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Prevalence and clinico-diagnostic evaluation of mixed gastrointestinal parasitic infections in dogs

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Abstract

The present study was undertaken to determine the prevalence and evaluate the clinical, diagnostic and haemato-biochemical features associated with mixed gastrointestinal (GI) parasitic infections in dogs. A total of 604 dogs presented to the Veterinary Clinical Complex, Proddatur, were screened for GI parasitic infections. Of the 604 dogs screened, 314 (51.98%) were found positive for GI parasitic infections, comprising 293 dogs with single infections and 21 dogs with mixed infections. Thus, the prevalence of mixed GI parasitic infections in the screened population was 21/604 (3.48%), while mixed infections accounted for 21/314 (6.69%) of the GI parasite-positive dogs. Among the 21 dogs with mixed infections, *Ancylostoma caninum* + *Toxocara canis* was the most frequently observed combination (9/21; 42.86%), followed by *Ancylostoma caninum* + *Dipylidium caninum* (7/21; 33.33%) and *Toxocara canis* + *Toxascaris leonina* (5/21; 23.81%). Breed and gender were not significantly associated with the occurrence of mixed GI parasitic infections, whereas age showed a highly significant association ($\chi^2 = 14.24$; $P = 0.0026$). The major clinical manifestations observed among dogs with mixed infections were anorexia (20/21; 95.23%), weakness (19/21; 90.47%), diarrhoea (18/21; 85.71%), vomiting (15/21; 71.43%), pale mucous membranes (14/21; 66.67%), poor coat condition (9/21; 42.85%), emaciation (7/21; 33.33%), abdominal bloating (5/21; 23.81%), pyrexia (5/21; 23.81%), lethargy (4/21; 19.05%) and congested mucous membranes (3/21; 14.29%). Among the copro-parasitological techniques used, direct faecal smear, sedimentation and zinc sulphate flotation detected mixed infections in 4/21 (19.05%), 8/21 (38.09%) and 9/21 (42.86%) dogs, respectively, with no significant difference among the methods. Haematological evaluation revealed significant reductions in haemoglobin, total erythrocyte count and packed cell volume, along with significant increases in total leucocyte, neutrophil and eosinophil counts in dogs with mixed GI parasitic infections compared with healthy dogs. Serum biochemical evaluation revealed significant increases in alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and a significant reduction in total protein, albumin and blood urea nitrogen, whereas creatinine and total bilirubin did not differ significantly. The findings highlight the occurrence of mixed GI parasitic infections in dogs and their associated clinical, diagnostic and haemato-biochemical alterations, emphasizing the importance of appropriate copro-parasitological examination for their detection.

Keywords: Mixed gastrointestinal parasitic infection, dogs, prevalence, copro-parasitological diagnosis, haemato-biochemical alterations

Introduction

Gastrointestinal (GI) parasitic infections are among the important health problems affecting dogs and may adversely influence their health and productivity. Dogs can harbour a variety of gastrointestinal parasites, some of which are of zoonotic importance, thereby posing a potential public health concern (Souza *et al.*, 2023; Wani *et al.*, 2017; Mathew *et al.*, 2016) [23, 24]. The occurrence and severity of parasitic infections may vary depending on several factors, including age, breed, management practices, environmental conditions and exposure to infective stages of parasites. (Ferreira *et al.*, 2016; Wani *et al.*, 2017) [9, 24]

Gastrointestinal parasitic infections may produce a wide range of clinical manifestations in dogs, including anorexia, diarrhoea, vomiting, weakness, weight loss, anaemia, abdominal distension and poor coat condition. The severity of clinical manifestations depends on the parasite species, intensity of infection and the general health status of the animal. Mixed infections involving two or more gastrointestinal parasites may result in overlapping clinical manifestations and may complicate their diagnosis and clinical management. (Mathew *et al.*, 2016) [13].

