Bacteria Causing Canine and Feline Hemorrhagic Gastroenteritis and Histopathological Studies in Experimentally Infected Dogs and Cats with *Salmonella* and *Escherichia coli* Strains

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Abstract:

Hemorrhagic gastroenteritis is a potentially fatal disease especially in untreated animals. Total samples of 202 rectal swabs collected from dogs and cats were subjected to bacteriological examination. One hundred four bacterial isolates were identified from the total 202 examined samples. The identified bacterial isolates were *E. coli* (46; 44.23%), *Proteus* species (22; 21.15%), *Klebsiella* species (9; 8.65%), *C. perfringens* (5; 4.80%), *Enterobacter* species (4; 3.84%), *Pseudomonas aeruginosa* (4; 3.84%), *Salmonella* species (3; 2.88%), *Shigella* species (3; 2.88%); *Citrobacter* species (2; 1.92%), *Providencia rettgeri* (2; 1.92%), *Serratia liquefaciens* (1; 0.96%), *Hafnia* species (1; 0.96%), *C. bifermentans* (1; 0.96%), *C. putrefaciens* (1; 0.96%). Serological identification for some *E. coli* isolates revealed that EHEC strains represent (12/26, 46.15%), EPEC (9/26, 34.61%), ETEC (4/26, 15.38%) and EIEC (1/26, 3.85%). Serotyping of *Salmonella* isolates detected *S*. Typhymurium, *S*. Heidelberg and *S*. Infantis. In case of canine *E. coli* isolates, resistance was recorded against amoxicillin/clavulanic acid, cephalexin, ceftriaxone, tetracycline, erythromycin and trimethoprim/sulphamethoxazole. Moderate resistance was recorded among feline *E. coli* isolates to

amoxicillin/clavulanic acid, tetracycline and trimethoprim/ sulphamethoxazole. *Salmonella* isolates were highly resistant to amoxicillin/clavulanic acid, cephalexin, erythromycin, tetracycline and trimethoprim/sulphamethoxazole. In experimentally infected puppies with *S*. Typhymurium, histopathological examination showed necrosis in the tips of the villi and leukocytic infiltration in the submucosa of the jejunum, degeneration and fibrosis of the liver. In experimental salmonellosis in cats, histopathological examination showed coagulative necrosis in the tips of the villi of the jejunum and multifocal necrosis in the liver. In experimental *E. coli* infection in cats, histopathological examination showed ulceration and necrosis of the small intestine. The liver showed congestion of the blood vessels and fibrosis around the hepatic areas.

Keywords: Hemorrhagic Gastroenteritis, histopathological studies, experimental infection, *Salmonella*, *Escherichia coli*, dogs and cats.

Introduction

Hemorrhagic Gastroenteritis (HGE) is a potentially life-threatening disease of dogs, characterized by sudden onset of bloody, watery diarrhea and vomiting ***(Unterer et al., 2011)***. If it is left untreated, the dog can be affected by shock then die. Understanding the enteritis pathophysiology is limited, because the microflora of the intestine in dogs and cats is complicated and incompletely understood organisms ***(Weese, 2011).*** Many bacteria causing HGE have a potential hazard to public health. Pets and domestic animals constitute natural reservoir for Attaching and effacing *E. coli* (AEEC) strains ***(Krause et al., 2005).*** Dogs and horses act as potential reservoir for EHEC O157: H7 in human ***(Trevena et al., 1996).*** Canine *Salmonella* shedding represents a potential public health hazard ***(Leahy et al., 2016).*** Dogs and cats represent potential source for the antimicrobial resistance spread due to their close contact with human and the massive use of antimicrobial drugs in dogs and cats' treatment ***(Weese, 2011).*** The community-related *Enterobacteriaceae* have extensive resistance attributed to the extended spectrum β-lactamases ***(Pitout, 2013).*** The study aimed to isolate bacteria causing HGE in dogs and cats, serological studies for some bacterial isolates, antimicrobial sensitivity test for some *E. coli*

and *Salmonella*, *P. mirabilis* and *K. pneumoniae* isolates, experimental study of salmonellosis in dogs and cats, experimental study of *E. coli* infection in cats, and histopathological studies for some experimentally infected cases.

Materials and methods

1. Samples

Two hundred and two rectal swabs were collected from housed dogs and cats (143 of dogs and 59 of cats) that suffered from bloody diarrhea and suspected to have hemorrhagic gastroenteritis. Samples were collected from private pet clinics and Governmental Pet Animals Units in Damietta and Dakahlia Governorates, Egypt during the period from February 2017 till April 2020, then subjected to bacteriological examination.

