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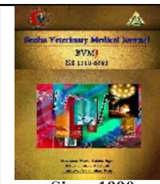

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Original Paper

Molecular characterization of *salmonella* species and *E. coli isolated from dogs and cats*

Reham Mekky[1], Abdel-Moneim Moustafa[2], Rania Abo-Sakaya[2]*, Khairy Abu-Zaid[3]

[1]*Department of Pet Animals, Awsim Veterinary Hospital, Giza*

[2]*Department of Animal Medicine, Faculty of Veterinary Medicine, Benha University, Egypt*

[3]*Department of Bacteriology, Animal Health Research Institute Dokki branch*

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ABSTRACT

In this study we aimed to investigate *salmonella* and *E. coli* serovars from fecal swabs collected from apparently healthy and diarrheic dogs and cats by bacteriological examination. 150 fecal samples of dogs and cats were examined for *salmonella* and *E. coli* species. *Salmonella* species were isolated from 40 cases out of 150, 30 of the *salmonella* positive samples were from dogs (31.6%), and 10 were from cats (18.2%) while 45 samples were positive for *E. coli*, 25 from dogs (26.4 %) and 20 from cats (36.4 %). *E. coli* and *salmonella* positive samples were subjected to antimicrobial disc diffusion susceptibility test by using 10 different antibiotic discs. Molecular investigation was done to detect the virulent gene of *salmonell*a (*inv* A) and *E.* coli (*eae* A) and antibiotic resistant gene for *both salmonella* and *E. coli* (*bla*TEM) using PCR.

1. INTRODUCTION

E. coli is the most popular gram-negative bacteria isolated and identified in clinical microbiology labs. (Trepeta and Edberg 1984). PCR is used to detect the virulence markers associated with E*nteropathogenic* and *Enterotoxogenic E. coli;* both VT2e gene and *eae*A gene are the most common virulence genes of *E. coli* in dogs and cats. The *eae*A gene of *Enter pathogenic E. coli* (EPEC) is necessary for intimate attachment to epithelial cells. Also, detection of *Escherichia coli* resistance gene; *bla*TEM gene was carried out (Coque*et al* 2002).

Pets are important reservoirs of antibiotic resistant bacteria (Guardabassi*et al.*, 2004). Rapid, cost-effective, real-time PCR methods is available, but few clinical diagnostic laboratories have fully embraced this technology and there have been no multicenter validations for the use of PCR to detect *Salmonella* in infecting animals (Ward *et al* 2005). Antibiotic resistance may be naturally occurring or acquired. Natural or intrinsic antibiotic resistance is due to internal structural or physiological nature of microbes. It is chromosome encoded and non-transferable. It is plasmid or chromosome encoded and transferable to other bacteria (Davies and Davies 2010). Antibiotic resistance increases the cost of treatment and causes mortality and morbidity. Due to the close contact of pet animals and owners, pets are alarming reservoir of antibiotic resistance. Antibiotic resistant bacteria- of zoonotic importance pose substantial threat to public health as well (Damborg*et al.*, 2015). For detection of *Salmonella* a harmonized method is used with several modifications, including the use of Rappaport-siliadis (RV) broth instead of modified semisolid Rappaport Vassiliadis (MSRV) for enrichment and the use of xylose-lysine-tergitol 4 (XLT4) and brilliant green with novobiocin (BGN) instead of xylose lysine deoxycholate (XLD) for the selective medium. Media in identical lots were obtained from the same vendor. *Salmonella* O antisera were used to confirm the presence of *Salmonella* (Reimschuessel, *et al* 2017). The aim of this study was to investigate *salmonella* and *E. coli* in dogs and cats by isolation, identification and determine their antibiotic resistance pattern.

2. MATERIAL AND METHODS

2.1 Material:

2.1.1. Animals and Fecal swabs:

From January 2018 to January 2019 about 150 fecal samples were collected from dogs and cats of different ages (in **dog's** age ranges from 6 months to 5 years and in **cats** from 3 months to 3 years) of both sexes; males and females.

