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Some Bacteriological studies on enteritis in pet animals

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Abstract
A total of 60 fecal swabs were collected from diarrheic cats and dogs coming to the clinic of Veterinary Medicine Faculty in Cairo University and examined bacteriologically to determine the bacterial causes of diarrhea. The samples were examined for the presence of pathogenic *E.coli*, *Salmonella* and other members of family *enterobacteriaceae* as well as enterotoxin of *C.perfringens* and *Campylobacter jejuni*. The incidence of each isolate was determined. *C.perfringens* enterotoxin production was detected using vero cell assay. Moreover, gens of some virulence factors in 7 *E.coli* isolates were also detected as an evidence for the severity of the disease. *C.perfringens* and *E.coli* were the most predominant isolates in an incidence of 51.4% in cats and 76% in dogs and 67.9% in cats and 56% in dogs respectively. However, *Campylobacter* could not be detected. Antimicrobial sensitivity test was made against the most predominant isolates using 12 different antibiotics to determine the most effective one to be recommended for treatment.

Key words: *enteritis, pet animals, virulence genes, enterotoxin.*

Introduction

Chronic intestinal diseases are common in small animals and can present a considerable challenge to the clinician. The main problem is that clinical signs-which may include vomiting, diarrhea and weight loss, are shared by many conditions that have either a primary or secondary effect on the gastrointestinal tract. A less obvious, but significant problem, is that intestinal disease may be completely overlooked in an animal with weight loss and no obvious vomiting or diarrhea in the history (**Batt, 2009).**

Many primary bacterial enteropathogens have been implicated as a cause of gastrointestinal disease in dogs and cats and particular attention has been focused on *Salmonella* spp. and *Campylobacter* spp. which can be readily identified by fecal culture. Detection of enterotoxins has been used to identify potentially enteropathogenic strains of Clostridia (**Marks *et al.,* 2002)**, but their significance particularly in chronic intestinal disease is not clear. Canine *C.perfringens* –associated diarrhea has been attributed to *C.perfringens* enterotoxin (CPE),which has been shown to induce fluid accumulation and diarrhea when administered orally or directly into intestinal lumen. (**Bartlett *et al.,* 1972).** Although several studies have shown an association between the immunodetection of CPE in fecal specimens and canine diarrhea, the pathogenesis of *C.perfringens* associated diarrhea in dogs and cats is not fully understood , because CPE also is detected in up to 14% of non-diarrheic dogs **(Marks *et al.,* 2002).**

The high prevalence of *C.perfringens* in healthy animals is a major limitation of fecal PCR assays, particularly those assays that target the ά- toxin gene that is of questionable virulence and that is present in all *C.perfringens* strains. Assays targeting other genes of potentially greater virulence e.g. (cpe,cbp2) might be useful. Analysis of fecal CPE with isolation and PCR detection of enterotoxigenic *C.perfringens* after a heat shock

treatment were performed in 32 diarrheic and non-diarrheic dogs (**Marks *et al.,* 2002**)

However, there is evidence that enteropathic *E.coli* should be considered as strains carrying pathogenic genes have been identified in dogs and cats by use of molecular biology techniques **(Beutin, 1999; Goffaux *et al.,* 1999** and **Sancak *at al.,* 2004).**

Escherichia coli is a predominant component of the intestinal microbiota of human and other mammals. Some *E.coli* srains represent primary pathogens having an enhanced potential to cause diseases, especially diarrhea (**Nataro and Kaper, 1998**).

Shiga toxin-producing *E.coli* (STEC) of the serogroup 0157, designated as enterohemorrhagic *E.coli* (EHEC), has been considered to be responsible for many outbreaks of hemorrhagic colitis and hemolytic uremic syndrome. Two types of Shiga toxins, stx1 and stx2, are known and constitute factors in STEC strains (**Paton and Paton, 1998).** Attaching and effacing *E.coli* (AEEC) are a group of *E.coli* strains that are able to colonize the intestinal mucisa of humans and animals with a characteristic histopathological lesion known as attaching and effacing (A/E). A\E lesions are initiated by the intimate attachment of bacteria to enterocytes, mediated by intimin, an adhesion encoded by the eae gene (**Nataro and Kaper, 1998)**. Detection of this gene has been taken as an indication of the presence of the A\E pathogenicity factor in a given bacterial strain.