2. Bacteriological examination

2.1. Isolation and identification of *Enterobacteriaceae*

For *E. coli* and *Klebsiella* isolation, inoculation of fecal swabs was made into buffered peptone water (Lab M), incubation at 37°C for 24 h, then streaking of inoculum was made onto the surface of MacConkey's agar (Oxoid) and incubation at 37°C for 24 h. The colonies suspected to be lactose fermenter were streaked onto EMB (eosin methylene blue) agar plates (Hi-Media). For *Salmonella* isolation, pre-enrichment of samples was made by inoculation into Rappaport-Vassiliadis broth (Lab M), incubation at 41.5°C for 24 h, then plating on XLD (Xylose Lysine Deoxycholate) agar (Hi-Media), and incubation at 37°C for 24 h. Identification of *Klebsiella* isolates was done morphologically on EMB agar. For *Klebsiella* isolates, detection of mucoviscosity was done according to ***Shon et al. (2013)*** by inoculation of a loopful taken from the suspected culture on nutrient agar (Lab M). Any produced viscous string longer than 5 mm was considered positive result and the isolate was identified as HVKP (hypermucoviscous *K. pneumoniae*), while negative result indicated CKP (classic *K. pneumoniae*). Identification of *E. coli*, *Klebsiella* and *Salmonella* isolates was made morphologically and microscopically according to ***Cruickshank et al. (1975),*** and biochemically according to ***Kreig and Holt (1984).***

For isolation and identification of *Proteus*, pre-enrichment of samples was made by inoculation into Rappaport-Vassiliadis broth (Lab M), incubation at 41.5°C for 24 h, then plating on XLD (Xylose Lysine Deoxycholate) agar (Hi-Media), and incubation at 37°C for 24 h. For isolation and

identification of *Shigella*, samples were enriched in Sodium bi-selenite broth (Hi-Media) according to ***Morris (1984)*** at 37°C for 24 h, then plating of isolates on XLD (Xylose Lysine Deoxycholate) agar (Hi-Media) at 37°C for 24 h. Streaking of inoculum was made on S-S agar (Lab M) for differentiation between *Salmonella* and *Shigella*. Identification of isolates was made morphologically and biochemically according to ***Kreig and Holt (1984).***

2.2. Isolation and identification of *Pseudomonas aeruginosa*

Inoculation of fecal swabs was made into buffered peptone water, incubation at 37°C for 24h, then streaking of inoculum on Cetrimide agar (Eur. Pharm). Biochemical Identification was made using biochemical tests according to ***Quinn et al. (2011) and Carter and Wise (2004).***

\2.3. Isolation and identification of *C. perfringens*

Enrichment was made by inoculation of samples in BHI (brain heart infusion) broth (Oxoid), then anaerobic incubation in an anaerobic jar at 37°C for 24 h. Enriched samples were streaked on SPS (sulphite polymixin sulphadiazine) agar plates (Hi-Media) and incubating anaerobically. Staining of suspected colonies was done with Gram's stain and subculturing on BHI (brain heart infusion) agar plates until obtaining pure culture. Biochemical tests were done as the methods defined by ***Merchant and Packer (1967), OIE (2000) and Calnek et al. (1997).*** The pure colonies suspected to be *C. perfringens* were streaked on 5% sheep blood agar (Hi-Media) and egg yolk agar (Hi-Media) plates and anaerobically incubated at 37°C for 24 hr. The colonies producing double zone of hemolysis on blood agar and forming opalescence zone around the colonies on egg yolk agar; were identified as *C. perfringens*.

3. Serological identification

3.1. *E. coli* and *Salmonella* isolates

Serological identification of *E. coli* isolates was done according to ***Kok et al. (1996)*** using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the Enteropathogenic types. Serological identification of *Salmonella* isolates was made using *Salmonella* antiserum (DENKA SEIKEN Co., Japan) according to Kauffman – White scheme ***(Kauffman, 1974)*** for the determination of (O) somatic and (H) flagellar antigens.

3.2. *Klebsiella* isolates

Quellung test "Neufeld reaction" was used for serological identification of capsular antigen

according to ***Edmondson and Cooke (1979).*** The used kit was purchased from **(Statens Serum Institute, Copenhagen, Denmark)**. Quellung test was carried out according to the producer instructions. The antigen-antibody reactions are observed microscopically. A positive quellung reaction is the result of the binding of the capsular polysaccharide with type specific antibody contained in the typing antiserum.

4. Antimicrobial sensitivity test

Some *E. coli* and *Salmonella* isolates were tested using 11 antimicrobial discs (Oxoid) involving amoxicillin/clavulanic acid (20/10µg), ampicillin-/sulbactam (10/10µg), cephalexin (30µg), ceftriaxone (30µg), cefotaxime (30µg), gentamicin (10µg), tetracycline (30µg), chloramphenicol (30µg), ciprofloxacin (5µg), trimethoprim/sulfamethoxazole (1.25/23.75µg) and erythromycin (15µg). Antimicrobial sensitivity test was done using disk diffusion method and interpretation of the results was done according to ***Clinical and Laboratory Standards Institute guidelines (2015).***

5. Experimental infection

5.1. Preparation of animals before the experiment:

Two kittens were used for experimental salmonellosis and two kittens were used as control. Two kittens were used for experimental *E. coli* infection and two kittens were used as control. Two puppies were used for experimental salmonellosis and two puppies were used as control. All animals were treated for external and internal parasites.