Some animals are fed naturally homemade food and others are fed artificial dry or wet food.

Samples were collected from different vet clinics and hospitals in Giza governorate.

2.1.2. Media used:

A) Media used for isolation of Salmonellae and E. coli:

Non- selective enrichment media (pre- enrichment) (Oxoid, UK) Buffered peptone water (BPW) broth. It was distributed as 9 ml tubes and was used for the optimal recovery of *Salmonella* and *E. coli* also for the reactivation.

Selective enrichment broth (Oxoid, UK):

* Corresponding author: Rania Abo-Sakaya,Department of Animal Medicine, Faculty of Veterinary Medicine, Benha University, Egypt. Email: rania.aboskayah@fvtm.bu.edu.eg

a. Rappaport-Vassiliadis broth: distributed as 10 ml tubes. This medium was used for selective isolation of *Salmonella* and *E. coli*.
b. Muller- Kauffmann tetrathionate/ novobiocin broth (MKTTn broth): distributed as 10 ml of tubes. A selective medium that allows *bacteria* to grow and multiply.

Selective plating media (Oxoid, UK)
1.Xylose Lysine Deoxycholate agar (XLD agar).
2.Brilliant Green agar (BGA).

B) Media used for biochemical identification of Salmonella and E. coli:
1.Christensen's Urea agar medium (Oxoid, UK). It was used to test the production of urease.
2.Glucose phosphate broth (Oxoid, UK). It was used for the methyl red (MR) and voges-proskaure (VP) tests.
3.Lysine iron agar medium (Oxoid, UK). It was used to test the production of lysine decarboxylase.
4.Simmon's citrate agar (MERCK, Germany). It was used citrate utilization test.
5.Triple Sugar Iron agar medium "TSI"(Oxoid, UK). It was used to detect the production of hydrogen sulfide (H2S) and glucose, lactose and sucrose fermentation.
6.Tryptone broth (Oxoid, UK). It was used for detection of indole production using Kovac's reagent.

C) ***Media used for preservation of Salmonella and E. coli:***
1.Brain heart infusion broth (Oxoid, UK). It was used to propagate bacterial isolates and to cryopreserve isolates with 16 % glycerol until further use.
2.Nutrient agar (LAB M, UK). It was used to preserve *Salmonella* isolates by repeated subculture for serotyping.
3.Semi-solid (soft) nutrient agar medium 0.4% was used for preservation and for detection of motility.
4.Tryptic soya agar (TSA) (Oxoid, UK). It was used to spread bacterial isolates as a non-selective medium.

D) Media and chemicals used for antibiogram assay:
1. Nephelometer barium sulphatestandardstube No (0.5) (PRO-LAB, UK). It was used for determination of the approximate number of bacteria by turbidity standard that corresponding to1.5x10^8 organisms/ml.
2. Mueller Hinton agar medium (Oxoid, UK). It was used for studying the antibiotic sensitivity of the isolated *Salmonellae*.
3. Mueller Hinton broth (Oxoid, UK). Antimicrobial susceptibility testing medium was used for propagation of the isolates.

2.1.3. Chemicals and reagents used for biochemical and serological identification of Salmonella and E. coli:
Hydrogen peroxide (H_2O_2)3% solution.
Kovac's Indole Reagent (Oxford, India).
McFarland nephelometer barium sulphate standards tube No (2.0) (Pro-Lab Diagnostic, U.K).
Oxidase test strips (Oxoid, UK).
Methyl red solution 0.04 % (Sigma-Aldrich).
Voges-Proskauer reagent.
Sterile normal saline solution 0.85%.
Urea solution 40% (Oxoid, UK).

2.1.4. Diagnostic Salmonella antisera:
Diagnostic omnivalent, polyvalent and monovalent *Salmonella* O and H (phase 1 and phase 2) antisera (DENKA SEIKEN CO., LTD) and (Pro- Lab Diagnostic, U.K).

