Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2022.009

RESEARCH ARTICLE

Molecular prevalence with associated risk factors and haemato-serum electrolyte analysis of *E. coli* O157:H7 in Canine pups with diarrhoea

Asfand Yar Khan[1], Syed Saleem Ahmad*[1], Muhammad Avais[1] and Kamran Ashraf[2]

[1]Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore, Pakistan, 54000
[2]Department of Parasitology, University of Veterinary and Animal Sciences, Lahore, Pakistan, 54000
*Corresponding author: vetsalim@uvas.edu.pk

ARTICLE HISTORY (21-340)

Received: August 17, 2021
Revised: January 23, 2022
Accepted: January 24, 2022
Published online: February 02, 2022

Key words:
Canine diarrheic puppies
E. coli O157:H7
Hematological
PCR
Serum electrolytes

A B S T R A C T

This study was conducted on canine puppies (n=600) with diarrhoea during October 2019-20 in district Lahore, Pakistan to know the molecular prevalence of *E. coli* O157:H7, its associated risk factors and the impact of bacterial diarrhea on various blood parameters and serum electrolytes. PCR-based positive (n=21) and negative (n=21) cases were sampled for their blood, and serum for the haemato-serum electrolyte analysis. The results showed that the overall prevalence of *E. coli* O157:H7 was 3.5% (P<0.05) in canine diarrheic pups. The highest prevalence of *E. coli* O157:H7 was found within the UVAS pet center (10 %, 10/100). Season, food, drinking water plus animal breed, sex, co-habitation with other animals and contact with animals`feces, were significantly (P<0.05) associated with the *E. coli* O157:H7 prevalence. Mean value of Hb (P<0.01), RBCs (P<0.05), lymphocytes (P<0.01) and platelets (P<0.05) were reduced significantly, while neutrophils, PCV and TLC were increased significantly(P<0.01) in the infected group as compared to the control healthy group. A significant decrease was found in sodium mEq/l (P<0.01) and potassium mEq/l (P<0.05), while a significant (P<0.01) increase was found in chloride mEq/l and bicarbonate mmol/l in the infected group as compared to control healthy group. In conclusion, the *E. coli* O157:H7 was present in the canine puppies of district Lahore Pakistan and had a high effect on blood parameters and serum electrolytes.

To Cite This Article: Khan AY, Ahmad SS, Avais M and Ashraf K, 2022. Molecular prevalence with associated risk factors and haemato-serum electrolyte analysis of *E. coli* O157:H7 in Canine pups with diarrhoea. Pak Vet J, 42(2): 161-166. http://dx.doi.org/10.29261/pakvetj/2022.009

INTRODUCTION

Dogs are evolved from grey wolves (Zhenxin *et al.*, 2016). In Pakistan, dogs are kept for many purposes like sniffing in routine forces security operations (Sniffer dog), fighting purposes (Bull Terrier), racing purposes (Hounds), etc. (Shah *et al.*, 2017). Some shepherds utilize dogs for the herding of sheep (Bhattacharjee *et al.*, 2013), hence dogs have deep involvement in humans' life.

Mostly, the presence of *E. coli* is beneficial, however, sometimes it causes infection in new born puppies due to lack of systemic immunity. On the basis of virulency factors, the enteric *E. coli* is categorized as Enterohaemorrhagic (EHEC), Enteropathogenic (EPEC), Enteroinvasive (EIEC), Enteroaggregative (Eagg EC), and Verotoxigenic (VTEC) strains (Croxen *et al.*, 2013).

The most pathogenic serotype of Enterohaemorrhagic *E. coli* group is *E. coli* O157:H7 (Kiranmay *et al.*, 2010). *E. coli* O157:H7 is the main cause of recent worldwide outbreaks of hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS) in dogs as well as in humans (Kwon and Cho, 2015). The bacteria express virulent genes which cause diarrhoea in canine puppies and in adults as well (Razzaq *et al.*, 2021). Virulency is initiated by two types of toxins (Stx-1 or Stx-2, or both) characterized by *E. coli* O157:H7 bloody diarrhoea if intestine is infected (Hassan *et al.*, 2020).

Fewer past studies have been performed to know the overall prevalence of *E. coli* O157:H7 in canine diarrheic puppies estimating15.8% by Ojo *et al.* (2014), 72.72% by Hassan *et al.* (2016), 22.64% by Yousif *et al.* (2016) and 9.7% by Abdulrazzaq *et al.* (2021). Generally, *E. coli* O157:H7 infection in canine puppies progresses with a varying intensity that can be frequently associated with many factors such as age (Yousif *et al.*, 2016), warm weather (especially summer months), breed, housing condition, drinking water contamination, immune response, competition for microbial flora, correlated with

an increase in rates of *E. coli* O157:H7 faecal shedding (Hassan *et al.,* 2016).

