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Diagnostic utility of selected faecal biochemical parameters in the determination of acute diarrhoea and associated defecation stooling characteristics in dogs: An observational study

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ABSTRACT

Diarrhoea, which is a clinical manifestation of various illnesses, is frequently observed in dogs. Regrettably, many dog owners find it difficult to provide comprehensive case histories, primarily because of limited interaction with their canine companions. This study aimed to evaluate the potential of faecal biochemical analytes in detecting and characterizing acute diarrhoea in dogs. Sixty-two domestic dogs were selected using the proportionate stratified sample technique. A structured questionnaire was used to collect demographic and clinical data. Faecal stool specimens from the dogs were obtained using the colon flush technique. The specimens were taken through biochemical analysis to determine urea, creatinine, total bilirubin, total cholesterol, triglycerides, gamma-glutamyl transferase and uric acid levels. Results showed a significant association between the diarrhoea status of the participants and their age, weight, breed, body size, source of last diet, period of inappetence, and other gastrointestinal signs ($p < 0.050$, respectively). Dogs that had not eaten in at least three days were five times more likely ($p < 0.05$) to have diarrhoea. Furthermore, miniature breeds were about six times more likely to develop diarrhoea ($p < 0.05$). Of the seven selected biochemical parameters, total faecal cholesterol was the most predictive index in diagnosing acute diarrhoea in dogs, with a likelihood ratio of 6.5, and it was the most accurate in predicting defecation stooling frequency and texture. In summary, in situations of inadequate case histories, measuring total faecal cholesterol could assist veterinarians in detecting diarrhoea and predicting its faecal stooling texture and frequency in dogs.

1. Introduction

Diarrhoea is a common clinical sign in dogs and is characterized by loose or liquid faeces, with or without an increase in the frequency of daily defecation (Guarino et al., 2018). It is estimated that about a third of dogs sent to veterinary clinics either have diarrhoea or have had the condition within a month of their presentation. Acute diarrhoea in dogs can often be caused by multiple reasons, including infections and lifestyle risk factors (Berset-Istratescu et al., 2014; Stavisky et al., 2011). When examining the presence of disease-causing microorganisms using electron microscopy, researchers found that 44% of the 936 dogs with haemorrhagic diarrhoea at a veterinary hospital had possible pathogens in their faeces stools. In comparison, 18% of the 200 healthy control dogs had these pathogens (Schulz et al., 2008). There is a paucity of data on the prevalence of diarrhoea among dogs in Ghana. Unpublished data from the Small Animal Teaching Hospital, University of Ghana, showed that an estimated 30% of cases presented at this facility are associated with acute diarrhoea. Ghana has a man-to-dog ratio of 5:1, with 80% of the dog population not under strict confinement within households (Tasiame et al., 2019). The dogs often roam within the communities of their owners. This implies inadequate owner-dog contact time. It is, therefore, not surprising that dog owners do not provide adequate anamnesis about their dogs when they visit veterinary hospitals (Maddison, 2014).

Diarrhoea can be said to be acute when it has a brief duration, often lasting less than 14 days (Armstrong, 2013; Brandt et al., 2015; Guarino

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et al., 2018; Marks, 2013). Dog owners must be able to provide information on the defecation stooling frequency and faecal texture of their dogs with acute diarrhoea. This information is critical for the successful management of such cases. Reports indicate that diarrhoea causes changes in faeces' content, and the faeces' biochemical components can impact the faecal matter's overall look (Guard et al., 2015). The intestinal presence of biochemical parameters such as urea, creatinine, total bilirubin, total cholesterol, uric acid, triglycerides, and gamma-glutamyl transferase (GGT) has been reported in dogs (Forster et al., 2018; Kilburn et al., 2020). These parameters are commonly measured in serum to evaluate the health condition of humans (Gazzola et al., 2004; Weiner et al., 2015) and the general well-being of domestic animals (Kozat & Sepehrizadeh, 2017). Enterocytes and gut bacteria have been found to collectively generate certain quantities of urea through their metabolic processes (Patra & Aschenbach, 2018). In patients with chronic renal illness, creatinine undergoes an enteric cycle to facilitate its removal through the gastrointestinal tract (De Santis et al., 2022; Dunn et al., 1997). Bilirubin undergoes metabolism by intestinal microflora to become urobilinoids or stercobilin, which are then eliminated or reabsorbed (Hamoud et al., 2018).

