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HEMATOLOGICAL,BIOCHEMICAL AND HISTOPATHOLOGICAL STUDIES ON THE CORRELATION BETWEEN ANTIOXIDANT TRACE ELEMENTS (Se, Zn,Cu) AND OXIDATIVE STRESS IN PYODERMA INFECTED DOGS

By

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ABSTRACT

Canine dermatological problems chiefly of bacterial etiology are among the most commonly seen disorders in canine practice that may lead to increased risk of oxidative stress. This work aimed to study the relationship between antioxidant trace elements (Zn-Cu and Se) and oxidative stress in dogs affected with pyoderma**.** This clinical study was applied on 31 dogs, 20 dogs used as control and 11 dogs affected with pyoderma to determine hematological, biochemical, histopathological and antioxidant trace elements alterations**.** Results from infected dogs showed leukocytosis accompanied by neutrophilia and lymphpenia**.** Along with statistically significant decrease in Plts and insignificant decrease in (PCV) %. Affected dogs also showed presence of hypothyroidism as demonstrated by reduced levels of free T4 and TSH. Along with statistically significant increase in the cortisol level**.** Hyperadrenocorticism were the most recorded haemato-biochemical alterations. The antioxidant trace elements indicated statistically significant decrease in selenium level (Se) together with insignificant decrease in zinc level (Zn)**.** The study revealed that pyoderma infection represents a stress factor against dogs affecting their antioxidant trace elements, consequently antioxidant defense system.

Keywords:

Canine pyoderma, hemato- biochemistry, histopathology, oxidative stress and antioxidant trace elements

INTRODUCTION

Skin is the largest and important organ of the body. It has many vital functions including protection from the extreme environment, thermoregulation and maintenance of the biochemical homeostasis besides its metabolic, immunological, and sensory effects **(Kutlay and Hoştürk, 2005 and Noli, 1999).**

Dermatological problems are reported to be the most commonly encountered and can be a nightmare for the unfortunate dog and frustrating to the veterinarian and dog owner in small animal practice **(Muller *et al*. 1989).**

Infectious skin diseases of dogs include contagious and non-contagious diseases. Contagious infections include bacterial, parasitic, fungal and viral skin diseases. Non-contagious skin infections can develop when normal cutaneous micro flora is allowed to proliferate resulting in clinical signs **(Munjal, 2012).**

Bacterial skin infections represent the most common disorders and are often recurrent. Canine pyoderma is literally means pus in the skin and caused by infectious, inflammatory, impaired local defense mechanisms,which permit secondary bacterial invasion of the skin **Fondati (2007), MacDonald (2013), Sykes *et al*. (2013) and Reddy *et al*. (2014b).**

Pyoderma was characterized by erythema, papules, pustules, scaling, collarettes, alopecia, nodules. Acute moist posttraumatic dermatitis (Hot Spot); hyperpigmentation folliculitis, plaques, ulcers and crusts, with/without draining tracts. Pruritus was variable, ranging from none to intense levels **Fondati (2007) Schissler (2009), Zabel (2011) and Hnilica (2011).**

Diagnosis can be made based on a thorough physical examination. clinical signs (Multifocal areas of alopecia, papules, pustules and epidermal collarettes), diagnostic procedures include skin scrapings, cytologic examination, swab for aerobic bacterial culture and susceptibility testing is playing a more important role in diagnosis of canine pyoderma **Zabel (2011), Sykes *et al*. (2013)and Bloom (2013).**

Skin considers as a major target of oxidative stress due to reactive oxygen species (ROS) that originate in the environment and in the skin itself **Trouba *et al.* (2002).**

Oxidative stress occurs because of elevated Reactive oxygen species (ROS) levels which cause bio molecular damage manifested by lipid, protein peroxidation, enzyme inactivation **(Özben, 1998), Prado *et al.* (2008) and (Trouba *et al.* 2002).**

Excess ROS generates oxidative stress, resulting in damage of lipid/protein/DNA and

pathological changes in cells, tissues as exemplified by various skin diseases. **Garcia-Bailo *et al*. (2011), Lenaz (2012) and Mailloux and Harper (2012) and Wagener *et al*. (2013).**

Oxidative stress has been implicated to play an important role in etiopathogenesis of various infectious, and degenerative skin disease **Singh *et al.* (2011).**