In Pakistan, until now there was no research conducted on *E. coli*O157: H7 in dogs and it is progressively prevailing among diarrheic animals and the children as they are highly connected with canine puppies. In Pakistan, keeping in view the zoonotic importance, the study was designed to investigate the molecular prevalence of *E. coli* O157:H7 in canine diarrheic pups in district Lahore, Pakistan along with its associated risk factors and to determine the impact of bacterial diarrhoea on various blood parameters and serum electrolytes.

MATERIALS AND METHODS

Epidemiological information: The present study was conducted to know the actual Fig. of *E. coli* O157:H7 infected animals in the district Lahore, Pakistan by screening seven different localities including Wapda Town, Township, Gulberg Town, UVAS pet center, Model Town, Johar Town and Defense. The data recorded during the sample collection corresponded wisely to breed (German Shepherd, Labrador, Doberman, Pug, Rottweiler, Pointer, Schnauzer, Bully, Non-Descriptive and poodle), sex (male and female), age (<1 month, 1-2 months, 2-3 months and 4 months), emesis (present or absent), diarrhoea (bloody and without blood diarrhoea), co-habitation with other animals (yes or no), food (processed, raw and home-cooked), season (spring, summer, autumn, and winter), contact with other animals` faeces (cattle, buffalo , sheep, goat , birds , cat or equine faeces contacted and never contacted), and drinking water (clean and dirty) were used to establish association with samples suggestive of *E. coli* O157:H7 infection. The current study was approved by UVAS Ethical Review Committee for use of animals.

Inclusion criteria of sampling animal: The fecal samples were collected from canine diarrheic pups (n= 600) with or without blood characterized by anorexia, dehydration, congested mucus membrane and vomiting.

Culturing and conservation of isolates: Approximately 1 g of faeces was cultured on Eosin Methylene blue Agar (EMB; TM MEDIA, India "M3D6JQ01") and was incubated at 37°C for 24 hrs. The metallic sheen color colonies were further analyzed for microscopy and biochemical tests such as IMViC (Indole test, Methyl red test, Voges-Proskauer test and Citrate utilization test) were done as described by (Hassan *et al.,* 2016) as the traditional method of identification.

The three to four positive colonies from each positive sample cultured on EMB agar were sub cultured on Sorbitol MacConkey agar (CT-SMAC, China) supplemented with cefixime and potassium tellurite. The smooth colorless colonies indicated sorbitol non-fermented colonies of *E. coli* O157:H7 (Hasan *et al.,* 2016). The pure isolates of *E. coli* O157:H7 were maintained as frozen stock cultures in Luria Bertani broth at -70°C.

DNA extraction and Polymerase Chain Reaction (PCR): Bacterial DNA was extracted by using a commercial DNA extraction kit (Thermo scientific USA) as per |manufacturer's instruction, while the concentration of the DNA was checked through a Nanodrop spectrophotometer (Thermo scientific USA NanoDrop 1000). A pair of primers used for the rfbO157 gene and H7 gene of *E. coli* O157:H7 are presented in Table 1.

To confirm the rfbO157 and H7 gene of *E. coli* O157:H7, PCR was performed by using 25µl reaction mixture having 1µl of DNA template, 1µl of each primer, 15µl of PCR Master Mix and 7µl of PCR water. Thermocycler temperature at different stages for both genes were adjusted as described by (Samad *et al.,* 2018).

Blood collection and haemato-serum electrolytes analysis: Blood samples were collected from PCR-based positive canine diarrheic pups (n=21) and negative (n=21) healthy pups from cephalic or saphenous vein. The blood was stored at 4ºC in EDTA coated vacutainer tube till further analysis. The samples were processed in the laboratory for hematological examinations through a VET hematology analyzer (Model No. DW-3680/DW-36 (China)). The serum samples were processed for various parameters including Sodium mEq/l, Potassium mEq/l, Chloride mEq/l, Bicarbonate mmol/l at UVAS pet center Lahore.

Statistical analysis: The statistical analysis was performed using SPSS version 20. Chi-Square $\chi 2$ test was applied for risk factors analysis. Odd ratios were determined to know the significant association of risk factors. Haemato-serum electrolytes were analyzed by independent sample t-test. P-values less than 0.05 and 0.01 were considered as significant.