2.1.5. Antimicrobial susceptibility discs materials (Oxoid, UK):
TenAntimicrobial discs containing six antimicrobial types. Every antimicrobial disc was selected to represent the corresponding class of antibiotics (β-lactam, amino glycosides, tetracycline, fluroquinolones, potentiated sulfonamides and phenicols). In this study we used the group of aminoglycosides (gentamicin and streptomycin), the group of tetracyclines (doxycycline), the group of fluroquinolones (norfloxacin, enrofloxacin, nalidixic acid and levofloxacin), the group of β-lactam (ampicillin), the group of potentiated sulfonamides (sulphamethaxozle / trimethoprim) and the group of phenicols (chloramphenicol)(Table 1).

Table 1 Antimicrobial discs used in this study

Antimicrobial agents	Disc code	Concentration (μg)
Gentamicin	G	10
Streptomycin	S	10
Doxycycline	DO	30
Norfloxacin	NOR	10
Enrofloxacin	ENR	5
Nalidixic acid	NA	30
Levofloxacin	LEV	5
Ampicillin	AM	10
Sulphamethaxozle/trimethoprim	SXT	25
Chloramphenicol	C	30

2.1.6. Material used for DNA extraction:
QIAamp DNA Mini Kit (Catalogue no.51304):
Thehe QIAamp DNA Mini Kit provides silica-membrane-based nucleic acid purification from different types of samples. The spin-column procedure does not require mechanical homogenization, so total hands-on preparation time is only 20 minutes.
Ethanol 96% (Applichem)
PCR Master Mix used for cPCR:
Emerald Amp GT PCR master mix (Takara) Code No. RR310A Contains:
A) Emerald Amp GT PCR master mix (2x premix).
B) PCR grade water.
Oligonucleotide primers used in cPCR: Source: Metabion, Germany (table 2).
DNA Molecular weight marker: 100 bp DNA ladder (cat. NO. SM0243) supplied from Fermentas**.**
Material used for agarose gel electrophoresis:
a. Agarose1.5% (Sambrook et al., 1989)
A multi-purpose, high gel strength agarose suitable for a wide range of molecular biology techniques.
Agarose powder (AB gene) 1.5 g
TBE 100 ml
b. Ethidium bromide solution 10 mg / ml (Sambrook *et al*., 1989)
Ethidium bromide powder (Sigma) 10 mg
Sterile DDW 1.0 ml
It was mixed and stored covered at 4˚C
It was added to melted agarose to reach a final concentration of 0.1-0.5 μg/ml.
Tris borate EDTA (TBE) electrophoresis buffer (1x) (WHO, 2002)

Table 2 Oligonucleotide primers sequences investigating of *Salmonella* and *E. coli* spp isolated from pets

Primer	Sequence	Amplified product	Reference
Salmonella invA	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	284 bp	Oliveira *et al*., 2003
E. coli eaeA	ATGCTTAGTGCTGGTTTAGG GCCTTCATCATTTCGCTTTC	248 bp	Bisi-Johnson *et al*., 2011
*bla*TEM	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516 bp	Colom *et al*., 2003

2.2. Methods:

2.2.1. Clinical Examination of animals:

All animals were examined clinically for temperature, pulse and respiratory rates and for presence of any abnormal clinical signs. (AL-Kubaisi *et al* 2020).

2.2.2. Fecal swabs:

One hundred and fifty rectal swabs were collected from household dogs and cats at Giza governorate from different vet. Clinics and hospitals.

Swabs were transported to Animal health institute in Dokki under complete hygienic condition.

2.2.3. Isolation of Salmonella and E coli:

The method for *enterobacteriacae* detection, isolation and identification was applied according to (**ISO 6579 2002**), isolation requires three successive stages:

Stage 1: pre-enrichment in non-selective liquid broth:

Initially, the rectal swabs were inoculated into a non-inhibitory liquid medium to improve the repair and development of stressed or sub-lethal *enterobacteriacae* due to heat exposure, freezing, large temperature fluctuation, and high osmotic pressure. Inoculated in tubes of 9 ml of buffered peptone water for 1/10 dilution (weight to volume) incubation at 37°C ±1°C for 18± 2 hours.

