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Prostaglandin potentiates the effect of antibiotics in the clinical recovery of pyometra in bitches

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Abstract

This work aimed to study the effect of administration of cloprostenol (PGF_{2α} analog) on the efficacy of treatment with levofloxacin ornidazole combination in bitches with pyometra. Twelve bitches presented in the clinics with vaginal discharge were diagnosed for pyometra by ultrasonography, vaginal cytology and hemato-biochemistry. Bitches (n=12) were divided into two groups viz. LO (n=6) and LO+Clp (n=6). LO and LO+Clp group received a course of 2% levofloxacin and 4% ornidazole combination at the dose rate of 5 ml per 20 kg body weight twice daily for five days; in addition LO+Clp group received cloprostenol sodium at the dose rate of 5 µg/kg body weight sub-cutaneously thrice daily for five days. Serum progesterone level was measured on day 0, 1, 2 and 3 from the start of treatment (day 0). Vaginal cytology and hemato-biochemistry was repeated after 7 days from the start of the treatment (day 0). The progesterone level dropped sharply on day 1 in LO+Clp group compared to LO group (4.8±0.28 ng/ml and 1.8±0.07 ng/ml respectively; *P*<0.01). Clinical parameters like anorexia, vomiting, malaise and neutrophils in vaginal cytology returned to normal levels in all six bitches of LO+Clp group 7 days after treatment but LO group showed partial recovery. Hemato-biochemistry revealed significant increase in hemoglobin and RBC count and significant decrease in WBC count, total protein, BUN, creatinine and ALP level in LO+Cl group compared to LO group. It can be inferred from the results that cloprostenol sodium, potentiates the action of levofloxacin probably by reducing the level of progesterone or by stimulating strong myometrial contraction and flushing of uterine contents.

Keywords: Pyometra, bitch, levofloxacin, progesterone, cloprostenol

Introduction

Pyometra is a very common reproductive disorder in old bitches with high mortality rates if not diagnosed and treated at the proper time. The incidences of pyometra in bitches increase with age due to hormonal imbalances and untimely ovulation. During late pro-estrus and early estrus the reproductive tract is under estrogen dominance leading to widely open cervix that facilitates the entry of pathogens from the vagina into the uterus. Thus longer the cervix remains open, greater is the chances of ascending infection and taking into account that pro-estrus and estrus are normally prolonged in bitches, they are more pre-disposed to pyometra. Further in bitches the progesterone level rises before ovulation and LH Surge, thus if ovulation is delayed the bitch remains in progesterone and estrogen dominance. Progesterone promotes glandular secretion that acts as an ideal media for bacterial proliferation (Smith, 2006) [16].

Due to high incidences of recurrence and incomplete recovery in cases of canine pyometra, ovario-hysterectomy had remained the treatment of choice in the past. But due to increasingly high market demand of puppies of certain breeds, many dog owners refrain from spaying and insist on medical recovery. In the recent years there are increasing cases of pyometra in bitches at much younger age (2-4 years), thus a long breeding life is lying ahead for spaying to be warranted. Microbial resistance to antibiotics depends on the frequency of use (Greene, 2006) [6]. One of the challenges in the treatment of pyometra is the emergence of multi-drug resistant strains of bacteria (Coggan, 2005; Otto, 2007) [2, 13] the other being the local factors e.g. glandular secretions, cellular debris, anaerobic environment, etc. that decreases the efficacy of antibiotics and promotes bacterial growth. This fact is supported in our clinical practice by the number of canine pyometra cases that did not completely recover after antibiotics treatment selected after culture and sensitivity test.

We have therefore taken up this work to test the efficacy of cloprostenol sodium (PGF_{2α} analog) in the treatment of pyometra in bitch along with standard treatment protocol with antibiotics. PGF_{2α} has been reported to cause regression of canine corpus luteum, increase myometrial contraction thus facilitating flushing of uterine content (Fieni *et al.*, 2014) [4].