Faecal fats and lipids serve as indicators to assess intestinal and pancreatic function in maldigestion/malabsorption diseases (Piccione et al., 2004). Enteric bacteria convert unabsorbed cholesterol from digested food into coprostanone and coprostanol (Lye et al., 2010). Without this, cholesterol can trigger apoptosis in intestinal cells (Gazzola et al., 2004). Enterocytes further release gamma-glutamyl transferase, while the gut microbiota generates uric acid through the breakdown of purines (Shao et al., 2017). While previous research has been conducted on these biochemical indicators, their ability to diagnose and predict gastrointestinal health has not been extensively explored in resource-limited clinical settings. Thankfully, the techniques for quantifying urea, creatinine, total bilirubin, total cholesterol, uric acid, triglycerides, and GGT have been established and are often employed in evaluating faecal samples. In addition, the equipment used for conducting these analyses is predominantly fully automated, allowing for efficient processing and standardization of results. processed efficiently. These criteria are the basis for investigating the possibility of using their faecal levels to measure gut health. This study aimed to analyse and evaluate the levels of urea, creatinine, total bilirubin, total cholesterol, and uric acid in the faeces of dogs with and without diarrhoea. Additionally, the study aimed to assess the diagnostic value of these parameters in detecting and predicting diarrhoea in dogs. We hypothesize that elevations in these faecal biochemical analytes could predict diarrhoea. This will be especially beneficial for veterinarians who need to handle dogs suspected of having diarrhoea but whose owners cannot provide relevant medical histories due to a lack of observation.

2. Materials & methods

2.1. Study design, area and patient characteristics

The study was a case-control design conducted at the Small Animal Teaching Hospital (SATH), University of Ghana School of Veterinary Medicine (UGSVM), Accra, Ghana. A total of sixty-two (62) domestic dogs were selected to participate in the study, with 31 dogs presenting with diarrhoea and 31 without. The sampling frame consisted of dogs brought to the SATH for veterinary attention. Faecal samples of the dogs were categorized according to the presence or absence of diarrhoea. A random sample was initially chosen from each stratum, and then every second case was included until the desired sample size for each stratum was reached. The dogs included in the study were at least four months old. This ensured the completion of their vaccination protocols. Permission was sought from dog owners or their designated representatives to be included in the study.

2.2. Demographic and clinical data collection

The demographic and clinical indices of dogs were determined using a structured questionnaire. Age and body weight were measured as continuous variables. Categorical variables were breed type (by body size), sex, last kind of meal, rectal temperature, other gastrointestinal signs, period of inappetence and diarrhoea status. Breed type (body size) was measured as miniature, medium, large or giant based on their external body structure and conformation. Diarrhoea status was measured as present or absent. Diarrhoea was defined as the presence of loose or liquid faeces, with or without an increase in the frequency of defecation (Guarino et al., 2018). The faecal texture was categorized as formed, semiformed or liquid. This information was obtained through history from the dog owners (clients) and observation from the attending clinician if the dog defecated at the study site. Diarrhoea frequency/day was measured as not applicable, once or at least twice. Participants' most recent meal was classified as exclusively commercial, homemade, or a combination of both. The period of inappetence/days' was assessed in days of observed reduced appetite of the participant and expressed as not applicable, one, and at least two. The rectal temperature of the participants was measured using a digital thermometer and read as hypothermic (<38.0 °C), euthermic (38.0–39.5 °C) or hyperthermic (>39.5 °C). Gastrointestinal (GI) symptoms were evaluated for additional GI indicators, including vomiting, abdominal discomfort, borborygmus, excessive salivation, and diarrhoea. These were assessed as absent or present.