Oxidative stress has been linked to various inflammatory diseases. Inflammation is an essential response in the protection against injurious insults and thus important at the onset of wound healing. However, hampered resolution of inflammation can result in a chronic, exaggerated response with additional tissue damage. The prolonged release of excess ROS in the skin can aggravate inflammatory injury and promote chronic inflammation in which the antioxidant system may be depleted and prolonged oxidative stress occurs, **Wagener *et al*. (2013).**

Defense mechanisms including both enzymatic and non-enzymatic antioxidants Zn and Se are non-enzymatic antioxidant biomarkers. **Cárdenas (1997) and Dröge (2002).**

In case of skin diseases, the body possesses an array of a potent antioxidants that act synergistically to cause degradation of free radicals therefore combating oxidative damage **Bickers and Athar (2006) and Portugal *et al*. (2007).**

Antioxidants attenuate, delay or prevent ROS-induced cellular damage. However, increased or prolonged free radical action can overwhelm ROS defense mechanisms, contributing to the development of cutaneous diseases **(Trouba *et al.*2002) and (Paulsenand Carroll, 2013).**

Antioxidant trace elements (Zinc, Copper, Selenium) are required for the activity of antioxidant enzymes so their deficiency result in production of free radicals, tissue damage and oxidative stress **AL-Qudah *et al*. (2011) and Donia *et al.* (2014).**

Certain trace elements such as selenium (Se) and zinc (Zn) are common antioxidants **Spears, (2011); Abou-Zeina *et al*., (2013); Abou-Zeina *et al*., (2014).**

Selenium acts as antioxidant neutralize free radicals. It acts as integral part of antioxidant enzyme Glutathione peroxidase (GPX), which protects the skin against oxidative damage that caused by free radicals. It also inactivates harmful H_2O_2 via its dissociation to water and oxygen **Maryland Heights *et al.* (2011) and Dr. Richard Butterwick *et al*., 2015).**

Zinc has the ability to retard oxidative mechanism through protection of protein sulfhydryl groups or reduction of HO• formation from H_2O_2 **(Powell 2000).** Copper is an antioxidant element, essential for the activity of antioxidant enzymes, like superoxide dismutase (SOD)

and ceruloplasmin**. Hefnawy and El-Khaiat (2015).** It is the active center of more than 20 metalloid enzymes and metalloproteinase that are connected with free radicals destruction, synthesis of connective tissue formation of myelin and pigmentation **McDowell, (1999); Ortolani *et al.*, (2003) and Sousa *et al.*, (2012).**

Zinc and Copper are involved in multiple enzymatic pathways as (Cu/Zn SOD1). The activity of enzyme (Cu/Zn SOD1), and GPx is decreased in animals with Cu deficiency and increased in animal with Cu supplementation **Genther and Hansen (2014) and Ighodaro and Akinloye (2018).**

It could be concluded that bacterial infection (Pyoderma) represents a stress factor that affects the antioxidant defense system. So, this work aimed to study the relationship between antioxidant trace elements (Se- Zn-Cu) and oxidative stress in dogs affected with pyoderma**.**

MATERIAL AND METHODS

Animals:

This study was conducted on 31 dogs of different breeds and sex. Their ages ranged from 2 months to 6 years. Out of 31 dogs there were 20 apparently healthy dogs served as control group and 11 were pyoderma infected dogs that attended to the small animal medicine teaching hospital, Faculty of Veterinary Medicine,Cairo University, Egypt and small animal private veterinary clinics in Egypt from October 2020 to December 2021.

Clinical Examination:

At time of admission,case history, general clinical examination were applied for all dogs, clinical signs were recorded,animals exposed to a comprehensive special clinical examination of skin,stool and skin scraping samples were taken and examined microscopically.

Skin Swabs Samples:

Skin swab for bacterial culture were taken from suspected animals and cultured on Mannitol Salt Agar (MSA) according to technique described by **(Bannerman 2003).**

Blood Samples and Hematological Analysis:

Whole Blood Samples:

Blood samples were collected on EDTA tube from each dog from the cephalic vein or saphenous vein for examination of hematological parameters including Total erythrocyte count (TEC), packed cell volume (PCV), hemoglobin (Hb), Total Leucocyte count (TLC), Differential leucocyte count (DLC) and platelets by using Diatom hematology analyzer, USA.