Such an effect is expected to potentiate the action of antibiotics and hasten clinical recovery.

Materials and Methods

Twelve Labrador bitches of 4-6 years of age presented in the Teaching Veterinary Clinical Complex, Bihar Veterinary College, Patna with purulent vaginal discharge after a month of mating were included in this study. These bitches were completely off-fed, showed general malaise, vomited if force fed but did not have fever. Pyometra was confirmed by ultrasonography (figure 2) and presence of degenerating neutrophils in vaginal cytology (figure 1). Blood samples were taken on day 0, 1, 2, 3 and 7 for the estimation of the hemato-biochemical parameters (day 0 and 7) and progesterone assay (day 0, 1, 2 and 3). On day 0 the blood samples were taken before the start of treatments.

The following hemato-biochemical parameters were tested-hemoglobin (g/dl), RBC, WBC, neutrophil, band neutrophil and lymphocyte count, blood urea nitrogen (BUN), creatinine, total protein and alkaline phosphatase. Hemoglobin was estimated by Sahli's hemoglobinometer, RBC and WBC counted by hemocytometer and percentage of neutrophil, band neutrophil and lymphocyte determined by counting these cells in Giemsa stained blood smears. BUN, creatinine, total protein and alkaline phosphatase was measured by using colorimetric kits (AUTOSPAN[®], ARKRAY health care Pvt. Ltd., Surat, INDIA) following manufacturer's protocol. Progesterone level was estimated by ELISA kit (Biogenix, India) following manufacturer's protocol.

Pus samples were collected from anterior vagina by means of sterile insemination sheath and syringe and sent to Department of Microbiology, BVC, Patna for bacterial isolation and antibiotics sensitivity. All bitches had *E. coli* as major pathogen and was sensitive to levofloxacin.

The bitches were divided into two treatment groups viz. LO and LO+Clp each consisting of six bitches each. LO group was administered a combination of levofloxacin (2%) and Ornidazole (4%); Griptol N[®], Intas Pharmaceuticals Limited, India at the dose rate of 5 ml per 20 kg body weight twice daily for five days. LO+Clp group received the same antibiotics combination as LO group but in addition received Cloprostenol sodium (Pragma[®], Intas Pharmaceuticals Limited, India) at the dose rate of 5 µg/kg body weight subcutaneously thrice daily for five days. All bitches in the LO+Clp group received atropine sulphate at the dose rate of 25 µg/kg body weight (1 ml/25 kg body weight) subcutaneously 15 minutes before the administration of cloprostenol sodium.

All bitches received Ringer's lactate (Rintose[®], Vetoquinol, India) 50 ml/kg body weight daily along with ad lib access to water till they started to take solid food. Besides all bitches received vitamin B-complex supplement (Livoferol Pet[®], Petcare, India) 10 ml twice daily orally for 10 days. The data was analysed by independent and paired t-test using the software SPSS version 16.

Results and Discussion

No significant difference in hemato-biochemical parameters were observed before and after treatment in LO group but in LO+Clp group, the differences were highly significant (table 1). Highly significant difference in all hemato-biochemical parameters barring neutrophil % was observed between LO and LO+Clp group seven days after the start of treatment (table 1).

Among the clinical parameters studied, all bitches showed complete anorexia, vomited if force fed, were dull or depressed and had neutrophils in vaginal cytology (figure 1). After seven days of treatment there was partial improvement in clinical symptoms in LO group (1 bitch showed anorexia, 5 bitches had reduced food intake, 3 bitches still vomited after food intake, 4 were depressed and had neutrophils in cytology; table 2). LO+Clp group showed complete recovery after 7 days from the start of the treatment (all started taking food, did not vomit, looked active and did not have neutrophil in their vaginal cytology; table 2).

