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List of abbreviation

Abbreviation	Elaboration
СЕН	Cystic endometrial hyperplasia
CNF	Cytotoxin necrotizing factor
DLC	Differential leukocyte count
EDTA	Ethylene diamine tetra acetate
EGFs	Epidermal growth factors
EPC	endometritis-pyometra complex
ESR	Erythrocyte sedimentation rate
GF	Growth factor
Hb	Hemoglobin
HCl	Hydrochloric acid
IGCs	Insulin like growth factors
LH	Leutinizing hormones
MODS	Multiple organ dysfunction syndrome
PAMP	Pathogen associated molecular patterns
PMNs	Polymorpho nuclear cells
RBC	Red blood cells
SIRS	Systemic inflammatory response syndrome
TEC	Total erythrocyte count
TGFs	Transforming growth factors superfamily
TLC	Total leukocyte count
TLR	Toll-like receptors

Normal Blood Value- Dog

Parameter			Absolute count		Percentage		
Erythrocytes	Range	Avg.	Leukocytes	Range	Avg.	Range	Avg.
RBC	5.5-8.5	6.8	WBC	6000-17000	11.5	-	-
(×10 ⁶ ⁄ul)			(×10³⁄ul)				
Hb (gm%)	12-18	15	Neutrophil	0-300	70	0-3	0.8
				(Band)			
				300-11500	7000	16-77	70
				(Mature)			
PCV (%)	37-55	45	Lymphocyte	1000-4800	2800	12-30	20
MCV (%)	60-77	70	Monocyte	150-1350	750	3-10	5-2
MCH (%)	19.5-	22.8	Eosinophil	100-1500	550	2-10	4
	24.5						
MCHC (%)	32-36	34	Basophil	Rare	0	Rare	0

ABSTRACT

Pyometra is a life threatening, acute or chronic, polysystemic disorder of endometrium of adult bitch characterized by elevated WBC, toxic degeneration of neutrophils with normocytic, normochromic anaemia. The present study was conducted at Veterinary Clinics in Madras Veterinary College, Tamilnadu, India to determine the relationship of pyometra with breed and age of dog, along with their hematological parameters. A total number of 35 blood sample were collected with the case history to estimate different hematological parameters like Hb, PCV, TEC, TLC, Lymphocyte, Monocyte, Neutrophil, Eosinophil and Basophil. 82.86% cases were found to have Neutrophilia. Anemia was found in 62.86% cases. Breed susceptibility of Labrador retriever and spitz were highest and found 43% and 26% respectively. 6-10 years was the mostly recorded age of susceptibility. Pyometra is a distinct disease in pathogenesis, signs, diagnosis and treatment but hemograms may also help in diagnosing pyometra at an easy method.

Key words: Pyometra, Endometrium, Hematological, Neutrophilia, Anemia, Hemograms

CHAPTER 1 INTRODUCTION

The word pyometra is derived from the Latin words "pyo" meaning pus and "metra" meaning uterus. Thus pyometra literally means pus filled uterus and that is exactly what the condition is. 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 (**Hidalgo, et al., 1986**). It is a common, potentially life threatening condition affecting unneutered bitches. It is estimated that up to 24 per cent of entire bitches will be affected before the age of 10.

The infection normally occurs in middle-aged or older, non-spayed female dogs. Parity and age are significant risk factors in the development of pyometra (Hardy et al., 1977, Niskanen et al., 1998).

Pyometra is associated with hormonal alterations and bacterial infections. The incidence of pyometra in the bitch is high, and is recognized as one of the main causes of disease and death in this species. It is postulated that intrauterine bacteria, which ascend from the vagina during pro-oestrus and oestrus, induce the disease during metoestrus by acting on the progesterone-primed endometrium directly *via* toxin production, or indirectly by the release of inflammatory mediators (**Noakes et al., 2001**). The endometrial hyperplasia is induced by progesterone and is normally observed before the occurrence of pyometra. The bacterial infection is a secondary condition (**Grooters et al., 1994, Hidalgo, et al., 1986**). *Escherischia coli,* beta-haemolytic *Streptococci, Pasteurella multocida,* coagulase positive *Staphylococcus* spp. and *Klebsiella spp.* are amongst the organisms mostly isolated from pyometra cases, either in pure culture or mixed infections (**Brown JM 1985 , Coster et al., 1979, Dhaliwal et al., 1998, Lagerstedt et al., 1987**).

The disease is developed by two sequential process, that are- First Step is Cystic Endometrial Hyperplasia (CEH): Thickening of uterine endometrial lining occurring of canine. Escherichia coli subtypes are the most common isolates found in infection cases. Development of cystic endometrial hyperplasia is continuous but it remains a mystery why some bitches develop pyometra with minimal (**Kustritz, 2010**). Second step is infection: Infection generally occurs

due to microorganisms that are normal vaginal microflora effect probably at endometrial level

(Kustritz, 2010).

Pyometra is most commonly associated with polydipsia, polyuria, abdominal distension, vomition, poor appetite, weight loss and anaemia (Hardy et al., 1977). A purulent vaginal discharge may, however, be the only symptom in some cases of pyometra when the cervix is open. It appears that the degree of patency of the cervix dictates the severity of the clinical signs to a large extent (Dow C, 1958). In pyometra there are also notifiable haematological changes. These changes include elevated total white blood cell counts (WCC), marked left shift, toxic degeneration of neutrophils (Frasson et al., 1997).

There is a clear association between cystic endometrial hyperplasia (CEH) and pyometra (**Dow, 1958, Hardy et al., 1977, Low D G, 1954**). CEH is a progressive degenerative process and is generally accepted to be the initiating lesion and contributes to failure to shed normally transient uterine infections by compromising the uterine environment and/or local defence mechanisms (**Verstegen et al., 2008**).

Haematological studies are very effective to know about the physiological and pathological status of any individual. Clinical haematology also plays an important role in differential diagnosis and disease monitoring in man and is becoming increasingly important for the same purpose in other species (**Ghai, C.L., 2001**).

As pyometra may have fatal complications, clients opting to retain their bitch's reproductive capacity should be warned of potential risks of medical or surgical treatments. Clinical improvement of pyometra cases has been associated with reduction in plasma progesterone concentrations, increased vaginal discharges, reduction in diameter of the uterus as determined by ultrasonography and normalization of haematological and biochemical profiles (Gábor et al., 1999, Meyers-Wallen et al., 1986).