Serum Samples:

Blood samples were collected from diseased and healthy dogs in plain tubes for separation of serum which analyzed for estimation of alanine amino transferase (ALT), blood urea nitrogen (Bun), creatinine, thyroid stimulating hormone (TSH) and free thyroxin (FT4), cortisol level, copper (Cu) and zinc (Zn) level using specific kits according to manufactures instructions (Spectrum diagnostic, Egypt) using Diatom chemistry analyzer (USA). Selenium (Se) measured in serum samples according to method described by **Lavu *et al.* (2012) and Van *et al.* (2015**) using Thermo scientific ICE 3300 Atomic Absorption spectrometer, Germany.

Skin Biopsy and Histopathological Examination:

Histopathological Examination:

Skin biopsy specimens were collected (Using 3 mm circular punch at a depth of 2 mm) and fixed in 10% neutral buffer formalin, washed, dehydrated, cleared and embedded in paraffin. The paraffin embedded blocks were sectioned at 4-5 micron thickness and stained with Haematoxylin and Eosin (H & E) for light microscopic examination (Olympus BX50, Tokyo, Japan **(Bancroft *et al.* 2012).**

Statistical Analysis:

Statistical analyses were performed by SPSS version 20 (IPM INC., Chicago). Descriptive statistics were measured as mean ± SE (Standard Error). Variables of diseased animals, blood parameters and trace elements were compared to that of control animals using student T-Test, P value was considered significant at < 0.05 level of probability.

RESULT AND DISCUSSION

a) Clinical Examination:

During carful scrutinization of pyoderma infected dogs. The most consistent clinical signs in pyoderma affected dogs recognized as a focal lesion in dog's trunk were pustules, papules, puritus, epidermal collarettes,hyperpigmentation,and alopecia,while erythema,edema,vesicles and erosion along with alopecia. Our results came in accordance to **(Wildermuth *et al.* 2006) and Jaheen (2015)** who mentioned that superficial pyoderma is associated with pruritus, erythema, pustules, papules, epidermal collarettes, and multifocal alopecia.

b- Microscopic Examination:

Skin scraping were taken from lesion of infected animals and examined microscopically for exclusion of fungal and external parasitic infection. Fecal samples were taken for examination of internal parasites **(Houston 2000).**

c- Bacteriological Culture's Finding:

Yellow colonies surrounded by yellow medium caused by coagulase-positive Staphylococcus auras were found in samples confirming the presence of pyoderma.This results came in accordance to **(Rich 2005) and Kubasy *et al.*, (2017)** who reported that S. *aureus* consider one of the most common species isolated from pyoderma lesion.

d- Histopathological Finding:

Sections of skin biopsy from lesions showed pronounced histopathological alterations described as large focal area of epidermal liquifactive necrosis associated with dense neutrophils infiltration.The dermis showed severe dermatitis, folliculitis, perifollicular and perivascular inflammatory cells infiltration mainly neutrophils,lymphocytes and macrophages as shown in photos (6&8).These finding come in accordance with **Arbaga *et al.* (2021), Baumer *et al*. (2016), Rafatpanah, *et al.* (2020) and Rojko *et al.* (1978)** who demonstrated epidermal necrosis and edema, dermal collagen necrosis, and severe inflammatory cells infiltration, mainly neutrophils in the epidermis, dermis, and around the hair follicles either aggregate or in a diffuse manner.

e- Hematological Examination Finding:

Regarding the erythrogram as showed in (Table 1), our findings revealed statistically non-significant decrease in the value of PCV and Hb without any significant difference in the other**, Lodh and Das, (2014) and Farag (2016).** With reference to leukogram, (Table 1), leukocytosis was common due to statistically significant increase in the neutrophils (Neutrophilia), in addition there was lymphpenia.Ourfindings werecompatible with **Weiss and Wardrop (2010)** who explained that inflammation is the most frequent cause of neutrophilia, left shifts of great magnitude may occur in dogs that have pyoderma. Also agreed with **Aujla *et al*. (1997)**,**Ihrke (2006),Lodh and Das (2014) and Farag (2016)** who mentioned that dogs with bacterial pyoderma often reveal neutrophilia, lymphpenia but not similar to results of **Shyma and Vijayakumar (2011)** who found that pyoderma infected dogs had significantly lower mean value of total leukocyte count (TLC) and **Min *et al.* (2014)** who found leukopenia with neutropenia in dogs with pyoderma.

