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Clinico-haemato-biochemical and Pathological Alteration of Pyometra in Canines

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ABSTRACT

Background: Pyometra is a pathological state of the compromised uterus of grown-up to old, diestrum bitches. Pyometra is easy to identify but can be more challenging when there is no vaginal discharge. In the present study, an attempt was made to find out the pathogen involvement and clinico-haemato-biochemical alteration in bitches affected with Pyometra.

Methods: Twelve bitches with a history of inappetence, polydipsia, dirty vaginal discharge, occasional vomition irrespective of breeds and age groups were clinically examined and diagnosed as pyometra. The presumptive clinical diagnosis was based on case history, clinical signs, vaginal cytology, abdominal ultrasonography or radiography, gross examination of a pus-filled uterus after the ovariohysterectomy.

Result: Vaginal examination revealed brown chocolate malodorous vaginal discharge with discolouration of the perivulval tissue. Abdominal ultrasound showed hyperplasia with echogenic material in the uterine wall along with distended uterine horns containing a large volume of cellular contents. Radiographic imaging revealed cranio-dorsal displacement of the small intestine and a twisted uniform tubular opacity with a distended uterus. The vaginal cytology revealed a large number of degenerated neutrophils, intermediate and parabasal cells. *Escherichia coli* were the major pathogen isolated from the pyometra uteri of bitches. The surface of the endometrium was columnar ciliated epithelium, with hyperplasia of subepithelial tissue and thick muscularis. The haemato-biochemical evaluation in pyometra infected bitches revealed decreased haemoglobin (Hb), packed cell volume (PCV), total erythrocytic count (TEC) and lymphocyte count indicating the microcytic hypochromic type of anaemia. Leucocytosis with a predominant absolute neutrophilia and lymphocytosis were consistently found in canine pyometra. There was a significantly elevated profile of blood urea nitrogen, creatinine, transaminases and alkaline phosphatase as well as total proteins observed in pyometric female dogs as compared to healthy ones which may have diagnostic and prognostic importance, which also indicated damage of vital organ caused by bacterial endotoxins. The haemato-biochemical indices correlating with imaging techniques, vaginal cytology and histopathological examination can serve as good prognostic markers in bitches with pyometra.

Key words: Canine, Haemato-biochemical, Imaging technique, Pyometra, Uterine pathology.

INTRODUCTION

Pyometra is a pathological condition of the compromised uterus of old to previous, diestrual bitches and should occur as results of an exaggerated and abnormal response to chronic and perennial progestogen stimulation (Corrada *et al*. 2006). The pyometra is developed due to invasion of *Escherichia coli (E. coli)* to the uterus from the vagina and started to proliferate inside the numerous cysts and crypts when the local immunity compromised (Batista *et al*. 2016).

To diagnose the condition, a history of recent heat sign and other clinical signs, especially vaginal discharge, should raise the suspicion. The diagnosis can be more challenging in certain condition when the sign of vaginal discharge is lacking in bitches. Haemato-biochemical evaluations are not specific for pyometra. But, the presence of inflammation and secondary metabolic disturbances are more often in pyometra cases. The haematological analysis will often demonstrate a neutrophilia with a degenerative left shift which may be also identified in animals with endotoxemia (Hardy and Osborne 1974). Pyometra may cause dehydration of the animal as a result BUN and creatinine level is elevated which is also found in kidney insufficiency in an older bitch (Asheim 1965). Generally, abdominal ultrasonography is the preferred method for evaluating pyometra in bitches and queen (Dennis and Brian 2012). It may also be accustomed to exclude alternative conditions which will cause female internal reproductive organ enlargement or discharge, like early physiological state

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(Dennis and Brian 2012). In the present study, an effort was made to evaluate the haemato-biochemical alteration along with histopathological changes of the uterus of bitches having pyometra.