2.3. Specimen collection, processing, and analysis

Faecal specimens were collected using the colon flush technique as previously described (Hedgespeth et al., 2020). This involved flushing the colon with three push-and-pull cycles on the plunger of a 10 ml syringe pre-filled with deionized water via a foley catheter (10Fr/Ch, 3–5 ml/cc, 3.3 mm; Dansn Medical International Limited, China). Depending on the body size of the participant, 5 cm to 10 cm of the Foley catheter was introduced into the colon for specimen collection; the bigger the patient, the longer the catheter. Two to four push and pull cycles of the plunger of the 10 ml syringe were used to lavage the colon in short bursts. The specimens were stored standing in plain vacutainers on a test tube rack in a refrigerator until all particles were settled by gravity. The supernatant was aliquoted into cryotubes and stored at −20 °C. The stored specimens were thawed to room temperature before conducting biochemical analysis. About 100 mg of the thawed faecal sample is weighed on a balance scale and transferred into a plain tube. The sample is emulsified with physiological saline (0.85%) to create a uniform mixture and is subsequently centrifuged at a speed of 4000 rpm for 2 min. The supernatant portion is obtained and examined for biochemical parameters using an automated chemistry analyser (Selectra Pro-S, ELITechGroup, France) and manufacturer-recommended reagents and procedures (Elitech Clinical Systems, Reagent Package Insert). The biochemical indicators of interest were urea, creatinine, gamma-glutamyl transferase (GGT), total bilirubin, total cholesterol, triglycerides, and uric acid. A dipstick was used to measure faecal stool pH that was based on a double indicator system comprising methyl red for pH ranging 4 to 6 and bromothymol blue for pH ranging 6 to 9.

2.4. Ethical considerations

Ethical approval was obtained from the Ethical and Protocol Review Committee (EPRC) of the College of Health Sciences, University of Ghana (CHS-Et/M1-5.2/2019-2020, FWA: 000185779, IORG: 0005170, IRB: 00006220). Verbal and written consent was sought from dog owners before their dogs were recruited as study participants. This was after they had been taken through the aim of the research, specimen collection procedure, and benefits or otherwise for the study

participants. Data was kept confidential, and the dog owners were assured of this.

2.5. Statistical analysis

Data analysis was done using Statistical Package for Social Sciences (SPSS) version 20.0. The categorical variables were summarized as percentages. Continuous variables did not follow the Gaussian distribution and were summarized as median with interquartile ranges. Therefore, the reference interval was established by ranking the measurements for each parameter and using the 2.5th percentile as the lower limit and the 97.5th percentile as the upper limit. The binary regression model, with a threshold of 0.50, was employed to categorize individuals with diarrhoea and those without. Quality test indicators (sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio) were performed on the biochemical parameters. The values of the R square and odds ratio of multinomial regression models for each biochemical parameter were used to predict defecation stooling frequency and texture. A p-value < 0.05 was considered statistically significant. This study used various indices to assess the diagnostic utility of the selected biochemical parameters in determining acute diarrhoea. These indices were sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio. Sensitivity refers to the ability of a test to detect a condition when that condition is genuinely present. In contrast, specificity refers to the ability of a test to classify a condition as absent when that condition is genuinely absent (Parikh et al., 2008). The positive predictive value refers to the extent to which an individual characterized by a test as vivacious is genuinely positive. The negative predictive value refers to the extent to which an individual characterized by a test as unfavourable is genuinely hostile (Parikh et al., 2008). The likelihood ratio of a test gives a better assessment of its overall accuracy. These enable the evaluation of the precision of several tests that may be accessible for specific disease conditions (Grimes & Schulz, 2005; McGee, 2002).

3. Results

3.1. Demographic characteristics of study participants

Out of 62 participants, distribution by body size showed that only 3 (4.8%) were giant breeds. Most participants, 45 (72.6%), were large breeds. Male participants were 39 (60.9%). Only 1 (1.6%) of the participants was hypothermic. They were mostly euthermic, 40 (64.5%); however, an appreciable number, 21 (33.9%), were hyperthermic. The participants' most recent meal before sampling was solely homemade, 28 (45.2%), and exclusively commercial meals 23 (37.1%). Forty participants (64.5%) ate the last meal one (1) day before sampling, and 5 (8.1%) had not eaten in at least three (3) days. Most participants, 49 (79.0%) had no concurrent GI signs. The mean age of the participants was 30.8 ± 3.8 months, and the mean body weight was 24.4 ± 1.5 Kg. A summary of the demographic characteristics of the participants is shown in Table 1.