The aim of our study is to detect the bacterial causes of diarrhea in dogs and cats which could be a source of infection to in contact human.

Material and Methods

Samples.

A total of 60 fecal swabs were collected from diarrheic cats and dogs (35 cats and 25 dogs) coming to the clinic of Veterinary Medicine Faculty, Cairo University from the period of January 2013 to July 2013.

Isolation and characterization of the isolates. (Quinn, 1994)

The fecal swabes were inoculated into cooked meat and thioglycolate broths and onto McConkey agar for detection of *C.perfringens*, *Campylobacter jejuni* and members of family enterobacteriaceae respectively. Cooked meat broth tubes were incubated anaerobically at 37 ˚C for 24 hours and then transferred to Neomycin blood agar, while thioglycolate broth tubes were incubated in microaerophilic conditions using campylobacter kits (oxoid) at 42˚C for 24 hours then transferred onto campylobacter agar media. McConkey agar plates were incubated aerobically at 37˚C for 24 hours.

Identification of *C. perfringens*.

Colonies of double zone of hemolysis were picked up to be identified microscopically and typed serologically using neutralization test **(Stern and Batty, 1975)**.

Detection of *C.perfringens* type A enterotoxin using cytotoxicity test on Vero cells:

Sporulation medium was prepared according to **Duncan and Strong (1968).** Sporulation was initiated by inoculating 0.2 ml of over night cultures into 10 ml of Duncan and Strong (DS) media and incubated anaerobically at 37 ˚C for 8 hrs. The presence of sporulating cells in DS cultures was confirmed by microscopic examination. The cultures were then sonicated by sonic treatment (6 Hertz for 20 minutes using sonicator ultra-sonic) until more than 95% of the spores were free as determined by microscopic examination. The cultures were centrifuged at 1500 xg for 25 min at 4 ˚C and cytotoxicity of the supernatant was tested with a Vero cell assay according to **Sandvig and Olsnes (1982).**

The assay monitors the inhibition of protein synthesis in Vero cells after addition of toxic proteins, including CPE. The Vero cells were grown in a minimal essential medium supplemented with 10% fetal calf serum. The supernatants were precipitated in 80% saturated ammonium sulfate and kept at 4˚C before the Vero cell assay. The strain was defined as producing CPE when the inhibition of protein synthesis of Vero cell was more than 20%.

Identification of members of family enterobacteriaceae.

Lactose fermenting and non-fermenting colo-

nies were picked up onto blood agar plates and incubated at 42˚C onto nutrient agar sloap for biochemical examination (Indol, Methyl red, Voges Proskaeur and Citrate utilization). The isolates were further characterized by carbohydrate fermentation, urea hydrolysis, production of H2S on TSI according to **Edwards and Ewing (1972).**

Serotyping of *E.coli.*

Seven biochemically identified *E.coli* isolates were kindly serotyped at Serology unit in Animal Health Research Institute using specific polyvalent and monovalent antisera (**Seiken product, lot 350046, Japan Denka, Seiken co. LTD.)** The isolates were serologically investigated by slide agglutination technique.

Detection of different viulence genes in isolated *E.coli* strains by PCR (Cubbon *et al*., 1996)

DNA extraction. DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer. Primers used were supplied from **Metabion (Germany)** are listed in table (1).

PCR amplification. Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix **(Takara, Japan)**, 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in a T3 Biometra thermal cycler.

Analysis of the PCR Products.

The products of PCR were separated by electrophoresis on 1-1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. A 100 bp DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table (1). Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary den.	Sec. den.	Ann.	Ext.	Final ext.	Reference
stx1	ACACTGGATGATCTCAGTGG	614	94˚C 5 min.	94˚C 1 min.	58˚C 1 min.	72˚C 1 min.	72˚C 10 min.	Dipineto *et al.* (2006)
	CTGAATCCCCCTCCATTATG							
stx2	CCATGACAACGGACAGCAGTT	779						
	CCTGTCAACTGAGCAGCACTTTG							
hlyA	ACGATGTGGTTTATTCTGGA	165		94˚C 30 sec.	58˚C 30 sec.	72˚C 30 sec.		
	CTTCACGTGACCATACATAT							
eaeA	GACCCGGCACAAGCATAAGC	384		94˚C 45 sec.	54˚C 45 sec.	72˚C 45 sec.		Wen-Jie *et al*. (2008)
	CCACCTGCAGCAACAAGAGG							
CNF1	TATATAGTCGTCAAGATGGA	628		94˚C 1 min.	50˚C 1 min.	72˚C 1 min.		Kadhum *et al.* (2008)
	CACTAAGCTTTACAATATTGAC							
STa	GAAACAACATGACGGGAGGT	229		94˚C 30 sec.	57˚C 30 sec.	72˚C 30 sec.		Lee *et al.* (2008)
	GCACAGGCAGGATTACAACA							
LT	GGTTTCTGCGTTAGGTGGAA	605		94˚C 1 min.	57˚C 1 min.	72˚C 1 min.		
	GGGACTTCGACCTGAAATGT							