RESULTS

Based on assessment of bacteriological cultures, a total of 434 (72.3%; P<0.05) isolates were confirmed *E. coli*, while amongst 112 (25.8 %; P<0.05) were confirmed *E. coli* O157:H7. However, 21 (3.5%) isolates were confirmed *E. coli* O157:H7 through PCR (Fig. 1).

Our results revealed that the highest (P<0.05) prevalence of *E. coli* O157:H7 infection was found in UVAS pet centre 10/100 (10 %) and zero prevalence was observed in township 0 % (0/50) (Fig. 2).

A significant association (P<0.002) was recorded among breeds and highest prevalence was recorded in non-descriptive breed (OR 5.500; CI 0.690-43.809) compared with other breeds (Table 2). Sex of the animal was significantly (P<0.04) correlated with *E. coli* O157:H7 prevalence in canine. The Odd ratio (OR 2.500; CI 0.957-6.530) showed that female pups were more prone to infection than males. The pups with bloody diarrhoea had higher correlation with *E. coli* O157:H7 (OR 1.230 CI 0.501-3.018; P<0.0001). The results showed that *E. coli* O157:H7 was more prevalent in pups co-habitual with other animals than other pups (OR 3.200; CI 1.157-8.846; P<0.01). The type of food had a significant association (P=.005) with the prevalence of *E. coli* O157:H7 in canine diarrheic pups. Odd ratio suggested that the raw food-based diet was the highest potential risk factor (OR 13.000; CI 1.684-100.319) for *E. coli* O157:H7. The summer season had more potential for

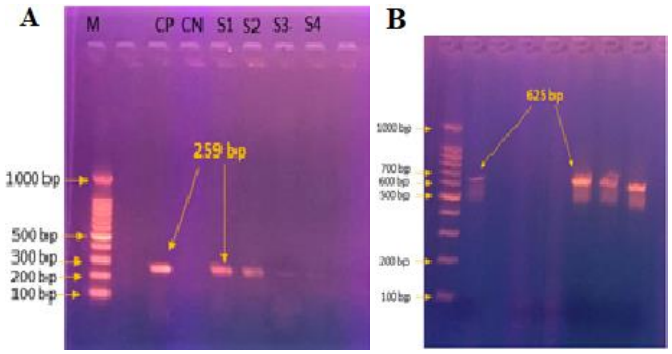

Fig. 1: (A & B) Gene O157 (259 bp) and H7 (625 bp) Bands of *E. coli* O157: H7 being Visualized after Gel electrophoresis (M was DNA marker, CP was Control Positive, CN was Control Negative, S1, S2, S3 and S4 were Samples).

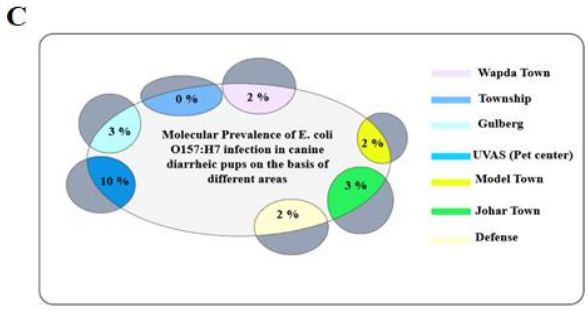

Fig. 2: (C) Area wise Prevalence of *E. coli* O157:H7.

Table 1: The primers with their sequences and product size

Primer	Sequence (5'-3')	Amplicon size (bp)	Reference
rfbO157	F: CGGACATCCATGTGATATGG R: TTGCCTATGTACAGCTAATCC	259	Paton and Paton, 1998
flic H7	F: GCGCTGTCGAGTTCTATCGAGC R:CAACGGTGACTTTATCGCCATTCC	625	Gannon *et al.*, 1997

Table 2: Analysis of different factors with the Prevalence of *E. coli* O157:H7infection