Treatment of canine pyometra with prostaglandins (PG) is successful with clinical recovery rates approaching 80 % and post-treatment pregnancy rates attained varying from 40 % to 80 % (Gilbert et al., 1989, Meyers-Wallen et al., 1986). Prostaglandin F2 α (PGF2 α) facilitates smooth muscle contraction in the uterine wall leading to expulsion of uterine contents in cases of pyometra (Jackson, 1979). It is this expulsion of uterine contents that is suspected to be responsible in part for recovery (Wheaton et al., 1993). The duration of prostaglandin

administration is thus dictated by the uterine dimension, time it takes for vaginal discharges to subside and ultimately by return of the WCC to normal and the patient to clinical normality. To compare the success rate of treatment protocols for pyometra clinical recovery rates, pregnancy rates, litter size, recurrence rates and duration of treatment should be considered. In some cases, the duration of treatment is of concern. Combination therapy using prostaglandins with either dopamine agonists or progesterone receptor antagonists have been used to successfully treat spontaneously occurring cases of pyometra (**England et al., 2007**). The presentation of haemograms in pyometra provide an easier and differential way in diagnosing, treating of the disease.

Considering above views this study was taken with the following objectives:

- To study the physical condition of the diseased animals.
- To diagnose pyometra by measuring blood parametres (TEC, TLC, DLC, PCV & ESR).
- To know about the relationship between pyometra and blood parametres.

CHAPTER 2 REVIEW OF LITERATURE

History:

Similar to this questions "where did we come from and what are we doing here?" since before the time when Aristotle approached these question in a scientific manner, like these questions it came to veterinarians about the pathogenesis of canine pyometra since 1930's. However, after long study of six decades it is still not completely known about the pathogenesis of canine pyometra to veterinarians (**Fransson B, 2000**).

Earlier the concept of CEH or cystic endometrial hyperplasia was given by (**Dow, 1957**) and he stated that hormonal changes led to CEH and this ultimately leads to secondary infection causing pyometra.

Definition of pyometra:

Pyometra is an acute or chronic polysystemic disease in mature bitches (Hardy & Osborne, 1974).

According to **Nelson and Feldman, 1986** pyometra is a disease occurs in a bitch one to two months following estrus due to elevated progesterone level whether she was bred or not.

According to **Boerresen**, **1975** pyometra is an acute or chronic endometritis occurring in metestrus.

According to **Noakes et al., 2001** pyometra can be defined as an intrautertine collection of cell debris(pus) possibly due to an exacerbated influence of progesterone.

According to **De Bosschere et al., 2001** in some cases the development of pyometra is a separate entity from CEH, with a hormonal component in the pathogenesis but mainly triggered by bacterial infection.

According to **Bartosz Kempisty et al., 2012** it is defined as the acute or chronic, polysystemic, di-oestrual disorder of the adult bitch, characterised by hyperplasia of the endometrium and infiltration of inflammatory cells and it may occur in all layers of the uterus.

In the mid-1980s a disease entity was described, called the endometritis-pyometra complex, which consisted of three clinical forms: pyometra, chronic endometritis, and cystic glandular endometrial hyperplasia (**Zdunczyk et al., 2006**).

According to **Chastain et al., 1999** canine pyometra is a common pathological affliction of intact bitches (Predominantly nulliparous bitches) depicting pus in the uterus.

Physiological aspects of the oestrous cycle in bitches:

The dog is the most important pet, a very important patient and an increasingly more important laboratory animal. However, the physiology of molecular regulatory mechanisms in reproduction remains unexplored. Bitches have a single oestrous cycle during a breeding season so they are monoestrous and have spontaneous ovulation. The first oestrus appears at 6–14 months of age and its timing depends on the breed and size of a bitch. In bitches, the oestrous cycle lasts 5–12 months. It consists of 4 consecutive phases: anoestrus (depending on the breed lasting 80-240 days), pro-oestrus (3-16 days), oestrus (4-12 days) and a relatively long di-oestrus (60-90 days) (Concannon, 2009). In domestic dogs, there is no relationship between the reproductive season and season of the year (the length of daylight). Furthermore, the duration of the period between heat (di-oestrus and an-oestrus) depends on the breed of a bitch. In German Sheppards the period between heats is relatively short; this breed enters the oestrus phase every 4–5 months (Grundy et al., 2002). It is said that the reproductive cycle of the bitch is one of the most primitive in all mammals. Anoestrus is the time of sexual inactivity, it ranges from variable to regular within bitches and is terminated by poorly understood interactions of environmental and endogenous factors. Anoestrus is followed by pro-oestrus; the time of vulvar swelling and bleeding, vaginal epithelial proliferation and cornification and vaginal secretion of pheromones. In vaginal smears parabasal cells predominate, followed by small and large intermediate squamous and cornified cells, and the cornification reaches the maximum at 1-6 days before the LH peak. In examination per vaginam the mucosa is oedematous, white, and lined with serosanguinous fluid. Many of the regulatory mechanisms of canine reproduction differ distinctly from those in other species. The *corpus luteum* is the only source of progesterone in pregnant and non-pregnant bitches (Concannon, 2011).

Etiology:

According to **FO Smith, 2006** the exact etiology of pyometra is still unknown.

In the past, investigations of the bacteria associated with canine pyometra have been few. However, multiple authors have reported a predominance of *Escherichia coli* (Asheim, 1965; **Renton et al., 1971; Grindlay et al., 1973; Sandholm et al., 1975; Kivisto et al., 1977; Vandesplassche, 1991; Dhaliwal et al., 1998**). *E. coli* adheres to receptors in the progesterone-stimulated endometrium, which might be one explanation for the observed predominance of this bacterium (**Grindlay and co-workers, 1973**) found that certain serotypes of *E. coli, e.g.* 02, 04, 06 and 075 were more commonly associated with pyometra than others (**Sandholm et al., 1975**). **Dhaliwal and co-workers, 1998,** concluded that *E. coli* serotype 032 and 04 were the most commonly prevalent in their study population. In addition, they found that Cytotoxin Necrotizing Factor (CNF) was expressed in (44%) serotypes associated with pyometra. From surgically removed uteri in most cases (70–80%) *E. coli* was isolated, in some cases *Staphylococci, Streptococci* and *Pseudomonas aeruginosa*. Considering β-haemolysis as an indirect indicator of pathogenicity, β-haemolytic *E. coli* strains were isolated more often from diseased than from healthy specimens (**Arora et al., 2006**).

Escherischia coli, beta-haemolytic *Streptococci*, *Pasteurella multocida*, coagulasepositive *Staphylococcus* spp. and *Klebsiella* spp. are amongst the organisms mostly isolated from pyometra cases, either in pure culture or mixed infections (**Brown, 1985, Coster et al., 1979**). According to **Susi et al., 2006**, 90% of cases, *Escherichia coli* is a main causal agent organisms infecting uterus are normal inhabitants of vagina. *E. Coli* is found to be most common (70% cases) while the other isolates like *Streptococcus Spp., Staphylococcus Spp., Klebsiella Spp., Proteus Spp., Pseudomonas Spp., Corynebacterium Spp., Enerococcus Spp., Pasteurella Spp., Serratia Spp., Haemophilus Spp., and Bacillus Spp., are also reported (Vandeplassche et al., 1991).*

Route of infection:

The route of infection of the uterus was early on suggested to be hematogenic or lymphogenic as well as ascending (**Dow, 1959**). The ascending route was not supported by the work of **Meyers-Wallen and co-workers, 1986**, who observed that the type of bacteria isolated from the vagina did not necessarily represent the bacterial species isolated from the uterus in pyometra. **Sandholm and coworkers, 1975**, showed that cystitis was commonly associated with canine pyometra, and that the *E. coli* bacteria isolated from the urinary bladder and the isolate from the uterus showed many similarities. It was suggested that the urinary tract may serve as a bacterial reservoir, and bacteria ascend into the uterus during a susceptible stage in the estrous cycle (**Sandholm et al., 1975**).

