

Uterine secretions from healthy bitches: Uterine secretions were obtained from healthy bitches during sterilization for ovariohysterectomy. Uteri were brought to the laboratory for bacteria culture and identification.

Vaginal secretions from healthy bitches: Vaginal secretions were collected with sterilized cotton pledgets from healthy bitches. The pledget was immediately put into a sterilized test tube containing 1 mL physiological saline and was brought to the laboratory for bacteria culture and identification.

Sera from healthy and CEH-P bitches: About 32 sera samples were obtained for the measurement of progesterone and estrode concentrations. Sera from bitches with endometritis (13) and pyometra (9) were obtained from the above-mentioned patients and from among the nine healthy bitches, sera were obtained from bitches without any clinical disease in diestrum (5) and from bitches in estrus (4).

Culturing of isolated aerobes: The sample from uteruses and vaginas were cultured in nutrient broth. Broth opacity indicated aerobe growth. All samples were sectional streaked on a blood plate, MacConkey Agar, Salmonella Shigella Agar and Eosin-methylene blue agar and were cultured at 37°C for 24 h. Mixed colonies were streaked again to isolate a pure culture. The characteristics such as size, opacity, shape, superficies, altitude, fringe, burnish, hardness, smell, colorant and haemolyticus of every colony in each cultural medium were recorded in detail. Colonies were transferred into tubes containing culture medium and were cultured at 37°C for 18~24 h. Each strain was stored at 4°C for later biochemical identification.

Identification of aerobes: Colonies from the stored tubes were rejuvenated and proliferated in nutrient broth at 37°C for 8 h. Each strain was smeared and dyed with gram staining. Strains were initially categorized as gram-positive coccus and bacilli or gram-negative coccus and bacilli based on their chromaticity, shape, size and alignment. Bacteria were transferred onto solid media and were cultured aerobically at 37°C for 18~24 h.

For further identification, cocci were cultured aerobically at 37°C for 24 h in Staphylococci biochemical assessor, O-F microdosis assessor, sucrose assessor, lactose assessor, sorbin assessor, amylomaltose assessor and indole assessor. The culture media and conditions for the bacilli were the same for cocci except Enterobacteriaceae assessor was used instead of the Staphylococci biochemical assessor. Identified results were recorded in detail.

Pathological model of CEH-P with aerobes: About 6 healthy hybrid bitches were randomly divided into 3 groups and each group contained three bitches. All of the bitches were injected subcutaneously with diethylstilbestrol. Aerobe broth (5 mL, 2×10^5 CFU mL^{-1}) was introduced into their uteruses with a catheter and a syringe in the trial group three times every 2 days. The control animals were inoculated with broth without bacteria. Body temperature, vaginal secretion characteristics, vaginal mucosa characteristics and uterine ultrasonography results were recorded in detail for 1 month.

Pathogenicity of aerobes in mice: About 54 mice were randomly divided into 8 groups. Aerobe broth (1 mL, 2×10^5 CFU mL^{-1}) made from bacteria isolated from bitches with pyometra was introduced into the abdominal cavities of the mice. Broths with the following aerobes were used (one aerobe per group of mice): *Escherichia coli*, *Salmonella enterica*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus agalactiae*, Shigella and Corynebacteria. The group injected with broth without any bacteria served as a control. The experiment lasted 5 days and the morbidity of the mice was recorded.

Analysis of progesterone and estrode concentrations in sera: An iodine [^{125}I] Progesterone radioimmunoassay kit and an iodine [^{125}I] Estradiol radioimmunoassay kit were used to measure the progesterone and estradiol concentrations in sera. SPSS 13.0 was used for data analysis.

RESULTS

The number of species and strains of aerobic bacteria: No bacteria were isolated from healthy uteruses. All of the aerobes isolated from vaginas and from the uteruses of bitches with CEH-P are shown in Table 1. About 8 aerobes (99 colonies) were isolated and identified. The most frequent aerobe was *Escherichia coli* (33/99); *Salmonella enterica* (14/99), *Proteus mirabilis* (13/99), *Pseudomonas aeruginosa* (13/99), *Staphylococcus aureus* (12/99), *Streptococcus agalactiae* (7/99), Shigella (6/99) and Corynebacteria (1/99) were also found.