MATERIALS AND METHODS

Twelve numbers of diseased bitches presented to Teaching Veterinary Clinical Complex, College of Veterinary Sciences and A.H., Central Agricultural University, Aizawl, Mizoram with a history of lethargy, depression, inappetence, polydipsia, occasional vomition and vaginal discharge were included in this study. All the animals were subjected to clinical examination with standard procedure. Six healthy dogs were also included in this study for comparison. For confirmatory diagnosis, each bitch was subjected to ultrasonography (Esaotemylab 40, Italy), X-ray, vaginal cytology (Haji *et al*. 2018) and haemato-biochemical analysis. Five milliliters of blood was collected aseptically from each animal with and without EDTA vial to study the haemato-biochemical changes of pyometric and healthy bitches. The haematological parameters were determined using an automated blood counter (MS4E, French). The levels of blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total protein were determined by using standard procedures and assay kits of Crest Biosystem, Goa, India on an autoanalyzer. The experimental plan of study was duly approved by the Institution Animal Ethics Committee, College of Veterinary Sciences and A.H., Central Agricultural University, Aizawl, Mizoram. Ovario-hysterectomy was carried out following the diagnosis of pyometra (Konwar *et al*. 2020). Stabilizing the animals' conditions was tried before surgery by giving fluid by intravenous route and antibiotics. Supportive therapy was followed for at least seven days in the postoperative stage.

For histopathological examination, the pyometric uterus, uterine tissues and ovary was collected in 10% formalin after the ovariohysterectomy and sent to the Pathology Department. The tissues were embedded in paraffin and Paraffin blocks were sectioned at five ìm, stained with Hematoxylin-Eosin (H&E) and examined below a lightweight magnifier.

Data analysis

The data was analysis by using SPSS software version 16.0 and presented as mean ± standard error (SE). The haemato-biochemical parameters of pyometra affected bitches were compared with healthy ones by using paired t-test.

RESULTS AND DISCUSSION

The clinical examination of pyometra infected dogs revealed lethargy/depression (100%, 12/12), inappetence (100%, 12/12), vaginal discharge (83.33%, 10/12), polydipsia (83.33%, 10/12), abnormal mucous membranes (75%, 9/12), uterine distension (75%, 9/12), polyuria (66.66%, 8/12), vomition (41.66%, 5/12), fever (41.66, 5/12) and dehydration (33.33%, 4/12) (Fig 1). Vaginal examination revealed brown chocolate malodorous vaginal discharge.

The progesterone stimulation during diestrus period, make a suitable environment for bacterial proliferation which lead to accumulating the uterine secretions, prominent endometrial gland crypts and immune suppression and as a result pyometra developed (Dow 1957). Presence of mature corpora lutea in pyometra infected bitches can supporting a synergistic hormonal effect (Strom *et al*. 2001). Progesterone-mediated pathologic proliferation leads to the growth of endometrial glands. When there is no bacterial infection of the uterus, the infection may be subclinical or mild (Hagman *et al*. 2006). In open pyometra cases, a continuous or intermittent mucopurulent to hemorrhagic vaginal discharge is often observed but absent in closed pyometra (Borresen *et al*. 1979). In closed pyometra, the systemic illness is more severe than those with open-cervix pyometra and the uterus becomes severely distended (Pretzer 2008; Jitpean *et al*. 2017). In closed pyometra, closed cervix prevents the elimination of the infectious uterine material and as a result it is complicated with septicemia, endotoxemia (most commonly associated with *E. coli* infection) and septic peritonitis (Nelson *et al*. 1982).

Major clinical signs observed in the present study were Fever, dehydration, vomiting, abdominal pain on palpation, anorexia, depression/lethargy, polydipsia, polyuria, tachycardia, tachypnea, increased pulse rate, abnormal visible mucous membranes and gait abnormalities which was also supported by Borresen *et al*. (1979). The increased level of rectal temperature, heart rate, pulse rate and respiration rate of pyometra affected bitches might be due to uterine inflammation and septicemia, bacteremia and toxaemia (Nath *et al*. 2009; Jena *et al*. 2013). However, shallow respiration rate was also reported in pyometra infected bitches (Feldman and Nelson 2004).

Diagnosis of pyometra by imaging techniques

The radiographic projections in most cases revealed a craniodorsal displacement of the small intestine and a twisted uniform tubular opacity in the caudoventral abdomen at lateral recumbency (Fig 2: A1). There was a cranial and medial displacement of the small intestine when the animal was in the ventro dorsal position (Fig 2: A2). Here in Fig 2 (A1 and A2), the largely distended uterus was visualized. Cranial and dorsal displacement of the small bowel is apparent. The abdominal ultrasonographic examination revealed an anechoic to hyperechoic fluid filled uterus with distention (Fig 2: B1 and B2).