5.2. Experimental Salmonellosis in dogs and cats

It was made using the previously isolated and serologically identified *S.* Typhimurium (group B, (O) antigen: 1,4,5,12; (H) antigen: i: 1,2), and *S.* Heidelberg from dog during this study. Refreshment of the preserved strain was made by inoculating into BPW (Buffered Peptone Water), incubating at 37°C at 24 hr, then inoculating into Rappaport-Vassiliadis Broth and incubating at 41.5°C at 24 hr, and then streaking onto the Xylose Lysine Deoxycholate (XLD) agar and incubating at 37°C for 24 hr. Serial dilution of the bacteria was made to obtain $5x10^8$ CFU of *S.* Typhimurium. Oral administration of 20 ml of $5x10^8$ CFU of *S.* Typhimurium according to ***Stone et al. (1995)*** to the 2 puppies designated as CES1 and CES2 (Canine Experimental Salmonellosis 1 and 2), followed by 20 ml of $5x10^8$ CFU *S.* Heidelberg. Oral administration of 10 ml of $5x10^8$ CFU of *S.* Typhimurium only to the 2 kittens designated as FES1

and FES2 (Feline Experimental Salmonellosis 1 and 2) according to ***Timoney et al. (1978).***

5.3. Experimental *E. coli* infection in cats

It was made using the previously isolated and serologically identified *E. coli* (EPEC O55:H7) from dog during this study. Refreshment of the previously preserved strain was made by inoculating into BPW (Buffered Peptone Water), incubating at 37°C at 24 hr, then inoculating onto MacConkey's agar and incubating at 37°C for 24 hr. Serial dilution of the bacteria was made to obtain 10^8 CFU. Oral administration of 10 ml of 10^8 CFU of *E. coli* was made to the 2 kittens designated as FEEC1 and FEEC2 (Feline Experimental *E. coli* 1 and 2) according to ***Watson et al. (2019).***

5.4. Observation of animals during the experiment

Daily observation of vital signs for animals during the experiment.

5.5. Euthanasia at the end of the experiment

Euthanasia was made for FEEC1, FEEC2, CES1 and CES2. Sedation was made for cats with Xylazine hydrochloride and Atropine sulphate, followed by overdose of Ketamine Hydrochloride 5%. Sedation was made for dogs with Xylazine hydrochloride and Atropine sulphate, followed by Intra-cardiac injection of Deltamethrin.

5.6. Necropsy

All cases were subjected to necropsy immediately after death for naturally infected cases and the cases died from infection (FES1) and (FES2), and after euthanasia for other experimentally infected cases (FEEC1), (FEEC2), (CES1) and (CES2). All samples were taken under aseptic conditions using sterile instruments.

6. Histopathological examination

Specimens from small intestine, colon and liver were obtained from each animal and fixed in 10% neutral buffer formalin. Then sampled tissues were washed in water, embedded in paraffin ***(Luna, 1968),*** sectioned by a thickness of 5µm, then subjected to staining with hematoxylin and eosin. Microscopic examination was performed for each tissue ***(Watson et al., 2017).***

Results

The total identified bacterial isolates were 104 from the total 202 examined samples, as shown in Table (1). The identified isolates were *E. coli* (46; 44.23%), *P. vulgaris* (12; 11.53), *P. mirabilis* (10; 9.62%), *K. pneumoniae* (8; 7.69%), *K. oxytoca* (1; 0.96%), *C. perfringens* (5; 4.80%), *S. Typhimurium* (1; 0.96%), *S.*

Infantis (1; 0.96%), *S.* Heidelberg (1; 0.96%), *Citrobacter freundii* (1; 0.96%), *C. diversus* (1; 0.96%), *Serratia liquefaciens* (1; 0.96%), *S. dysenteriae* (2; 1.92%), *Shigella flexneri* (1; 0.96%), *Enterobacter* aerogenes (2; 1.92%), *Enterobacter cloacae* (2; 1.92%), *Providencia rettgeri* (2; 1.92%), *Hafnia* species (1; 0.96%), *Pseudomonas aeruginosa* (4; 3.84%), *C. bifermentans* (1; 0.96%) and *C. putrefaciens* (1; 0.96%). Twenty-six *E. coli* isolates were identified serologically, *E. coli* serotypes represents EHEC (12/26; 46.15 %), EPEC (9/26; 34.62%), ETEC (4/26; 15.38%) and EIEC (1/26; 3.85%), as shown in Table (4). Seven *K. pneumoniae* were identified serologically; involving 4 isolates carried K1 and 2 isolates carried K2, as shown in Table (3). By string test, 4 *K. pneumoniae* were identified as Hypermucoviscous *K. pneumoniae* (HVKP) and 3 isolates were identified as Classic *K. pneumoniae* (CKP). Antimicrobial sensitivity test revealed that canine *E. coli* isolates were resistant to Amoxicillin/Clavulanic acid (66.67%), Cephalexin (66.67%), Ceftriaxone (66.67%), Cefotaxime (33.33%), Trimethoprim/sulphamethoxazole (66.67%), Tetracycline (100%) and Erythromycin (100%), while feline *E. coli* isolates had moderate resistance (50%) to Amoxicillin/Clavulanic acid, Trimethoprim/sulphamethoxazole, and Tetracycline. *Salmonella* isolates from dogs were resistant to Amoxicillin/Clavulanic acid and Cephalexin by a percentage of 100%. *Salmonella* isolates from dogs were resistant to Trimethoprim/sulphamethoxazole, Tetracycline and Erythromycin by a percentage of 66.67%. *P. mirabilis* isolate was resistant to Amoxicillin/Clavulanic acid, Cephalexin, Cefotaxime, Erythromycin and Chloramphenicol. *K. pneumoniae* isolate was resistant to Erythromycin. These results were illustrated in Table (5).