Types of pyometra:

There are three different types of pyometra although most studies have described only the general presentation of pyometra without distinguishing between the open-cervix and closedcervix types (Smith, 2006; Pretzer, 2008). 1) Closed - this is an extremely dangerous condition in which the closure of the cervix causes pus to pool in the uterus with no escape. As this happens your dog may become increasingly "off" and her symptoms may worsen over a short period of time. Because there is no discharge and other symptoms of infection aren't easily noticeable, owners may decide to wait it out before seeking veterinary attention. Unfortunately, this increases the risk that uterus will continuously expand and eventually rupture within the body, or that the kidneys will fail. Up to 1/3 of pyometra victims will fall into this category. 2) Open - this type of pyometra occurs when the cervix remains open following infection. There will usually be tell-tale discharge around the vulva. However this can easily be mistaken as a continuation of the heat cycle. Though the uterus may not swell or expand, an open pyometra is still classed as an emergency due to the threat of organ damage especially to the kidneys. The sooner your pet receives veterinary attention the better the prognosis will be. 3) Stump- though rare, a stump pyometra is dangerous in that it is often unexpected: it occurs in spayed animals. Unbeknownst to many owners, stump infections may occur in remnants of tissue left behind during a routine spay, which fill with infected fluid. The symptoms and risks are similar to those of a true pyometra, therefore it is important that owners of spayed pets consult their vet as soon as any of the previously-mentioned symptoms crop up.

Pathogenesis of pyometra:

The cystic endometrial hyperplasia-pyometra complex (CEH/P), earlier called the endometritis-pyometra complex (EPC), is one of the most serious and most common uterine diseases in bitches (**Kida et al., 2006**). The ovarian abnormality and hormonal disorders resulting from the prolonged influence of estrogens (prolonged heat, ovarian cysts and active ovarian neoplasia) are thought to be the main cause of endometritis-pyometra complex (**Kida et al., 2006**). Changes in the concentration of ovarian steroid hormones in the blood and in their receptors in uteri with pyometra were investigated (**de Bosschere et al., 2002**). The estrogens used to prevent nidation also influence the development of pyometra because these hormones promote overproliferation of endometrium and lengthen the period in which the uterine cervix remains open (**Bartosz et al., 2012**).

Nolte et al., (1990) reported that all bitches under two years of age affected by pyometra had earlier been treated with hormones. The action of progesterone on canine uterus consists in decreasing its local immune reactivity, promotion of secretion in endometrial glands, a decrease in its motility and closing of the uterine cervix (Bartosz et al., 2012). Intrauterine introduction of *E. coli* cultures, ascending from the vagina in the luteal phase, causes inflammation within this organ (Johnston et al., 1985). It has been proven that progesterone induces the development of endometrium receptors which allow for the adhesion of *E. coli* colonies (Bartosz et al., 2012).

According to **Gama et al., 2009** growth factors(GF) which may play a significant role in the appearance and development of various genital pathologies in bitches, manifesting autocrine and paracrine activity.

Recent work by Nomura and co-workers (**Nomura et al., 1998**) and the clinical observations of Koguchi et al. have suggested that the classical sequence of progesterone leading to CEH and subsequently, CEH to pyometra may not be correct, and that the sequence may in fact be

reversed with bacteria being the initiating factor. A subtle (sub-clinical) uterine infection or endometrial irritation by foreign bodies may first occur at the end of estrus or during the first half of diestrus, providing the stimulus for an excessive endometrial hypertrophy and hyperplasia and the resulting increase in endometrial glandular proliferation and luminal epithelial cellular secretions can initiate the development of a pyometra.

According to **Gerstenberg et al., 1999, Komatsu et al., 2003; Kida et al., 2010,** the level of expression of receptors responsible for induction of growth hormones play important role for the production of pyometra. These growth factors include Insulin-like growth factors (IGFs), Epidermal growth factors (EGFs), Transforming growth factors superfamily (TGFs).

According to **De Cock et al., 2002,** insulin-like growth factor I located in and around the epithelial cells of endometrium in dogs with CEH may play an important role in development of pyometra.

Predisposing factors of pyometra:

Age:

Pyometra is a common disease. Nulliparous bitches and bitches of more than 4 years of age seem to be predisposed (**Chastain et al., 2012**).

According to **Hagman, 2000** the incidence of pyometra in dogs is approximately 24% before 10 years of age.

Dow (1958, 1959) reported that the mean age of clinical case was 8.2 years, with only, 12% of the cases under 6 years of age.

Breed variation:

Golden Retriever Rottweiler, Saint Bernard, Chow Chow, Bernese Mountain dog, Rottweiler, rough-haired Collie, Cavalier King Charles and Spaniel are listed as predisposed breeds (**Hagman, 2004**). Breeds with low risk for pyometra includes Drevers, German Shepherd, Daschunds, Swedish hounds etc.

Parity:

Previously it was suggested that nulliparity, abnormal estrous cycle and pseudopregnancy increase the risk of pyometra. But recent literature has suggested that there is no association between pseudopregnancy and abnormal estrus cycle and pyometra. However there is a modest relationship between nulliparity and pyometra (**Dow, 1957**).

Fiddler et al., 1966, however found no relationship to parity or estrous characteristics; this opinion now widely accepted pyometra is a disease of the luteal phase, with most bitches showing clinical signs between 5 and 80 days after the end of oestrous.

Stages of estrous cycle:

Most bitches present to pyometra within 8 weeks of last estrous. However, it may occur at any stage of estrous cycle or during pregnancy, **Haque et al., 2003**

Hormone:

Progesterone has a role in initiating the pathogenesis of cystic endometrial hyperplasia (CEH)- Pyometra complex. Endometrial hyperplasia that resulted into CEH caused by progesterone is more pronounced when it has been primed with estrogen. Thus administration of estrogens, when progesterone levels high may predisposes bitches to pyometra. Estrogen keeps the cervix relaxed for longer period in the luteal phase and also enhancing the stimulatory effects of progesterone on the uterus. The hormonal therapies that include either progesterone for estrous suppression or estrogen for estrous induction or pregnancy termination may explain the development of pyometra in young bitches (**Dow, 1957**). Pyometra is common in bitches with abnormal oestrous cycles and pseudopregnency (**Whitney, 1967**).

