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Study the prevalence of exocrine pancreatic insufficiency in dogs in and around tarai region of Uttarakhand

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Abstract
The present research work was undertaken to assess the prevalence of exocrine pancreatic insufficiency (EPI) in dogs. Out of total 1503 dogs registered during the study period from Pantnagar and adjoining areas, 961dogs were presented with gastro intestinal disorders. For the epidemiological study, 386 dogs irrespective of age, sex and breed with the history of anorexia, vomition, weakness, abdominal pain, diarrhoea and dehydration were examined. Out the 386 dogs examined preliminary for EPI, 15 dogs were reported to be positive for EPI on the basis of faecal trypsin test and presence of starch and neutral fat in faeces. Serum lipase and canine pancreatic lipase (cPL) were also estimated the study. Biochemical parameters estimation revealed a three to five times increase in serum lipase values of seven out of fifteen dogs. Serum canine pancreatic lipase was above the cutoff range (400 ng / ml) in six dogs. The overall prevalence of EPI was found to be 0.99% on faecal analysis basis. However on serum lipase and canine pancreatic lipase activity it was found to be 0.46 and 0.40 %, respectively. The clinical prevalence on faecal analysis was recorded to be 3.88 % and on serum lipase and cPL basis was 1.82 and 1.56 %, respectively. The maximum prevalence was observed in dogs aged 5 years and above and maximum prevalence was recorded in German shepherd dogs.

Keywords: Prevalence, exocrine pancreatic insufficiency, serum lipase, canine pancreatic lipase

Introduction
Dogs are considered one of the most intelligent and loyal pet animal to the mankind. Dogs get benefits like companionship, protection, shelter and a reliable food source out of the deal. Now a day, dogs render there services in areas like forensic medicine, tracking, mining, sports, guarding, space science, medical science and many more. During their service to the mankind, they suffer with many ailments. Exocrine pancreatic diseases are rapidly emerging problem leading to life threatening outcomes in canines. Canine exocrine pancreatic insufficiency is an alimentary tract disorder which is characterized by inadequate production of digestive enzyme from pancreatic acinar cells and hence leading to the characteristic clinical signs of polyphagia, weight loss and increased faecal volume [1-3]. The acute form of the disease is believed to be much more common in dogs, whereas chronic pancreatitis is thought to be the primary form in cats. The clinical diagnosis of chronic pancreatitis is challenging as the disease is often subclinical or is associated with mild and nonspecific clinical signs. Therefore, most cases of canine and feline chronic pancreatitis probably go on undiagnosed. The functional reserve capacity of the pancreas is enormous, so these diseases only develop if more than 80% to 90% of functional mass is lost [4]. Though all canine breeds are susceptible to EPI, but some breeds appear to be more predisposed than others. The German Shepherds, followed by Rough-coated Collies, Chow Chows, and Cavalier King Charles Spaniels are some of the major breeds commonly affected by EPI [5-8]. German Shepherds (GSD) represented 60% of all the cases of EPI [8]. The relationship between gender was not clear and no clear sex predisposition was identified [8-10]. Most dogs suffered with pancreatitis are more than 5 years and overweight [11-13]. For a long time, amylase and lipase activities have been well thought-out markers of pancreatic inflammation; however, these enzymes originate from many tissues and hence are not specific markers of pancreatic diseases, making their specificity for pancreatitis fairly low [14-16]. Furthermore, non-pancreatic diseases like renal failure, hepatic diseases, intestinal diseases and lymphoma may also lead to elevations in serum amylase and/or lipase activities [16, 17].

According to the official recommendations of the American Gastroenterological Association the diagnosis of acute pancreatitis should be established within 48 hours, considering an increase in amylase or lipase activity greater than 3 times the URL in the absence of impaired renal function [18]. Several tests have been made available sequentially for the pancreas-specific lipase measurement in dogs [19]. This enzyme is very specific in dogs [19] and no interference of bilirubin, lipid and hemoglobin has been reported [20]. The performance canine pancreatic lipase (cPL) is reported to be superior to amylase activity, lipase activity and trypsin-like immune reactivity assays [20, 21]. The reported sensitivities of cPL vary in a wide range from 21% to 72%, while specificities vary from 78% to 100% [22, 23] .In the diagnosis of canine acute pancreatitis, the sensitivity and specificity of cPL is 93% and 78%, respectively. In evaluating the diagnosis of histologically confirmed canine chronic pancreatitis, the sensitivity of cPL was reported to vary from 26% or 58% depending on the cutoff values [24]. Hence, there is a need of correlation between different noninvasive parameters to diagnose the subclinical cases to detect the severity of defect at an earlier stage, so that not only to avoid maldigestion related problems but also a normal nutritional status can be ensured.