Serological identification of *Salmonella*, *K. Pneumoniae* and *E. coli* isolates was illustrated in Table (2), (3) and (4), respectively. Necropsy of Experimental canine salmonellosis (CES1) showed enlarged cecum, severe congestion of intestinal blood vessels, hemorrhagic inflammation of intestine and several necrotic foci in the small intestine (jejunum). Histopathological examination of CES1 small intestine (jejunum) showed coagulative necrosis of the tips of villi along with sloughing of some epithelial cells in the lumen,

moderate focal leukocytic infiltration in the submucosa. CES1 colon showed mucinous degeneration and coagulative necrosis of the tips of villi. CES1 Liver showed diffuse vacuolar degeneration, mild congestion of central veins and portal blood vessels, and mild fibrosis. Results were illustrated in Figure (1).

Necropsy of Feline Experimental salmonellosis (FES1) revealed congestion of liver and intestinal blood vessels, hemorrhagic enteritis of intestine, enlarged mesenteric lymph nodes and pericardial edema. Histopathological examination of FES1 small intestine (jejunum) showed coagulative necrosis of the tips of villi. FES1 colon showed degeneration, necrosis of the tips of villi along with massive leukocytic infiltrations. FES1 liver showing multifocal necrosis, congestion of blood vessels and hyperplasia of bile ducts, as shown in Figure (2).

Necropsy of Feline Experimental *E. coli* infection (FEEC1) revealed congestion of intestinal blood vessels and megacolon. Histopathological examination of FEEC1 small intestine showed ulceration and discontinuation of intestinal mucosa with degeneration and necrosis. FEEC1 colon showed congested blood vessels and vacuolation of epithelial cells lining the villi. FEEC1 liver showed congestion of blood vessels and fibrosis around the hepatic areas, as shown in Figure (3).

Table (1): *Number and percentage of bacterial isolates* from the examined dogs and cats:

Bacterial Isolate	Total Number	Percentage (%)	Animal species	
			Cats	Dogs
E. coli	46	44.23	18	28
S. Typhimurium	1	0.96	0	1
S. Infantis	1	0.96	0	1
S. Heidelberg	1	0.96	0	1
Proteus mirabilis	10	9.62	2	8
Proteus vulgaris	12	11.53	2	10
Klebsiella pneumoniae	8	7.69	2	6
Klebsiella oxytoca	1	0.96	0	1
Shigella dysenteriae	2	1.92	2	0
Shigella flexneri	1	0.96	0	1
Citrobacter freundii	1	0.96	0	1
Citrobacter diversus	1	0.96	0	1
Serratia liquefaciens	1	0.96	0	1
Enterobacter cloacae	2	1.92	0	2
Enterobacter aerogenes	2	1.92	1	1
Providencia rettgeri	2	1.92	0	2
Hafnia species	1	0.96	1	0
C. perfringens	5	4.80	0	5
C. putrefaciens	1	0.96	0	1
C. bifermentans	1	0.96	0	1
Pseudomonas aeruginosa	4	3.84	2	2
Total Number of isolates	104	51.48%	30	74

Table (2): *Salmonella serotypes from the examined dogs and cats:*

Serial No.	Key No.	Identified strain	Group	Antigenic structure	
				O	H
1	D24	*Salmonella* Typhimurium	B	1,4,5,12	i: 1,2
2	D25	*Salmonella* Infantis	C1	6,7,14	r: 1,5
3	D35	*Salmonella* Heidelberg	B	1,4,5,12	r: 1,2

Table (3): *Serotypes of K. pneumoniae:*

Serial No	Key No	Identified bacterium	Biotyping	String test	Serodiagnosis
1	C46	*Klebsiella pneumoniae*	B1	HVKP	K1
2	C59	*Klebsiella pneumoniae*	B1	CKP	Untypable
3	D90	*Klebsiella pneumoniae*	B1	HVKP	K1
4	D103	*Klebsiella pneumoniae*	B1	HVKP	K2
5	D122	*Klebsiella pneumoniae*	B4	CKP	K1
6	D125	*Klebsiella pneumoniae*	B1	HVKP	K1
7	D133	*Klebsiella pneumoniae*	B3	CKP	K2

CKP:** Classic *Klebsiella pneumoniae*** **HVKP:** Hypermucoviscous *K. pneumoniae*