Stage 2: Enrichment on a selective liquid broth:

Each culture of pre-enrichment has been inoculated into two enrichment media to promote microbial proliferation by selectively inhibiting the growth of competing micro-organisms. From the pre-enrichment cultures 0.1ml was transferred to tubes containing 10 ml Rappaport Vassiliadis broth and then incubated at 41.5ºC±1ºC for 24±3 hour. One ml of the pre-enrichment culture was also transferred to tube contain10ml Muller-Kauffman tetrathionate /novobiocin broth and incubated at 37ºC for 24±3 hour.

Stage 3: Plating into selective agar media:

Each enrichment culture was streaked into a minimum of two agars for isolation. 10µ loop-full of the inoculated Muller-Kauffman tetrathionatenovobiocin broth and Rappaport Vassiliadis broth were spread on the surface of Xylose Lysine Deoxycholate agar (XLD) and the surface of Brilliant Green agar and incubated at 37.0±1 ºC for 24±3 hour.

2.2.4. Identification of salmonellae and E. coli*:*

a. Detection of *salmonella* suspected colonies according to (**ISO 6579 2002**):

The suspected colonies on Xylose Lysine Deoxycholate agar and Brilliant Green agar were picked up and motility was tested by 0.4% soft nutrient agar then colonies were transferred into semisolid Tryptone soya agar (TSA) slope for preservation and further identification.

b. Biochemical identification of the isolates pure culture:

Biochemical identification was performed according to (Quinn *et al* 2002).

2.2.5. Serological confirmation and serotyping of suspected salmonella isolates:

The biochemically positive colonies of *salmonella* were primarily confirmed by Omni valent antisera and serologically classified using slide agglutination tests, according to the modified Kauffman-White scheme as described by **(WHOCC-Salm 2007).** Diagnostic polyvalent and monovalent *Salmonella* O and H (phase 1 and phase 2) antisera. (Denka Seiken co., LTD) and (Pro-lab diagnostic, U.k) were provided by serology unit, Animal health Research Institute, Dokki, Giza.

2.2.6. Antimicrobial Sensitivity Test:

Subculture from each *salmonella* serovars were prepared and the test was applied according to (Cruickshank *et a2l* 1975). The results were reported and compared to the standard levels to decide if salmonella isolates were sensitive, intermediate and resistant and the results were interpreted according to CLSI (2014) Clinical and Standards Institute.

Table 3 Zone diameter interpretive standards of different antimicrobial agent used for *salmonella* spp:

Antibiotic	Symbol	Disc Conc. (µg)	Zone diameter (mm)		
			Resistant	Intermediate	Susceptible
Gentamicin	G	10	≤12	13-14	≥15
Streptomycin	S	10	≤11	12-14	≥15
Doxycycline	DO	30	≤10	11-13	≥14
Norfloxacin	NOR	10	≤12	13-16	≥17
Enrofloxacin	ENR	5	≤15	16-20	≥21
nalidixic acid	NA	30	≤13	14-18	≥19
Lamofloxacin	LEV	5	≤13	14-16	≥17
Sulphamethaxozle /trimethoprim	SXT	25	≤10	11-15	≥16
Chloramphenicol	C	30	≤12	13-17	≥18

2.2.7. Confirmation of results by PCR:

a. Extraction of DNA according to QIAamp DNA mini kit instructions

b. Preparation of PCR Master Mix: according to **Emerald Amp GT PCR master mix (Takara)** Code No. **RR310A**kit

The PCR reaction mixture consisted of 12.5 µL of Emerald Amp GT PCR master mix (Takara),1 µL of each set of forward and reverse primers (20 pmol), 5 µL of DNA as a template and nuclease free water to make 25 µL of reaction volume.