3.2. Association of diarrhoea status of participants with demographic and clinical indices

An association was found between diarrhoea status of the participants, breed type (body size), source of recent meal consumed before sampling, the period of inappetence, and other gastrointestinal signs ($p < 0.05$). No association was observed between diarrhoea status versus the sex or rectal temperature of the participants ($p > 0.05$). The distribution and association of demographic and clinical indices with diarrhoea status are presented in Table 1. An odds ratio was used to determine the exact groups within the categorical variables that exhibited significant associations with the diarrhoea status of the participants. The results showed that of the four breed types (body size), miniature dogs were about six times more likely to develop diarrhoea than the other breed types (OR=6.16, $p = 0.002$). It was also observed that a dog that ate a solely commercial meal was about seven times more likely to develop diarrhoea than those fed exclusive homemade meals or a mixture of homemade and commercial meals (OR=6.62, $p = 0.001$). Furthermore, a dog not eating for at least three days is about five times more likely to develop diarrhoea (OR=5.26, $p = 0.005$). A dog with concurrent gastrointestinal signs was about ten times more likely to have diarrhoea (OR=10.12, $p < 0.001$).

Table 1
Distribution and association of demographic and clinical indices with diarrhoea status of domestic dogs.

Variables	Diarrhoea status			χ^2 (p-value)	OR (p-value)
	Present n = 31 (%)	Absent n = 31 (%)	Total n = 62 (%)		
Body Size:				8.06 (0.045)	
Miniature	5 (16.13)	1 (3.23)	6 (9.68)		**6.16 (0.002)**
Medium	4 (12.90)	4 (12.90)	8 (12.90)		1.00 (1.000)
Large	20 (64.52)	25 (80.65)	45 (72.58)		0.44 (0.011)
Giant	2 (6.45)	1 (3.22)	3 (4.84)		2.43 (0.195)
Sex:				0.92 (0.338)	
Male	17 (54.84)	21 (67.74)	39 (62.90)		–
Female	14 (45.16)	9 (29.03)	23 (37.09)		–
Rectal temperature:				1.91 (0.384)	
Hypothermic	1 (3.23)	–	1 (1.61)		–
Euthermic	21 (67.74)	19 (61.29)	40 (64.52)		–
Hyperthermic	9 (29.03)	12 (38.71)	21 (33.87)		–
Type of last meal:				7.31 (0.026)	
Commercial	16 (51.61)	7 (22.58)	23 (37.10)		**6.62 (0.001)**
Homemade	9 (29.03)	19 (61.29)	28 (45.16)		0.26 (0.003)
Mixed	4 (12.90)	5 (16.13)	9 (14.52)		0.78 (0.688)
Period of inappetence/day:				8.94 (0.030)	
N/A	6 (19.35)	4 (12.90)	10 (16.13)		1.78 (0.132)
One	15 (48.39)	25 (80.65)	40 (64.52)		0.28 (0.001)
Two	3 (9.68)	1 (3.23)	4 (6.45)		**3.99 (0.027)**
≥ Three	4 (12.90)	1 (3.13)	5 (8.06)		**5.26 (0.005)**
Other GI signs:				13.29 (0.001)	
Present	13 (41.94)	1 (3.23)	14 (22.58)		**10.18 (<0.001)**
Absent	18 (58.06)	30 (96.77)	48 (77.42)		0.04 (<0.001)

n = sample size, NA = not applicable, GI = gastrointestinal∞ = infinity, χ^2 = Chi-square ≥ = at least, OR = Odds Ratio, p-value < 0.05 was considered statistically significant.

3.3. Determination of acute diarrhoea in dogs using faecal biochemistry data

The Mann-Whitney U test was used to compare the levels of the biochemical parameters of participants with and without diarrhoea (Table 2a). Differences in the means of urea, creatinine, cholesterol,

Table 2a
Comparison of the levels of the biochemical parameters among domestic dogs.

parameter	Diarrhoea status		*p*-value
	Present ($n = 31$)	Absent ($n = 31$)	
	Median (IQR)	**Median (IQR)**	
Urea (mmol/L)	0.54 (1.27)	0.19 (0.32)	0.001
Creatinine (μmol/L)	14.52 (10.14)	9.98 (4.58)	0.012
T. Bilirubin (μmol/L)	0.85 (2.40)	0.32 (0.18)	0.001
T. Cholesterol (mmol/L)	0.06 (0.16)	0.02 (0.01)	0.001
Uric acid (μmol/L)	7.94 (5.90)	3.01 (2.59)	0.001
Triglyceride (mmol/L)	0.03 (0.07)	0.01 (0.01)	0.001
GGT (U/L)	98.17 (92.44)	27.76 (37.159	<0.001

n = Sample size.
IQR= interquartile range.

bilirubin, GGT, triglyceride and uric acid between the two groups were statistically significant ($p < 0.05$ for all).