The progesterone concentration of bitches with pyometra averaged 5 ng/ml before the start of the treatments (figure 3). On the first day of treatment the progesterone concentration of LO+Clp group dropped significantly (1.8 ± 0.07 ng/ml; figure 3) compared to LO group (4.8 ± 0.28 ng/ml; figure 3). On the third day of treatment the progesterone concentration of LO+Clp group was 0.8 ± 0.07 ng/ml that was significantly lower than that of LO group (4.6 ± 0.8 ng/ml; figure 3).

The parameters including increased WBC count, band neutrophil, BUN, creatinine, total protein, alkaline phosphatase and decreased hemoglobin, RBC count, lymphocyte count in pyometra groups were consistent with the findings of Kuplulu *et al.* 2009^[10] and Shah *et al.* 2017^[15]. Culture and sensitivity test of 12 Labrador bitches in our investigation had *E. Coli* as the major pathogen and were sensitive to levofloxacin and is consistent with the findings of Kuplulu *et al.* 2009^[10] and Fransson *et al.* 1997^[5]. The increase in BUN and creatinine level in cases of canine pyometra as found in our study and also by other workers (Kuplulu *et al.*, 2009; Shah *et al.*, 2017)^[10, 15] is due to glomerulonephritis due to immune complex deposition in the glomerular basement membrane (Pretzer, 2008)^[14]. Degenerating neutrophils in vaginal cytology is an excellent aid to the diagnosis of pyometra in bitches along with ultrasonography and hemato-biochemistry as has been found in our study and reported by Haji *et al.* 2018^[7].

The progesterone level of bitches in our case was greater than 2 ng/ml, indicating the presence of active corpus lutea. In fact these bitches were discharging pus for more than a week almost for 4 to 6 weeks since last heat. Chaffaux and Thibier 1978^[1] reported the progesterone concentration in bitches with pyometra those had functional corpus lutea to be in the range of 2-13 ng/ml, much greater variation than observed in our case. This could be due to the time of diestrus when the pyometra was reported as there is continuous decline in blood progesterone level from day 21 to 51 from LH surge. That pyometra did not result in extension of luteal lifespan was strongly rejected from the findings of Chaffaux and Thibier 1978^[1] where they reported that pyometra after 70 days of mating had progesterone level less than 2 ng/ml. Further, the high progesterone level found in our cases was of luteal origin was reiterated by the fact that administration of cloprostenol sodium, a luteolytic agent resulted in sharp decline in progesterone level.

During the late pro-estrus and the early estrus phase the cervix remains open due to high estradiol concentration thus facilitating ascending infection from the vagina and during the diestrus phase, progesterone secreted by the corpus luteum results in endometrial proliferation and uterine glandular secretion, decreased myometrial contraction and induces closure of the cervix (Smith, 2006)^[16] creating an ideal environment for bacterial growth. Since the major pathogen in cases canine pyometra is *E. coli*, there is release of endotoxins

from the uterus that is responsible for toxemia and multiple organ failure.

Administration of cloprostenol along with levofloxacin ornidazole combination resulted in dramatic improvement of clinical parameters and reduction in pus formation compared to when the antibiotics was used alone. The purulent discharge first became mucus then serous and reduced in quantity with vaginal cytology revealing no neutrophil. Similar finding have been reported by Felman and Nelson 1996 [3] who treated 103 bitches with metritis and open pyometra with natural prostaglandins and observed complete resolution in 93% of the cases that corroborates well with our results wherein we observed sharp decline in progesterone concentration a day after first administration of PGF_{2α} analog cloprostenol sodium at dose rate of 5 µg/kg body weight s.c. thrice a day and level below 1 ng/ml on the third day.

The fact that cloprostenol potentiated the action of levofloxacin ornidazole in the treatment of bitches suffering from pyometra could be due to removal of progesterone and stoppage of glandular secretion that provided excellent media for bacterial growth. In fact progesterone has been found to increase the binding of *E. coli* to the endometrial epithelium (Ishiguro *et al.*, 2007; Leitner *et al.*, 2003) [8, 11] and suppress the local immunity (Sugiura *et al.*, 2004) [17] thus creating an ideal environment for proliferation. Further insufficient penetration of levofloxacin in the glandular secretion might have resulted in the growth of more resistant bacteria and incomplete recovery in levofloxacin ornidazole treated group. Fieni *et al.* (2014) [4] reported a 60% recovery rate in bitches with pyometra when treated with progesterone receptor antagonist aglepristone and they attributed this success to the fast opening of cervix. Thus it is clear from the above

discussion that removal of progesterone support is the key for potentiating the effect of antibiotics in cases of canine pyometra.

