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Occurrence of Canine Skin Disorder and its Haematobiochemical Alterations

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A B S T R A C T

The present study was conducted to investigate a detailed occurrence of different dermatological problems in dogs in and around Kolkata and to determine the changes in the haematological and biochemical parameters of affected dogs. During a period of 6 months 156 dogs with primary complain of dermatological discomfort were studied, out of which the maximum number of animals were affected with skin disease caused by parasites i.e. 48 (30.78%) followed by bacteria 45 (28.85%). Dogs below 1 year of age were mostly susceptible (35.54%) to various dermatological disorders and the least susceptible group were more than 4 years of age (16.03%). Higher occurrence was observed in male dogs (61.02%) in comparison to females. Results revealed that spitz were more predisposed to various dermatological disorders (27.56%) followed by Labrador (19.87%). On examination of skin scrapping sarcoptic mange and demodectic mange were the commonly identified mites. The bacteria isolated from pyoderma of dogs were mostly *Staphylococcus spp.* followed by *E.coli* and *Klebsiella sp.* The haemoglobin, PCV and TEC values of affected animals had significantly decreased. The TLC significantly increased with neutrophilia and eosinophilia was recorded. The total protein and albumin had significantly increased while serum globulin had decreased in affected animals.

Introduction

Skin disorder is one of the most commonly encountered problem in pet animals. Dogs are susceptible to various skin problems be it parasitic, fungal, bacterial skin disease or allergies of various origin. These conditions become worse in hot and humid climate therefore becoming difficult to resolve. This not only deteriorates the cosmetic value of the animal but also poses a risk to its general health and utility. The present study was conducted with the objective of determining a detailed occurrence of canine dermatological problems in Kolkata and to investigate the changes in haematological and biochemical parameters of such affected dogs.

Materials and Methods

Occurrence study

The occurrences of different nature of dermatological disorders were tabulated from the total number of dogs brought to the Teaching Veterinary Clinical Complex,

Belgachia and some private clinics in Kolkata with primary complain of skin problems. The dog's breed, gender, age was recorded to establish whether they were associated dermatological problems. The dog breed were assessed according to the official breed standard from the American Kennel club.

Thorough history was collected from the owners and the information thus obtained along with the observations made on the patient was recorded. Diagnosis was done by collecting skin scraping from dogs with complain of pruritus, alopecia, scaling or crusting, erythema, macular, pustular eruptions, erosive or ulcerative lesions for the identification of causative agent.

Skin scrapping of dogs suspected for mange infestation was collected and examined by sedimentation method and by direct smear method. For the direct smear method a few amount of skin scraping was placed on the centre of a clean microscopic slide. 2-3 drops of 10% potassium hydroxide was poured on to it. The scrapping was then triturated with a needle and the materials were dispersed in a thin layer by gently pressing the cover slip. The slide was gently warmed over a spirit lamp to soften the skin material and digest the keratin debris. The slide was allowed to cool and examination was done under low power microscope for the detection of parasites. For the sedimentation method skin scraping was transferred to a 15 ml centrifuge tube and 5 ml of 10% potassium hydroxide solution was added to it. Then the treated material was heated gently until the keratin debris dissolved. The material was then centrifuged at 3000 rpm for 5 mins. The tube was allowed to cool for 5 mins. The supernatant fluid was discarded with a pipette. 1-2 drops of the sediment was transferred to a glass slide using a pipette. A cover slip was placed carefully and the slide was then examined under low power magnification.

For the fungal identification a pinch of infected materials collected was put on a clean dry slide along with one to two drops of 10% potassium hydroxide solution (KOH) and then covered with a cover slip. The slide was then heated gently over a flame making sure that it does not boil and was examined after 15 mins under a microscope as suggested by Beneke (1966). It was first examined under low power microscope and then under high power microscope for the presence of any mycelia and spores. For the detailed study of fungus all the skin scrapping collected were examined culturally by using Sabourad's Dextrose agar fortified with cyclohexamide and chloramphenicol. Sabourad's dextrose agar slants were used initially. For the study of detailed morphology and colony characters, agar plates were used and incubated for a maximum period of 30 days. A portion of the colony was detached by the tip of a long sterile needle and then placed on a clean slide having a drop of Lacto phenol cotton blue. Then a thin coverslip was placed over it. This was now examined under a low power and then under high power objective of a microscope.