The PCR cycling conditions were programmed according to the reference of the primer (Table 1).

The amplified PCR products were resolved by agarose gel electrophoresis, using 1.5% agarose gel stained with ethidium bromide (0.5 µg/mL) and visualized and documented using UV gel documentation system (Alpha Innotech, Biometra).

3. RESULTS

3.1. Infection rate of Salmonella and E. coli serotypes among dogs and cats:

Some animals that were clinically examined were apparently healthy and others were diseased with signs of fever, diarrhea, vomiting and loss of appetite.

Bacteriological culturing of 150 fecal samples (95 of dogs and 55 of cats) revealed a presence of different types of *Enterobacteriaceae* as *salmonella*, *E. coli* and some cases were negative for *Enterobacteriaceae.*

These results revealed that *salmonella* species were isolated from 40 cases out of 150, 30 from the *salmonella* positive samples were collected from dogs (31.6%) and 10 were collected from cats (18.2%) but 45 samples were positive for *E. coli*, 25 from dogs (26.3 %) and 20 from cats (36.4 %).

Our results showed that *salmonella* shedding in dogs was increased in adults (42.9%) than young (Figure 1), while *E. coli* shedding was increased in young dogs, (88%) than adults (Figure 5). Otherwise, *salmonella* shedding was increased in German Shephered (46.7%) than Pitbull and Husky (Figure 3), while *E. coli* shedding was increased in Husky (34.3%) than Pitbull and German Shephered (Figure 7).

For sex factor in dogs, *salmonella* shedding was increased in females (62.2%) than males (Figure 2), while *E. coli* shedding was increased in females (53.3%) than males (Figure 6). Otherwise, seasonal factor *salmonella* shedding was increased in winter (81.8%) than summer and autumn

(Figure 4). While *E. coli* shedding was increased in winter (66.7%) than summer and autumn (Figure 8).

Infection rate in cats, *salmonella* was increased in adults (28.6%) than young (Figure 9), *E. coli* was higher in young (100%) than adult (Figure 13). Otherwise, *salmonella* was increased in males, (35%) than female (Figure 10) and *E. coli* was increased in females (51.4%) than males (Figure 14). While *salmonella* was increased in Persian cat (25%) than Himalaya (Figure 11), *E. coli* was increased in Persian (45%) than Hemalaya (Figure 15).

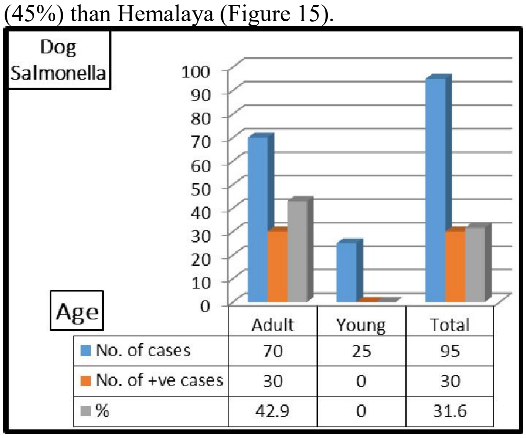

Figure 1 Age prevalence of *Salmonella* in dogs.

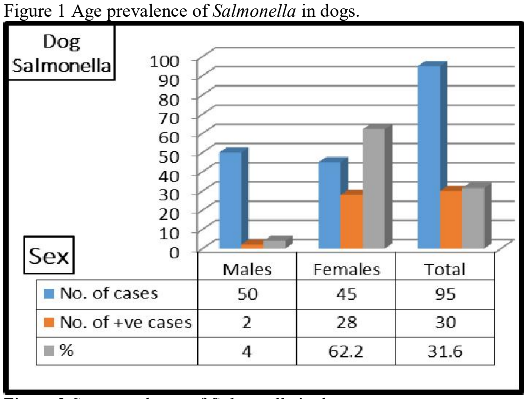

Figure 2 Sex prevalence of *Salmonella* in dogs.