Diagnostic imaging is efficacious for crucial the female internal reproductive organ size and to rule out alternative causes of female internal reproductive organ enlargement. X-ray and ultrasonography have the advantage of detecting intrauterine fluid, even when the uterine diameter is within the normal range and of revealing additional pathologic changes of the uterine tissue and ovaries, such as ovarian cysts or CEH, which may affect the outcome of medical treatment negatively. Additional sophisticated diagnostic

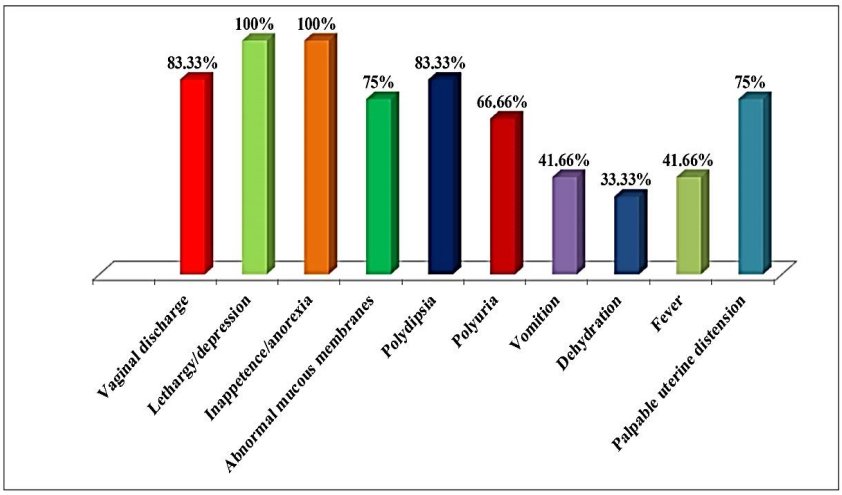

Fig 1: Clinical signs in bitches with pyometra.

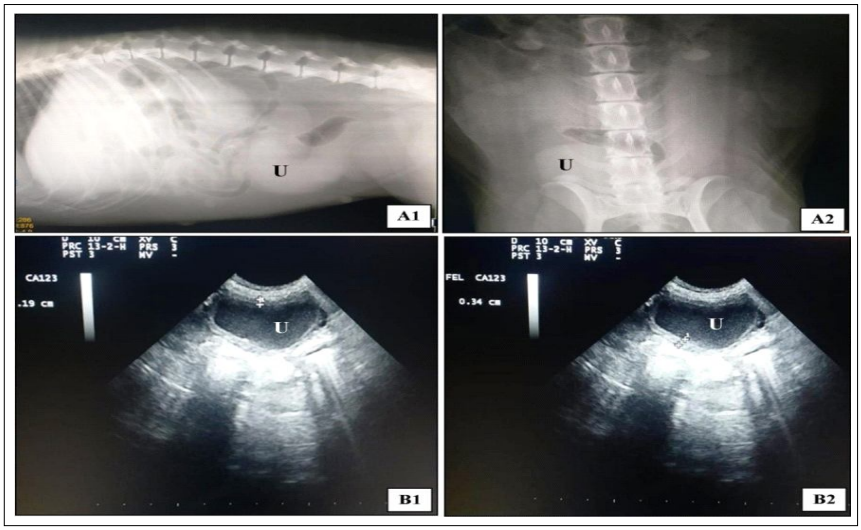

Fig 2: A1: lateral and A2: ventrodorsal radiographic projection of pyometric bitch with distended uterus (U); B1 and B2 showed ultrasonographic imaging of pyometric uterus with irregular thickening of the uterine wall and distended uterus (U).

imaging techniques are hardly ever necessary. Differential diagnoses include mucometra, hydrometra and hematometra that may have similar clinical presentation and ultrasonography findings (Bigliardi *et al*. 2004; Haji *et al*. 2017). The radiographic projections in most cases at lateral recumbency revealed that there was the craniodorsal displacement of the small bowel and a twisted uniform tubular opacity in the caudoventral abdomen (Root 2002). Radiography wills insensible for sleuthing pyometra as female internal reproductive organ distention generally can't be detected till the diameter of the womb is larger than that of the adjacent tiny bowels (Root 2002). Additionally, radiography cannot distinguish pyometra from alternative causes of female internal reproductive organ distension, like mucometra or early maternity (before craniates skeletal mineralization). The irregular and thickening of the uterine wall revealed during USG examination might be due to prolonged or repeated stimulation of progesterone hormone on the endometrium. An increased level of progesterone concentration (>40 ng/ml) during pyometra promotes endometrial hyperplasia (Haji *et al*. 2017). In a case of uterine rupture, there may be free fluid within the abdominal cavity and the omentum may be hyperechoic secondary to bacterial peritonitis (Dennis and Brian 2012). Abdominal prenatal diagnosis also can be wont to exclude alternative conditions that may cause female internal reproductive organ enlargement or discharge, like early maternity.