Table (4): *The identified E. coli serotypes from the examined dogs and cats:*

Type	EHEC		EPEC		ETEC		EIEC	
Prevalence	NO %		NO	%	NO %		NO %	
	12/26 46.15		9/26 34.62		4/26 15.38		1/26 3.85	
Serotypes	O6:H11 (3 isolates) O91:H21 (6 isolates) O26:H11 (2		O159:H4 (1 isolate) O6 (3 isolates) O45:H2 (2 isolates) O2:H7 (1 isolate) O55:H7 (1		O128:H2 (3 isolates) O127:H6 (1 isolate)		O159 (1 isolate)	
Species	Dogs	Cats	Dogs	Cats	Dogs	Cats	Dogs	Cats
NO	9	3	7	2	1	3	0	1
%	75	25	77.78	22.22	25	75	0	100

Table (5): *Prevalence of some resistant bacterial isolates from the examined dogs and cats:*

Species / Antimicrobial	Resistant bacterial isolates number (%)						
	E. coli (n=5)		*Salmonella* isolates (n=3)			*Proteus mirabilis* (n=1)	*Klebsiella pneumoniae* (n=1)
	Dogs (n=3)	Cats (n=2)	S. Typhimurium (n=1)	S. Infantis (n=1)	S. Heidelberg (n=1)		
Amoxicillin/ Clavulanic acid	2(66.67)	1(50)	3(100)			1(100)	0
Ampicillin/ Sulbactam	0	0	0			0	0
Cephalexin	2(66.6)	0	3(100)			1(100)	0
Ceftriaxone	2(66.6)	0	0			0	0
Cefotaxime	1(33.3)	0	0			1(100)	0
Ciprofloxacin	0	0	0			0	0
Gentamicin	0	0	0			0	0
Trimethoprim/ Sulfamethoxaze	2(66.6)	1(50)	2(66.67)			0	0
Tetracycline	3(100)	1(50)	2(66.67)			0	0
Erythromycin	3(100)	0	2(66.67)			1(100)	1(100)
Chloramphenid	0	0	0			1(100)	0

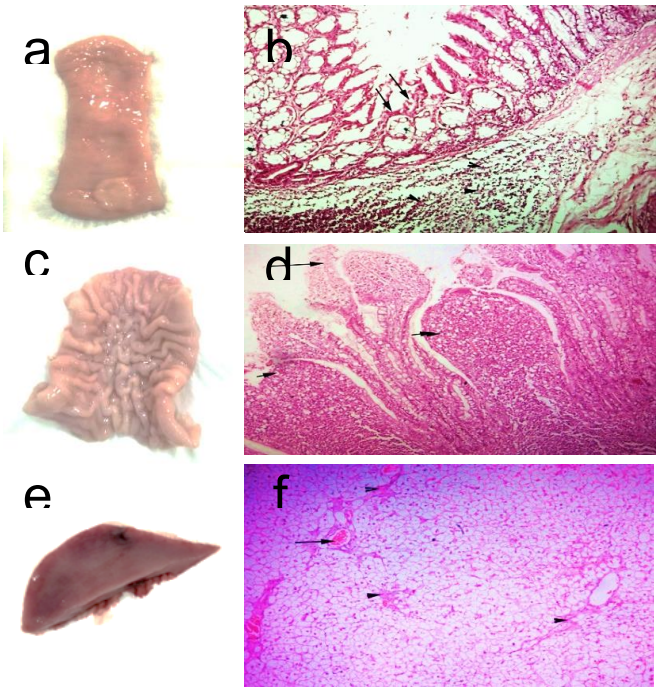

Figure (1): *Macroscopic and microscopic picture of Canine Experimental Salmonellosis 1 (CES1)*

(a) Jejunum: showed necrotic foci in jejunum **(c) Colon:** severe inflammation **(e) Liver:** showed congestion
(b) CES1 jejunum: showing coagulative necrosis of the tips of villi (arrows) along with sloughing of some epithelial cells in the lumen, moderate focal leukocytic infiltration in the submucosa (arrow heads). H&E, X 200.
(d) CES1 colon: showing mucinous degeneration and coagulative necrosis of the tips of villi (arrows) along with sloughing of some epithelial cells in the lumen. H&E, X 200.
(f) CES1 liver: showing diffuse vacuolar degeneration, mild congestion of central veins and portal blood vessels (arrows), and mild fibrosis (arrow heads). H&E, X 200.