Immunological overlook on pyometra:

It is thought that the cause of development of pyometra is the result of interaction between potential pathogen and variable level of progesterone (**Bartosz et al., 2012**).

Innate mechanisms are based on the activity of Toll-like receptors (TLR), which recognise and cooperate with pathogen-associated molecular patterns (PAMP) synthesised by microbes, initiating a reaction cascade including the early inflammatory response (**Horne et al., 2008**).

Haematological changes:

Pyometra cases show marked haematological and biochemical changes. These changes include elevated total white blood cell counts (WCC), marked left shift, toxic degeneration of neutrophils and detection of plasma endotoxin in some cases (**Fransson et al., 1997**).

Hagman et al., (2006) investigated hematological and blood chemistry data from bitches and reported that pyometra is associated with several hematologic parameters. According to

Wheaton et al., 1989 peripheral leukocytosis and normocytic, normochromic anemia is common in canine pyometra with PCV ranging from 21-48%.

According to **J. Verstegen et al., 2008** pyometra cases are regarded as being accompanied by marked leukocytosis characterized by neutrophilia with a left shift and toxic degeneration of neutrophils, as well as a monocytosis. However, this is not always present, since as many as 25% of pyometra cases may have leukograms within the normal range. Many affected bitches have a mild to moderate normocytic, normochromic anemia (PCV 30–35%).

According to **Asheim**, **1964**; **Renton et al.**, **1971** total leukocyte counts of 25000 or greater, with mature neutrophils outnumbering immature form. In pyometra associated with a closed cervix, the total leukocyte count may approach $100,000/\mu$ l. The PCV level also increase which range 36-40%.

According to the (**Bloom, et al., 1944**) there is usually marked leucocytosis (20,000 to 10, 0000 or more), with the average total leukocyte count being 50,000/microliter or over. There is marked neutrophilic shift to the left.

Clinical signs:

Canine pyometra is a life-threatening disease (Marretta et al., 1989; Pretzer, 2008). Common clinical signs of pyometra are not limited to the genital tract, *e.g.* vaginal discharge, but include systemic signs such as vomiting and/or inappetence, polyuria /polydipsia and lethargy (Nelson & Feldman, 1986, Stone et al., 1988). It has been suggested that the clinical signs are more severe in cases where the cervical canal is occluded (Dow, 1958, Borresen, 1975). The cervix may spontaneously open or close during the disease, causing intermittent vaginal discharge or a sudden deterioration in the clinical status of the bitch (Studdert, 1971).

According to **Barton**, **1992** CEH is not associated with clinical signs unless the uterine content becomes infected; this is referred to as pyometra.

The disease often causes general signs/symptoms, such as anorexia, weakness, polydipsia/polyuria, vomiting, increased body temperature, tachycardia and tachypnoe (Bedrica and Sacar, 2004; Fransson et al., 1997).

There are found hepato-cellular damage in response to toxemia, or diminished hepatic circulation due to dehydration. Hyperproteinemia may develop in response to dehydration, and hyperglobulinemia reflects the chronic antigenic stimulation present with this disease (**J**.

Verstegen et al., 2008).

Pyometra is most commonly associated with polydipsia, polyuria, abdominal distension, vomition, poor appetite, weight loss and anaemia (Hardy, 1977).

In a few cases, a urinary tract infection with the same organism infecting the uterus (usually E. coli) is found.

However, cystocentesis is not recommended in bitches suspected of having a pyometra, as there is a high risk of perforating the distended uterus (**J. Verstegen et al., 2008**).

Histo-pathological lesions:

Endometrial hyperplasia is the result of cystic deformation of endometrial glands and stromal proliferation of fibroblasts with inflammatory reaction (**De Bosschere et al., 2001**).

CEH is also associated with mucometra; it results from endometrial thickening with the accumulation of viscid uterine fluid caused by hyperplastic and hypertrophic endometrial glands (**D. Biswas et al., 2012**).

Histo-pathologically the affected uterus reveals multifocal erosions and ulcerations in the superficial epithelial layers of the endometrium. Sometimes the endometrial layer is infiltrated with a large number of viable and degenerated neutrophils, tissue debris and extravascular erythrocytes (**D. Biswas et al., 2012**). Characteristically the endometrial glands become expanded and haphazardly arranged with a hyperplastic, crowed epithelial lining.

D. Biswas et al., 2012 also reported that the hyperplastic uterine glands are thought to be the cause of excessive secretion that causes voluminous fluid in pyometra.

Dow, 1958 histologically classified canine endometrial hyperplasia-pyometra complex into four stages: 1. Stage-I (Uncomplicated CEH): This stage is marked by thickened endometrial surface with multiple irregular cystic lesions giving it cable stone appearance. 2. Stage-II: Infiltration of plasma cells occurs in cystic endometrial tissue. 3. Stage-III: (CEH overlying acute endometritis): Gross lesions include ulceration and haemorrhage of endometrium and uterus and may contain redbrown to yellow-green pus. Acute endometritis is characterized by congestion, oedema and superficial and deep infiltration of neutrophils into endometrium.

Myosis may also be seen in some cases. 4. Stage-IV (CEH with chronic endometritis): Open cervix pyometra is characterized by collapse and grossly thick walled uterus wall with minimum discharge. The endometrium is atrophied while the myometrium is hypertrophied and fibrotic. While in closed cervix pyometra both endometrium and myometrium of distended uterus are atrophied.

Diagnosis of pyometra:

A variety of signs may be detected by the owners. The most obvious one is vaginal discharge, which may vary from serosanguinous to mucopurulent. In some bitches, the amount of discharge is minimal and fastidious grooming by the bitch makes that discharge difficult to detect. In other cases, signs of vaginal discharge may not be externally apparent, but vaginal smears and vaginoscopic examination reveal the presence of uterine exudate in the cranial vagina. The amount of vaginal discharge is also partially dependent on the degree of cervical patency. Many bitches with a "closed" pyometra and apparently less obvious discharge are presented in a more advanced stage of the disease and in a more serious clinical condition.

Cytology of vulvar exudates reveals degenerative polymorphonuclear cells (PMNs), bacteria and non-cornified epithelial cells (small intermediate epithelial cells, large intermediate epithelial cells). Usually the onset of clinical signs is gradual and insidious. Common signs include lethargy, depression, and inappetance. Vomiting may be present and is more common in the more severely affected patients (**Verstegen et al., 2008**).

Despite modern treatment routines, the mortality rate due to pyometra is still approximately 4% (Egenvall et al., 2001). Myocardial injury secondary to endotoxemia, inflammation, disseminated bacterial infection, or infarction is suspected to be a contributing factor in unexpected deaths (Marretta et al., 1989).