3.4. Prediction of defecation faecal frequency

An analysis of the biochemical parameters revealed a positive correlation between defecation faecal frequency and the levels of each parameter (Table 2b). The Kruskal-Wallis H test revealed a statistically significant disparity in the levels of each biochemical parameter among a minimum of two groups ($p < 0.05$). The Dunn-Bonferroni posthoc tests showed that total bilirubin, total cholesterol, GGT, urea and uric acid levels were statistically different among the three categorized groups ($p < 0.05$ for all).

3.5. Prediction of faecal texture

A comparison of the faecal texture (formed, semiformed and liquid) and levels of biochemical parameters is presented in Table 2c. The Kruskal-Wallis H test showed a statistically significant difference in the levels of each biochemical parameter between at least two groups ($p < 0.05$). The Dunn-Bonferroni posthoc tests showed that for all the biochemical parameters, their levels were statistically significantly different with regard to faecal texture ($p < 0.05$) (Table 2c).

3.6. Diagnostic accuracy of biomarkers in predicting acute diarrhoea

Quality attributes were used to evaluate the utility of biochemical indicators as diagnostic predictors for acute diarrhoea (Table 3). Total cholesterol exhibited the highest sensitivity (83.4%), specificity (87.1%), positive predictive (86.7%) and negative predictive (88.4%) values with a likelihood ratio of 6.5. Further, urea showed the lowest predictability, with a likelihood ratio of 2.4.

Table 2b
Comparison of the levels of the biochemical parameters and defecation faecal frequency in dogs.

Parameter	Defecation faecal frequency/day			*p*-value
	Nil: $n = 31$	One: $n = 17$	≥ 2: $n = 14$	
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	
Urea (mmol/L)	0.18 (0.32)	0.45 (0.69)	0.82 (6.61)	0.001
Creatinine (μmol/L)	9.89 (3.58)	14.44 (10.28)	15.48 (34.88)	0.017
T. Bilirubin (μmol/L)	0.22 (0.16)	0.63 (2.09)	0.68 (3.17)	0.001
T. Cholesterol (mmol/L)	0.01 (0.01)	0.05 (0.03)	0.09 (0.20)	0.001
Uric acid (μmol/L)	2.99 (2.59)	6.08 (3.00)	16.32 (31.77)	0.001
Triglyceride (mmol/L)	0.01 (0.01)	0.02 (0.03)	0.04 (0.26)	<0.001
GGT (U/L)	27.16 (17.15)	92.28 (95.74)	213.25 (314.75)	<0.001

n = sample size.
IQR= interquartile range.

Table 2c
Comparison of the levels of the biochemical parameters between the faecal texture groups.

Parameter	Faecal texture			*p*-value
	Formed $n = 31$	Semiformed $n = 17$	Liquid $n = 14$	
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	
Urea (mmol/L)	0.18 (0.22)	0.34 (0.42)	1.07 (4.56)	0.001
Creatinine (μmol/L)	9.89 (4.58)	14.41 (9.26)	18.08 (34.28)	0.013
T. Bilirubin (μmol/L)	0.22 (0.16)	0.53 (1.16)	0.92 (3.27)	0.001
T. Cholesterol (mmol/L)	0.01 (0.01)	0.03 (0.02)	0.07 (0.24)	0.001
Uric acid (μmol/L)	2.98 (2.59)	6.57 (5.51)	15.27 (35.51)	0.001
Triglyceride (mmol/L)	0.01 (0.01)	0.02 (0.01)	0.04 (0.37)	<0.001
GGT (U/L)	27.78 (44.15)	79.19 (93.30)	183.43 (264.5)	<0.001

n = sample size.
IQR= interquartile range.