Materials and Methods

The present study was carried out at Department of Veterinary Medicine and Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, G.B.P.U.A. & T, Pantnagar U.S. Nagar (Uttarakhand) during the period of September 2016 to April 2017. In addition, referral cases from the practicing veterinarians in and around the tarai region of Uttarakhand were also included during the study period. Screening of dogs for exocrine pancreatic defects was based on patient's history, clinical signs, faecal examination, hematology and biochemical examination**.** Clinical examination of all the dogs was carried out to evaluate rectal temperature, pulse rate, heart rate, respiratory rate and level of dehydration. A total of 961 dogs out of 1503 dogs were presented with gastro intestinal disorders, during the study period of which 386 dogs were suspected and examined for exocrine pancreatic insufficiency (EPI) among them 15 dogs were found positive for EPI.

Collection and examination of faecal samples

Approximately, 5 gm of fresh faecal sample were collected either directly from the rectum of each dog or defecated naturally. Immediately after collection of faecal sample it was subjected for the gross examination like consistency, colour, odour and microscopic examination like presence of fat, starch and muscle fibres and faecal trypsin.

Microscopic examination of faeces

Fat (Steatorrhea)

Small amount of faeces was taken and 2-3 drops of water was added to it on a clean grease free glass slide. Sudan III (equal parts of 70% alcohol with excess of Sudan III stain) dye was added to it and the slide was examined under 10 X under coverslip. Neutral fat appeared orange or red globules with varying diameter of EPI affected dogs.

Starch (Amylorrhea)

Faecal samples were stained with Lugol's solution and were observed under microscope at 10 X magnification. Blackish bluish colour was observed in faecal samples containing starch of dogs suspected for EPI.

Film test (Faecal Trypsin)

Total 9 ml of 5% solution of sodium bicarbonate solution was taken in a cylinder and the solution was made 10 ml by adding 1 ml of faeces. A strip of exposed X- RAY film was submerged in it. The solution was incubated at 37^0 C for 1 hour. The strip was washed under gentle stream of tap water. Cleared area indicated presence of trypsin in faeces which is normally not present in the faeces of normal dogs.

Serum biochemical estimation

The harvested sera was analyzed for different biochemical parameters i.e. serum lipase and canine serum pancreatic lipase immunoreactivity (cPL).

Serum Lipase

Lipase activity was measured using Quanti Chrome™ Lipase Assay Kit (DLPS- 100) based on Rapid Colorimetric Determination of Lipase Activity at 412 nm. Colour reagent was mixed into assay buffer and vial was shaked to mix well in a vial. 0.8 ml BALB Reagent was mixed in the vial. The Working Reagent was prepared freshly and was used within one hour. 150 µl of water and 150 µl of calibrator were transferred into wells of clear bottom 96 well plate.10 µl of samples were pipetted into separate wells and 140 µl of working reagent was added to each sample and mixed well. O.D. on a plate reader at 10 minutes (OD_{10min}) and at 20 min (OD_{20min}) was read and lipase activity was calculated as

Activity = $[(OD_{20min} - OD_{10min}) / (OD_{calibrator} - OD_{water})]$ X 735 (U/L)

Canine Pancreatic Lipase (c PLI)

PL ELISA kit is based on the competitive enzyme immunoassay technique. The kit utilizes a monoclonal anti-PL antibody and a PL-HRP conjugate. The pre coated plate with PL-HRP conjugate was incubated with assay sample and buffer for one hour. The wells were decanted and washed five times, after the incubation. The substrate for HRP enzyme was added and wells were then again incubated. A blue colored complex was formed by the product of the enzyme-substrate reaction. Finally, the reaction was stopped by adding a stop solution, which turns the solution yellow. A microplate reader is used to measure the intensity of colour spectrophotometrically at 450 nm.