Although history and clinical signs may be very suggestive of pyometra, definitive diagnosis is achieved by ultrasound. This should reveal a distended, fluid-filled uterus with variable echogenicity (signs will be less severe with open pyometra). Alternatively radiography can be performed - this should show a soft tissue opacity in the caudal abdomen, but it is hard to differentiate from pregnancy, pseudopregancy and other conditions affecting the uterus on the basis of these radiographic signs. (**Verstegen et al., 2008**).

According to **Christiansen**, **1984** the definitive of pyometra become very difficult when estrus and breeding history of bitches is not properly known. It may be confused with pregnancy, renal failure, cystitis, vaginal neoplasia and polyarthritis .

According to **Bigliardi and Pamigiani**, 2004 ultrasound and X-ray examinations can reveal a fluid-filled uterus in a "closed" form of pyometra.

According to **Renton et al., 1991 and Ayyappan et al., 1997** 100% visualization of the uterus in pyometra is possible through radiography.

Treatment:

In principal, pyometra can be treated by surgical or medical therapy. However, the decision depends on several factors like: medical treatment in young bitch intended for breeding, general condition, side effects after spaying, presence or absence of endometrial glandular cysts by ultrasonography. In ovariohysterectomy the following factors are related- Elderly bitches, Non-breeding bitch, Presence of systemic disease, Open or Closed cervix, Endometrial glandular cysts (**Wykes and Olson, 1993**). In certain cases, such as young, valuable breeding bitches with an open-cervix pyometra that

are not critically ill, medical management may be considered. Injections of a hormone called *prostaglandin f 2 alpha* are given for 3-5 days, along with antibiotics and fluids. There are side effects to the prostaglandin, including panting, drooling, diarrhea and vomiting. If the animal has not responded well to medical management, surgery needs to be considered **(Wilasrusmee et al., 2002).**

Haque and Ahmad, 2003, reported that the early treatment of pyometra was effective whereas late treatment of pyometra was always fatal. The medical treatment of pyometra with PGF2alpha is not popular due to its side effects (Wykes and Olson, 1993)

Historically, pyometra has been most commonly treated by ovariohysterectomy (OHE). This remains the recommended treatment in all cases for bitches without significant reproductive value, or when the owner has no strong desire to breed the bitch. The main advantage of OHE is the exclusion of any risk of recurrence but surgical treatment has its limits when the risks of anesthesia and surgery are life threatening (**J. Verstegen et al., 2008**). The earliest proposed medical therapy employed simply the use of systemic and local single antibiotics (**Threlfall WR. et al., 1995**).

According to **Powell et al., 2003** herbal remedies are effective in medical treatment. Some powdered mixtures are suggested in this case for treating purpose, like *Panax pseudoginseng* that helps in immune-mediated actions in resolving urinary infections (**Wilasrusmee et al., 2002**). Intravenous fluids (Lactated Ringer's sol. / Glucose 5%), H2-blocker (in case of gastric injury / vomiting), Anti inflammatory drugs (NSAIDs) are supplied supportive therapy.Close observation of the bitch during the first days of treatment has to be ensured. Close monitoring is essential for medical treatment of pyometra. Repeated general condition examination (once or twice daily), repeated ultrasound – decrease of uterine content (every 2 – 3 days), repeated complete blood count (every 1 – 2 days), repeated intravenous fluids suppliment should be done **Kustritz, 2010**.

Complications of pyometra:

If pyometra is allowed to continue untreated for a significant period of time, it can affect the entire body, leading to critical disease, shock and death. Because the infection can be so overwhelming, the reasons for presentation are not limited to the genital tract. The animal can become so overwhelmed by the inflammation associated with the infection that the animal may die from its own uncontrolled inflammatory process. The severity of the resulting illness is greatly influenced by the degree of drainage from the uterus. If the cervix is closed, then fluids and toxins accumulate, with potential for toxic effects. Rupture or slow leakage from one of the uterinr horns can release inflammatory products into the abdominal cavity, causing peritonitis (Wheaton et al., 1989).

According to **Musal et al., 2005** Bacterial toxins have urine concentrating mechanism. Bacterial infection and toxins may cause secondary damage to the liver as well. Endotoxic shock alters blood supply to all tissues and can disrupt normal bloos clotting mechanisms. The most life threatening complications associated with pyometra are sepsis and systemic inflammatory response syndrome (SIRS)/multiple organ dysfunction syndrome (MODS).

According to **Kustritz**, **2010** renal complications are common sequel to pyometra. Endotoxins released from the cell wall of gram negative bacteria inhibit normal renal tubular function. These changes being reversible are controlled as soon as infection is taken care.

Prognosis:

Prognosis is better in open pyometra cases and in animals that present with less severe signs. However, following successful surgery to remove the uterus, prognosis is very good (Marretta et al., 1989).

Recurrence of pyometra:

The incidence of pyometra recurrence after medical treatment is still controversial, with contradictory results published. However, the percentage of recurrence is obviously decreasing over time with improvements in therapeutic approaches and treatments. **Meyers-Wallen et al., 1986** described therapeutic success in 10 of 10 treated animals with recurrence in 40% of the bitches within 1 y, and 77% within 27 months. According to **Johnston et al., 2001** rate of success and recurrence rates after conservative treatment of pyometra with prostaglandins and reported that recurrence rates averaged 10%. After surgery, patients with pyometra may develop wound infections, fistulous tracts, or local swelling of the surgical incision site or may hemorrhage.

Public health significance:

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.

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 (Franco et al., 1996).

CHAPTER 3

MATERIALS AND METHODS

3.1 **Study area and duration:** The study was conducted in Madras veterinary college,India during my internship placement at pathology laboratory from 03.07.2014 to 17.07.2014. In pathology lab I had done my laboratory work.

3.2 **Study population:** I had collected 37 blood samples from the patient came at Madras Veterinary College with the history of continuous vaginal discharge (sanguinipurulent to mucopurulent), lethargy, vomiting, polydipsia and/or fever. In closed pyometra vaginal discharge is not evident but other signs were found.

3.3 **Case definition:** Pyometra is an important disease which is characterized by sanguinipurulent to mucopurulent vaginal discharge, lethargy, polyuria, polydipsia with or without fever.

3.4 **Methods of study**: A total 37 blood samples were collected and were studied by DLC of blood samples.

Collection of blood and processing of blood:

Approximately 5 ml of blood for each case was drawn aseptically from the radial vein in case of dog and 3 ml was transferred to a sterile vial containing anticoagulant (ethylene diamine tetra acetate(EDTA), 5-10 mg for 5 ml of blood) to determine hematological parameters. All samples for hematological analysis were processed immediately after the collection of blood samples.