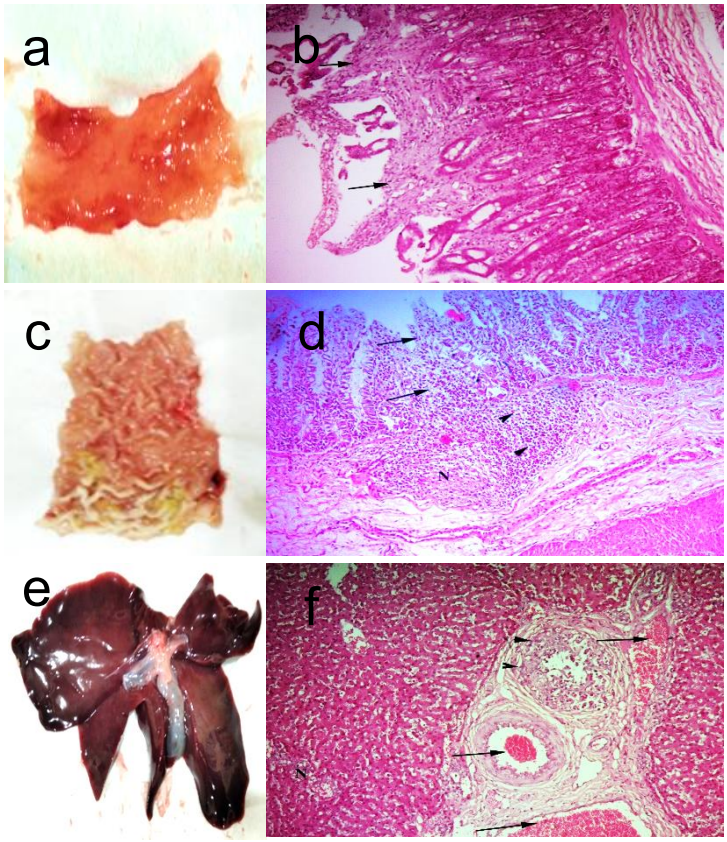

Figure (2): *Macroscopic and microscopic picture of Feline Experimental Salmonellosis 1 (FES1)*
(a) Jejunum: showing hemorrhagic inflammation **(c) Colon:** showing hemorrhagic inflammation **(e) Liver and gall bladder:** showing congestion of liver

(b) FES1 jejunum: showing coagulative necrosis of the tips of villi (arrows) along with sloughing of some epithelial cells in the lumen. H&E, X 200.
(d) FES1 colon: showing degeneration (arrows), necrosis (N) of the tips of villi along with massive leukocytic infiltrations (arrow heads). H&E, X 200.
(f) FES1 liver: showing multifocal necrosis (N), congestion of blood vessels (arrows) and hyperplasia of bile ducts (arrow heads). H&E, X 200.

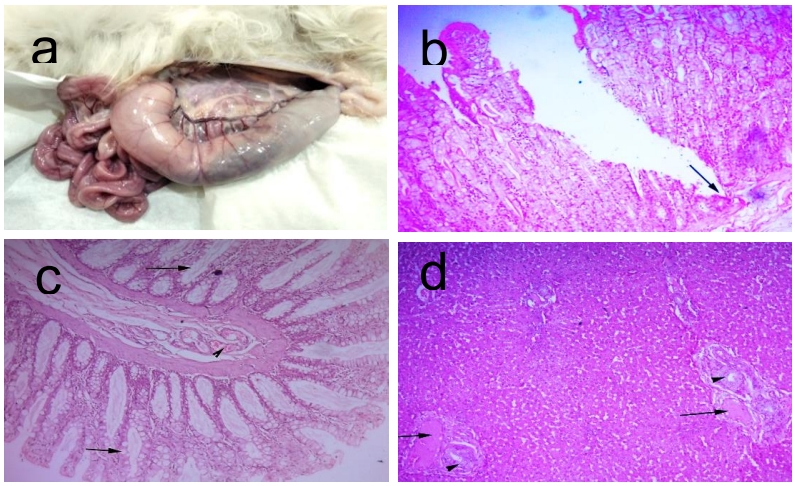

Figure (3): *Macroscopic and microscopic picture of Feline Experimental E. coli infection 1 (FEEC1)*
(a) Intestine of FEEC1: showing congestion of intestinal blood vessels and megacolon.
(b) FEEC1 jejunum: showing ulceration and discontinuation of intestinal mucosa (arrow) along with degeneration and necrosis. H&E, X 200.
(c) FEEC1 colon: showing congested blood vessels (arrow head), vacuolation of epithelial cells lining the villi (arrows). H&E, X 200.
(d) FEEC1 liver: showing multiple congestion of blood vessels (arrows), hyperplasia of bile ducts (arrow heads) and fibrosis around the hepatic areas. H&E, X 200.

Discussion

Hemorrhagic gastroenteritis (HGE) is a disease typically affects small breed dogs, young to middle-age, although dogs of any breed or age can be affected. The disease characterized by a per acute onset of clinical signs that can develop rapidly to death without proper treatment ***(Trotman, 2014).*** At necropsy, the main intestinal lesions of