Risk Factor	Groups	Total	Positive %	Odd Ratio	95 % CI for OR	P-Value
Breed	German Shepherd	100	5 (5)	2.500	0.284-21.977	0.002
	Labrador	50	2 (4)	2.000	0.175-22.770	
	Doberman	50	0	0.333	0.0133-8.379	
	Pug	50	1 (2)	1.000	0.060-16.435	
	Rottweiler	50	0	0.333	0.0133-8.379	
	Pointer	50	0	0.333	0.0133-8.379	
	Schnauzer	50	0	0.333	0.0133-8.379	
	Bully	50	1 (2)	1.000	0.060-16.435	
	Non-Descriptive	100	11 (11)	5.500	0.690-43.809	
	Poodle	50	1 (2)	Ref.	-	
Age	< 1 month	150	10 (6.66)	5.000	1.077-23.206	0.07
	1-2 month	150	5 (3.33)	2.500	0.477-13.087	
	2-3 month	150	4 (2.66)	2.000	0.122-11.084	
	3-4 month	150	2 (1.33)	-	-	
Sex	Male	300	6 (2)	-	-	0.04
	Female	300	15 (5)	2.500	0.957-6.530	
Vomiting	Absent	300	8 (2.66)	-	-	0.2
	Present	300	13 (4.33)	1.625	0.663-3.977	
	Without Blood	400	13 (3.25 %)	-	-	0.000
Diarrhoea	With Blood	200	8 (4 %)	1.230	0.501-3.018	
Co-habitation with	Yes	300	16 (5.33)	3.200	1.157-8.846	0.01
other animals	Not	300	5 (1.66)	-	-	
	Processed	200	1 (0.5)	-	-	0.005
	Raw/offal's	200	13 (6.5)	13.000	1.684-100.319	
Food	Home cooked food	200	7 (3.5)	7.000	0.853-57.419	
Season	Spring	150	2 (1.33)	-	-	0.02
	Summer	150	10 (6.66)	5.000	1.077-23.206	
	Autumn	150	2 (1.33)	1.000	0.139-7.192	
	Winter	150	7 (4.66)	3.500	0.715-17.123	
Contact with other	Sheep & Goat Feces (n= 100)	100	1 (1)	-	-	0.000
Animal's Feces	Cattle & Buffaloes Feces (n= 100)	100	10 (10)	10.000	1.256- 79.587	
	Birds Feces (n=100)	100	2 (2)	2.000	0.178-22.412	
	Cats Feces (n= 100)	100	0	0.333	0.0134-8.281	
	Equine Feces (n= 100)	100	0	0.333	0.0134-8.281	
	Never Contacted (n=100)	100	8 (8)	8.000	0.982-65.152	
Drinking Water	Clean	300	4 (1.33)	-	-	0.004
	Dirty	300	17 (5.66)	4.250	1.413-12.779	

E. coli O157:H7 infection than other seasons. Moreover, the odd ratio values (OR 4.250; CI 1.413-12.779) suggested that dirty water is one of the major potential risk factors for the prevalence of *E. coli* O157:H7. Age and vomiting condition exhibited non-significant (P<0.07) association with the prevalence of *E. coli* O157:H7 (Table 2).

In hematological analysis of *E. coli* O157:H7 infected canine diarrheic puppies, the mean values of Hb (P<0.01), RBCs (P<0.05), lymphocytes (P<0.01) and platelets (P<0.05) were reduced significantly in the infected group as compared to the control healthy group.On the contrary, neutrophils (P<0.01), PCV (P<0.01), and TLC (P<0.01) were increased significantly in the infected than the control group (Table 3).

The serum electrolytes' analysis revealed a significant decrease in sodium mEq/l (P<0.01) and potassium mEq/l (P<0.05), whereas an increase (P<0.01) in chloride mEq/l and bicarbonate mmol/l in infected puppies compared with control.

Table 3: Hematological analysis of *E. coli* O157:H7infected pups and Healthy pups while various signs showed, P<0.01** Significant, P<0.05* Significant, P>0.05 ^{NS} Non-significant

Parameters	Control (n=21) Healthy Pups Mean±Std deviation	Diarrheic (n= 21) E. coliO157 :H7Infected Mean±Std deviation	Statistical analysis (T-test)
Hb (G/dL)	13.56±0.32	11.61±0.04	P<0.01**
RBCs x10^6/uL	8.20±0.20	6.94±0.35	P<0.05*
PCV (%)	37.65±0.85	47.55±0.55	p<0.01**
MCV fl	54.09±1.60	55.40±0.90	p>0.05 ^{NS}
MCHC (G/dl)	31.07±1. 11	29.01±1.20	p>0.05 ^{NS}
TLC (x103 /µL)	11.25±0.66	14.17±0.20	P<0.01**
Neutrophils %	70.81±1.38	78.95±1.45	P<0.01**
Monocytes %	3.76±0.49	2.31±o.34	p>0.05 ^{NS}
Eosinophils %	1.29±0.33	0.95±0.14	p>0.05 ^{NS}
Lymphocytes %	21.06±1.16	15.08±0.94	p<0.01**
MCH Pgs	26.20±1.14	23.10±1.12	p>0.05 ^{NS}
Platelets(x 105/µL)	370.30±31.15	290.10±18.10	P<0.05 *

Table 4: Serum electrolyte analysis of *E. coli* O157:H7infected pups and Healthy pups while various sign of it showed, P<0.01** Significant, P<0.05* Significant