For the diagnosis of pyoderma, samples were collected in sterile cotton swabs Swabs collected from dogs affected with pyoderma were inoculated into Nutrient Broth and incubated at 37 for 24 hours and then a loopful of broth culture was streaked on nutrient agar plates. Based on morphology and Gram's staining properties, cultures were inoculated into specific/ selective media like Mannitol Salt Agar, Eosin Methylene Blue and MacConkey Agar.

Haematological and Biochemical alterations

For haemato-biochemical study the blood samples were collected from dogs with dermatological disorders and also from 6

healthy animals for reference values. From each dog 7 ml of blood was drawn from the recurrent tarsal vein or radial vein. Out of 7 ml, 5 ml of blood was collected in sterilized test tubes without any anticoagulant for the separation of serum. Another 2 ml of blood was collected in dried vials containing EDTA as anticoagulant (1mg/ml) and mixed by rotating the tubes. This was then used for the determination of parameter like haemoglobin (Hb) by Sahil's method as described by Schalm *et al.,* (1986) and is expressed in gm %, packed cell volume (PCV) by micro haematocrit method and was expressed as percentage of the total volume as described by Schalm *et al.,* 1986,total erythrocyte count (TEC) by haemocytometer as described by Schalm *et al.*,1986 and was expressed in per cubic millimeter (106/cmm), total leucocyte count (TLC) by haemocytometer as described by Schalm *et al.,* 1986 and was expressed as thousands per cubic millimetre (103/cmm) of blood and differential leucocyte count (DLC) was estimated as described by Schalm *et al.,* 1986 and expressed as percentage. Total serum protein and serum albumin levels were estimated by Biuret method of Reinhold (1953) in a VU-VIS Spectrophotometer at the wavelength of 550-600 nm and the values were expressed as gm/dl. Serum total globulin content was calculated as the difference between serum total protein and albumin levels.

All the data obtained in respect to the different parameters in this study have been statistically analysed as per Snedecor and Cochran, 1967.

Results and Discussion

Occurrence study

During the period of study (December 2016 – May 2017), a total of 6,530 canine cases was registered at the Teaching Veterinary Clinical Complex, Belgachia, run round the clock including holidays, out of which 788 (12.06%) had the complications of dermatological disorders. From the 788 dermatological cases a total of 156 cases were randomly selected on the basis of easy access to the cases and support of the owners for the present study for a detailed category wise occurrence.

The results of the present study are corroborated with the study of Shyma and Vijayakumar (2012) who reported 12% of dermatological problems in one year in dogs. Prevalence of skin disorders ranging from 15-25% in dogs had earlier been reported by Scott *et al.,* 2001 and Hill *et al.,* 2006.

On the contrary, the percentage of prevalence of dermatological affections as reported by Sharma *et al.,* 2013 and Summers *et al.,* 2014 were just 5.6% and 1.3%. Higher prevalence of dermatological disorders may be due to season, climatic factors and management factors adopted in a particular area as stated by Sharma *et al.,* (2008).

The occurrence of dermatological disorders according to the pathogens (Fig. 1) in this study showed that the maximum no. of dermatological problems were due to parasitic (30.78%) followed by bacterial (28.85%), fungal (18.58%) and mixed skin infections (12.82%). It was interesting to note that a substantial percentage (8.97%) were of the non-infectious type which might be due to hypothyroidism, nutritional deficiencies or canine atopic dermatitis (Fig. 5–7).

It was observed that the occurrence of scabies was 38 (24.36%) and that of canine demodicosis was 10 (6.41%) out of the total of 156 skin affected dogs.

All the 156 cases were subjected to Wood's lamp screening, direct microscopic examination of hair pluckings, skin scrapping and cultural examination of fungal isolates, 29

dogs were found to be positive for dermatomycosis.

45 out of the 156 cases were found positive in bacteriological culture were subjected to Gram's staining, 38 showed the presence of gram positive cocci while the remaining 7 revealed gram negative rods. Detailed cultural examination was carried out in 45 pyoderma samples from which 90 isolates were obtained and identified. 76 isolates were found to be positive for *Staphylococcus* species, 10 were positive for *E. coli* and 4 isolates were positive for *Klebsiella*.

A similar findings regarding the clinico-epidemiological studies in canine dermatitis was reported by Singh *et al.,* 2012 with the highest prevalence of parasitic dermatitis (34.82%) followed by bacterial (25%), fungal (18.75%), non-specific (14.28%) and nutritional (7.14%).