Analysis of Blood sample:

Hemoglobin (Hb), packed cell volume (PCV), erythrocyte sedimentation rate (ESR), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) were determine as per procedure set by **Shastry (1983).**

Methods of estimation of differentials leukocyte count (DLC):

3.4.1 Apparatus:

- Microscope
- Glass slide
- Blood lenset or needle
- Staining stray
- Cotton
- Blowing pipe

3.4.2 Reagents:

- Wright's stain (Na phosphate-3.2g, K-6.63g, Distilled water-100cc)
- \circ Immersion oil
- Distilled water
- o Spirits

3.4.3 Procedure:

a. Preparation of blood smears:

1. A drop of blood was placed at the right end of the slide.

2. The slide was kept on the table and hold firmly by thumb and fore fingers of the left hand. Then the even edge of the 2nd slide (spreaders) was placed near the drop of the blood towards the middle of the slide.

3. The 2nd slide was drawn towards the drop of blood of an angle of 45° and the blood was spread along its edge. This brought the blood forward and a smear was produced.

b. Staining of blood film:

1. When blood film was dried, it was placed on the staining rake. Enough Wright's stain (8-10 drops) were poured over the slide to cover the blood smear and allowed to stand for 1 minute.

2. Distilled water about double of the quantity of the stain was added and rinsed by blowing pipe and allowed to stand for 5 minutes.

3. The film was then washed with distilled water with out much distributing the slide till the film become pinkish.

4. The slide was put against a support in an inclined position, stained smear facing down and allowed to dry in the air.

3.4.4 Counting of WBC:

- 1. The slide was placed under microscope and a drop of immersion oil was placed on the slide and cell was identified by using high power objective (100X) and following parallel strip method.
- 2. Different types of cells were counted by tally method. Here 200 cells counting in the best method.

There are mainly two type of WBC

• Granulocytes

Eosinophils

Neutrophil

Basophile

• A granulocytes

Lymphocyte

Monocyte

3.5 Method of estimation of RBC

3.5.1 Materials required:

• Microscope

- Haemocytometer- It includes Newberg counting Chamber, red pipette (RBC diluting pipette) covers lip
- Syringe with needle
- Cotton and spirit
- Diluting fluid- Hayemi's solution, Grower and toison solution .
- Blood

Composition of Hayem's solution:

- o Nacl- 2.0gm
- o Na2SO4-5.0gm
- o Hg2cl2-0.5øgm
- \circ Distilled Water 200cc

3.5.2 Procedure:

i) A dry and clean counting chamber was placed under the microscope. After words, the chamber was examined under low power objective (10x) without the cover slip in order to understand the ruling.

ii) Blood mixed properly with EDTA was drawn into the RBC pipette up to the mark 0.5 keeping the pipette nearly horizontal.

iii) The diluting fluid was sucked into the pipette until the minter of blood and the fluid reaches the 101 mark about the bulb. The pipette was rotated and inverted (8 knot motion) several times to ensure mixing of minute of blood with the diluting fluid.

- iv) Charging the counting Charging
- a. A few drops of mixture were discarded from the pipette and wiped out its tip. Diluted fluid mixture was allowed to form at the tip of the pipette. The tip was placed on the surface of the chamber touching the edge of the cover slip at an angle of 45° to the horizontal. The diluted blood was allowed to flow evenly and slowly under the cover slip by capillary action.
- b. A few minutes were spent before starting the count to allow the corpuscles to settle.

V) Counting of RBC:

a) All corpuscles (RBC) were counted in 80 smallest squares i.e 5 mall square (4 corner squares and a central square).

b) In counting the cells which touched the left hand lines on the upper lines of the square were taken to be with in the square and those which touched the lowered right lines were omitted as outside the square. The definite technique was followed to avoid the duplicate counting and counting was started from the upper left chamber.

Dilution obtained:

The volume of the bulb is 100 (101-1=100) from the tip of the pipette to the mark 1 the pipette contains diluting fluid only and which docs not take part in the dilution. Thus 100 volumes of diluted blood (in the bulb) contain 0.5 volume of blood and 99.5 volumes of diluting fluid.

3.5.3 Calculation:

The total number of cells was multiplied by the calculation factor i.e 10,000 and expressed the result in million/mm³ by dividing the total number of cell by 1 million.

Calculation factor:

Area of the chamber: $5/25 \text{mm}^2 + 1/5 \text{mm}^2$

Depth of the chamber: 1/10 mm.

Dilution of pipette: 1:200=1/10,000 mm²

3.6 Methods of estimation of hemoglobin:

3.6.1 Apparatus and reagents:

1. Sahli's (sahli - Adams) anemometer or hemoglobinometer- it consist of

- Color measuring box or com palter
- Sahli- Adams hemoglobin tube

- Hemoglobin pipette
- A thin glass rod or jitter.
- Dropper.
- 2. Sterile blood lancet
- 3. Reagent: N/10 HCl
- 4.70% alcohol and distilled water.

3.6.2 Procedure:

N/10 HCl was taken in the graduated to be of the hemoglobinometer up to mark 20 percent or 3ml of the blood was sucked into hemoglobin pipette (holding it rarely horizontal) up to the 20 micro liter. There should be no air bubble in the pipette. The blood of the pipette was transferred slowly into acid of the mining up to the pipette and returned it to the pipette mining tube.

Next little distilled water was sucked up to the pipette and there was added to the mixture.

The mining tube was kept inlets place in the matching box for the formation of acid haematin which give dark blow color to the solution. Now distilled water was added gradually drop by drop to acid haematin solution in gradually tube and stirred for through mixing. In this way distilled water was added till color to acid haematin solution exact match with the color at standard

The graduated mining to be was removal from the matching box and the volume of the build was red both in purchase and in gm.

3.7 Methods of determination of packed cell volume:

3.7.1 Apparatus and Reagents:

- Wintrobe's of haematocrit tube
- Centrifuge machine
- \circ Test tube
- Capillary pipette

- Disposable syringe
- o Cotton.

Paul hellers Reagents:

Composition:

Potassium-oxalate=0.8gm

 NH_4 oxalate = 1.2gm

Distilled water = 100ml.

3.7.2 Procedure:

1.0.2ml of Paul holler's mixture was taken in a test tube. The 2ml of blood was added into the test tube containing anticoagulant fluid.

2. The blood was thoroughly mixed with the fluid by gently shaking the test tube.

3.With the help of a capillary pipette Wintrob's tube was filled with blood starting at the bottom s' with drawing the pipettes' the tube was filled from below upwards. The tube was filled up to the mark 10. It any air bubble it may removed from the top of the column of blood so that it stood exactly at 10.

4. The Wintrobe's tube was centrifuged by a rate of 2500-3000 RPM for about 30mins.

3.7.3 Observation:

After centrifugation the blood was separate into 3 layers

- 1) A tall bottom layer of packed red cells.
- 2) A very thin middle layer of WBC & platelets. (called Buffy coat)
- 3) Top coyer of clear plasma.