Aerobes in bitches with CEH-P: The aerobes isolated from the CEH-P contents are shown in Table 2. About 8 aerobes species were identified from the 87 colonies that

Table 1: The results of microscopic examination and biochemical identification of the aerobes

Bacteria	*Escherichia coli*	*Salmonella enterica*	*Proteus mirabilis*	*Pseudomonas aureus*	*Staphylococci aeruginosa*	*Streptococcus agalactiae*	Shigella	Corynebacteria
Colony amount	33	14	13	13	12	7	6	1
$G^{+/-}$	G^-	G^-	G^-	G^-	G^+	G^+	G^-	G^+
Haemolyticus	+/-	-	-	+	+	+	-	-
O/F test	Fermentation	Fermentation	Fermentation	Fermentation	Fermentation	Fermentation	Fermentation	Fermentation
Triple sugar iron	bevel acid (+) bevel acid(+) button acid (+) aerosis (+) H_2S (-)	bevel alkali (+) button acid (+) aerosis (+) H_2S (-)	bevel acid (+) alkali (+) aerosis (+) H_2S (+)	bevel alkali (+) button acid and alkali (+) aerosis (-) H_2S (+)	bevel acid (+) button acid (+) aerosis (-) H_2S (-)	bevel acid (+) button acid (+) aerosis (-) H_2S (-)	bevel alkali (+) button acid (+) aerosis (-) H_2S (-)	button acid (+) aerosis (+) H_2S (-)
Benzopyrrole test	+	-	-/+	-	-	-	-	+
Methyl red test	+	+	+	-	+	+/-	+	+
VP test	-	-	-	-	-	-	-	-
Gelatin test	-	-	-/+	+	-	-/+	+	-/+
Esculin	-/+	-	-	-	+	+	+	-
40% bile	-	-	-	+	-	-	-	-
Citral test citric acid test	-	-	-/+	+	-	-	-	-
Sorbite	+	-	-	-	-	-	+	+
Manicol	+	-	+/-	-	+/-	-/+	+	+
Glucose	+	-	+	+	+	+	+	+
Lactose	+	-	+/-	-	-	+/-	-	+
Sucrose	+	-	+/-	-	-/+	-/+	-	+
Urea	-	-	-/+	+	-	-/+	-	+

+positive, -negative, +/-many strains positive, -/+many strains negative

Table 2: The identification results for the aerobes isolated from pyometra contents

	Pyometra contents		Vaginal secretion		Total	
Bacterium	Colony amount	Percent	Colony amount	Percent	Colony amount	Percent
Escherichia coli	30	30.3	3	3.0	33	33.0
Salmonella enterica	11	11.1	3	3.0	14	14.1
Proteus mirabilis	11	11.1	2	2.0	13	13.1
Pseudomonas aeruginosa	12	12.1	1	1.0	13	13.1
Staphylococcus aureus	10	11.1	2	2.0	12	13.1
Streptococcus agalactiae	7	7.0	-	-	7	7.0
Shigella	5	5.0	1	1.0	6	6.0
Corynebacterium	1	1.0	-	-	1	1.0
Total	87	87.8	12	12.2	99	100.0

were isolated from bitches with CEH-P. The aerobes were the same as those listed above. The total number of colonies was 87 (87/99) and the most frequent bacterium was *E. coli*.

Aerobes in vaginas: About 6 aerobes (*Escherichia coli*, *Salmonella enterica*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and Shigella) were isolated from vaginal secretions in healthy dogs (Table 2). Researchers did not detect *Streptococcus agalactiae* or Corynebacteria and the number of colonies was low (12/99).

Pathogenicity of aerobes in bitches: The bitches treated with diethylstilbestrol and bacteria exhibited vaginal endomembrane hyperemia, estus and yellow mucous secretions. However, they recovered 3 days later. The bitches without treatment only exhibited vaginal endomembrane hyperemia and estus and they recovered on the 2nd day. None of the bitches showed any abnormality after 1 month.

Pathogenicity of aerobes in mice: Mice inoculated with bacteria started to die on the 2nd day; their morbidity and time of death are shown in Table 3. All of the mice treated with *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* died on the 2nd day; all of the mice treated with *Salmonella enterica* died on the 3rd day. The frequencies of mortality in the other groups ranged from 66.7-88.3%. There was no death in the control group. The bacteria isolated from the ascites of dead mice were the same as those from the inoculation.