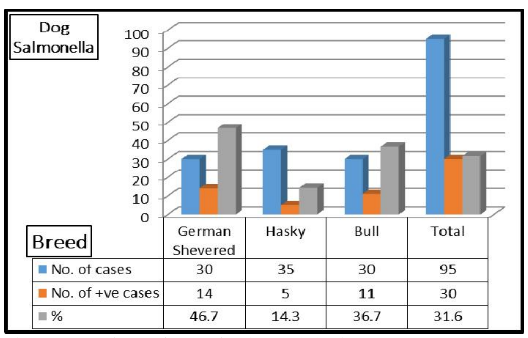

Figure 3 Breed prevalence of *Salmonella* in dogs.

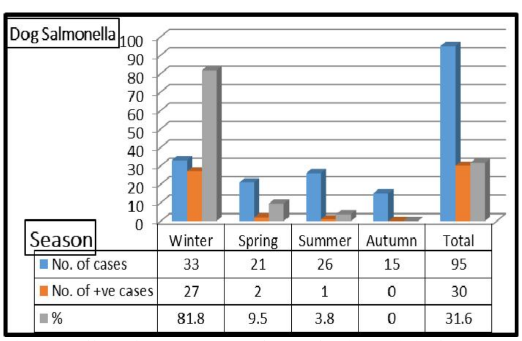

Figure **4** Effect of season on prevalence of *Salmonella* in dogs.

3.2. Identification of isolated strains:

The biochemical reactions (MR test-VP-Indole Test-Citrate utilization –Urease –Lysine decarboxylase –Sugar fermentation –Oxidase-Catalase tests) revealed that *salmonella* gives positive reaction with (MR- citrate utilization-Lysine decarboxylase –Sugar Fermentation-Catalase, and in TSI test the positive result is H_2S production and gas formation) and *E. coli* gives positive reaction with (Indole- MR- Lysine decarboxylase-Catalase test) (Table 4).

Table 4 Biochemical reactions of *Salmonella* and *E. coli* serovars

Biochemical Reaction / M.O	Indol test	MR test	VP test	Cit. utilization	Urease test	Lysine	Sugar fermentation	Oxidase test	Catalase test	TSI test
Salmonella	-	+	-	+	-	+	+	-	+	A/K +H_2S and gas producton
E. coli	+	+	-	-	-	+	-	-	+	A/A+gas production

By performing PCR for five *salmonella* positive samples and five *E. coli* positive samples, we detected the virulence gene of *salmonella* (*inv*A gene) and of *E. coli* (*eae*A gene) Figure 18. Also, we detected the antibiotic resistant gene for *salmonella* and of *E. coli (bla*TEM) Figure17.

S*almonella* strains are *S.Typhemurium, S.Entertidis, S.Nitra, S.Bokher,* and *S.Santipool* respectively and *E.coli strains* are O26, O157, O119, O55, and O111 respectively. The *E. coli* sample number 3 has no virulence gene. The antigenic structure of the Salmonella serotypes was illustrated in table 5

Table 5 Antigenic Structure of *Salmonella* serotypes

Salmonella types	Antigenic structure of *salmonella*: Somatic antigen (O)	Flagellar antigen (H) Phase I	Phase II
S.nitra	2,12	g,m	
S.bocker	[1],6,14,[25]	lv.	1,7
S.typhemurium	1,4,[5],12	I	1,2
S.Entertidis	1,9,12	g,m.	1,7
S.ibargi	21	Y	1,2

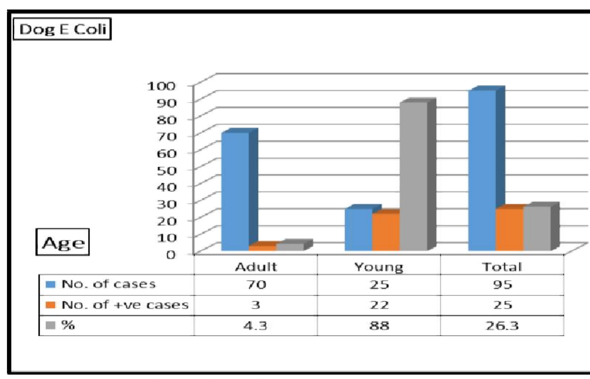

Figure 5 Age prevalence of E. coli in dogs.