F-Biochemical Analysis Finding:

Rgarding evaluation of ALT, BUN, and creatinine as showed in (Table 2) our results revealed no statistical significance difference. Our findings come in accordance with **Sentürk *et al.* (2005), Jaheen (2015) and farag (2016**) who found that no statistical significance difference recorded in (ALT), (Bun) and creatinine levels in in dogs with pyoderma. Respecting evaluation of endocrine function as showed in (Table 2), our results demonstrate presence of significant decrease in the level of TSH with insignificant decrease in level of ft4. Hypothyroidism is the most common endocrine disorder in dogs with non-specific clinical signs beside presence of numerous factors that influence thyroid function **Daminet (2002).** Dermatological changes such as dry skin, changes in coat quality or color, alopecia or seborrhoea were described in 60-80 % of hypothyroid dogs **(Panciera, 1997; Dixon *et al*. 1999 and Scott-Moncrieff and Guptill -Yoran, 2000)**. Recent study by **Srikala and Kumar (2014)** demonstrated that dermatological disorders were very high (80%) in hypothyroidism. Combined measurements of the concentrations of serum thyroid-stimulating hormone (TSH) and free T4 (fT4) are recommended **(Dixon *et al*, 1999).** However, determination of the fT4 concentration in canine serum samples has several advantages over traditional measurements of the tT4 concentration **(Larsson, 1988).**Our findings come in accordance with **Mederle *et al.* (2010), Bate (2013), Mctaggart (2013) and Farag (2016)** who considered presence of an association between hypothyroidism and pyoderma. Thyroid gland plays a critical role in regulating the body immune system, and hence, when it is depressed or compromised, the body becomes increasingly vulnerable to the assault of the pathogens, as is seen in recurrent bacterial infections of the skin such as folliculitis, pyoderma and furunculosis **(Bansal *et al*.2007). Chastain and Panciera (1995)** had observed that hypothyroidism might impair neutrophil and lymphocyte function thereby causing abnormal systemic immune responses and alterations in local immunity resulting in pyoderma and other allergic dermatitis.

Our results also demonstrated presence of statistically significant increase in the cortisol level (Hyperadrenocorticism) and this agreed with **Zur and White(2011),Coyner (2012) and Kirby (2016)** who demonstrated that Cushing's syndrome can be challenging to identify because of its variable clinical manifestations in canine patients and the trickier clinical presentations for veterinarians to keep in mind is recurring pyoderma.

G-Antioxidant Trace Elements Analysis:

Results of oxidative stress markers as showed in (Table 3) indicated statistically significant decrease in selenium level (Se) together with insignificant decrease in zinc level (Zn). Referring to zinc, our results agreed with **Walaa *et al.* (2008) and Kubesy *et al*. (2017).** Zinc is a major component of Cu-Zn SOD and the fact that it plays an important role in steadying of lipid membrane content against oxidation **(Zago and Oteiza 2001**) may explain that a low zinc level may occur as a result of the high demand of zinc due to over production of ROS. **Shyma and Vijayakumar (2011)** disagreed and mentioned that the zinc value was significantly higher in the infected group than the normal control value. No previous data reported about selenium value in pyoderma infection so our figures are so far the first in this context but **Farag (2016) and Kubesy *et al*. (2017)** reported significant decrease in GPx **a**ctivity in pyoderma infected dogs. Selenium (Se) is an essential trace metal with beneficial properties and is principally known for its antioxidant capacity,which protects cell from free radicals **Parızek (1990).**Selenium helps protect against peroxidation as part of the glutathione peroxidase (GPx) antioxidant system and the antioxidant capability of selenium can measured by GPx activity **Smith *et al.* (2009).**

CONCLUSION

The study revealed that pyoderma infection represents a stress factor affecting the antioxidant trace elements consequently antioxidant defense system.

Recommendation:

With regard to serum antioxidant trace element selenium, our figures are so far the first in this context. It however, necessitates further studies.

Photo (1): Pyoderma infected dog.

Photo (2): Pyoderma infected dog.