Influence of pyometra on vaginal cytology

The vaginal cytology revealed that there was a large number of neutrophils, intermediate and parabasal cells (Fig 3) in the open cervix pyometra of bitch.

The cytological examination is not a proper diagnostic tool for pyometra without additional confirmatory testing as same findings are also observed in vaginitis. The absence of excessive neutrophils and bacteria in the vaginal discharge would warrant reconsideration of the cause (Dennis and Brian 2012). Meanwhile, vaginal cytology

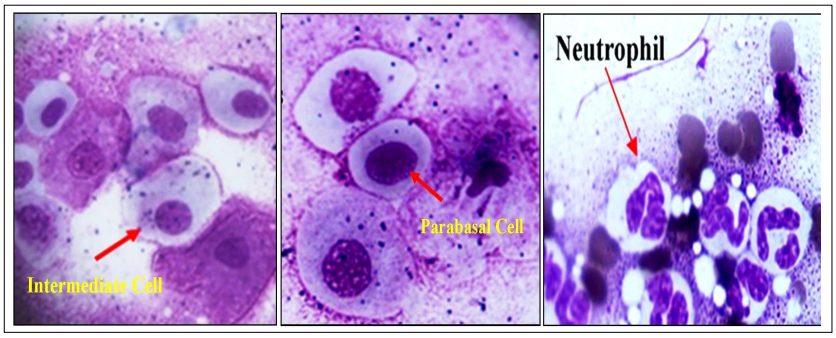

Fig 3: Vaginal cytology showed the presence of intermediate cell, parabasal cell and neutrophils from a bitch with open-cervix pyometra.

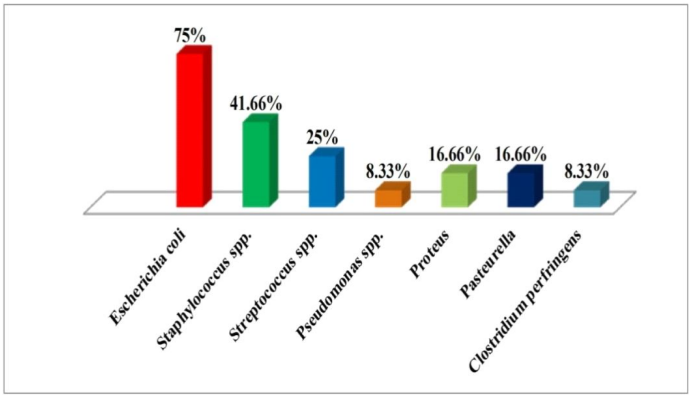

Fig 4: Different microbial species isolated from the uterus of bitches with pyometra.

findings in bitch with completely closed-cervix pyometra may only reflect the stage of the estrous cycle. Intermediate and parabasal cells are the predominance cells during diestrus stage and an influx of non-degenerate neutrophils in early diestrus (Dennis and Brian 2012). The presence of neutrophils in vaginal cytology might be due to increased progesterone concentration during pyometra which promotes endometrial hyperplasia and provides excellent media for bacterial growth (Corrada *et al.* 2006).

Microbial species isolated from the uterus of bitches with pyometra

The coli form bacteria *i.e. Escherichia coli* was the leading pathogen isolated from pyometra uteri of bitches (75.00%), other than that other species of *Staphylococcus, Streptococcus, Pseudomonas, Proteus, Pasteurella* and *Clostridium perfringens* were also responsible for the cause, the percentage is depicted in Fig 4.