Results

Sixty fecal samples from diarrheic cats and dogs were bacteriologically examined for detection of bacterial causes of enteritis in these animals that may be a source of infections to humans. *C.perfringens* is one of the most common wide world pathogen causing enteritis in humans and animals. Table (2) shows the incidence of *C.perfringens* isolates in diarrheic cats and dogs. Eighteen *C.perfringens* isolates were recovered from 35 diarrheic cats in an incidence of 51.4% from which eight isolate were type A (44.4%) and 10 were non-toxigenic (55.6%). On the other hand, out of 25 dogs, 19 *C.perfringens* isolates were recovered (76%): 10 were type A (52%), 1 was type C (5.2%) and 8 were non-toxigenic (42.1%).

Table (2). Incidence of *C.perfringens* in diarrheic dogs and cats.

C.perfringens isolates / No. of Diarrheic animals	No. of *C.perfringens* isolates		Type A		Type C		Non toxic	
	No.	%	No.	%	No.	%	No.	%
35 cats	18	51.4	8	44.4	----------		10	55.6
25 dogs	19	76	10	52	1	5.2	8	42.1

Clostridium perfringens enterotoxin (CPE) is an important virulence factor for both *C.perfringens* type A food poisoning and several non-food-born gastroenteritis. Enterotoxin producing *C.perfringens* isolates were detected and tabulated in table (3). Out of 37 *C.perfringens* isolates from diarrheic cats and dogs, only 10 isolates were found to produce enterotoxin (27%): 4 isolates from cats (22.2%) and 6 from dogs (31.6%).

Table (3). Incidence of enterotoxigenic *C.perfringens* type A in studied cats and dogs.

No. of *C.perfringens* isolates	Enterotoxin products					
	Type A		CPE production			
			+ve		-ve	
	No	%	No	%	No.	%
Cats (18)	8	44.4*	4	22.2*	4	22.2*
Dog (19)	10	52.6*	6	31.6*	4	21.1*
37	18	48.6*	10	27*	8	21.6*

*According to number of *C. perferingens* isolates.

Vero cell assay is rapid technique that measures the biological activity of *C.perfringens* enterotoxin. This method involves the rapid killing of vero cells by enterotoxin produced by *C.perfringens* grown in Duncan and Strong sporulation medium. The cytotoxicity of Vero cell revealed that 10 out of 18 *C.perfringens* isolates succeded in causing ≥ 20% inhibition of protein synthesis suggesting CPE production. Photo (1) shows the cytopathic effect of enterotoxin on Vero cells where detachment of the cells is clear comparing with the normal Vero cell sheet (photo 2).

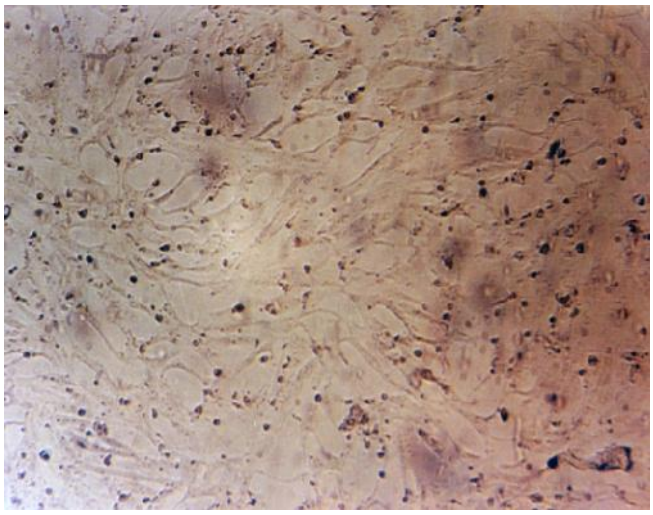

Photo (1). Shows the cytopathic effect of enterotoxin on vero cells.