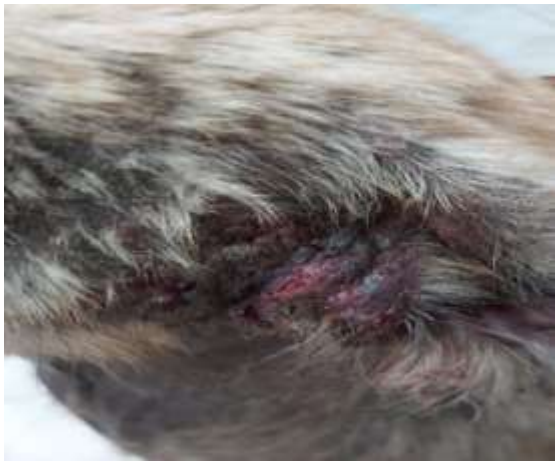

Photo (3): *pyoderma lesions*.

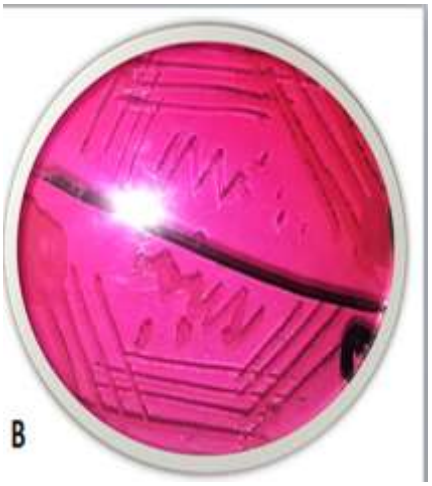

Photo (4): *Staphylococcus aureus* on manitol salt agar media.

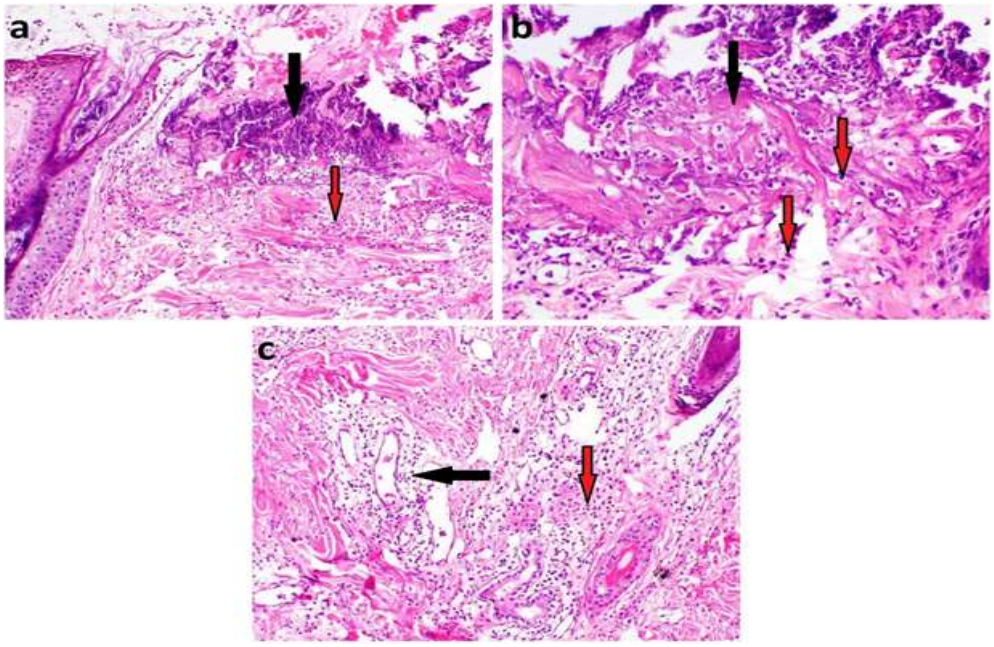

Photo. (5): Photomicrographs of a skin biopsy of pyoderma infected dog showing **(a & b)** large focal area of epidermal liquifactive necrosis (Black arrow) associated with dense neutrophils infiltration (Red arrow). **(c)** severe dermatitis exhibited by perifollicular and perivascular massive neutrophils, lymphocytes and macrophages infiltration **(H & E stain, X 100 (a & c) & X 200 (b).**