Several gastrointestinal parasites of dogs, including *Ancylostoma caninum*, *Toxocara canis*, *Toxascaris leonina*

and *Dipylidium caninum*, are commonly encountered, and some have zoonotic significance. *Toxocara canis* is associated with visceral and ocular larva migrans in humans (Dogan *et al.*, 2007) [7], while hookworm infections may cause cutaneous larva migrans and other zoonotic manifestations. Therefore, accurate detection and appropriate identification of gastrointestinal parasites in dogs are important from both veterinary and public health perspectives. (Souza *et al.*, 2023) [23]

Copro-parasitological examination remains an important approach for the diagnosis of gastrointestinal parasitic infections. Different techniques, including direct faecal smear, sedimentation and flotation methods, may vary in their ability to detect parasitic stages. Evaluation of haematological and serum biochemical alterations may additionally provide useful information regarding the systemic effects associated with gastrointestinal parasitism. (Qadir *et al.*, 2011; Shahat *et al.*, 2022; Payghan *et al.*, 2023) [17, 20, 15].

Although gastrointestinal parasitic infections in dogs have been widely reported, information specifically concerning mixed gastrointestinal parasitic infections, their prevalence, clinical manifestations, diagnostic findings and associated haemato-biochemical alterations is comparatively limited in the study region. Therefore, the present study was undertaken to determine the prevalence of mixed gastrointestinal parasitic infections in dogs and to evaluate their clinical manifestations, copro-parasitological diagnostic findings and associated haemato-biochemical alterations.

Materials and methods

Study Area and Study Population

The study was conducted at the Veterinary Clinical Complex, College of Veterinary Science, Proddatur. A total of 604 dogs presented to the clinical complex were screened for gastrointestinal parasitic infections based on clinical examination and relevant clinical history.

Faecal Sample Collection and Parasitological Examination

Fresh faecal samples were collected from the dogs in clean, labelled containers and subjected to copro-parasitological examination. Three diagnostic techniques, namely direct faecal smear, sedimentation and zinc sulphate flotation, were employed for detection and identification of gastrointestinal parasitic ova and other parasitic stages. Identification was carried out based on the morphological characteristics of the parasitic eggs as described by Soulsby (1982) [22].

Of the 604 dogs screened, 314 were positive for gastrointestinal parasitic infections. Among these positive dogs, 293 had single parasitic infections, and 21 had mixed gastrointestinal parasitic infections. The 21 dogs with mixed infections constituted the study group for evaluation of their clinical, diagnostic and haemato-biochemical findings.

Clinical Examination

Detailed clinical examination was performed in dogs diagnosed with mixed gastrointestinal parasitic infections. Clinical findings including anorexia, diarrhoea, vomiting, weakness, pale mucous membranes, poor coat condition, emaciation, abdominal bloating, pyrexia, lethargy and congested mucous membranes were recorded.

Haematological and Serum Biochemical Examination

Blood samples were collected from the affected dogs for haematological and serum biochemical evaluation. Haematological parameters included haemoglobin, total erythrocyte count, total leucocyte count, packed cell volume, differential leucocyte count and platelet count. Serum biochemical parameters evaluated included alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, blood urea nitrogen, creatinine, total bilirubin, total protein and albumin. The obtained results were evaluated and interpreted based on the standard reference values reported by Jain (1993) [11].

Statistical Analysis

The data were analysed using SPSS software. The association of breed, age and gender with the occurrence of mixed gastrointestinal parasitic infection was evaluated using the chi-square test. Haematological and serum biochemical parameters of healthy and mixed gastrointestinal parasitic-infected dogs were compared using a two-sample t-test. A probability value of $P < 0.05$ was considered statistically significant.

Results

Prevalence and Pattern of Mixed Gastrointestinal Parasitic Infections

A total of 604 dogs were screened for gastrointestinal parasitic infections during the study period. Of these, 314 dogs (51.98%) were found to be positive for gastrointestinal parasitic infections. Among the 314 positive dogs, 293 had single gastrointestinal parasitic infections, whereas 21 had mixed gastrointestinal parasitic infections. Thus, mixed gastrointestinal parasitic infections were recorded in 21/604 dogs, giving an overall prevalence of 3.48% in the screened population. Mixed infections constituted 21/314 (6.69%) of the gastrointestinal parasite-positive dogs.

Among the 21 dogs with mixed gastrointestinal parasitic infections, *Ancylostoma caninum* + *Toxocara canis* was the most frequently detected combination, occurring in 9/21 dogs (42.86%). This was followed by *Ancylostoma caninum* + *Dipylidium caninum* in 7/21 dogs (33.33%) and *Toxocara canis* + *Toxascaris leonina* in 5/21 dogs (23.81%). The distribution of mixed gastrointestinal parasitic infections is presented in Table 1 and representative photomicrographs of gastrointestinal parasitic ova identified during faecal examination are shown in Plate 1.