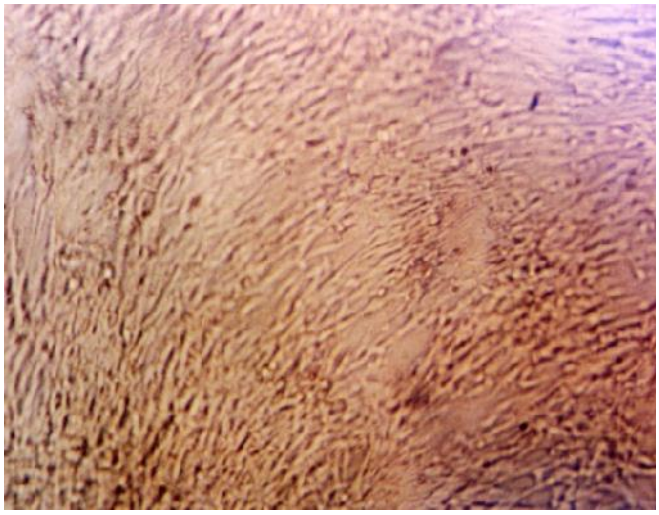

Photo (2). Shows the normal vero cells sheet.

Escherichia.coli and other members of family *enterobacteriaceae* were incriminated in several cases of enteritis in pets. Table (4) shows the prevalence of some enteric bacteria belonging to family *enterobacteriaceae* in diarrheic cats and dogs. *E. coli* was recovered from cats and dogs in an incidence of 67.9%and 56% respectively, while 2.8% of diarrheic cats and 20% of dogs shed *Proteus vulgaris.* Moreover, *Proteus mirabilis* was detected in diarrheic cats and dogs in an incidence of 8% and 2.8% respectively. Only one strain of *Klebsiella pneumoniea* and one strain of *Yersenia spp.* were recovered from two diarrheic cats (2.86%)for each. Finally, One *Enterobacter* strain was recovered from a dog (4%). *Salmonella* as well as *Campylobacter* species could not be detected.

Table (4). Prevalence of some enteric bacteria belonging to family enterobacteriaceae in diarrheic cats and dogs.

No. of animals	*E.coli*		*Salmonella spp.*		*Proteuse*				*Klebsialla pneumoniea*		*Ensterobacter spp.*		*Shigella spp.*		*Yersenia Spp.*	
					P.vulgaris		P.*mirabilis*									
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
35 cats	22	67.9	—	–	1	2.86	1	2.86	1	2.86	—	—	1	2.86	1	2.86
25 dogs	14	56	_	_	5	20	2	8	—	_	1	4	—	—	—	—
Total 60	36	60	—	_ _	6	10	3	5	1	1.7	1	1.7	1	1.7	1	1.7

Seven *E.coli* isolates were serologically typed using specific antisera and the results are shown in table (5).

Table (5). Serological typing of 7 *E.coli* isolate.

Isolate number	Polyvalent	Monovalent
1	7	O136
2	6	O115
3	7	O136
4	6	O 8
5	1	O127a
6	1	O119
7	2	O55

The virulence of *E.coli* depends on the presence of different virulence attributes that enable them to cause disease. In this study, seven serologically identified *E.coli* isolates were examined for the presence of seven virulence factors genes that may explain the severity of the disease. It was found that all isolates encoded attaching and effacing gene (eaeA), while, 5 *E.coli* isolates had cytotoxic necrotizing factor (CNF1) gene and only one isolate carried heat stable enterotoxin (STa) gene. However, none of the *E.coli* isolates had shiga toxins 1 or 2 or heat labile (LT) or hemolysin (hylA) genes.(table 6 and photos 3 through 9).

Table (6). Prevalence of virulence factors genes in 7 *E.coli* isolates.

Sample	Gene						
	eaeA	CNF1	STa	STX1	STX2	LT	hlyA
1	+	-	+	-	-	-	-
2	+	-	-	-	-	-	-
3	+	+	-	-	-	-	-
4	+	+	-	-	-	-	-
5	+	+	-	-	-	-	-
6	+	+	-	-	-	-	-
7	+	+	-	-	-	-	-

Photoes (3-9). Show detection of different virulence genes in 7 *E.coli* isolates using PCR.