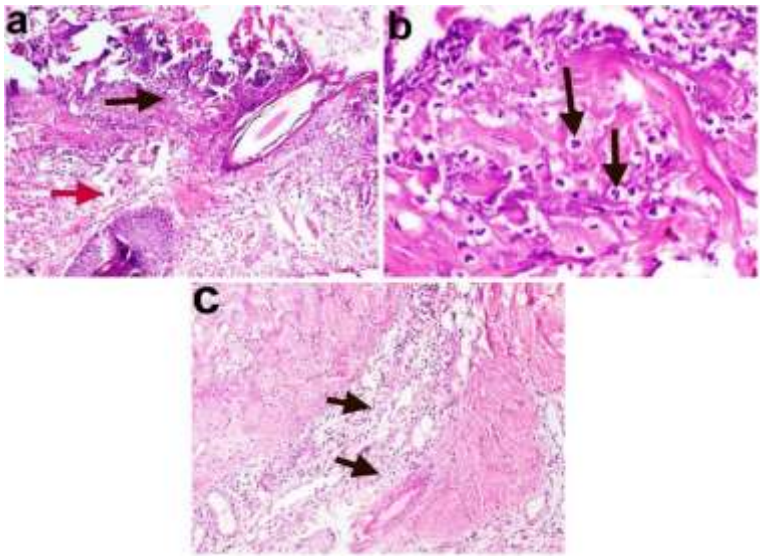

Photo. (6): Photomicrographs of a skin biopsy of pyoderma infected dog showing **(a)** large focal area of epidermal liquifactive necrosis (Black arrow) associated with dense neutrophils infiltration (Red arrow). **(b)** heavy neutrophils infiltration (Blab arrow) **(c)** severe dermatitis exhibited by perifollicular and perivascular massive neutrophils, lymphocytes and macrophages infiltration **(H & E stain, X 100 (a & c) & X 400 (b).**

Table (1): Hematological profiles in pyoderma infected dogs.

Parameters	Control (n = 20) Mean±SE	Pyoderma (n = 11) Mean±SE	P-Value (SIG)
HB (g/dl)	14.685±0.344	13.5±2.23	0.63
RBCs count ($\times 10^6$U/l)	6.98± 0.15	6.04± 1.03	0.42
PCV % (HCT)	45.84± 1.06	38.8± 6.54	0.35
MCV (fl)	65.22± 0.94	64.68± 1.05	0.71
MCH (pg)	21.23± 0.25	22.82± 0.95	0.17
MCHC (g/dl)	33.55± 0.30	34.73± 0.69	0.17
RDWcv %	15.26± 1.20	16.98± 0.33	0.18
Plts count ($\times 10^3$ U/L)	297.8 21.53	114±* 39.39	0.005
WBCscunt($\times 10^3$U/l)	11.24± 0.57	18.3± 3.59	0.12
Neutrophils %	66.89± 1.30	94± 9.72	0.15
Staff %	2.7± 0.20	3± 0	0.16
Seg %	64.19±1.23	46.94±9.72	0.15
Lymphocytes%	39.28±10.5	22.04±1.11	0.9
Eosinophils %	7.2±0.53	6.46±0.64	0.4
Monocytes %	3.88±0.28	4.32± 0.69	0.57

SE =Std. Error M.V=Mean Value P.V= Significant Difference

***Means Significant at p<0.05.**

Table (2): Serum biochemical findings in pyoderma infected dogs.

Parameters	Control (n = 20) Mean±SE	Pyoderma (n = 11) Mean±SE	p-value (SIG)
ALT (U/L)	50.9± 5.50	41.46±7.79	0.34
BUN (mg/dl)	19.9± 1.18	16.5± 2.16	0.21
Creatinine(mg/dl)	1.015± 0.05	0.98± 0.11	0.81
TSH (ng/ml)	0.33± 0.04	0.12± *0.07	0.04
Free T4 (pmol/l)	21.15± 2.17	15.38± 3.48	0.20
Cortisol (nmol/l)	197.6± 9.85	568.27± 136.06*	0.05

M.V = Mean Value S.E = Std. Error P. Value = Sig. Differance

***Means Significant at P< 0.05.**

Table (3): Serum antioxidant trace elements of pyoderma infected dog.

Parameters	Control (n = 20) Mean±SE	Pyoderma (n = 11) Mean±SE	P-Value (SIG)
Selenium (mg/dl)	0.256± 0.01	0.05± *0.01	000
Copper (mg/dl)	111.15± 3.34	111.8± 9.53	0.93
Zinc (mg/dl)	102.7± 2.38	93.48± 6.58	0.12

M.V = Mean Value S.E = Std. Error P. Value = Sig. Difference

***Means Significant at P< 0.05**

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