Estradiol and progesterone concentrations in sera: The levels of estrode in bitches with endometritis, bitches with pyometra and bitches in estrus were higher than that in diestrus bitches ($p<0.01$). In addition, the level of estrode was higher in pyometra bitches than in estrus bitches ($p<0.05$). The estrode concentration in endometritis bitches was higher than that in estrus bitches ($p<0.01$). No significant difference in the estradiol levels were found between endometritis and pyometra bitches. Progesterone concentrations were different among all 4 groups ($p<0.01$)

Table 3: The morbidities of mice treated with different bacteria

Bacterium	No. of deaths at 12 h	No. of deaths at 24 h	No. of deaths at 48 h	No. of deaths at 72 h	No. of deaths at 96 h	Total no. of deaths	Morbidity (%)
Staphylococcus aureus	0	2	4	0	0	6	100.0
Escherichia coli	0	4	2	0	0	6	100.0
Pseudomonas aeruginosa	3	2	1	0	0	6	100.0
Salmonella enterica	0	0	4	2	0	6	100.0
Streptococcus agalactiae	0	3	2	0	0	5	83.3
Shigella	0	3	2	0	0	5	83.3
Corynebacteria	0	4	0	0	0	4	66.7
Proteus mirabilis	0	4	0	0	0	4	66.7
Control	0	0	0	0	0	0	0.0

Table 4: The levels of estrode and progesterone in the blood

Groups	Samples	Estrode concentration ($pg\,mL^{-1}$)	Progesterone concentration ($ng\,mL^{-1}$)
Diestrus bitches	5	$34.77\pm14.52^{a\,A}$	0.52 ± 0.29^{A}
Estrus bitches	4	$535.37\pm213.24^{b\,B}$	4.94 ± 0.24^{B}
Endometritis	13	$1818.55\pm745.10^{c\,C}$	9.53 ± 3.31^{C}
Pyometra	9	$1318.89\pm619.74^{c\,BC}$	641.00 ± 330.00^{D}

The same letter in a column indicates no difference ($p>0.05$); a different lower case letter indicates a significant difference at $p<0.05$; a different upper case letter indicates significant difference at $p<0.01$

in a descending order of progesterone levels, from high to low, the groups were pyometra, endometritis, estrus and diestrus bitches (Table 4).

DISCUSSION

Aerobic pathogens: Certainly, bacteria were a direct factor in bitches developing CEH-P (Hagman *et al*., 2006; Hagman and Kuhn, 2002; Pretzer, 2008; Smith, 2006; Siqueira *et al*., 2009). Although, the most common bacterium in bitches with CEH-P was *E. coli* (Dhaliwal *et al*., 1998; Fransson *et al*., 1997; Siemieniuch *et al*., 2005), other anaerobic and aerobic bacteria such as *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Streptococcus canis*, *coagulase-negative Staphylococcus* sp., *Enterobacter aerogenes*, *Streptococcus dysgalactiae* ssp., *equisimilis*, *Proteus mirabilis*, *Serratia marcescens*, *Staphylococcus intermedius* and *Pseudomonas aeruginosa* were also detected in bitches with CEH-P (Gibson, 1998; Lee *et al*., 2006; Zdunczyk *et al*., 2006).

Moreover, *Citrobacter* sp. was isolated from canines with pyometra and hydrometra (Lee *et al*., 2006). Even *Clostridium perfringens* was isolated from the uterine lumen of a bitch with emphysematous pyometra (Hernandez *et al*., 2003).

The results indicated that the primary aerobes in most cases were similar but the dogs living at different sites could be infected by diverse bacteria. Bacteria were isolated from all of the bitches with vaginitis and the aerobes were *E. coli*, *Streptococcus canis*, beta-hemolytic Streptococcus, *Staphylococcus aureus*, *Staphylococcus intermedius*, *Proteus mirabilis* and *Pasteurella multocida* (Zdunczyk *et al*., 2006; Kustritz, 2006; Watts *et al*., 1996, 1997, 1998). Intrauterine bacteria that ascended from the vagina during proestrus and estrus induced cystic endometrial hyperplasia/ pyometra in bitches during metestrus (Noakes *et al*., 2001). Nevertheless, more information was necessary to explain the relationship between the bacterium species found in bitches with CEH-P in healthy vaginas and in the uterus.