Prostaglandin (cloprostenol) facilitates smooth muscle contraction in the uterine wall leading to expulsion of uterine contents in cases of pyometra (Jackson 1979) [9]. It is this expulsion of uterine contents that is suspected to be responsible in part for recovery. This view has been supported by Fieni *et al.* 2014 [4] who reported a recovery rate of 84% with cloprostenol sodium as against 60% with aglepristone. Thus the flushing effect of uterine content by the contraction of myometrium by cloprostenol leads to lesser absorption of endotoxins into blood stream and this effect is largely responsible for quick and marked improvement of clinical symptoms in bitches with open pyometra. In fact in our cases the owners reported improvement of clinical symptoms on the third day from the start of treatment. Another theory that can be put forward in favor of cloprostenol in the treatment of pyometra is the recent finding that exogenous prostaglandin administration stimulates local uterine defence against the offending pathogen thus hastening recovery (Lewis, 2006) [12].

Conclusion

Pyometra following estrus is a common complication in bitches, incidences increasing with age. Among more advanced technology for the diagnosis like ultrasonography, vaginal cytology still remains an important tool for confirmation of open pyometra. For the treatment of pyometra, the antibiotics should not only be chosen after culture and sensitivity of pus but it should be accompanied by a course of sub-cutaneous dose of prostaglandins to hasten recovery.

Table 1: Hemato-biochemical parameters in cases of pyometra in bitches following treatment with levofloxacin ornidazole combination along with cloprostenol sodium

Parameters	Unit	Normal Range	Significance (between LO & LO+Clp treated groups post-treatment)	Levofloxacin Ornidazole (LO) Treated		Significance (before and after LO treatment results; paired- t)	Levofloxacin Ornidazole + Cloprostenol (LO+Clp) Treated		Significance (before and after LO+Clp treatment results; paired- t)
				Before Treatment	After Treatment*		Before Treatment	After Treatment*	
Hemoglobin	g/dl	12-16	P<0.01	5.5±0.07	8.05±0.21	NS	5.48±0.11	10.87±0.17	P<0.01
RBC	×10 ⁶ /µl	5-7	P<0.01	4.23±0.06	5.35±0.13	NS	4.08±0.11	6.55±0.11	P<0.01
WBC	×10 ³ /µl	6-17	P<0.01	44.7±0.95	38.42±0.64	NS	45.05±0.35	17.62±0.53	P<0.01
Neutrophils	%	60-70	NS	65.82±0.63	63.92±0.57	NS	64.9±0.59	65.62±2.06	NS
Band Neutrophil	%	<3	P<0.01	15.38±0.3	9.7±0.39	NS	15.12±0.34	3.93±0.36	P<0.01
Lymphocytes	%	30-40	P<0.01	11.03±0.33	21.25±1.11	NS	11.62±0.58	32.95±1.84	P<0.01
BUN	mg/dl	10-20	P<0.01	74.75±0.63	61.2±2.38	NS	74.13±0.98	74.13±0.98	P<0.01
Creatinine	mg/dl	<1.4	P<0.01	2.02±0.1	1.77±0.02	NS	2.07±0.07	1.57±0.02	P<0.01
Total Protein	g/dl	5-8	P<0.01	10.17±0.52	8.1±0.2	NS	10.03±0.23	6.27±0.29	P<0.01
ALP	IU/L	<90	P<0.01	182.03±2.25	142.22±2.75	NS	177.78±1.83	102.9±1.03	P<0.01