Association of Mixed Gastrointestinal Parasitic Infection with Breed, Age and Gender

The occurrence of mixed gastrointestinal parasitic infection was evaluated in relation to breed, age and gender. Among the 21 dogs with mixed infections, mongrel dogs showed the highest occurrence (6/21; 28.57%), followed by Labrador (4/21; 19.04%), Pomeranian (3/21; 14.28%), German Shepherd (3/21; 14.28%), Golden Retriever (2/21; 9.52%), Dachshund (2/21; 9.52%) and Shih Tzu (1/21; 4.76%). However, no statistically significant association was observed between breed and occurrence of mixed gastrointestinal parasitic infection ($\chi^2 = 5.33$; $P = 0.50$) as shown in Table 2.

Age showed a highly significant association with the occurrence of mixed gastrointestinal parasitic infection ($\chi^2 =$

14.24; $P = 0.0026$). The highest proportion of mixed infections was recorded in dogs aged 0-6 months (12/21; 57.14%), followed by 7-12 months (6/21; 28.57%), 13-36 months (2/21; 9.52%) and 37-60 months (1/21; 4.76%) as given in Table 3.

Regarding gender, mixed gastrointestinal parasitic infection was recorded in 11/21 male dogs (52.38%) and 10/21 female dogs (47.62%). The association between gender and occurrence of mixed infection was not statistically significant ($\chi^2 = 0.0476$; $P = 0.83$) as indicated in Table 4.

Copro-parasitological Diagnosis of Mixed Gastrointestinal Parasitic Infections

Three copro-parasitological techniques, namely direct faecal smear, sedimentation and zinc sulphate flotation, were employed for the detection of mixed gastrointestinal parasitic infections in the 21 affected dogs. Zinc sulphate flotation detected mixed infections in 9/21 dogs (42.86%), followed by sedimentation in 8/21 dogs (38.09%) and direct faecal smear in 4/21 dogs (19.05%). Zinc sulphate flotation showed the highest detection rate among the three techniques. However, the difference in detection among the diagnostic methods was not statistically significant ($\chi^2 = 2.024$; $P = 0.3635$) as summarized in Table 5.

Clinical Manifestations

The major clinical manifestations observed in dogs with mixed gastrointestinal parasitic infections were anorexia (20/21; 95.23%), weakness (19/21; 90.47%), diarrhoea (18/21; 85.71%), vomiting (15/21; 71.43%), pale mucous membranes (14/21; 66.67%), poor coat condition (9/21; 42.85%), emaciation (7/21; 33.33%), abdominal bloating (5/21; 23.81%), pyrexia (5/21; 23.81%), lethargy (4/21; 19.05%) and congested mucous membranes (3/21; 14.29%) as presented in Table 6.

Anorexia and weakness were the most frequently recorded clinical manifestations, followed by diarrhoea and vomiting. The occurrence of these clinical manifestations may be associated with the gastrointestinal effects and systemic consequences of parasitic infection.

Haematological Alterations

Haematological parameters were evaluated in 21 dogs with mixed gastrointestinal parasitic infections and compared with those of 12 healthy dogs as detailed in Table 7. The mean haemoglobin, total erythrocyte count and packed cell volume values were significantly reduced in infected dogs compared with healthy dogs ($P \leq 0.01$). A highly significant reduction in lymphocyte count was also observed in infected dogs. In contrast, total leucocyte count, neutrophil count and eosinophil count were significantly increased ($P < 0.01$), indicating leucocytosis, neutrophilia and eosinophilia, respectively. No statistically significant differences were observed in monocyte and platelet counts ($P > 0.05$).

The observed reduction in haemoglobin, erythrocyte count and packed cell volume indicates anaemia associated with mixed gastrointestinal parasitic infection, whereas the increases in leucocyte, neutrophil and eosinophil counts reflect the haematological response associated with parasitic infection.

Serum Biochemical Alterations

Serum biochemical parameters were evaluated in 21 dogs with mixed gastrointestinal parasitic infections and compared with those of 12 healthy dogs as depicted in Table 8. The mean serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities were significantly higher in infected dogs than in healthy dogs ($P < 0.01$). Mean blood urea nitrogen (BUN) concentration was significantly lower in infected dogs ($P < 0.05$).