HGE in dogs were exhibited superficial hemorrhagic necrosis of the mucosa ***(Cave et al., 2002; and Unterer et al., 2014).*** The most common bacterial species isolated from dogs with Acute Hemorrhagic Diarrhea Syndrome (AHDS) was *E. coli* that is considered an enteropathogen. Gastrointestinal diseases in dogs specially in young puppies are attributed to *E. coli*, notably, enteropathogenic (EPEC), enterohemorrhagic (EHEC), and enterotoxigenic (ETEC) strains ***(Marks and Kather, 2003)***. In this study, the most prevalent isolate was *E. coli* (46; 44.23%). From the 26 isolates subjected to serological examination, the percentages of isolation of EHEC, EPEC and ETEC strains were 12/26 (46.15%), 9/26 (34.62%), and 4/26 (15.38%), respectively. The previous results were in agreement with that recorded by ***Marks and Kather (2003);*** who said that the three pathotypes enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), and enteropathogenic *E. coli* (EPEC) had been studied in dogs. In the present study, the three *Salmonella* isolates were serotyped as *Salmonella* Typhimurium, *S*. Heidelberg and *S*. Infantis, while in a study performed by ***Ojo and Adetosoye, (2009),*** all the *Salmonella* isolates were serotyped as *S*. Typhimurium. In this study, the prevalence of *K. pneumoniae* isolates was (8; 7.69%) and *K. oxytoca* (1; 0.96%). In a report documented by ***Roberts et al. (2000);*** a severe *K. pneumoniae* enteritis outbreak occurred in Bordeaux mastiffs, producing septicemia and death. The symptoms began with vomiting and diarrhea. Seven adult dogs had symptoms, and 4 died. Vomiting and bloody or watery diarrhea were present in all affected animals. Serological identification of 7 *K. pneumoniae* isolates showed that 4 isolates carried K2 and 2 isolates carried K2 indicating their virulence; these results were in agreement with that reported by ***Holt et al. (2015) and Effah et al. (2020);*** that the Hypermucoviscous *K. pneumoniae* (HMKP) or Hypervirulent *K. pneumoniae* (hyKp) is known to carry virulence factors including capsular types K1, K2 and K20. *E. coli* isolates from dogs were resistant to Amoxicillin/Clavulanic acid by a percentage of (66.67%), Cephalexin (66.67%), Ceftriaxone (66.67%), Trimethoprim/sulphamethoxazole (66.67%), Tetracycline (100%) and Erythromycin (100%). These results were not similar to that reported in a study performed by ***Habib et al. (2016);*** who recorded that *E. coli*

isolates had high to moderate resistance to tetracycline (54.33%) and ceftriaxone (44.88%). Similar *E. coli* resistance pattern has been reported previously ***(Minton et al., 1983).***

Also, feline *E. coli* isolates had moderate resistance to Amoxicillin/clavulanic acid (50%), trimethoprim/sulphametoxazole (50%), and tetracycline (50%). These results were similar to that reported by ***Habib et al. (2016).*** Also, similar pattern of *E. coli* resistance had been reported previously by ***(Minton et al., 1983). Monaghan et al. (1981);*** reported moderate to high level of antibiotic resistance to different antibiotics in *E. coli.* Also, ***Pedersen et al. (2007);*** reported high level of resistance to tetracycline and sulphonamides in *E. coli.*

In the current study, the antimicrobial sensitivity pattern of the three *Salmonella* isolates was determined. High susceptibility rate was demonstrated to Ampicillin/Sulbactam (100%), Ceftriaxone (100%), Cefotaxime (100%), Ciprofloxacin (100%), Gentamicin (100%) and Chloramphenicol (100%). This result was nearly similar to that reported by ***Ojo and Adetosoye, (2009);*** where they recorded a high susceptibility to Ciprofloxacin (100%) and Chloramphenicol (89.2%). Resistance was shown to Tetracycline (66%), Erythromycin (66%), Trimethoprim/sulphamethoxazole (66%), Cephalexin (100%) and Amoxicillin/Clavulanic acid (100%). ***Ojo and Adetosoye, (2009);*** reported that resistance was to Tetracycline (70.6%) and Amoxicillin (35.3%).

In the present study, in case of feline experimental salmonellosis, FES1 liver showing multifocal necrosis, congestion of blood vessels and hyperplasia of bile ducts, these results were similar to that reported by ***Stiver et al. (2003);*** who found that histopathological examination revealed necrotizing hepatitis with random, multifocal small areas of hepatic cells necrosis and associated neutrophilic and histiocytic inflammation. Also, it showed subacute to chronic enteritis with lymphoplasmacytic, histiocytic, and neutrophilic infiltrates in the lamina propria. Clinical Salmonellosis is rare in adult dogs despite presence of some serotypes in healthy animals, but the disease is more dangerous in young animals and animals exposed to stress condition ***(Kallo and Hasso, 2001).*** In the present study, this was very clear as cats and dogs infected by experimental salmonellosis showed signs of infection when

immunosuppression occurred as a result of fungal infection. In the present study, in case of canine experimental salmonellosis, CES1 liver showing diffuse vacuolar degeneration, these results were similar to that recorded in a study performed by ***Giuliano et al. (2015),*** in a dog that the histopathology of the liver showed acute hepatic necrosis. Identified areas of diffuse liver necrosis were defined, with only some normal hepatic cells exist in the portal areas. Inflammatory infiltrates of lymphocytes, neutrophils, and plasma cells were identified with areas of multifocal cholestasis. Areas of multifocal hemorrhage were also present. In the present study, the re-isolated strain from fecal sample of canine experimental infection was *S.* Typhimurium group B of serotype 1,4,5,12, i: 1,2. This result was in agreement with that documented in a study performed by ***Giuliano et al. (2015);*** where the microbiological examination detected the isolation of *Salmonella enterica* of group B from the liver, while in the present study, it was isolated from the intestinal tract. Bacteria were cultured by an enrichment technique. The result reported the presence of Typhimurium-like *S. enterica* of serotype I 4,5,12: -:1,2 **(*Giuliano et al., 2015).*** In the present study, in case of feline experimental *E. coli* infection, FEEC1 small intestine showed ulceration and discontinuation of intestinal mucosa along with degeneration and necrosis, these results were similar to that recorded in a study performed by ***Waston et al. (2017),*** where the results of histopathology of kittens, that died or euthanized due to diarrhea, revealed a significant relation between aEPEC isolates detection from kittens and lesions in the colon and small intestine. Lesions were characterized by the presence of injury in the small intestinal epithelium with presence of an inflammatory infiltrate in the small intestine and colon.