Lodh and Das (2014) also reported that dermatitis caused by ectoparasites were the maximum (28.01%). Auijla (2000) and Sharma *et al.,* 2008 reported 31.31% and 28.43% the prevalence of bacterial dermatitis respectively. Sumathi and Vasu (2009) observed 20.50% of fungal dermatitis in canines. The incidence of parasitic dermatitis was reported as 36.53% and 33.33% by Ayodhya *et al.,* 2006 and Sharma *et al.,* 2009respectively. All these findings are in close agreement to the findings of the present study.

The animals in this study were divided into 4 groups viz. group I (below 1 year),group II (in between 1-2 years), group III (in between 2-4 years) and group IV (more than 4years) (Fig. 2). The highest percentage was recorded in dogs of age group less than 1 year (36.54%) followed by the age group 1-2 years (26.92%), then 2-4 years (20.51%) and the least in the age group more than 4 years (16.03%).

A very similar finding was noticed by Singh *et al.,* 2012 who reported the highest occurrences of dermatitis in dogs in the age group of 0-1 year (42.17%) followed by the age group 1-2 years (22.44%) then the age group 2-3 years (21.08%) and the lowest in the age group 3-4 years (14.28%). Poor development of epithelium and lack of specific immunity acquired after first exposure could be the possible reason for increased skin infection in young dogs (Hay, 1992). Moreover high body temperature in young animals, high nutritive demand and overcrowding may also increase the susceptibility of young animals to skin infection (Scott, 1995). Further, biochemical properties of skin and skin secretions, especially low fatty acids in the sebum may also be responsible for the infection in young ones, as these fatty acids are highly fungistatic (Hay, 1992).

The findings of the present study is also in close agreement to Khurana *et al.,* 2016 who reported the maximum occurrence of dermatological problem in canines at the age group of below 1 year (40.90%) followed by the age group of 1-2 years, then between 2-4 years and the least in the age group of above 4 years of age. However, Kumar *et al.,* 2006 also reported that the maximum no. of cases of all skin disorders was observed in the age group of 1-3 years. Among the skin disorders-affected dogs, maximum numbers were of males (61.02%) whereas females constituted only 38.98% (Fig. 3). The findings of the present study is similar to that of Kumar *et al.,* 2006 who reported that males dogs were found to suffer from various skin diseases more as compared to female dogs. Khurana *et al.,* 2016 also reported a close finding i.e. 63.22% of male dogs and 36.78% of female dogs were affected with various skin disorders. The difference between male and female as far as the occurrence according to the sex did not draw any conclusion. This warrants further study.