Total protein and albumin concentrations were also significantly reduced in infected dogs compared with healthy dogs ($P < 0.01$). No statistically significant differences were observed in serum creatinine or total bilirubin concentrations between healthy and infected dogs ($P > 0.05$).

The observed increases in ALT, AST and ALP, together with reductions in total protein and albumin, indicate alterations in serum biochemical parameters associated with mixed gastrointestinal parasitic infection.

Discussion

Mixed gastrointestinal parasitic infections are of clinical and epidemiological importance in dogs because simultaneous infection with more than one parasite may result from exposure to multiple sources of infection. In the present study, 21 of 604 dogs screened were affected with mixed gastrointestinal parasitic infections, corresponding to an overall prevalence of 3.48%. Among the 314 gastrointestinal parasite-positive dogs, mixed infections accounted for 6.69% (21/314). The prevalence observed in the present study was comparable with the prevalence reported by Souza *et al.* (2023) [23], whereas higher prevalences were reported by Ahmed *et al.* (2018) and Wani *et al.* (2017) [24]. Variation among studies may be associated with differences in geographical location, management practices, environmental conditions, host population and diagnostic methods.

Among the mixed infections identified in the present study, *Ancylostoma caninum* + *Toxocara canis* was the most frequently detected combination, followed by *Ancylostoma caninum* + *Dipylidium caninum* and *Toxocara canis* + *Toxascaris leonina*. The occurrence of these combinations may reflect simultaneous exposure of dogs to different infective stages present in the environment.

Breed was not significantly associated with the occurrence of mixed gastrointestinal parasitic infections ($P > 0.05$). Although mongrel dogs represented the largest proportion of affected animals, this difference was not statistically significant. These findings are similar to those of Dubie *et al.* (2023) [8]. Breed-related variation in parasitic infection may be influenced by management practices, housing, nutrition, veterinary care and roaming or scavenging behaviour.

A highly significant association was observed between age and occurrence of mixed gastrointestinal parasitic infection ($\chi^2 = 14.24$; $P = 0.0026$). The highest proportion of mixed infections was recorded in dogs aged 0-6 months, followed by those aged 7-12 months. The higher occurrence among younger dogs may be associated with their comparatively immature immune response and, in some parasitic infections, transplacental or transmammary transmission of infective larvae. (Bowman, 2009 and Ambily *et al.*, 2011) [5, 2] Similar age-related susceptibility has been reported by Ferreira *et al.* (2016) [9] and Matthew *et al.* (2016), while differences among

studies may reflect variations in study population and environmental exposure.

Gender was not significantly associated with mixed gastrointestinal parasitic infection ($P > 0.05$). Male dogs accounted for 11/21 (52.38%) cases, whereas female dogs accounted for 10/21 (47.62%). Similar findings were reported by Nguyen *et al.* (2022) [14] and Souza *et al.* (2023) [23], although some studies have reported higher infection rates in males. Such variations may be influenced by differences in behaviour, management, hormonal status and environmental exposure.

Among the copro-parasitological techniques evaluated, zinc sulphate flotation showed the highest detection rate (9/21; 42.86%), followed by sedimentation (8/21; 38.09%) and direct faecal smear (4/21; 19.05%). However, the difference among the three diagnostic techniques was not statistically significant ($\chi^2 = 2.024$; $P = 0.3635$). The higher detection observed with zinc sulphate flotation may be related to its ability to concentrate parasitic stages and facilitate their microscopic detection. Similar observations regarding the usefulness of flotation techniques have been reported by Rodriguez *et al.* (2023) [18].

The major clinical manifestations recorded in affected dogs were anorexia, weakness, diarrhoea, vomiting and pale mucous membranes, followed by poor coat condition, emaciation, abdominal bloating, pyrexia, lethargy and congested mucous membranes. These findings were comparable with those reported by Kumar *et al.* (2014) [12], Pradeep (2016) [16], Campos *et al.* (2017) [6] and Gayatri and Soya (2018). The clinical manifestations may vary according to the parasite species, intensity of infection and the general health status of the affected animal.