*: 7 days after the start of treatment

Table 2: Number of bitches with pyometra showing different clinical symptoms before and after treatment with levofloxacin ornidazole and/or cloprostenol combination

Clinical Parameters	Levofloxacin Ornidazole		Levofloxacin Ornidazole + Cloprostenol	
	Before Treatment	After Treatment	Before Treatment	After Treatment
Completely off-fed	6	1	6	
Partial Intake		5		
Full Intake				6
Vomition after food intake	6	3	6	0
Lethargy	6	4	6	0
Neutrophil in vaginal cytology	6	4	6	0

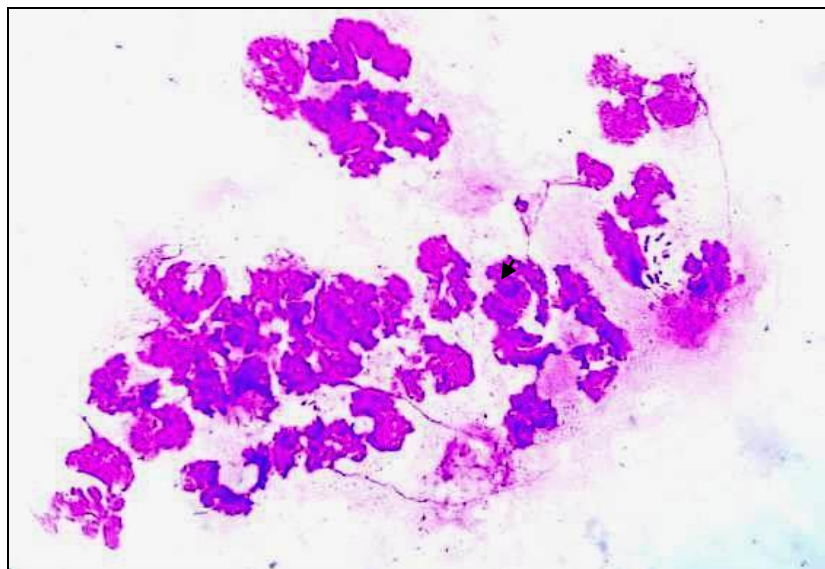


Fig 1: Vaginal cytology smear of bitch with pyometra showing degenerating neutrophils. Arrow indicates engulfed bacteria



Fig 2: Ultrasound image of hypoechoic pus filled uterine horn in a bitch with pyometra. The diameter of the uterine horn was 2.12 cm compared to less than 1 cm in normal bitch.

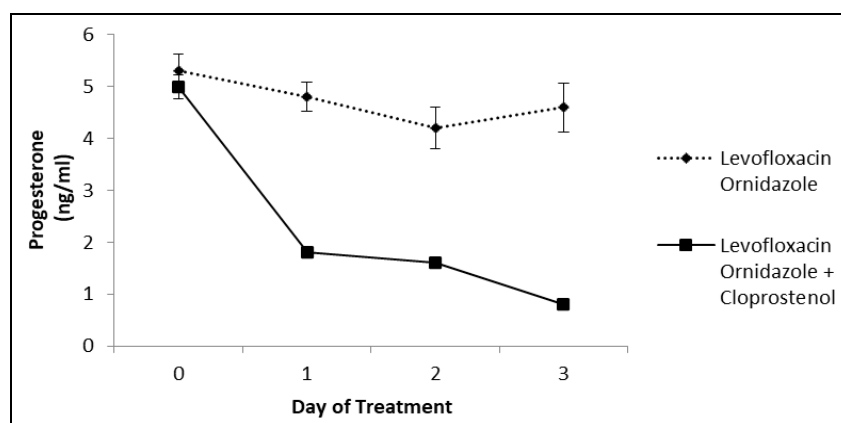


Fig 3: Serum progesterone level in bitches with pyometra treated with levofloxacin ornidazole and cloprosteonl sodium combination for five days. Day '0' is the start of treatment and on that day the blood sample was collected before the start of treatment.

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