Fig.1

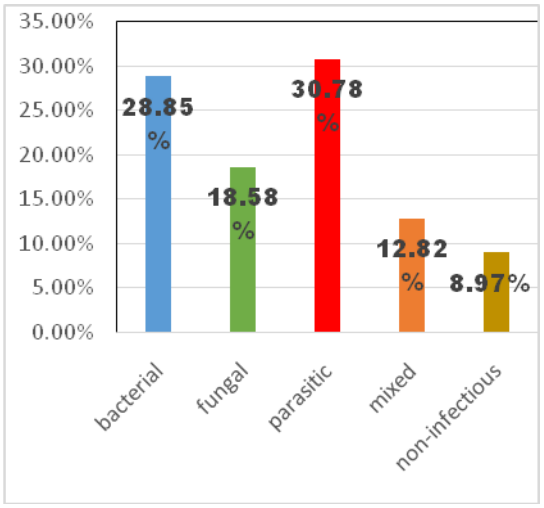

Fig.2

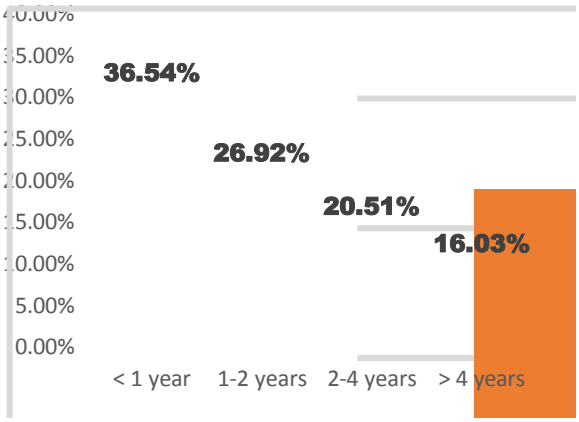

Fig.3

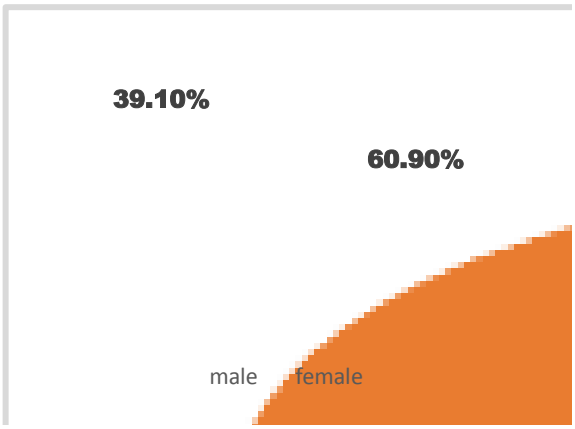

Fig.4

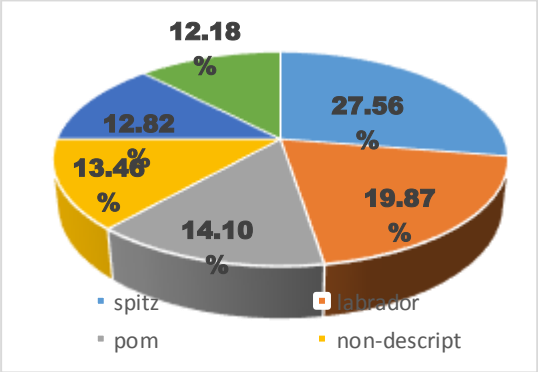

Fig.5 Photograph showing symptomatology of skin disorders (a) Papules (b) Pustules (c) hot spots (d) patchy alopaecia with dry scales (e) typical ringworm lesions

Fig.6 Photograph showing (a) *Sarcoptes scabiei* on microscopic examination (b) *Demodexcanis* on microscopic examination. (c) fluorescence of hair produced by *Microsporum canis* on Wood's lamp examination (d) *E.coli* in EMB agar (e) *Klebsiella* in MacConkey agar (f) Mannitol fermenting colonies of *Staphylococcus sp.*