To determine the source of the bacteria, it was compared aerobes from bitches with CEH-P, bitches with vaginitis and bitches with healthy vaginas and uteruses. Researchers did not isolate any aerobes from healthy uteruses and the bacterial species in the uteruses of bitches with CEH-P were mostly like those in the vagina. *Escherichia coli*, *Salmonella enterica*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus agalactiae*, Shigella and Corynebacteria were isolated from bitches with CEH-P and *Escherichia coli*, *Salmonella enterica*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and Shigella could be identified in healthy vaginas. Although, *Streptococcus agalactiae* and Corynebacteria were not found in the vagina, they accounted for 8% of the total isolates. These results suggested that the aerobes found in the uteruses of bitches with CEH-P ascended from the vagina and that the aerobes in vagina came from environmental contamination. Likewise, contamination of different sites in bitches with CEH-P occurred passively. The viewpoint could be also supported by other infectious diseases in animals.

The aerobes found in soft tissue abscesses, mastitis, pericarditis, pyometra and joint and foot abscesses in cattle, sheep and goats include *Microaerophilic coccus*, *Corynebacterium pyogenes*, *Fusobacterium necrophorum*, *Peptostreptococcus indolicus* and *Bacteroides* sp. (Slee, 1985). However, the above-mentioned bacteria were isolated less frequently in healthy cows than in cows with pyometra and cows with retained fetal membranes.

In cows with pyometra and retained fetal membranes, coliform were only found in the early stages of disease and the most common bacteria were *Corynebacterium pyogenes*, *Fusobacterium necrophorum* and *Bacteroides melaninogenicus* (Olson *et al*., 1984). These results indicated that the bacteria species in affected uteruses are related to those in the vagina and other body places.

Progesterone and estrode: CEH-P is typically secondary to pathological changes that are induced in the endometrium due to overexposure to progesterone in successive heat cycles (Noakes *et al*., 2001; Hardy and Osborne, 1974). The hypothesized pathogenic mechanism was that bacteria ascended into the uterus while the cervix was open because of estrode during proestrus and estrus (Sandholm *et al*., 1975; Noakes *et al*., 2001; Verstegen *et al*., 2008). After that time, progesterone causes endometrial hyperplasia and luminal fluid accumulation, suppresses leukocyte activity and decreases myometrial activity. Reduced contractility and fluid accumulation favor ascending bacterial infections which are typically due to enteric bacteria from the vagina (Lee *et al*., 2000; Noakes *et al*., 2001; Smith, 2006). Analysis of progesterone receptor expression supports the above hypothesis (De Cock *et al*., 1997; Noakes *et al*., 2001; Sugiura *et al*., 2004; Ververidis *et al*., 2004). The significance of uterine trauma to pyometra formation was also confirmed (Noakes *et al*., 2001).

A negative correlation has been demonstrated between progesterone and estrode receptor expression within both the canine CEH-P and control groups. Generally, progesterone was the main uterine receptor regulator for both the progesterone and estrode receptors during diestrus and early anestrus in healthy and affected uteruses. High progesterone levels suppress the expression of the progesterone and estrode receptors. It has been shown that the estrode receptor is expressed at a low level in the squamous metaplastic epithelium in the uterus of dogs with CEH-P (Ververidis *et al*., 2004). However, the same expression was also found in the columnar epithelium during the normal estrus cycle of dogs (De Cock *et al*., 1997). As for progesterone, it was proposed that the level of estrode was also increased in CEH-P bitches.

Although, the receptor expression has not been analyzed, the stronger hormone roles of progesterone and estrode could be argued. The amount and strength of a receptor is usually regulated depending on the functional demand of the animal and the expression can be directly adjusted by the concentration of regulators and by the response level.

It has been shown that progesterone and estrode levels increased in peripartum women and those who delayed to the 3rd day after delivery (Klier *et al*., 2007). The results obviously showed that progesterone levels increased in bitches with CEH-P and were even higher than those in estrus bitches. In particular, estrode levels in CEH-P bitches were higher than in estrus bitches. These hormone levels in CEH-P bitches should be similar to the endocrinal characteristics of peripartum bitches. The signaling pathways in both normal and pathological conditions should be investigated.