Parameters	Control (n=21) Healthy Pups	Diarrheic (n= 21) *E. coli* O157:H7Infected	Statistical analysis (T-test)
Sodium mEq/l	131.7±6.17	128.21±5.07	p<0.01**
Potassium mEq/l	5.1±0.57	3.11±0.44	P<0.05 *
Chloride mEq/l	100.7±1.28	87.6±2.27	p<0.01**
Bicarbonate mmol/l	20.2±2.23	27.35±1.54	p<0.01**

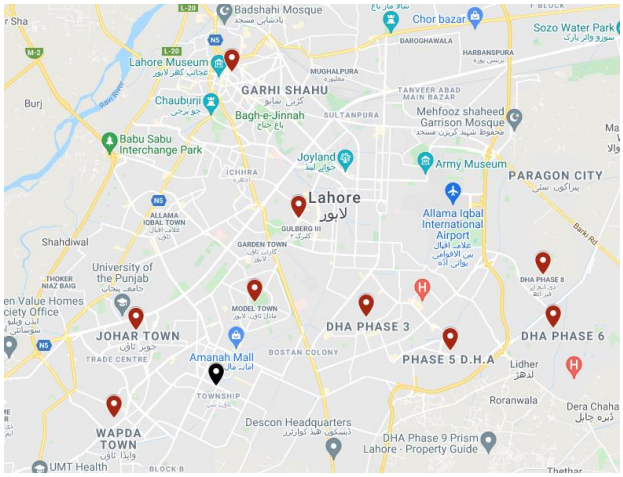

Fig. 3: Map of epidemiological study area.

The serum electrolytes' analysis revealed a significant decrease in sodium mEq/l (P<0.01) and potassium mEq/l (P<0.05), whereas an increase (P<0.01) in chloride mEq/l and bicarbonate mmol/l in infected puppies compared with control.

DISCUSSION

In the present study, the molecular prevalence of *E. coli* O157:H7, its associated risk factors and the impact of bacterial diarrhea on various blood parameters and serum electrolytes were evaluated. The samples were preliminarily screened for *E. coli* and the bacteriological culturing based on eosin methylene blue (EMB) agar revealed metallic sheen colonies similar to the findings of (Abdulrazzaq *et al.*, 2021). The metallic sheen color of the colonies might be due to the change in pH, which was acidic pH resulting in specific color for *E. coli* diagnosis. After the culturing of pathogenic *E. coli* from EMB agar on CT-SMAC, the smooth colorless colonies of *E. coli* O157:H7 were observed which were compatible with the finding of Garcia *et al.* (2010). The plausible reason might be the fermentation of lactose by *E. coli* O157:H7 without affecting sorbitol, producing colorless colonies.

The current study showed the overall prevalence of *E. coli* O157:H7 in canine diarrheic pups 3.5%. In previous reports, 0.16% and 1.1 % prevalence of this bacteria was observed in dogs (Bantancor *et al.*, 2007; Kataoka *et al.*, 2010). The extremely low rates in said studies might be due to difference in techniques used for detection of *E. coli* O157:H7 in dogs. On the other hand, the prevalence of *E. coli* O157:H7 in canine diarrheic puppies had also been reported as 15.8% (Ojo*et al.*, 2014), 72.72 % (Hassan *et al.*, 2016), 22.64 % (Yousif *et al.*, 2016), and 9.7% (Abdulrazzaq *et al.*, 2021) possibly due to higher exposure to the organism (Hassan *et al.*, 2016).

In this study the area wise prevalence of *E. coli* O157:H7 in canine diarrheic puppies was studied in the district Lahore and observed a significantly (P<0.05) higher prevalence of *E. coli* O157:H7 in canine pups from UVAS pet center higher prevalence might be due to the fact that it is a teaching hospital where people bring their dogs from variety of nearby and far areas including villages where people mostly keep non-descriptive breeds, practices less hygienic measures and they only bring their dogs at UVAS pet center when they are sick. In contrast, in other study areas of the district Lahore, mostly people keep exotic breeds, stay indoors, provide commercial feeding and take good care of their pets which resulted in lesser cases in those areas. Gebremedhin *et al.* (2021) reported that leftover feed, dead poultry, and other animals might serve as a vehicle for *E. coli* transmission to dogs.