The haematological findings demonstrated significant reductions in haemoglobin, total erythrocyte count and packed cell volume in infected dogs compared with healthy dogs, consistent with the findings of Qadir *et al.* (2011) [17], Kumar *et al.* (2014) [12], Baba *et al.* (2017) [4], Shahat *et al.* (2022) [20] and Payghan *et al.* (2023) [15]. The reduction in erythrocytic parameters may be associated with blood loss, nutritional deficiencies and the effects of gastrointestinal parasites (Smith *et al.*, 2005; Da Silva *et al.*, 2015). A significant decrease in lymphocyte count was observed, in agreement with Qadir *et al.* (2011) [17] and Baba *et al.* (2017) [4], whereas Kumar *et al.* (2014) [12] and Shahat *et al.* (2022) [20] reported no significant alteration. Total leucocyte, neutrophil and eosinophil counts were significantly increased, with similar findings reported by Kumar *et al.* (2014) [12], Shahat *et al.* (2022) [20] and Audu *et al.* (2024) [3] for leucocytosis, Audu *et al.* (2024) [3] for neutrophilia, and

Qadir *et al.* (2011) [17], Kumar *et al.* (2014) [12], Baba *et al.* (2017) [4], Shahat *et al.* (2022) [20], Audu *et al.* (2024) [3] and Payghan *et al.* (2023) [15] for eosinophilia. These changes may reflect inflammatory and host immune responses to parasitic infection. No significant differences were observed in monocyte and platelet counts, consistent with Qadir *et al.* (2011) [17], Kumar *et al.* (2014) [12], Baba *et al.* (2017) [4], Shahat *et al.* (2022) [20] and Payghan *et al.* (2023) [15].

Serum biochemical analysis showed significantly increased ALT, AST and ALP activities, along with significant reductions in BUN, total protein and albumin in infected dogs. These findings were comparable with the observations of Kumar *et al.* (2014) [12], Shahat *et al.* (2022) [20], Saichenko *et al.* (2022) [19], Campos *et al.* (2017) [6] and Payghan *et al.* (2023) [15]. The increased hepatic enzyme activities may reflect hepatic alterations associated with parasitic infection, whereas reduced protein and albumin concentrations could be related to intestinal protein loss, poor nutrient absorption and reduced feed intake (Kumar *et al.*, 2014; Campos *et al.*, 2017) [12, 6]. Creatinine remained comparable between groups, consistent with Baba *et al.* (2017) [4] and Saichenko *et al.* (2022) [19], while total bilirubin showed no significant variation, contrary to the findings of Saichenko *et al.* (2022) [19]. Overall, the observed biochemical changes suggest systemic effects of gastrointestinal parasitism involving hepatic function and protein metabolism.

Conclusion

Mixed gastrointestinal parasitic infections were recorded in 21/604 dogs screened, with an overall prevalence of 3.48%, and accounted for 6.69% of the gastrointestinal parasite-positive dogs. *Ancylostoma caninum* + *Toxocara canis* was the most frequently observed mixed infection. Age showed a highly significant association with the occurrence of mixed gastrointestinal parasitic infection, whereas breed and gender were not significantly associated. Zinc sulphate flotation showed the highest detection rate among the copro-parasitological techniques evaluated, although the difference among diagnostic methods was not statistically significant. Mixed gastrointestinal parasitic infections were associated with significant haematological and serum biochemical alterations, including anaemia-associated changes, leucocytosis, neutrophilia, eosinophilia, increased hepatic enzyme activities and reductions in total protein and albumin. The findings emphasize the importance of routine copro-parasitological examination and appropriate diagnostic evaluation for the detection and management of mixed gastrointestinal parasitic infections in dogs.

Table 1: Distribution of mixed gastrointestinal parasitic infections among affected dogs

No.	GI parasite	No. of dogs(n=21)	% Positive
1.	<i>Ancylostoma caninum</i> + <i>Toxocara canis</i>	9	42.86
2.	<i>Ancylostoma caninum</i> + <i>Dipylidium caninum</i>	7	33.33
3.	<i>Toxocara canis</i> + <i>Toxascaris leonina</i>	5	23.81
Total		21	100

Table 2: Breed-wise distribution of mixed gastrointestinal parasitic infections in dogs (n=21)

Sl.No.	Breed	No. of Positive dogs (n=21)	% Positive
1.	Mongrel	6	28.57
2.	Labrador	4	19.04
3.	Pomeranian	3	14.28

4.	German Shepard	3	14.28
5.	Golden Retriever	2	9.52
6.	Dachshund	2	9.52
7.	Shih Tzu	1	4.76
	Total	21	100
Chi- square test = 5.33: P=0.50 ^{NS}			