Several bacterial species can be involved in pyometra, but the cultures are sometimes negative (Grindlay *et al*. 1973). In a normal physiological state, the uterus eliminates bacteria those able to enter through the cervical opening, but the capacity may compromise in the sick uterus. *E coli* infection leads to CEH/pyometra during the luteal phase compared to other oestrous cycle stages (Nomura *et al*. 1988). The infection is most likely ascending because the same strains are present in the gastrointestinal tract, but haematogenic spread could also occur (Agostinho *et al.* 2014). The natural inhabitants of *E. coli* in vaginal flora (Watts *et al*. 1996), may have an increased ability to adhere with specific receptors in a progesterone-stimulated endometrium (Sandholm *et al*. 1975). These bacteria and bacterial products play a major role as a potent inducer of local and systemic inflammation which induces fever, lethargy, tachycardia and Tachypnea (Van Miert and Frens 1968).

Uterine pathological alterations of the bitches affected with pyometra

Macroscopic findings

The uterine horns were swelled, hyperemic and having full of whitish pus in the lumen and thickening of the uterine mucosa in most of the cases **(**Fig 5**)**. The uterine mucosa of pyometra infected bitches showed longitudinal folds with ulcerated areas. There was congestion and ecchymotic haemorrhage of the body of the uterus of bitch.

The macroscopic examination revealed swollen and hyperemic uterine horns with full of whitish pus in the lumen. Longitudinal folds and certain ulcerated areas were also observed in the uterine mucosa. There was congestion and ecchymotic haemorrhage of the body of the uterus of bitch which was also observed by Coggan *et al.* (2008). They also reported that the epithelial cells and glandular uteri were degenerated which was also observed in this study.

Histopathological changes

The surface of the endometrium was a pseudostratified

ciliated columnar epithelium; with hyperplasia of subepithelial tissue and thick muscularis with primary and secondary folds (Fig 6). The degenerated surface epithelial cells and cystic degeneration on the glandular uteri were markedly observed. The stratum vasculature showed the evidence of leukocytosis. In other cases, the glandular uterus having degenerative hyperplasia and atrophy and the stromal connective tissue was characterized by congestion and haemorrhage.

Effect of pyometra on physiological parameters of bitches

In the present study, there was an increase in mean rectal temperature, heart rate (bpm), pulse rate (bpm) and respiration rate of pyometra affected bitches (102.32±0.23 °F, 125.83±3.14 beats/min, 135.46±3.07 beats/min and 32.4±1.44 per min, respectively) in comparison to healthy bitches (99.76±0.23°F, 102.91±2.91 beats/min, 110.0±2.33 beats/min and 22.33±0.53 per min, respectively).

Effect of pyometra on haematological parameters

In the present study, it was observed that the haematological parameters *viz*. Hb, PCV, RBC, were significantly decreased (P<0.01) in pyometric bitch in comparison to healthy which indicated anaemia (Table 1).

The DLC analysis revealed neutrophilia, lymphocytosis with eosinophilia in pyometra affected bitches (Table 1) as compared to healthy.

A microcytic hypochromic type of anaemia observed in pyometric bitch might be due to the loss of red blood cells by diapedesis into the uterine lumen apart from depressed feed intake and impaired erythropoiesis under the toxaemic condition in severely affected cases. The findings of the present study are in concordance with the previous reports (Nath *et al*. 2009; Jena *et al*. 2013). Different degree of leucocytosis observed in pyometra infected dogs might be due to varying degree of the inflammation (Dabhi *et al*. 2009) which is in agreement with the previous reports (Dabhi *et al*. 2009; Babu *et al*. 2018).

The observed neutrophilia, lymphocytosis with eosinophilia in pyometra affected bitches might be due to uterine infection, which exerts a chemotactic effect on neutrophils resulting in accelerated granulopoiesis and lymphocytosis (Leib and Monnie 1997).

Effect of pyometra on blood metabolites

Total protein

The mean total protein level (9.17±0.35 g/dl) of pyometra affected bitches was significantly (p<0.01) higher, than healthy ones (7.75±0.35 g/dl) (Table 1).

BUN and Creatinine

The kidney function test *viz*. blood urea nitrogen (BUN) and creatinine level (66.12±6.51 mg/dl and 1.95±0.24 mg/dl, respectively) was significantly (P<0.01) elevated in pyometra affected bitch as compared to healthy (35.41±3.53 mg/dl and 1.30±0.18 mg/dl, respectively) (Table 1).

Liver enzymes activity in pyometric bitches

The respective mean activities of liver enzymes *viz*. serum alanine aminotransferase (ALT), serum aspartate

Table 1: Level of physiological and haemato-biochemical parameters of pyometra affected and healthy bitches (mean ± SE), *n*= 12.