3.8 Methods of determination of erythrocyte sedimentation rate:

3.8.1 Materials and reagents require:

- 1) Wintrobe's tube
- 2) Test tube
- 3) Test tube rake

Paul Heller's mixture or Double oxalate mixture.

Normal Composition:

K-oxalate: 0.8gm

NH4-oxalate 1.2gm

Distilled water: 100 ml

Procedure:

1. 2ml of blood was collected and transferred to the tube containing the anticoagulatnt in the proportion of 1-2 mg of dried powder for each .ml of blood .

2. Blood was drawned in a capillary tube and wintrobe's tube was filled up to mark o cm from above to the bottom.

3. The tube was placed in vertical position and reading was taken after 1 hrs.

4. At the end of 1 hrs the height at the clear plasma was taken from the graduation, this reading give the ESR in mm in lst hrs.

Statistical analysis:

The data obtained were recorded in the Microsoft Excel and transfer into the statistical software STATA-7 for analysis. A descriptive analysis was carried out for the obtained data. The data were expressed as mean and standard Deviation.

CHAPTER 4

RESULTS

A total number of 35 samples were collected (3 Dalmatian, 2 Doberman, 2 German shepherd, 15 Labrador, 9 Spitz and 4 other breeds). All the samples were collected from Madras veterinary college clinics, from the owners of the breeds. All the samples were collected from the animals having signs shown in pyometra.

4.1 Results for breed variation of blood parameters:

There were found hematological variation for different breeds. In the present study there were 35 samples having 6 categories of dog breeds. Here are the following results:

Name of breed	Number of cases	Total samples	Percentage
	found		
Dalmatian	3	35	8.57
Doberman	2	35	5.7
German shepherd	2	35	5.7
Labrador	15	35	43
Spitz	9	35	26
Others	4	35	11

Table 2: Breed percentage of the study

Table 2 shows that 8.57% of the total samples were from Dalmatian, 5.7% Doberman, 5.7% German shepherd, 43% Labrador, 26% Spitz and 4% were other breeds. Other breeds included 1 pug, 1 Rottweiler, 2 Non-descriptive breeds.

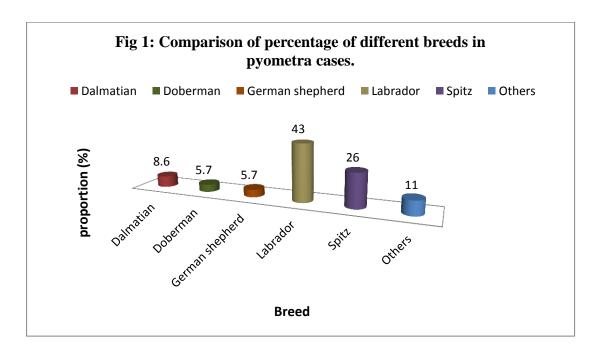


Fig 1 shows the comparison of percentage of different breeds in the study. Percentage of Labrador was highest as shown in the figure.

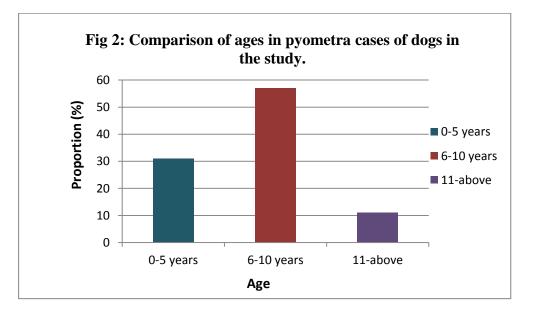


Fig 2 shows the comparison of ages in pyometra cases of dogs in the study. Age category of 6-10 years was highest as shown in the figure.

Age categorical study for pyometra:

4.2 Results for age variation in pyometra:

All the 35 samples were also categorized as 1, 2, 3 for age 0-5, 6-10, 11-above respectively. Age limit for causing disease is also an important factor.

Table 3: Age categorical percentage of pyometra

Age(years)	Number of samples	Total samples	Percentage
0-5	9	35	31
6-10	22	35	57
11-above	4	35	11

Table 3 shows that 37% of the samples were within 0-5 years age limit, 6-10 years and 11above years breed were found 57% and 11% respectively. There were found highest 57% of the cases in 5-10 years age category.

4.3 Results for hematological parameters in pyometra:

Hematological	Samples	Total samples	Percentage	
findings				
Neutrophilia	29	35	82.86	
Hypochromatia	6	35	17.14	
Polychromatia	2	35	5.71	
Anisocytosis	2	35	5.71	
Poikilocytosis	1	35	2.86	
Acanthocytosis	2	35	5.71	
No abnormality	5	35	14.23	
detected(NAD)				

Here the table shows the hematological findings for blood parameters:

Table 4: Hematological findings of pyometra cases of the study.

Table 4 shows that in these 35 samples there are 82.86% neutrophilia, 17.14% hypochromatia, 5.71% polychromatia, 5.71% anisocytosis, 2.86% poikilocytosis, 5.71% acanthocytosis and in 14.23% there were found no abnormality. The highest percentage was found for neutrophilia and the lowest percentage was found in poikilocytosis.

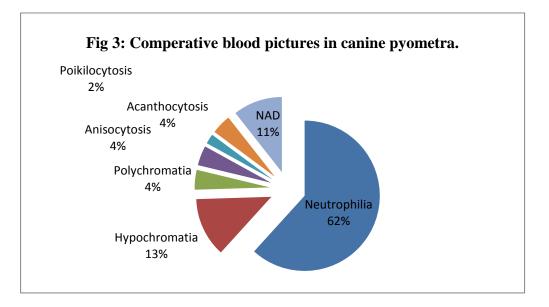


Fig 3 shows the comparative blood pictures in canine pyometra. There was found neutrophilia in most of the cases.

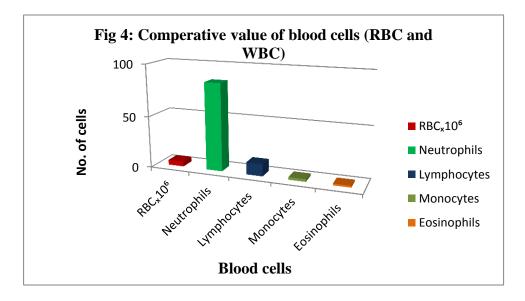


Fig 4 shows the comparative values of different blood cells where Neutrophils value was highest.