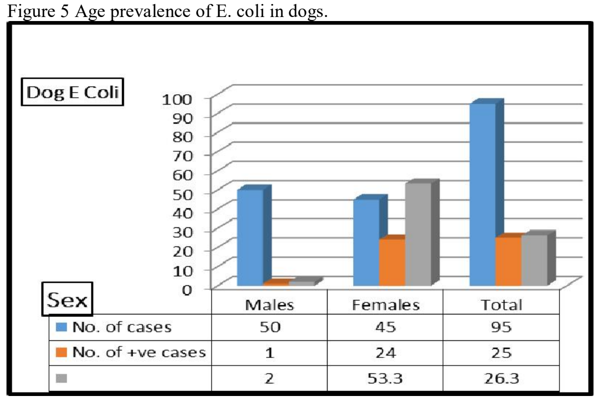

Figure 6 Sex prevalence of *E. coli* in dogs.

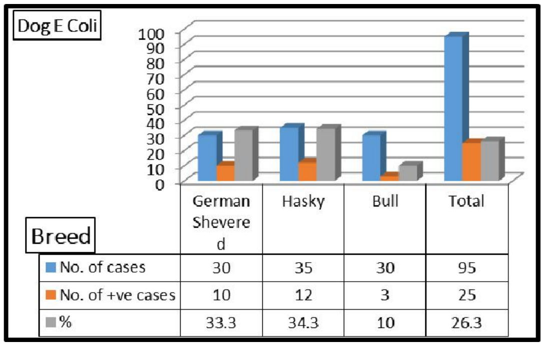

Figure 7 Breed prevalence of *E. coli* in dogs.

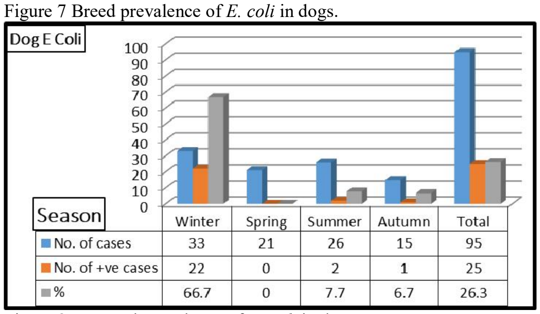

Figure 8 Seasonal prevalence of *E. coli* in dogs.

4. DISCUSSION

Salmonellosis is a disease of major zoonotic importance, and all *Salmonella* organisms, with the exception of those causing human typhoid fever, infect humans and animals. Foodborne outbreaks of non-typhoid salmonellosis can occur in people through contaminated products of animal origin (meat, eggs, and milk) that have been improperly prepared, stored, or handled before consumption. (Marks *et al* 2011). Pathogenic *E. coli* are transmitted from infected human or animal feces to new susceptible hosts via environmental reservoirs such as hands, water, and soil (Navab-Daneshmend *et al* 2014). This study was planned to investigate the role of *salmonella* and *E. coli* in dogs and cats and determination of the effect of some factors such as age, sex, breed and the seasons on infection rates and detection of the antibiotic resistance gene of *salmonella* and *E. coli* (*bla*TEM).