CONCLUSION

In this study, the results support the hypothesis; the most frequent aerobic pathogens in the uteruses of bitches with CEH-P ascended from the vagina and bacteria in healthy vaginas came from the environment. Additionally, both progesterone and estrode levels were elevated in bitches with CEH-P and these hormone levels in CEH-P bitches could be similar to the endocrinal characteristics of peripartum bitches.

In addition, researchers hypothesize that estrode and progesterone pretreatment and uterine mucosa damage were preconditions of the bacterial infections.

ACKNOWLEDGEMENTS

Samples were collected from Hua Shou Da Animal Hospital and the Teaching Hospital of the Veterinary College, Huazhong Agricultural University. Researchers greatly appreciate the collaboration of associate professor Deng Lixin from Henan Agricultural University, China who analyzed the hormone levels with radioimmunoassays.

REFERENCES

Ajadi, T.A., R.A. Ajadi, M. Agbaje and A.A. Abiabe, 2008. Cystic endometrial hyperplasia-pyometra complex in bitches: A report of three cases. Indian J. Vet. Surg., 29: 123-125.

Bigliardi, E., E. Parmigiani, S. Cavirani, A. Luppi, L. Bonati and A. Corradi, 2004. Ultrasonography and cystic hyperplasia-pyometra complex in the bitch. Reprod. Domest. Anim., 39: 136-140.

De Cock, H., H. Vermeirsch, R. Ducatelle and J. de Schepper, 1997. Immunohistochemical analysis of estrogen receptors in cystic-endometritis-pyometra complex in the bitch. Theriogenology, 48: 1035-1047.

Dhaliwal, G.K., C. Wray and D.E. Noakes, 1998. Uterine bacterial flora and uterine lesions in bitches with cystic endometrial hyperplasia (pyometra). Vet. Rec., 143: 659-661.

Fransson, B., A.S. Lagerstedt, E. Hellmen and P. Jonsson, 1997. Bacteriological findings, blood chemistry profile and plasma endotoxin levels in bitches with pyometra or other uterine diseases. J. Vet. Med. Ser. A, 44: 417-426.

Gibson, S., 1998. Bacterial and Mycotic Diseases: Non-Human Primates in Biomedical Research. Academic Press, San Diego, pp: 59-110.

Hagman, R. and I. Kuhn, 2002. *Escherichia coli* strains isolated from the uterus and urinary bladder of bitches suffering from pyometra: Comparison by restriction enzyme digestion and pulsed-field gel electrophoresis. Vet. Microbiol., 84: 143-153.

Hagman, R., H. Kindahl, B.A. Fransson, A. Bergstrom, B.S. Holst and A.S. Lagerstedt, 2006. Differentiation between pyometra and cyctic endometrial hyperplasia/mucometra in bitches by prostaglandin $F_{2\alpha}$ metabolite analysis. Theriogenology, 66: 198-206.

Hardy, R.M. and C.A. Osborne, 1974. Canine pyometra: Pathophysiology, diagnosis and treatment of uterine and extrauterine lesions. J. Am. Anim. Hosp. Assoc., 10: 245-268.

Hernandez, J.L., J.G. Besso, D.N. Rault, A.H. Cohen, A. Guionnet, D. Begon and Y. Ruel, 2003. Emphysematous pyometra in a dog. Vet. Radiol. Ultrasound, 44: 196-198.

Iglesias-Nunez, M., K. Sousa-Oliveira, J. Oliva-Hernandez, A. Rodriguez-Bertos, G.H. Toniollo and J.F. Perez-Gutierrez, 2008. Immunohistochemical study of the epidermal growth factor, transforming growth factor alpha and the epidermal growth factor receptor in the cystic-endometrial-hyperplasia-pyometra complex in the bitch. Reprod. Domestic Anim., 43: 80-80.

Klier, C.M., M. Muzik, K. Dervic, N. Mossaheb, T. Benesch, B. Ulm and M. Zeller, 2007. The role of estrogen and progesterone in depression after birth. J. Psychiatr. Res., 41: 273-279.

Kustritz, M.V.R., 2006. Collection of tissue and culture samples from the canine reproductive tract. Theriogenology, 66: 567-574.

Lee, C.M., F.M. Wu and P.C. Liu, 2006. A retrospective study on canine pyometra and hydrometra in central Taiwan. Taiwan Vet. J., 32: 17-23.