Our results showed the highest prevalence in non-descriptive breeds of puppies as compared to other exotic breeds. Previous reports also reported higher prevalence of *E. coli* O157:H7 infection in the non-descriptive breed as compared to the Exotic breed (Yousif *et al.*, 2016). In the present study, the *E. coli* O157:H7 was more prevalent in pups of 1 month of age than other ages which was in accordance with the findings reported by Yousif *et al.*, 2016 and (OJo *et al.*, 2014). Day *et al.* (2007) stated that the higher rate of infection in canine puppies in their early life is due to the immuno-incompetence or lack of adequate maternal antibodies in the early aged puppies. Furthermore, our results showed the higher prevalence of

E. coli O157:H7 in female puppies as compared to males. The similar finding had been documented by Yousif *et al.* (2016), reporting the highest prevalence of *E. coli* O157:H7 infection (20.63 %) in female dogs as compared to male (12.20 %) dogs. The most plausible reason might be the less immune stability of female puppies compared to males. Moreover, the canine pups with vomiting conditions showed the highest prevalence of *E. coli* O157:H7 as compared to dogs without vomiting signs. These results are in agreement with Wang *et al.* (2006) who demonstrated that dogs with *E. coli* infection had higher vomiting conditions.

The number of cases of *E. coli* O157:H7 was noticed more prevalent in canine puppies with bloody diarrhoea as compared to puppies without bloody diarrhoea. Our findings are supported by Tortora *et al.* (2013) who stated that *E. coli* O157:H7 is one of the major causes of bloody diarrhoea due to its growth in the intestine. Moreover, Hassan *et al.* (2020) also observed that after inoculation of *E. coli* 0157:H7in puppies, just after 24 hrs all puppies showed diarrhoea. Additionally, canine puppies cohabitated with other animals exhibited the maximum (5.33%) number of cases of *E. coli* O157:H7 as compared to canine puppies that don't have any cohabitation (1.66 %). The possible reason may be the transmission of *E. coli* O157:H7 infection from other animals to canine puppies. Kataoka *et al.* (2010) reported similar results regarding transmission of *E. coli* O157:H7 from humans to animals. Further, the canine puppies having a history of feeding raw meat or offal had more chances (6.5 %) of *E. coli* O157:H7 infection as compared to feeding on home-cooked food (3.5%) and (0.5 %) processed food. Our findings are in close agreement with the survey of the Netherland conducted by (Bree *et al.,* 2018) who isolated *E. coli* O157:H7 pathogen around 20 % from raw diet samples.

The summer season showed the highest prevalence of *E. coli* O157:H7 than winter, spring and autumn. Our results are supported by Sanchez *et al.* (2002) who established that warm weather (Summer months) was positively correlated with *E. coli* O157:H7 in fecal shedding. Moreover, Luna *et al.* (2018) reported that cow manure was a potential source of *E. coli* O157:H7 infection. The finding of this study revealed that the canine pups having a history of direct contact with cattle and buffalo faeces were more susceptible to *E. coli* O157:H7 infection as compared to pups who did not have any history of contact with animal faeces.

The present study ascertained the highest prevalence in dirty water 5.66% as compared to clean water 1.33%, and our result is in-line with the finding of Nillian *et al.* (2019) who found 6.25% of *E. coli* O157:H7 in drinks collected from different poor hygienic localities, and also with Suardana *et al.* (2017) who concluded that use of non-tap water as a drinking source increased the risk for *E. coli* O157:H7 infection as many as 2.44 times. Taulo *et al.* (2008) conducted a study in Malawi to explore the prevalence of *E. coli* O157:H7 in stored water in Lungwena households and found 6.7% samples positive for *E. coli* O157:H7.

The hematological analysis of *E. coli* O157:H7 infected canine diarrheic puppies revealed that the mean values of Hb, RBCs, lymphocytes and platelets were reduced significantly in an infected group when compared with the control. Conversely, neutrophils, PCV and TLC were increased significantly in the infected group as compared to the control group. A decrease in Hb value and an increase in PCV and TLC values of our study are in accordance with the findings of Khanduri *et al.* (2021) who had reported a similar pattern in dogs suffering from vomiting conditions. Additionally, a significant decrease in Hb and TEC value may also indicate anemia and dehydration in canine puppies due to the damaged capillaries of the villi of the intestine leading to the loss of blood and iron deficiency (Sulthana *et al.,* 2015). We found a significant decrease in platelet values in infected animals than control group similar to the findings of Sharma *et al.* (2008) who stated that the loss of blood with faeces ultimately results in a decrease in platelets. In the current study, a significant increase in neutrophils, and decrease in lymphocytes might be due to the general reaction of the immune system to bacterial infection and inflammatory process in GIT (Berghoff *et al.,* 2011). Moreover, the mean value of TLC was significantly increased in our study similar to findings of Bhat *et al.* (2013), which might be due to hemorrhagic gastroenteritis.