4. Discussion

As is required for managing all conditions, a pertinent, adequate and accurate case history is also necessary for managing diarrhoea. Veterinarians rely on animal owners to provide information regarding their animals' health during clinical examinations. The owner's provision of inadequate or inaccurate information can lead to misdiagnosis (Otranto, 2015). Approximately 80% of dogs in Ghana are not confined to households [5], meaning owners will likely have little contact time with their dogs. As a result, health-related disorders in dogs may go unnoticed due to the lack of observation. Owners of such dogs are unlikely to give comprehensive and precise records for managing their pets at veterinary facilities.

Diarrhoea is a common reason dogs are presented to veterinary clinics for attention (Stavisky et al., 2011). If not well managed, diarrhoea could lead to severe dehydration, which is potentially life-threatening (Guard et al., 2015). Diarrhoea alters faecal composition and the gross appearance of faecal material (Jia et al., 2009). This study investigated the levels of selected faecal biochemical analytes and their usefulness in identifying the presence of diarrhoea. The study further examined the demographic and clinical factors that define diarrhoea in dogs. These measures could enable the timely identification of the condition for immediate intervention and guarantee optimal clinical results. Analysis of the annual caseload at the Small Animal Teaching Hospital, University of Ghana School of Veterinary Medicine, showed that 30% of the 1800 yearly attendances are diarrhoea-related conditions (unpublished data). This estimate corroborates a study conducted in the United Kingdom, which reported that a third of all dogs sent to veterinary clinics have diarrhoea or have had it within one month of presentation (Stavisky et al., 2011). This makes diarrhoea one of the common conditions in dogs, which veterinarians must manage even without adequate case histories. This study determined no inherent tendency for purebred or crossbred domestic dogs to have diarrhoea. Sex was also found not to be a predisposing factor for the occurrence of diarrhoea, as reported elsewhere (Pugh et al., 2017). On the contrary, other studies have reported that female dogs are more likely to have diarrhoea (Sævik et al., 2012; Stavisky et al., 2011).

Dogs categorized as miniature and underweight had a six-fold and fivefold increase in the likelihood of experiencing diarrhoea compared to dogs with different body condition scores. Diarrhoea causes dogs to become dehydrated (Battersby & Harvey, 2006; Unterer & Busch, 2021).

Table 3
Diagnostic accuracy of the biochemical parameters in predicting acute diarrhoea.

Observed	Predicted		Sensitivity	Specificity	PPV	NPV	LR
	Present	Absent	(%)	(%)	(%)	(%)	(ratio)
Urea			54.8	77.4	70.8	63.2	2.4
Present	17	14					
Absent	7	24					
Creatinine			48.4	83.9	75.0	61.9	3.0
Present	15	16					
Absent	5	26					
T. Bilirubin			43.3	87.1	76.5	61.4	3.4
Present	13	17					
Absent	4	27					
T. Cholesterol			83.4	87.1	86.7	84.4	6.5
Present	26	5					
Absent	4	27					
Uric Acid			60.0	83.9	78.3	68.4	3.7
Present	18	12					
Absent	5	26					
Triglycerides			71.9	86.2	81.2	75.8	5.0
Present	23	8					
Absent	4	25					
GGT			61.3	86.9	80.3	78.4	4.7
Present	19	12					
Absent	4	27					

PPV = Positive Predictive Value.
NPV = Negative Predictive Value.
LR = Likelihood Ratio.

Depending on the extent of dehydration, physical alterations in body form and contours of dogs, such as a sunken abdomen and pronounced visualization of the ribs and bone extremities, may be present. These physical alterations are characteristic of an underweight dog.

Most recent meal consumed, period of inappetence/day and other gastrointestinal signs were associated with diarrhoea status. A participant was five times more likely to have diarrhoea if the source of their most recent meal was commercial instead of homemade or a mixture of the two. The majority of commercially marketed dog diets undergo heat treatment before feeding. However, a few are in their raw state. The raw commercial feed may occasionally have enteropathogenic organisms like *Salmonella sp,* which cause diarrhoea (Duijvestijn et al., 2016; Marks et al., 2011). The heat-treated commercial feed may also become contaminated with such organisms during packaging, transportation and storage. These organisms, if present, remain viable and may potentially cause diarrhoea. Secondly, gluten, a cereal protein in some commercial feed, could trigger a sensitivity reaction associated with increased gastrointestinal permeability, culminating in diarrhoea in some dogs (Hall & Batt, 1992; Volta et al., 2017). Interestingly, when mixed with commercial feed, there appears to be a reduction in the "inoculum size" of the pathogenic bacteria [32], possibly reducing their likelihood of developing diarrhoea. On the other hand, homemade feed is usually fed warm with the heat likely destroying microbes that may be present.