Lee, S.H., J.K. Cho, N.R. Shin, H.S. Kim and H.Y. Yong *et al*., 2000. Identification and antimicrobial susceptibility of Bacteria from the uterus of bitches with pyometra. Korean J. Vet. Res., 40: 763-767.

Martinez-Jimenez, D., P. Chary, H.W. Barron, S.J. Hernandez-Divers and J. Basseches, 2009. Cystic endometrial hyperplasia-pyometra complex in two female ferrets (*Mustela putorius furo*). J. Exotic Pet Med., 18: 62-70.

Niskanen, M. and M.V. Thrusfield, 1998. Associations between age, parity, hormonal therapy and breed and pyometra in finnish dogs. Vet. Rec., 143: 493-498.

Noakes, D.E., G.K. Dhaliwal and G.C. England, 2001. Cystic endometrial hyperplasia/pyometra in dogs: a review of the causes and pathogenesis. J. Reprod. Fertil. Suppl., 57: 395-406.

Olson, J.D., L. Ball, R.G. Mortimer, P.W. Farin, W.S. Adney and E.M. Huffman, 1984. Aspects of bacteriology and endocrinology of cows with pyometra and retained fetal membranes. Am. J. Vet. Res., 45: 2251-2255.

Pretzer, S.D., 2008. Clinical presentation of canine pyometra and mucometra: A review. Theriogenology, 70: 359-363.

Sandholm, M., H. Vasenius and A.K. Kivisto, 1975. Pathogenesis of canine pyometra. J. Am. Vet. Med. Assoc., 167: 1006-1010.

Schlafer, D.H. and A.T. Gifford, 2008. Cystic endometrial hyperplasia, pseudo-placentational endometrial hyperplasia and other cystic conditions of the canine and feline uterus. Theriogenology, 70: 349-358.

Siemieniuch, M., T. Fronczek, M. Blaszkowska, W. Bielas and A. Dubiel, 2005. Bacterial flora of the genital-urinary tract in clinically healthy queens. Medycyna Weterynaryjna, 61: 1305-1307.

Siqueira, A.K., M.G. Ribeiro, S.L. Dda, M.R. Tiba and C. Moura *et al*., 2009. Virulence factors in *Escherichia coli* strains isolated from urinary tract infection and pyometra cases and from feces of healthy dogs. Res. Vet. Sci., 86: 206-210.

Slee, K.J., 1985. A microaerophilic coccus in pyogenic infections of ruminants. Aust. Vet. J., 62: 57-59.

Smith, F.O., 2006. Canine pyometra. Theriogenology, 66: 610-612.

Sugiura, K., M. Nishikawa, K. Ishiguro, T. Tajima and M. Inaba *et al*., 2004. Effect of ovarian hormones on periodical changes in immune resistance associated with estrous cycle in the beagle bitch. Immunobiology, 209: 619-627.

Verstegen, J., G. Dhaliwal and K. Verstegen-Onclin, 2008. Mucometra, cystic endometrial hyperplasia and pyometra in the bitch: Advances in treatment and assessment of future reproductive success. Theriogenology, 70: 364-374.

Ververidis, H.N., C.M. Boscos, A. Stefanaki, P. Saratsis, A.I. Stamou and E. Krambovitis, 2004. Serum estradiol-17 beta, progesterone and respective uterine cytosol receptor concentrations in bitches with spontaneous pyometra. Theriogenology, 62: 614-623.

Watts, J.R., P.J. Wright and K.C. Whithear, 1996. Uterine, cervical and vaginal microflora of the normal bitch throughout the reproductive cycle. J. Small Anim. Pract., 37: 54-60.

Watts, J.R., P.J. Wright, C.S. Lee and K.G. Whithear, 1997. New techniques using transcervical uterine cannulation for the diagnosis of uterine disorders in bitches. J. Reprod. Fertil. Suppl., 51: 283-293.

Watts, J.R., P.J. Wright and C.S. Lee, 1998. Endometrial cytology of the normal bitch throughout the reproductive cycle. J. Small Anim. Pract., 39: 2-9.

Wiebe, V.J. and J.P. Howard, 2009. Pharmacologic advances in canine and feline reproduction. Topics Companion Anim. Med., 24: 71-99.

Zdunczyk, S., T. Janowski and I. Borkowska, 2006. Vaginal and uterine bacterial flora in bitches with physiological and inflammatory conditions. Medycyna Weterynaryjna, 62: 1116-1119.