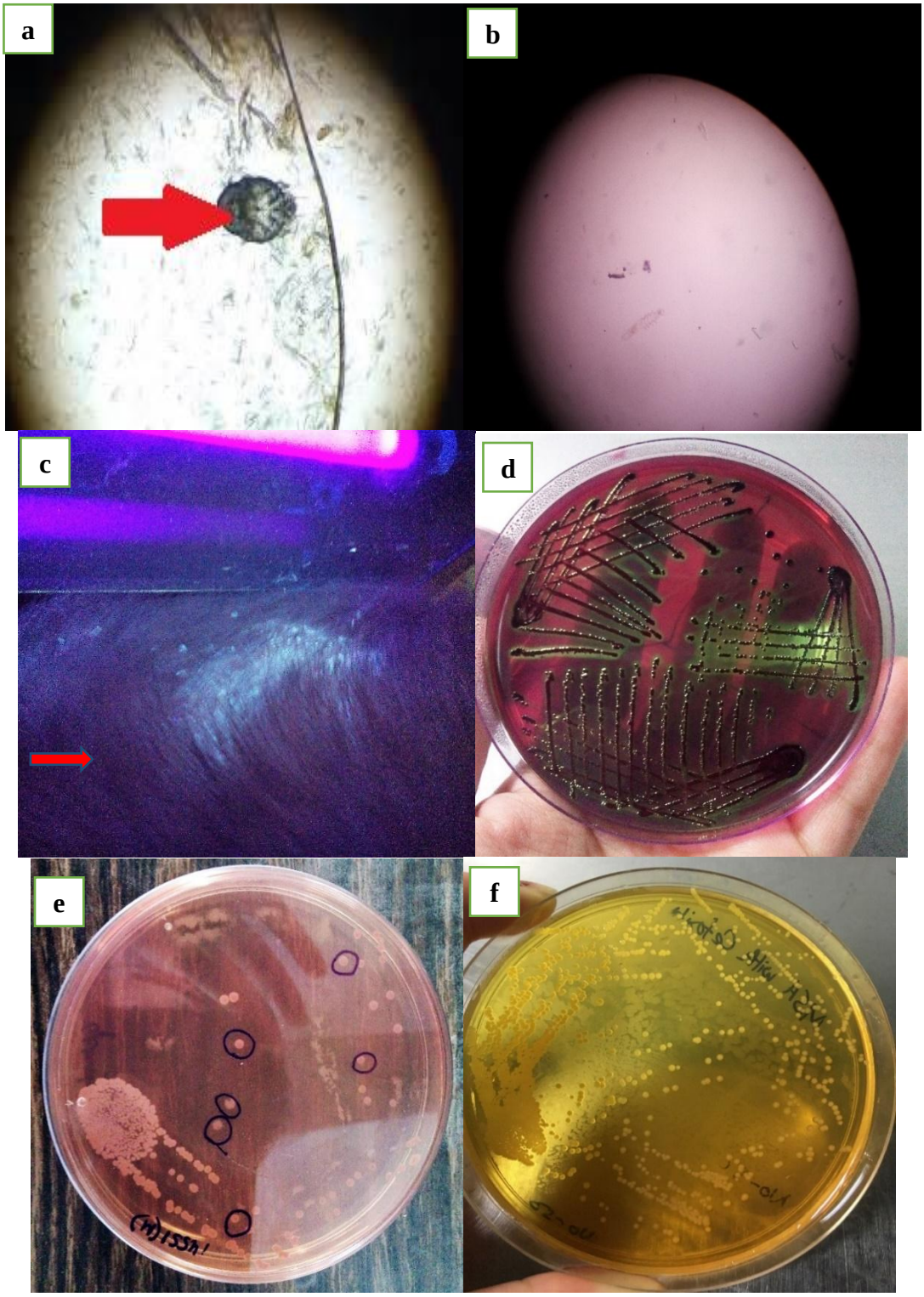

Fig.7 Photograph showing (a) cultures of *Tricophyton mentagrophytes* in Sabaroud's Dextrose Agar (b) culture of *Microsporum canis* in Sabaroud's Dextrose Agar. (c) fungal hyphae on lactophenol cotton blue staining. (d) macroconida