CHAPTER 5

DISCUSSION

The present study was targeted to find any correlation between breed of dog and prevalence of pyometra. In my study there were found that 57% of the cases were Labrador retriever breed, and lowest percentages were found in Doberman and German shepherd. **Boerresen, 1975** studied 119 cases of pyometra in Norway and found them to be randomly distributed among the different breeds. In a Swedish study 487 Swedish cases of pyometra were studied from a total of 10,993 postmortem examinations and detected a significant predisposition for pyometra in the Saint Bernard, Rottweiler, Chow Chow, Swedish Hound, French Bulldog, Collie, Pointer, Great Dane, and Skye Terrier. This finding is in accordance with the moderately higher risk for pyometra in the Rottweiler and rough Collie found in the present study, in which only 30 breeds were evaluated. Lower risks also were found for the German Shepherd Dog that was my finding in my study.

The interactions with biologic age could be interpreted to mean that in some breeds (eg, Rottweiler, Golden retriever, and Labrador retriever) the risk of pyometra actually increases more and at an earlier age compared with other breeds. These breeds may carry a higher genetic predisposition to pyometra than other breeds. Although normally increased age suggests more susceptibility but biologic age is the variation and breed susceptibility. In general, breed differences may reflect true genetic differences or may be a reflection of different life spans in different breeds (**Egenvall et al., 2001**).

In female dog pyometra although most common but age is inter connected with pathogenesis. In this study it was found that 57% of the cases were found at the age of 6-10 years of age limit. In a Swedish study, based on Animal insurance data, there were found that 23.24% of all bitches developed pyometra before the age of 10 years (**Hagman, 2004**).

In a colony of bitches 15.2% of the bitches more than 4 years of age were affected, whereas the mean age was 9.4 years at the time of clinical signs (**Fakuda**, **2001**).

According to Dow 1958 the mean age of bitches affected by the disease ranges between 6.4 and 8.5 years. However, pyometra has been reported in bitches from 4 months to 18 years of age (**Stone et al., 1988**)

Pyometra is associated with several hematological parameters. The highest value of erythrocyte was found 7.06 million/mm3 and lowest value was found 1.26 million/mm3. According to (**Schepper et al., 1987**) erythrocyte number in pyometral case is lower than the normal value (5-9million/mm3). The study here also shows anemia in these animals.

The decreased value of RBC and hemoglobin shows blood loss through vaginal discharge. But this anemic condition is mostly associated with open cervix pyometra (**Verstegen et al., 2008**).

The highest value of Hb% was found 16.5% and the lowest value was found 6%. But the range for normal Hb% in dog is 12-18%. This also indicates anemia. In case of pyometra there is mostly found normocytic and normochromic anemia (**Pretzer, 2008**). The anemia has been suggested to be caused by decreased erythropoesis, so called anemia of chronic disease and by loss of erythrocytes into the uterine lumen (**Sandholm et al., 1975**). According to **Nelson et al., 1998** anemia of chronic disease can be caused by a variety of disorders including chronic inflammation, in which lactoferrin and other acute phase reactants mediate an iron sequestration within the myeloid cells in the bone-marrow, withdrawing iron from the normal erythropoiesis.

Leukocytosis is a distinguishing hematologic indicator (Wheaton et al., 1989; Frasson et al., 1997). Normal range for leukocyte in dog is 1000-4800 (Andersen et al., 1958). Leukocytosis is found to be more dominant in closed cervix pyometra (Feldman et al., 1989). Our present study was also consistent with this. According to the (Khuen et al., 1940) the total number of leukocyte is frequently elevated in case of pyometra. Study also reveales in case of pyometra total leukocyte count has been increased in all the cases. The mean leukocyte value is found 24 thousand/cu mm, that is higher than the normal value.

In the study the highest value of PCV was found 48 and the lowest value was 17. In case of pyometra according to the (Asheim, 1964; Renton, et al., 1971) the PCV range (36-40) may accompany pyometra. From the picture we can say that PCV value was less than the normal. There is found 30.4% PCV as the mean value that ranges in the normal value. The decrease value in PCV indicates anemia that is coincided with **Pretzer**, 2008.

In the differential leukocyte count of the several types of domestic animals the most significant species characteristic is the neutrophil lymphocyte (N: L) ratio. The N: L ratio is 3.5 for the dog (**Schalm, 1962**). Here this ratio has been found high in all cases due to higher percentage of neutrophils.

The total number of leukocytes is frequently elevated in cases of pyometra (Khuen et al., 1940), although the degree is much less marked in case of open-cervix pyometra compared with closed-cervix pyometra, (Morris et al., 1942). However an elevated white cell counts is not always present (Sheridan, 1979).

There were found 17.14% hypochromasia, 5.71% polychromasia, anisocytosis was found as 5.71%, no abnormality was found in 14.23%. The value of hypochromasia indicates normochromic anemia that is associated with vaginal bleeding during pyometra.

CHAPTER 6

RECOMMENDATION

Pyometra is a common, potentially lifethreatening disease of bitch that is unnutered. Proper managemental strategies may be helpful in preventing this disease. Following strategies can be helpful in preventing pyometra and pet owners should be aware of these strategies:

Future Management Strategies for Pyometra

- Early diagnosis and therapeutic intervention may prevent severe complications associated with advancing sepsis in dogs with pyometra. For this purpose, it is crucial to identify diagnostic or prognostic biomarkers that can be used in clinical practice.
- To be able to detect and evaluate suitable biomarkers, it is necessary to obtain more knowledge of the aetiology, pathogenesis and systemic development of the disease.
- ◆ In the long-term perspective, preventive measures for pyometra need to be explored.
- Development of a vaccine aimed at certain virulence attributes of bacteria associated with pyometra could be applicable for susceptible individuals.
- A genetic predisposition for the disease might, if identified, provide the possibility of implementing breeding programs aimed at reducing the occurrence in high-risk breeds.
- Prevention of pyometra by elective neutering has the advantage of surgery being performed in a healthy individual, but unwanted side effects of the procedure occur.
 Possible health benefits of neutering will need further investigation because of large breed variations

CHAPTER 7 CONCLUSION

The study was conducted to clinically investigate canine pyometra and the hematological correlation with pyometra. In my study there were found that almost 83% of the cases showed neutrophilia, almost 63% of the patients were anemic . Here leucocytosis with neutrophilia and increase level of PCV correlate with tentative diagnosis In my study I have also tried to make a correlation between dog breed and prevalance of pyometra and age variation in my 35 samples. Most of the cases were for Labrador retriever that was 43% and 26% of the cases for Spitz. The study also revealed that after 6 years dog breeds become mostly affected by pyometra.

As day by day dog population as pet increasing, owners must become aware of this deadly disease. Managemental strategies may be helpful in prevention of pyometra. Proper hospitalization is needed as early as possible. So, "never let the sun set on a pyometra",

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ANNEX

A RECORD KEEPING DATA SHEET

Data sheet during examination of the animal:
Owners name:
Address of owner:
Registration no:
Date:
PATIENTS DESCRIPTION:
Species:
Sex:
Breed:
Age:
Body weight:
Owner's complaint:
Feeding habits:
Clinical findings:
Tentative diagnosis:
Blood collection:

Signature