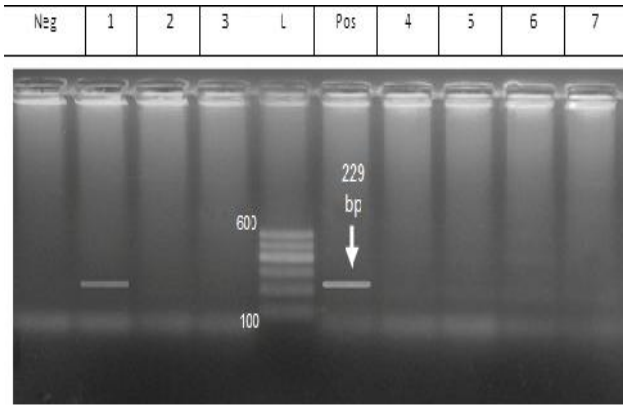

Photo (3). Detection of eaeA gene of *E.coli* in seven isolates at 384 bp .All tested isolates were positive.

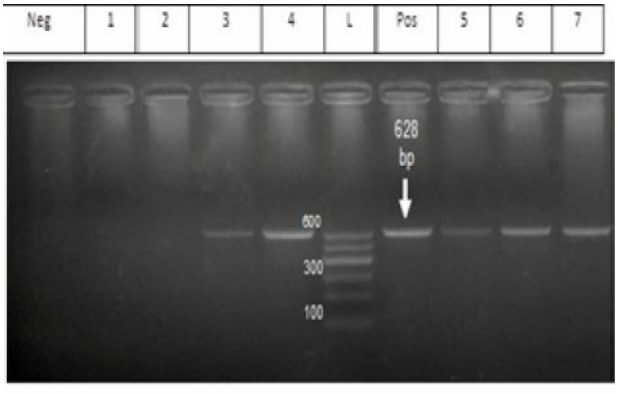

Photo (4). Sensitivity of PCR in detecting CNF1 gene at 628 bp. Lanes no.3,4,5,6 and 7 were positive.

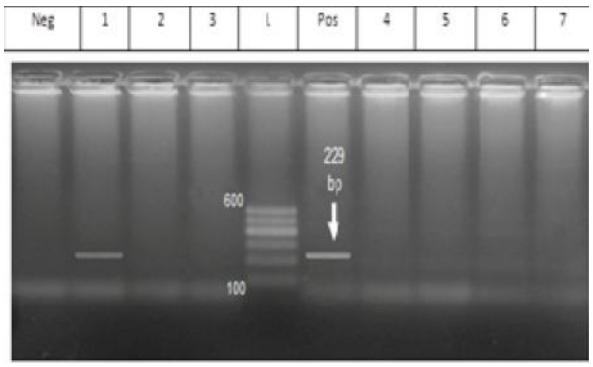

Photo (5). Shows the detection of heat-stable (Sta) gene at 229 bp by PCR where only one isolate was positive.

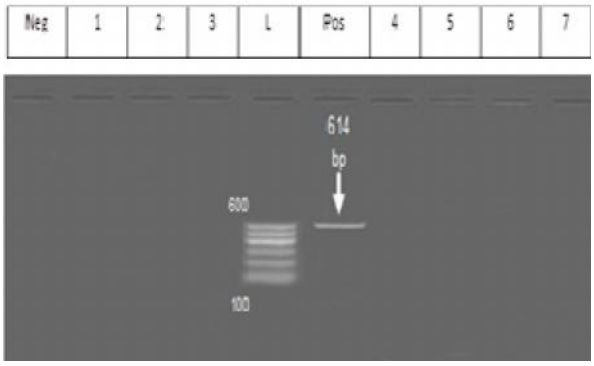

Photo (6). Shows that none of tested *E.coli* isolates have the shiga toxin (stx1) gene.

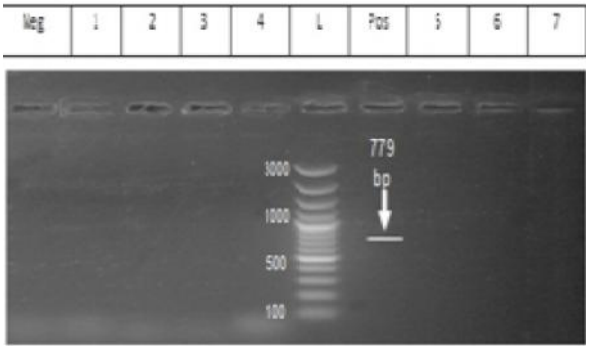

Photo (7). Shows that none of the E.coli isolates has stx2 gene.