In this study, the serum electrolytes' analysis revealed a significant decrease in K+ (hypokalemia) and Na+ (hyponatremia) level in an infected group when compared to the control. Our findings are in agreement with that of Schoeman *et al.* (2013). This might be due to anorexia, vomiting and diarrhoea contributing depression and weakness. An increase in the bicarbonate level might be due to hypokalemia in which intracellular shift in hydrogen ions can occur causing an imbalance in the buffer system leading to a relative increase in bicarbonate (Stimson and Reynolds, 2018). Comparable findings were also documented by Emejuo *et al.* (2020) who noticed hypokalemia, hyponatremia and an increase in serum bicarbonate level in (100, 75 and 100 %) in bloody diarrheic animals. We observed a significant decrease in chlorine (hypochloremia), which has been implicated with anorexia, gastrointestinal losses through vomiting, diarrhoea, and dehydration (Tello *et al.,* 2017).

Conclusions: We documented the prevalence (3.5% 21/600) of *E. coli* O157:H7 in canine puppies of district Lahore, Pakistan. This present study showed that canine puppies of district Lahore should be considered reservoirs of *E. coli* O157:H7 serotypes. Various risk factors such as breed, sex, co-habitation with other animals, unhygienic food and water, summer season and contact with cattle faeces were associated with the prevalence of *E. coli* O157:H7 in canine puppies. *E. coli* O157:H7 had an impact on hemato-serum electrolyte values of infected pups. In future, further investigations are required in different animal species to establish the appropriate methods of control.

Acknowledgments: This research was funded by Higher Education Commission (HEC) of Pakistan under National Research Programme for Universities (NRPU) project # (9719/NRPU/R& D/HEC). This project was under principal investigator Retired-Associate professor Dr. Syed Saleem Ahmad.

Authors contribution: Conceptualization, AY, SS; methodology, AY, MA; formal analysis, AY, SS, MA, and KA; writing original draft preparation, AY, SS; writing, review and editing, AY, SS and MA.

REFERENCES

Abdulrazzaq KM, Owain MS, Majeed HM, *et al.,* 2021. Molecular detection of rfbO157, shiga toxins and hemolysin genes for Escherichia coli O157: H7 from canine feces in Tikrit and Mosul cities, Iraq. Iraqi J Vet Sci 5:325-9.

Bhattacharjee K, Sarmah PC, *et al.,* 2013. Prevalence of haemoparasites in pet, working and stray dogs of Assam and North-East India: A hospital based study. Vet World 6:874-8.

Bentancor A, Rumi MV, Gentilini MV, *et al.,* 2007. Shiga toxin producing and attaching and effacing E. coli in cats and dogs in a high hemolytic uremic syndrome incidence region in Argentina. FEMS Microbiol Lett 267:251-6.

Berghoff N and Steiner JM, 2011. Laboratory tests for the diagnosis and management of chronic canine and feline enteropathies. Vet Clin North Am Small Anim Pract 41:311-28.

Bree FP, Bokken GC, Mineur R, *et al.,* 2018. Zoonotic bacteria and parasites found in raw meat-based diets for cats and dogs. Vet Rec 182:50-50.

Croxen MA, Law RJ, Scholz R, *et al.,* 2013. Recent advances in understanding enteric pathogenic Escherichia coli. Clin Microbiol Rev 26:822–80.

Day MJ, 2007. Immunoglobulin G subclass distribution in canine leishmaniosis: a review and analysis of pitfalls in interpretation. Vet Parasite 147:2-8.

Emejuo NT, Agina OA, Udeani IJ, *et al.,* 2020. Electrolytes, serum biochemical and acid-base disturbances in dogs with foul-smelling bloody diarrhoea. Alex J Vet Sci 66:1.

Gannon VP, D'Souza S, Graham T, *et al.,* 1997. Use of the flagellar H7 gene as a target in multiplex PCR assays and improved specificity in identification of enterohemorrhagic Escherichia coli strains. J Clin Microbiol 35:656-62.

García A, Fox JG and Besser TE, 2010. Zoonotic enterohemorrhagic Escherichia coli: a One Health perspective. Institute Lab Anim Resource 51:221-32.

Gebremedhin EZ, Merga D, Sarba EJ, *et al.,* 2021. Prevalence, risk factors and antibiogram of Escherichia coli isolated from dogs in Ambo, Gojo and Bako towns of Oromia region, Ethiopia. Ethiop Vet J 25:1-22.