Since PL from samples and PL-HRP conjugate compete for the anti-PL antibody binding site, the intensity of the color was inversely proportional to the PL concentration. As the number of sites is limited and more sites are occupied by PL from the sample leaving fewer sites to bind PL-HRP conjugate. A standard curve was plotted relating the intensity of the colour (O.D.) to the concentration of standards. The PL concentration in each sample was interpolated from this standard curve.

Results and Discussion

Out of these 386 dogs irrespective of sex, age, breeds with the history of dehydration, vomition, diarrhoea, abdominal pain and weight loss, 15 dogs were founded positive for EPI (Table 1). The overall Exocrine Pancreatic Insufficiency in dogs was recorded to be 0.99% on faecal analysis basis. The prevalence among cases suffering from gastrointestinal problems was found to be 3.89%.The maximum positive percentage for EPI was recorded in German shepherds 5.29% (Table 2 and Fig.1). Similar findings have been reported by

earlier workers [8].

Out of total 15 positive cases of EPI, 06 were male and 09 were female which represents 40 % and 60 % of the total positive cases respectively (Table 2). Similar findings have been previously reported by many workers [8-10].

The maximum prevalence of EPI cases were recorded in dogs of more than 5 years of age (5.81%) and minimum prevalence was recorded in below 1 years of age (Table 3 and Fig. 2). Dogs diagnosed with chronic pancreatitis are typically middle to older aged. Most dogs that present with pancreatitis are older than 5 years [11, 12, 13]. On serum lipase basis the overall prevalence of EPI was found to be 0.47% and 1.81% among the screened cases whereas on serum canine specific pancreatic lipase estimation 06 dogs were detected with EPI representing overall prevalence and prevalence among screened cases as 0.40% and 1.55% respectively. When Spec PL values were used for the diagnosis of pancreatitis, a cutoff value has been fixed (400 µg / L in dogs) and there is also a gray zone between the cutoff and the upper limit of the reference range (200 µg / L in dogs). i.e., dogs having Spec PL values above 400 µg/L are considered positive for pancreatitis whereas dogs having Spec PL values between 200 to 400 µg/L are in the gray zone.

Conclusion

From the present study it can be concluded that Exocrine Pancreatic Insufficiency in dogs is moderately present in and around Pantnagar region of Uttarakhand. The overall prevalence was recorded to be 0.99%. The prevalence of EPI in dogs, was founded to be 3.86% on faecal positive basis which was further reduced to 1.81% on serum lipase activity measurement (3-5 times above the reference range). On canine Pancreatic Lipase activities basis the prevalence was recorded to be 1.55%. Mostly the dogs of above 5 year age group were founded positive for EPI. Maximum prevalence was recorded in German Shepherds. Females were founded to be more predisposed for EPI than males. Diagnosis of EPI can be made on faecal examination on a regular basis, serum lipase and cPL values. However, it was also observed that no significant difference in serum lipase (three to five times above the reference range) and cPL was present in diagnosis of EPI. But keeping the economic factor in consideration serum lipase estimation (3 to 5 times above the reference range) can be considered as a better diagnostic kit over canine pancreatic lipase.

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Table 1: Area wise screening of dogs for Exocrine Pancreatic Insufficiency

S. No.	Area	No. of Dog examined	No. of Dog having digestive disorder	No. of Dog found positive for EPI
1	Pantnagar	872	171	3
2	Jawahar Nagar/ Shantipuri	163	54	2
3	Lalkaun	106	38	3
4	Haldwani	183	56	1
5	Rudrapur	92	42	5
6	Kichha	87	25	1
	Total	1503	386	15

Table 2: Breed wise and sex wise Exocrine Pancreatic Insufficiency prevalence in dogs

Breed	No. of dogs examined	Positive dogs	M	F	Positive (%)		Overall prevalence	Clinical prevalence	Breed wise clinical prevalence
					M	F			
Mongrel	54	02	01	01	1.85	1.85	0.133	0.518	3.70
Labrador	68	01	00	01	0.00	1.47	0.066	0.259	1.47
Spitz	67	01	00	01	0.00	1.49	0.066	0.259	1.49
German Shepherd	96	05	02	03	2.08	3.125	0.333	1.259	5.21
Doberman	8	01	01	00	12.50	0.00	0.066	0.259	12.5
Great Dane	6	00	00	00	0.00	0.00	0.000	0.000	0.00
Non-Descript	54	03	01	02	1.85	3.70	0.200	0.777	5.56
Saint Bernard	9	01	01	00	11.11	0.00	0.066	0.259	11.11
Rottweiler	7	00	00	00	00.00	0.00	0.000	0.000	0.00
Pug	17	01	00	01	0.00	5.89	0.066	0.259	5.88
Total	386	15	06	09	1.55	2.33	0.998	3.886	3.886