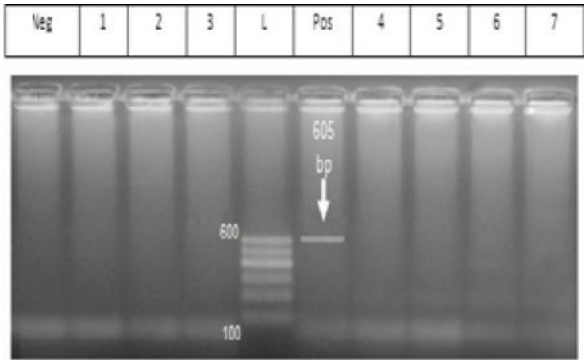

Photo (8). Shows that none of the tested isolates contain the heat-labile (LT) toxin gene.

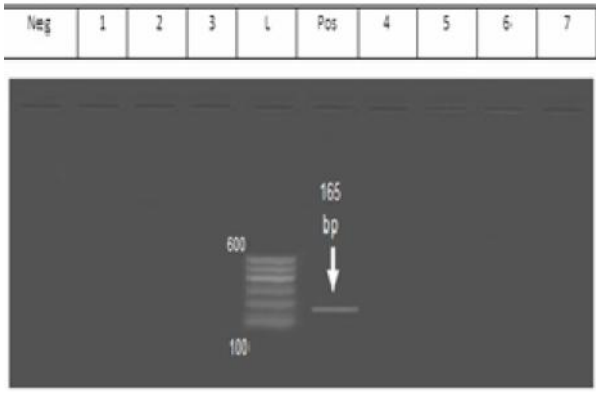

Photo (9). shows that none of tested E.coli isolates has hly.A toxin gene

Sensitivity test was made for the most predominant isolates(*C.perfringens* and *E.coli*) against 12 antibiotics to determine the most effective one for treatment. It was found that Amoxicillin and Ampiclox were the most effective antibiotics for *C.perfringens,* while, Cefoperazone and Cefepime were the most effective antibiotics for *E.coli* as shown in tables (7&8).

Table (7). Antimicrobial sensitivity result against *C.perfringens* isolates (CLSI, 2012).

Antibiotics	Code	Inhibition zone (mm)	Reference inhibition zone
Amoxycillin	AML 25	30	-
Cefoperazone	CFP 75	18	-
Vancomycin	VA 30	20	-
Oxytetracycin	OT 30	0	-
Amikacin	AK 30 c	0	-
Cefotaxime	CTX 30	0	-
Cefepime	FEP 30	0	-
Ampiclox	AX 30	30	-
Enrofloxacin	ENR 5	15	-
Ampicillin	AMP 10	0	-
Ciprofloxacin	CIP 5	0	-
Cefadroxil	CDX 30c	0	-

Table (8). Antimicrobial sensitivity result against *E.coli* isolates (CLSI, 2012).

Antibiotics	Code	Inhibition zone (mm)	Reference inhibition zone
Amoxycillin	AML 25	20	≥18
Cefoperazone	CFP 75	30	≥21
Vancomycin	VA 30	0	≥12
Oxytetracycin	OT 30	23	≥15
Amikacin	AK 30 c	25	≥17
Cefotaxime S	CTX 30	27	≥26
Cefepime	FEP 30	30	≥18
Ampiclox	AX 30	18	-
Enrofloxacin	ENR 5	23	-
Ampicillin	AMP 10	0	≥17
Ciprofloxacin	CIP 5	28	≥21
Cefadroxil	CDX 30c	12	-

S= sensitive, I= intermediate, R= resistant

Discussion

Dogs and cats belong to those species of animals which have been living in a close community with man for many thousands of years. As a consequence, contacts between humans, dogs and cats are numerous and possibility of transmission of microorganisms between these different host species is extremely high. In this study, sixty fecal samples from dogs and cats suffering from diarrhea were bacteriologically examined to determine the presumptive causes of diarrhea. Particular attention has been focused on *C.perfringens* and *E.coli.* Detection of enterotoxins has been used to identify potentially enteropathognic strains of clostridia. However, there is evidence that enteropathic *E.coli* - as strains carrying pathogenic genes- have been identified in dogs and cats by use of molecular biology techniques.