It was observed that dogs that had not eaten in two days were four times more likely to have diarrhoea, whereas those that had not eaten in at least three days were five times more likely. Such dogs may avoid food to minimize the discomfort associated with frequent defecation stooling. It was observed that dogs with diarrhoea are ten times more likely to have concurrent gastrointestinal signs. These concurrent signs are only secondary to diarrhoea; however, they are the signs most dog owners give as complaints (Walker & McMahon, 2019). These signs may include borborygmus, flatulence, dehydration, vomiting, abdominal distention and abdominal pain (Chandler, 2010; Marks et al., 2011). Thus, it is almost certain that a dog with these gastrointestinal signs also has diarrhoea. Well-characterized diarrhoea is important in any clinical setting. The frequency of diarrhoea, faecal texture, and faecal colour were identified as indices with significant associations with the diarrhoea status of the participants. Determining defecation stooling frequency and texture is essential and helps estimate fluid loss per episode of diarrhoea. Information on defecation stooling frequency and texture can help clinicians accurately estimate dehydration associated with acute diarrhoea in domestic dogs (Marks, 2013). Acute diarrhoea accompanied by moderate or severe dehydration should be regarded as potentially life-threatening (Armstrong, 2013). Using a dipstick that could only determine pH in ranges, 95.2% of the specimens had a pH between 6.0 and 6.9. This confirms the reported pH of 6.5 for colonic contents (Koziolek et al., 2019), validating our sampling technique.

Seven faecal biochemical parameters were selected to investigate their diagnostic utility in determining acute diarrhoea and associated defecation stooling frequency and texture. These were urea, creatinine, total bilirubin, total cholesterol, triglycerides, GGT and uric acid. Among other indices of diagnostic performance, the likelihood ratio was used to assess the overall diagnostic performance of each biochemical parameter and allows for comparing several tests that may be available for a specific condition (Grimes & Schulz, 2005; McGee, 2002). This study showed that faecal urea, creatinine, total bilirubin, total cholesterol, uric acid, triglycerides and GGT levels significantly differ between dogs with diarrhoea and those without. Previous studies have indicated the intestinal presence of urea, creatinine, total bilirubin, total cholesterol and uric acid (Forster et al., 2018; Kilburn et al., 2020). Both enterocytes and gut microflora are involved in the metabolism of urea (Patra & Aschenbach, 2018). Gut microbial ureases break down urea into ammonia, the accumulation of which can disturb gut homoeostasis. Pre-renal azotemia, like dehydration, is associated with diarrhoea and contributes to increased urea levels in proportion to creatinine levels in dogs (Mylonakis et al., 2016). Indeed, gut microbiota expresses specific enzymes mediating creatinine breakdown (Wallimann et al., 2021). Urea levels were higher in dogs with diarrhoea than those without. Intestinal ureases catabolize the conversion of urea to ammonia (Weiner et al., 2015), a toxic gas whose accumulation alters intestinal homoeostasis by inducing pathological processes that culminate in decreased nutrient absorption, epithelial cell proliferation, and impaired gut barrier function in monogastric animals (Hailemariam et al., 2021). This could perhaps be the mechanism underlying the association between urea and acute diarrhoea.

The accumulation of bilirubin in the intestines may indicate dysbiosis as this metabolite is either reabsorbed or metabolized into

urobilinoids or stercobilin and eliminated in faeces (Hamoud et al., 2018). A positive correlation existed between total bilirubin levels, acute diarrhoea occurrence, and defecation stooling frequency. Intestinal bacteria play an important role in breaking down bilirubin and producing urobilinoids in the intestines. This process is essential for controlling the composition and abundance of gut microflora. Excess bilirubin, the substrate involved in this process, indicates an imbalance in the gut microbiota (Hamoud et al., 2018).