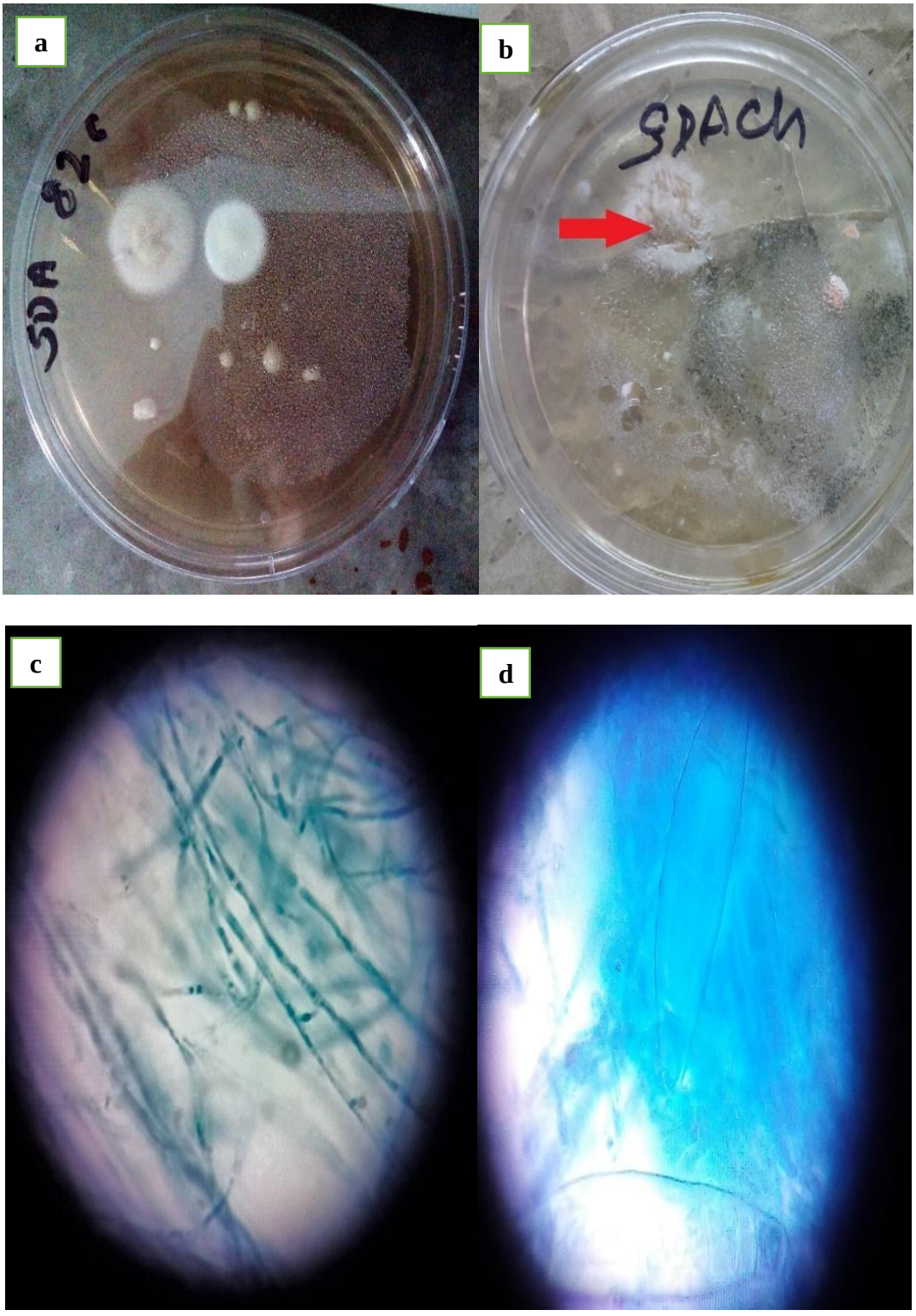

Table.1 Haematological and biochemical values of healthy and infected group of animals

Parameters \ Group	Healthy (n=6)	Infected (n=36)
Hb (gm/dl)	12.93±0.08	10.04±0.09**
PCV (%)	35.67±0.49	33.75±0.18*
TEC (10^6/ mm^3)	6.26±0.12	4.79±0.52**
TLC (10^3/ mm^3)	14.44±0.32	16.63±0.17**
Neutrophil (%)	62.5±0.43	70.78±0.19**
Eosinophil (%)	5.83±0.31	7.17±0.15**
Lymphocyte (%)	27.83±0.48	20.42±0.18**
Monocyte (%)	3.5±0.34	1.36±0.28**
Basophil (%)	0.33±0.21	0.25±0.07NS
Total protein (gm/dl)	6.80±0.14	6.54±0.031 NS
Albumin (gm/dl)	4.67±0.14	4.36±0.019 NS
Globulin (gm/dl)	2.13±0.07	2.18±0.011 NS

**: highly significant (P<0.01)
NS: non-significant

Breed wise occurrences of skin disorders in dogs revealed that Spitz (27.56%) were more predominantly affected with various kinds of skin disorders than other breeds followed by Labrador (19.87%), Pomeranian (14.14%), Non-descript (13.46%) and Pug (12.82%) (Fig. 4). The other breeds like Doberman Pinscher, Dalmatian, Boxer, Golden Retriever etc. collectively represented (12.18%) since the individual breeds did not significantly contribute to the overall percentage of skin disorders in dogs.

In the present study Spitz were predominantly found to be affected with skin disorders, this has earlier been reported by Kumar *et al.*, 2006 and Sarma *et al.*, 2013. The reason for spitzbeing more predisposed in the present study might be due to their long hair perpetuating the mistness favourable for the harbouring infection in hot and humid climate in majority of months in a year. However, Singh *et al.*, 2012 reported that prevalence of dermatitis was maximum in Mongrel (67.52%) followed by German Shepherd (9.02%), Labrador (6.51%), Spitz (4.27%) and Doberman (3%). The difference in the findings might be due to the popularity of a certain breed in an area as stated by Pocta and Svoboda (2007) that the breed composition of canine population in a particular region and popularity of the particular breed can affect the results of the breed predilection to dermatological disorders in the examined area.

Haematological and Biochemical alterations

Haemato-biochemical values in healthy and infected dogs were presented in table 1. The level of haemoglobin (gm/dl) in healthy control and affected animals were12.93+0.08 and 10.04+0.09 respectively which clearly shows that the level of haemoglobin was significantly lower (P<0.01) in dogs that were affected with various nature of skin disorders.