C.perfringens is a Gram-positive anaerobic spore-forming bacilli. It is one of the most wide-spread pathogenic bacteria, and inhabits the gastrointestinal tract of humans and animals. The organism is divided into 5 biotypes, A to E, based on the possession of 1 or more of 4 major toxin genes: alpha, beta, epsilon and iota. Each biotype also may express a subset of at least 10 other established toxins, including *C.perfringens* enterotoxin (CPE). Although all 5 biotypes can harbor the enterotoxin gene (cpe), the global distribution of enterotoxigenic strains is relatively low (~5%), and the majority of strains belong to type A. **Kokai-Kun *et al.,* (1994)** and **Songer and Meer (1996)** .

Limited information is available about the incidence of *C.perfringens*-associated diarrhea in dogs and cats, given the challenges of proving cause and effect after detection of CPE in diarrheic animals. **Weese *et al.,* (2001) and Marks *et al.,* (2002).**

In this study, out of 35 diarrheic cats, 18 *C.perfringens* isolates (51.4%) were recovered 8 of which were type A (44.4%) and 10 were non-toxigenic (55.6%),while 19 *C.perfringens* isolates were estimated from 25 diarrheic dogs (76%):10 of which were type A ,1 was type C and 8 were non-toxigenic (42.1%). The prevalence of *C.perfringens*-associated diarrhea in cats is much lower than in dogs (**Marks *et al.,* 2011).** Our findings agreed with **Marks *et al.,* 2011** as shown in table (2).

Canine *C. perfringens*-associated diarrhea has been attributed to *C. perfringens* enterotoxin, which has been shown to induce fluid accumulation and diarrhea when administered orally or directly into the intestinal lumen (**Bartlett *et al.,*1972).** The role of CPE in the development of diarrhea is unclear because CPE is detected in up to 34%of diarrheic dogs, and in 5-14% of nondiarrheic dogs (**Weese *et al.,* 2001** and **Marks *et al.,* 2002).** There appears to be an association between the detection of CPE in dogs with acute hemorrhagic diarrheal syndrome (AHDS), because CPE was detected in 8/12 dogs (67%) with AHDS. **Cave *et al.,* (2002).**

Four out of 18 *C.perfringens* isolates from diarrheic cats were shown to secrete enterotoxin (22.2%) ,while, 31.6% of *C.perfringens* isolates recovered from diarrheic dogs have CPE toxin. In CPE-associated diseases, the isolate usually represents type A, perhaps because isolates of type A account for more than 95% of global *C. perfringens* isolates **McClane, (2001).** CPE biosynthesis is associated temporally with sporulation and its synthesis begins after the induction of sporulation and increases progressively for at least 6-8 hours, thus sporulation in vitro is essential to measure the production of CPE from isolates.

Vero cell assay is rapid technique that measures the biological activity of *C.perfringens* enterotoxin. This method involves the rapid killing of vero cells by enterotoxin produced by *C.perfringens* grown in Duncan and Strong sporulation medium (**Mahony *et al.,*1989).** In this study, successful sporulation was achieved with 18 *C. perfringens* type A isolates on Ducan and Strong medium. The cytotoxicity of Vero cell revealed that 10 out of 18 *C.perfringens* isolates succeded in causing ≥ 20% inhibition of protein synthesis suggesting CPE production.

Escherichia coli is a predominant component

of the intestinal microbiota of humans and other mammals but can be associated with gastroenteritis in the presence of bacterial virulence factors and impaired local or systemic immunity **Marks *et al.*, (2011).** *E.coli* that cause diarrhea have specific characteristics called virulence attributes that enable them to cause disease. Therefore, the presence of the genes encoding for virulence attribute can be used to distinguish these pathogen from the nonpathogenic or commensal *E.coli* normally carried in the intestine **Leyla and Kadri, (2007).** The objective of this study was to determine the prevalence of enterotoxigenic *E. coli* (ETEC), Shiga toxin- producing *E. coli* (STEC), cytotoxic necrotizing factor type1 (CNF1) producing *E.coli* and attaching and effacing *E.coli* (AEEC) contribute to disease among dogs and cats suffering from diarrhea. Samples were cultured onto McConkey agar plates and identified biochemically using IMVC test, TSI and urea and serologically using specific poly and monovalent antisera.