Table 3: Age wise prevalence rate of exocrine pancreatic insufficiency

Age Group	No of sample examined	Positive cases	Positive %
< 1 year	51	01	1.96
1 – 2 year	47	01	2.13
2 – 3year	56	02	3.57
3– 4 year	64	02	3.12
4– 5 year	82	04	4.87
>5 years	86	05	5.81
Total	386	15	3.88

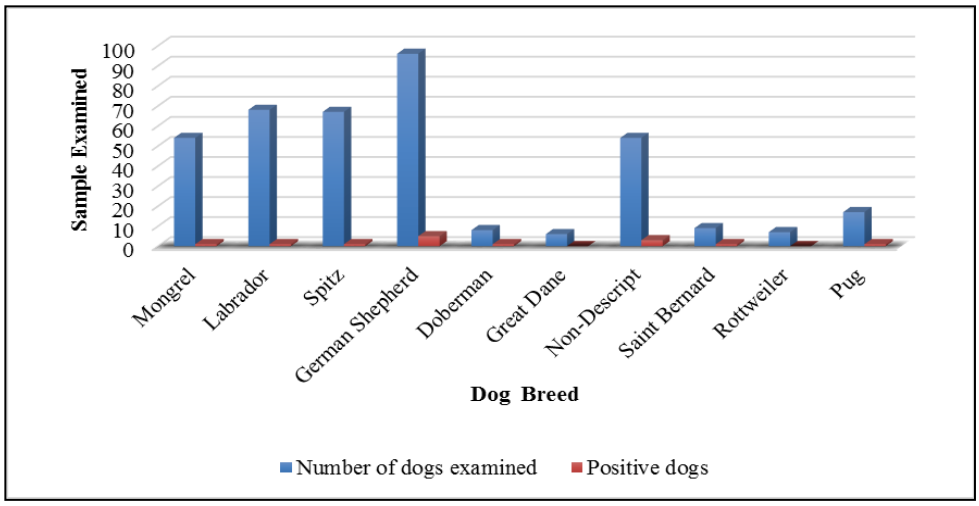

Fig 1: Breed wise prevalence rate for Exocrine Pancreatic Insufficiency (EPI)

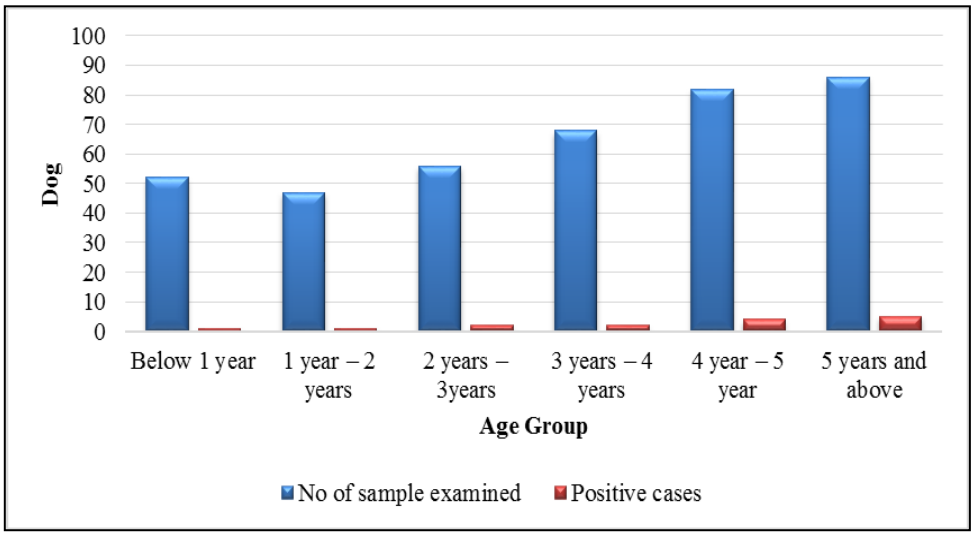

Fig 2: Age wise prevalence rate for Exocrine Pancreatic Insufficiency (EPI)

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