Hasan MS, Yousif AA and Alwan MJ, 2016. Detection of virulent genes in E. coli O157: H7 isolated from puppies and adult dogs by polymerase chain reaction. Res J Vet Pract 4:1-6.

Hasan MS, Yousif AA and Alwan MJ, 2020.Macroscopical and Microscopical Changes of Body Organs after E. coli O157: H7 Inoculation in Puppies. Al-Anbar J Vet Sci13:1.

Kataoka Y, Irie Y, Sawada T and Nakazawa M, 2010. A 3-year epidemiological surveillance of Escherichia coli O157:H7 in dogs and cats in Japan J Vet Med Sci 1001220138-1001220138.

Khanduri R, Raval SK, Rao N, *et al.,* 2021. Haemato-Biochemical Alterations in Dogs with Vomition. Indian J Vet Sci Biotech 17:52-5.

Kiranmayi CB, Krishnaiah N and Mallika EN, 2010. Escherichia coli O157: H7-An emerging pathogen in foods of animal origin. Vet World 3:382.

Kwon T and Cho SH, 2015. Draft genome sequence of Enterohemorrhagic Escherichia coli O157 NCCP15739, isolated in the Republic of Korea. Genome Announc 3:15.

Luna S, Krishnasamy V, Saw L, *et al.,* 2018. Outbreak of E. coli O157: H7 infections associated with exposure to animal manure in a rural community-Arizona and Utah, June–July 2017. Morb Mortal Wkly Rep 67:659.

Nillian E, Nur A, Nur D, Zakirah A, and Bebey G, 2019. Prevalence of Escherichia coli O157: H7 in Drink from Different Food Premises in Kota Samarahan Sarawak. Trends Undergrad Res 2:13-9.

Ojo O, Bello A, Amosun E, *et al.,* 2014. Multidrug resistant verocytotoxin-producing Escherichia coli O157: H7 in the faeces of diarrhoeic and non-diarrhoeic dogs in Abeokuta, Nigeria. Vet Arh 84:63-73.

Paton AW and Paton JC, 1998. Detection and characterization of Shiga toxigenic Escherichia coli by using multiplex PCR assays for stx 1, stx 2, eaeA, enterohemorrhagic E. coli hlyA, rfb O111 and rfb O157. J Clin Microbiol 36:598-602.

Samad A, Abbas F, Ahmad Z, 2018. Prevalence of foodborne pathogens in food items in Quetta, Pakistan. Pak J Zool 50:1-4.

Sanchez S, Stevenson MA, Hudson CR, *et al.,* 2002. Characterization of multidrug-resistant Escherichia coli isolates associated with nosocomial infections in dogs. J Clin Microbiol 40:3586-95.

Schoeman JP, Goddard A, and Leisewitz AL, 2013. Biomarkers in canine parvovirus enteritis. New Zeal Vet J 61:217-22.

Shah SSA, Khan MI, Rafiullah KM, *et al.,* 2017.Tick-borne diseases-possible threat to humans-dog interspecies bond. Adv Anim Vet Sci 5:115-20.

Sharma V, Jakhar KK and Dahiya S, 2016. Immuno-pathological studies on broiler chicken experimentally infected with Escherichia coli and supplemented with neem (Azadirachta indica) leaf extract. Vet World 9:735.

Stimson L and Reynolds T, 2018. Differential diagnosis for chronic hypokalaemia. Case Reports, 2018-2017.

Suardana IW, Widiasih DA, Nugroho WS, *et al.*, 2017. Frequency and risk-factors analysis of Escherichia coli O157: H7 in Bali-cattle. Acta trop 172:223-228.

Sulthana CM, 2015. Clinico-pathological findings in dogs affected with canine parvo virus (CPV) infections. Intas Polivet 16:443-4.

Taulo S, Wetlesen A, Abrahamsen R, Mkakosya R and Kululanga G, 2008. Microbiological quality of water, associated management practices and risks at source, transport and storage points in a rural community of Lungwena, Malawi. Afr J Microbiol Res 7:131-7.

Tello L and Freytes R, 2017. Fluid and electrolyte therapy during vomiting and diarrhoea. The Veterinary Clinics of North America. J Small Anim Pract 47:505.

Wang JY, Wang SS and Yin PZ, 2006. Haemolytic–uraemic syndrome caused by a non-O157: H7 Escherichia coli strain in experimentally inoculated dogs. J Med Microbiol 55:23-9.

Yousif AA, Hasan MS and Alwan MJ, 2016. Clinical and molecular study of E. coli O157: H7 isolated from Diarrheic and non-diarrheic dogs. Mir Res Vet Sc Anim 5:1-10.