It was found that *E.coli* was the most predominant isolate recovered from diarrheic cats and dogs in a total incidence of 60% but isolation in cats was more than that in dogs. However, *proteuse* species were common in dogs more than in cats. *Salmonella* and *Campylobacter* species play an important role in enteritis in dogs and cats **Marks *et al.*, (2011)** however, none of them could be detected in this study.

Attaching and effacing *E.coli* (AEEC) are characterized as leading to diarrhea due to their ability to cause A\E lesions in the gut mucosa mediated by intimin, an adhesion encoded by the eae gene **Nataro and Kaper, (1998).** Production of CNF1 was shown to be closely associated with alpha-hemolysin production and with certain serotypes of *E. coli* strains isolated from dogs and cats **Blanco *et al.*, (1993)** and **Cherifi *et al.*, (1991).**

Most of the enterotoxigenic *E.coli* ETEC had genes for more than one type of enterotoxin, with heat stable (STb) and heat labile(LT) being the most prevalent. ETEC were isolated from diarrheic dogs and were characterized by a number of methods including bioassays, such as the infant mouse assay and the ligated ileal loop assay, by serological tests and by DNA based detection methods **Beutin, (1999*)***.

In our study, polymerase chain reaction (PCR) assay was made to determine if the tested isolates carried genes for: Attaching and effacing activity (eae), cytotoxic necrotizing factor (CNF), *E.coli* enterotoxin of heat stable (STa) or heat labile (LT), Shiga toxin 1 and 2, and hemolysin toxin (hlyA). All tested *E.coli* isolates were found to encode eae gene responsible for colonization in the intestinal mucosa of the hosts causing characteristic histopathological lesions.

Moreover, cytotoxic necrotizing factor gene, causing intra and extra-intestinal lesions, was detected in 5 *E.coli* isolates, while heat- stable toxin gene was found in only one isolate. On the other hand, none of the tested *E. coli* isolates gave positive results for shiga toxins 1and 2, LT and hlyA toxin genes.

Further detection should be directed towards detecting and characterizing diarrheagenic *E.coli* types in dogs and cats, their host specificity and the possible exchange of pathogenic strains between animals and humans.

It has been shown that antimicrobials present in animals destined for alimentary purposes may contribute to selective antimicrobial resistance, posing risks to humans due to the transmission of resistant zoonotic bacteria via the food chain **Wegener *et al.*, (1999).** Such resistant bacteria could be acquired by humans via alternative pathways including person-to- person transmission, environmental and\or direct exposure to animals. Dogs and cats represent potential sources for the spreading of antimicrobial resistance in view of the extensive use of antimicrobial agents in these animals, and their close contact with human beings **Guardabassi *et al., (*2004).** This study also aimed to study the antimicrobial susceptibility to 12 antimicrobial agents against *C.perfringens* and *E.coli* isolates to make a recommendation for proper treatment of clinical cases. According to the Clinical Laboratory Standard Institute **(CLSI),** *C.perfringens* has not standard reference zones of inhibition with disc diffusion test

however, as its recommendation, penicillins group may be effective.

Conclusion

As pets can be natural reservoirs of several organisms potentially able to cause disease to humans who, in turn, may also be carriers of counless infectious agents specific for animals, care must be taken during manipulation of such animals and strict hygiene should be applied for cleaning, feeding, sanitary removal of wastes as well as following up the programs of vaccinations. All dogs and cats with idiopathic diarrhea or diagnosis of infections with any of bacteria described in this assay should be considered potentially contagious. Basic practices such as isolation, use of appropriate personal protective equipment, and proper cleaning and disinfection practices are the main control measures. Hand washing is preferred over alcohol-based hand sanitizers because spores of *C.perfringens* and also *C.difficile* are alcohol resistant. If affected animals need to be walked, they should be walkrd in an area where other patients are not walked and where feces can be promptly removed. Litter boxe should be cleaned and disinfected regularly. Gloves should be worn when handling litter boxes and hands washed after glove removal. Veterinarians should be cognizant of the fact that self-limiting diarrhea, and the injudicious administration of antimicrobials could be more harmful than beneficial. Supportive therapy and appropriate hygiene control should be considered in all animals with suspected or confirmed bacterial-associated diarrhea (with the exception of *E.coli* associated with granulomatous colitis in which antimicrobial therapy is warranted), and antimicrobials should only be administered to animals manifesting systemic signs of illness.

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