Table 3: Age-wise distribution of mixed gastrointestinal parasitic infections in dogs (n=21)

Sl.No.	Age	No of positives	% positive
1.	0-6 M	12	57.14
2.	7-12 M	6	28.57
3.	13-36 M	2	9.52
4.	37-60 M	1	4.76
Chi- square test = 14.24: P=0.0026 (Highly significant $P<0.01$)			

Table 4: Gender-wise distribution of mixed gastrointestinal parasitic infections in dogs (n=21)

Sl.No.	Gender	No. of positives	% positive
1	Male	11	52.38
2	Female	10	47.62
	Total	21	100
Chi- square test = 0.0476: P=0.83 ^{NS}			

Table 5: Detection of mixed gastrointestinal parasitic infections by different copro-parasitological diagnostic methods

Sl.No	Different copro parasitological diagnostic Procedure	Total No. of positive cases	% Positive
1.	Sedimentation technique	8	38.09
2.	Zinc sulphate flotation	9	42.86
3.	Direct faecal smear method	4	19.05
Chi- square test = 2.024; P=0.3635 ^{NS}			

Table 6: Clinical manifestations observed in dogs with mixed gastrointestinal parasitic infections (n=21)

Sl.No	Clinical sign	No. of dogs	Percentage (%)
1	Anorexia	20	95.23
2	Weakness	19	90.47
3	Diarrhoea	18	85.71
4	Vomiting	15	71.43
5	Pale mucous membranes	14	66.67
6	Poor coat	9	42.85
7	Emaciation	7	33.33
8	Abdominal Bloating	5	23.81
9	Pyrexia	5	23.81
10	Lethargy	4	19.05
11	Congested mucous membrane	3	14.29

Table 7: Haematological Alterations in infected dogs and compared with healthy dogs

Sl.No.	Parameter	Healthy Animals (n=12)	Infected Animals (n=21)	t-value	P-value
1	Hb (g/dL)	13.83±0.23	7.78±0.29**	15.11	3.79×10 ⁻¹⁶
2	TEC(10 ⁶ /μL)	6.625±0.10	3.42±0.15**	17.19	2.2×10 ⁻¹⁷
3	TLC (10 ³ /μL)	9.158±0.28	17.36±0.94**	8.28	8.4×10 ⁻⁹
4	Neutrophils (%)	67.67±0.31	71.28±0.94**	3.63	0.00066
5	Eosinophils (%)	2.33±0.19	8.76±0.47**	12.4	9.45×10 ⁻¹³
6	Monocytes (%)	1.91±0.91	1.95±0.20 ^{NS}	0.42	0.33
7	Lymphocytes (%)	27.91±0.29	18±1.02**	9.34	1.34×10 ⁻⁹
8	PCV (%)	39.65±0.54	23.86±0.90**	14.99	8.89×10 ⁻¹⁶
9	Platelets(10 ⁵ /μL)	3.28±0.057	3.09±0.185 ^{NS}	1.13	0.133
Two sample t-test interpretation: Non-significant ($P>0.05$)					
**Highly Significant at $P<0.01$					
*Significant at $P<0.05$					

Table 8: Biochemical alterations in infected dogs and compared with healthy dogs

Sl.No.	Parameter	Healthy Animals (n=12)	Infected animals (n=21)	t-value	P-value
1	ALT	58.08±3.28	138.19±5.37**	12.72	6.32x10 ⁻¹⁴
2	AST	50.67±3.04	137.52±4.23**	16.65	2.58x10 ⁻¹⁷
3	ALP	82.42±6.04	174.90±5.82**	11.023	5.37x10 ⁻¹²
4	BUN	19.67±1.11	14.76±1.73*	2.37	0.011
5	Creatinine	1.033±0.08	1.038±0.06 ^{NS}	0.043	0.48
6	Total bilirubin	0.2±0.04	0.19±0.02 ^{NS}	2.38	0.45
7	Total Protein	6.4±0.15	3.94±0.15**	11.44	2.24x10 ⁻¹²
8	Albumin	3.075±0.08	1.6±0.10**	10.42	1.27x10 ⁻¹¹
Two sample t-test interpretation: Non-significant ($P>0.05$)					
**Highly Significant at $P<0.01$					
*Significant at $P<0.05$					

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