These results are in accordance with the findings of Lodh and Das (2014). Similarly, the values of PCV (%) and TEC (106/ mm3) in healthy animals were 35.67+.49 and 6.26+.12 respectively. The corresponding values in affected dogs were 33.75+.18 and

4.79+.52 respectively which shows a significant decrease (P<0.01) in both the values in the affected group of animals as compared to the healthy animals. These findings corroborated with the findings with Lodh and Das (2014) and Prathibha (2000). Significant lower values of haemoglobin, PCV and TEC indicating anaemia in infected dogs might be due to loss of skin protein due to excessive scratching and stress arising from the diseases (Deb *et al.,* 2000). This is particularly seen in dogs suffering from pyoderma, scabies and demodex. While the reason of anaemia in dogs infected with dermatophytes might be due to less food intake caused by clinical illness and discomfort (Devi and Vijayakumar. 2013). The mean total leucocyte count (×103/mm3) in healthy and affected dogs was 14.44+0.32 and 16.63+0.17 respectively. The total leucocyte count in the affected group of dogs in this study was elevated significantly (P<0.01) as compared to the healthy dogs. The mean values (%) of neutrophil, eosinophil, lymphocyte, monocytes and basophils in healthy dogs were 62.5+0.43, 5.83+0.31, 27.83+0.48, 3.5+0.34 and 0.33+.21 respectively and in affected dogs the corresponding values were 70.78+0.19, 7.17+0.15, 20.42+0.18, 1.36+0.28 and 0.25+.07 respectively. The level of neutrophil, eosinophil was significantly higher (P<0.01) while the level of lymphocyte and monocyte was significantly lower (P<0.01) in the affected animals as compared to the healthy animals. This result agreed with the findings of Arlian *et al.,* 1995. Cellular and humoral responses to inflammation on account of host-parasite interaction are the reported reason (Huntley *et al.,* 1995). Skin affected dogs had significantly higher (P<0.01) neutrophil count (70.78+0.19). Elevated level of neutrophils was earlier reported by Gera *et al.,* 2012 and statistically higher levels of neutrophils in certain bacterial dermatitis were recorded by Pal *et al.,* 1991. The reason for neutrophilia might be due to the injury to the cells which causes the release of substances like leukotoxins and leucocytosis promoting factors from the blood to the site of injury resulting in the release of more neutrophils in the blood stream as stated by Lodh and Das (2014). The level of eosinophils in skin affected dogs were recorded significantly higher (P<0.01) (7.17+0.15) in comparison to the healthy animals. Significantly higher levels of eosinophils in caninedemodicosis, was reported by Chakraborty and Pradhan (2015). Increase in the level of eosinophil might be due to irritation of the skin tissues which stimulated the mast cells to release more histamine and since histamine is chemotactic for eosinophils, eosinophilia develops (Aujla *et al.,* 2000). The lymphocyte count and monocyte in the present study significantly decreased (20.42+0.18 and 1.36+0.28) which was in agreement of the findings of Lodh and Das (2014). Stressful condition to the animals affected with skin disorders might be the most probable reason behind it.

The mean serum total protein (gm/dl), albumin (gm/dl) and globulin (gm/dl) in healthy animals were recorded as 6.80+0.14, 4.67+0.14 and 2.13+0.07 respectively. While the in the infected group the values of serum total protein, albumin and globulin were recorded as 7.60±0.04, 3.61±0.05 and 3.99±0.06 respectively which indicated that there were significant (P<0.01) increase in the level of serum total protein, significant decrease in the serum albumin while a decrease in the serum globulin.

These findings are similar to the findings of Mason (1991), Shyma and Vijayakumar (2011) and Lodh and Das (2014). Hyperglobinamia and hypoalbuminemia in bacterial skin diseases were earlier reported by Shyma and Vijayakumar (2011). Increased serum protein in the present study might be

due to the increased inflammatory responses produced by various pathogens involved in skin disorders. The antigens were trapped in the Langerhan's cells, which were the prominent antigen presenting cells of the skin immune system and presented the antigens to T- lymphocytes (Devi and Vijayakumar (2013). The migration of antigen presenting cells was initiated by cytokines and this leads to dense infiltration of inflammatory cells in the infected area and subsequent increase in serum total protein (Gudding and Lund. 1995).

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