Conclusion

From the previous results of the study, it can be concluded that the prognosis of HGI is excellent with proper and rapid treatment. Clinical salmonellosis is rare in cats. *E. coli* isolates from the examined dogs and cats belonged to EHEC, ETEC, EPEC serotypes, and the most prevalent was EHEC serotypes. The MDR bacteria spread is a problem requires restriction. Histopathology of experimental salmonellosis in dogs and cats showed necrosis of the tips of the villi of the small intestine. Histopathology of experimental *E. coli* in cats showed ulceration

of intestinal mucosa with degeneration and necrosis.

Author contributions

MEE and ME designed the study. MEE, AW and MEA collected the samples, and applied bacteriological examinations. MEE, MEA and AW performed serological identification. MEE and MEA wrote the manuscript. MEE, ME, AW, WMH and AAD applied experimental and pathological studies. All authors have read and approved the final manuscript.

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البكتيريا المسببة لإلتهاب الأمعاء النزفى فى الكلاب والقطط ودراسات هيستوباثولوچية فى الكلاب والقطط المصابة بالسلمونيلا وإى كولاى التجريبية

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الملخص العربي

يُعد مرض مرض التهاب الأمعاء النزفي فى الكلاب والقطط مرض مهدد للحياة خاصة فى الحيوانات التى لم تخضع للعلاج. تم عمل الفحص البكتيريولوچى لعدد ٢٠٢ عينة عبارة عن مسحات شرجية تم أخذها من الكلاب والقطط المصابة. كان العدد الكلى للبكتيريا المعزولة ١٠٤، حيث تم عزل بكتيريا ايشريشيا كولاى ٤٦ (٤٤,٢٣%)، بروتياس ٢٢ (٢١,١٥%)، الكلبسيلا ٩ (٨,٦٥%)، كلوستريديوم بيرفرنجنز ٥ (٤,٨٠%)، سلمونيلا ٣ (٢,٨٨%)، شيجيلا ٣ (٢,٨٨%)، و سودوموناس اريچينوزا (زائفة زنجارية)(٣,٨٤%)، انتيروباكتر ٤ (٣,٨٤%)، بروفيدنسيا ريتجيري ٢(١,٩٢%)، سيتروباكتر ٢ (١,٩٢%)، هافنيا ١ (٠,٩٦%)، سيراتيا ١ (٠,٩٦%)، كلوستريديوم بيفيرمانتانز ١ (٠,٩٦%)،وكلوستريديوم بيوتريفاشينز ١ (٠,٩٦%). تم عمل اختبار الحساسية للمضادات الحيوية حيث كانت معزولات ايشيريشيا كولاى من الكلاب عالية المقاومة لكل من: أموكسيسيللين/كلاڤيولانيك، سيفاليكين، سيفترياكسون، تراى ميثوبريم/ سلفاميثوكسازول، تتراسيكللين، واريثرومايسين، بينما كانت معزولات اى كولاي من القطط متوسطة المقاومةلكلٍ من: أموكسيسيللين/كلاڤيولانيك، تراى ميثوبريم/ سلفاميثوكسازول، و تتراسيكللين. كانت معزولات السلمونيلا عالية المقاومة لكل من: أموكسيسيللين/كلاڤيولانيك، سيفاليكين، تراى ميثوبريم/ سلفاميثوكسازول، تتراسيكللين، واريثرومايسين. تم عمل عدوى تجريبية باستخدام بكتيريا السلمونيلا المعوية (تيفيموريوم) حيث أظهرت نتائج الهيستوباثولوچى موت فى أطراف الخملات وارتشاح كرات الدم البيضاء باللفائفي بالأمعاء الدقيقة، وتدمير وتليف خلايا الكبد. فى حالة عدوى السلمونيلا المعوية فى القطط، أظهرت نتائج الهيستوباثولوچى، موت تخثرى فى أطراف الخملات فى الأمعاء الدقيقة (اللفائفي)، وموت فى خلايا الكبد. تم عمل عدوى تجريبية باستخدام بكتيريا إى كولاي فى القطط، حيث أظهرت نتائج الهيستوباثولوچى تقرح وموت فى خلايا الأمعاء الدقيقة، واحتقان الأوعية الدموية مع تليف فى بعض أجزاء الكبد.

الكلمات المفتاحية: (التهاب الأمعاء النزفي- دراسات هيستوباثولوچية- عدوى تجريبية- سلمونيلا- إى كولاى- الكلاب والقطط).