Parameters	Pyometric bitches	Healthy bitches	t-value
Temperature (°F)	102.32±0.23	99.76±0.23	10.173^{NS}
Heart rate (bpm)	125.83±3.14	102.91±2.91	8.427**
Respiration (per minute)	32.41±1.44	22.33±0.53	6.811^{NS}
Pulse (bpm)	135.46±3.07	110.0±2.33	6.524**
Haemoglobin (gm/dl)	9.52±0. 69	12.25±0. 45	4.67**
RBC (×10^6/µL)	5.05±0.24	6.85±0.25	6.587**
PCV(%)	27.65±1.15	38.58±1.22	8.240**
TLC(× 1000/µL)	49.83±34.29	12.08±14.82	4.652**
Nutrophil (%)	94.00±1.91	48.87±1.45	12.493**
Lymphocyte (%)	36.95±2.75	28.87±3.45	3.172**
Monocyte (%)	6.82±0. 59	4.84±0.44	4.561**
Eosinophil(%)	1.80±0.17	0.91±0.13	7.454**
Basophil	Nil		
MCV (fL)	45.66±1.91	63.87±2.05	7.958**
MCH (pg)	16.20±0.93	21.08±0.56	6.962**
MCHC (%)	22.29±0.86	31.62±0.51	14.057**
BUN (mg/dl)	66.12±6.51	35.41±3.53	5.787**
Creatinine (mg/dl)	1.95±0.24	1.30±0.18	4.854**
AST (IU/L)	55.80±4.22	42.50±4.02	5.487**
ALT (IU/L)	39.62±4.30	30.33±3.69	5.124**
ALP (IU/L)	155.71±11.20	86.25±9.34	7.368**
Total protein (g/dl)	9.17±0.35	7.75±0.35	5.690**

**P<0.01; NS: Non-significant.

Fig 5: The swelled uterus and ecchymotic haemorrhage (A), ulcerated area (B) in the uterine mucosa of pyometric bitch.

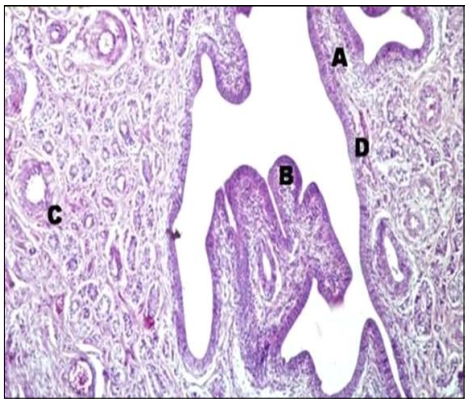

Fig 6: Pyometric uterus of bitch with primary folds (A), secondary folds (B), uterine gland (C) and pseudostratified ciliated columnar epithelium (D). H&E, 100X.

aminotransferase (AST) and alkaline phosphatase (ALP) in pyometric bitches (39.62±4.30 IU/L, 55.80 ± 4.22 IU/L and 155.71±11.20IU/L, respectively) were significantly (P<0.01) increased (Table 1) as compared to healthy bitches (30.33±3.69 IU/L, 42.50 ± 4.02 IU/L and 86.25±9.34IU/L, respectively).

The elevated total protein, BUN and creatinine concentrations in pyometra affected bitch might be due to chronic inflammation and dehydration (Dennis and Brian 2012). Bacterial endotoxemia and reduced liver perfusion are thought to contribute to elevated liver enzyme activities. The alterations of liver enzymes ALT, AST and ALP in pyometric bitches reflect the function of the liver, including hepato-cellular damage due to toxaemia or the impaired hepatic circulation due to dehydration (Borresen *et al*. 1979).

CONCLUSION

In conclusion, the results of the present study revealed that the prognosis of the disease may improve in bitches diagnosed as pyometra associated with alteration of haemato-biochemical parameters. The haemato-biochemical evaluation revealed decreased Hb, PCV and TEC in the bitches affected with pyometra. The leucogram revealed leucocytosis with neutrophilia. There was a significantly elevated profile of blood urea nitrogen, creatinine, transaminases and alkaline phosphatase as well as total proteins observed in pyometric bitches as compared to healthy ones which may serve as good prognostic markers in bitches with pyometra along with other diagnostic methods.

Conflict of interest: None.

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