Unabsorbed intestinal cholesterol is metaboliszd by gut bacteria into coprostanone and coprostanol for elimination in faeces (Lye et al., 2010) with the use of antibiotics or diet composition affecting the ability of enteric bacteria to convert cholesterol to coprostanol (Benno et al., 2005). We hypothesized that elevations in total cholesterol in the intestines can cause acute diarrhoea. This is believed to occur due to the upregulation of peroxisome proliferator-activated receptor-gamma (PPARg), which reduces intestinal cell growth by triggering apoptosis. This phenomenon has been observed in a laboratory experiment involving rat enterocytes (Gazzola et al., 2004; Ringseis et al., 2007). Analysis of the average intestine total cholesterol levels in study participants with and without diarrhoea (Table 2b) indicates that intestinal total cholesterol is strictly regulated to the extent that even a modest increase in its level can lead to diarrhoea. Among the seven biochemical parameters assessed, total cholesterol was the most accurate and sensitive by its likelihood ratio of 6.5 (Table 3). For the biochemical prediction of defecation stooling frequency and faecal stool texture associated with cases of acute diarrhoea, total cholesterol exhibited the most accurate predictability for defecation stooling frequency and faecal stool texture. Thus, the levels of faecal total cholesterol could predict participants without diarrhoea, those with one bout in a day, and those with at least two bouts in a day. For faecal stool texture, the levels of total faecal cholesterol could predict participants with firm, semiformed, and liquid faeces stools. Uric acid is excreted by the kidneys, and circulating uric acid levels have been reported to cause alterations in gut microbiota (Wang et al., 2022). A third of circulating uric acid is transported into the intestines, broken down by colonic bacteria and eliminated (Méndez-Salazar & Martínez-Nava, 2022). Gamma-glutamyl transferase is predominantly found in the cell membranes and in small portions in the cytoplasm of enterocytes. The levels of GGT increase when they lose their anchorage within the membranes of these cells (Bălăeţ et al., 2018). These descriptions of the intestinal metabolism of the faecal biochemical parameters in this study suggest that compromised gut health, as may manifest in diarrhoea, can affect the levels of these parameters. Further studies are recommended to ascertain the direct involvement of these biochemical parameters in the pathophysiology of diarrhoea in dogs. Future research must delineate the associated mechanisms underlying biochemical parameters and acute diarrhoea in domestic dogs. A limitation of the study was that we did not consider microorganisms present during acute diarrhoea. This study did not measure the gut microbiome of dogs, which could have strengthened our observations. The fact that this research was conducted using a cross-sectional study design limits its extent of generalization. Additionally, the expression of peroxisome proliferator-activated receptor--gamma (PPARg) could be considered as a diagnostic marker in future studies.

5. Conclusion

Results from this study have shown that among seven selected biochemical parameters, faecal total cholesterol is the most predictive of acute diarrhoea in domestic dogs. It is also a good indicator for predicting associated defecation stooling frequency and texture. The findings from this study should help veterinarians to detect diarrhoea and characterize it in dogs whose owners are unaware of the health status of their pets due to lack of observation.

Ethics statement

Ethical approval was obtained from the Ethical and Protocol Review Committee (EPRC) of the College of Health Sciences, University of Ghana (CHS-Et/M1-5.2/2019-2020, FWA: 000185779, IORG: 0005170, IRB: 00006220). Verbal and written consent of dog owners was sought before their dogs were recruited as study participants. This was after they had been taken through the aim of the research, specimen collection procedure, and benefits or otherwise for the study participants. Data was kept confidential, and the dog owners were assured of this. No human ethics approval was not needed for this study.

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CRediT authorship contribution statement

Obed D. Acheampong: Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Emmanuel K. Ofori:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Sherry A.M. Johnson:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Bill C. Egyam:** Software, Investigation. **Kweku Asare-Dompreh:** Resources, Formal analysis. **Seth K. Amponsah:** Writing – review & editing, Validation. **Henry Asare-Anane:** Writing – review & editing, Supervision, Methodology.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Obed D. Acheampong reports financial support was provided by The A. G. Leventis Foundation Fellowship Scheme. Obed D. Acheampong reports a relationship with The A.G. Leventis Foundation Fellowship Scheme that includes: funding grants. Obed D. Acheampong has patent N/A pending to N/A. N/A If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Emmanuel Kwaku Ofori and Obed D. Acheampong have full access to the data. They will make the dataset available to the interested party upon reasonable request.

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