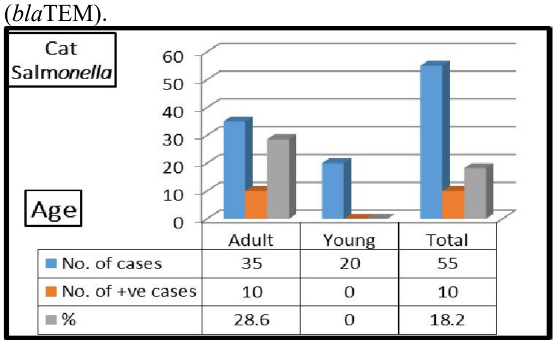

Figure 9 Age prevalence of *Salmonella* in cat.

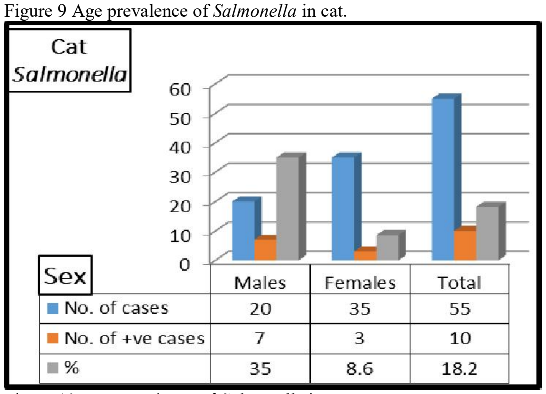

Figure 10 Sex prevalence of *Salmonella* in cat.

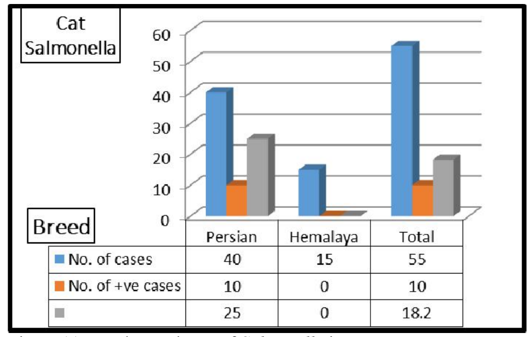

Figure 11 Breed prevalence of *Salmonella* in cat.

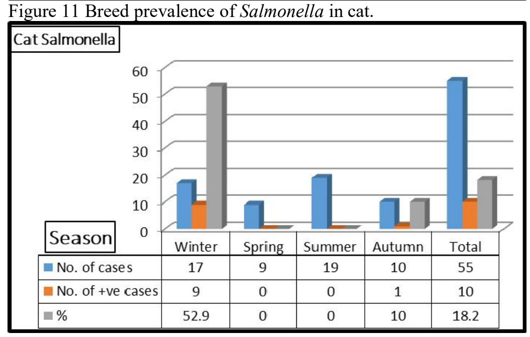

Figure 12 Seasonal prevalence of *Salmonella* in cat.

This study showed that, infection rate of *Salmonella* in pets in this survey is (31.6 % in dogs and 18.2 % in cats). Also, infection rate of *Salmonella* in dogs located in Holeta town of Central Ethiopia is 17.1% and first of its kind in the country that agree with our study (Aliyi *et al* 2018). The infection rate of *salmonella* was 2.5% in dogs (apparently healthy dogs from a variety of housing conditions) and 6% in cats in USA (this is very low) (Lowden *et al* 2015), this disagrees with our study. *The isolated Salmonella* species were *S. Ibargy, S.Entertidis, S.Bokher, S.Typhemurium* and *S.Nitra*, they were resistant to streptomycin, gentamycin and trimethoprim/sulphamethaxole, while, *S.ibargy* and *S.Entertidis* were sensitive to chloramphenicol, enrofloxacin and nalidixic acid. This result were disagreed with previous results of (Al kocabiyik *et al.* 2006) who isolated the *S.corvallis* from the stray dogs in Turkey that were sensitive to chloramphenicol, enrofloxacin and nalidixic acid, trimethoprim/sulphamethaxole and resistant to streptomycin.

Outbreak of *Escherichia coliO145:H28* infections in 2010 was the first known *Shiga toxin-producing E. coli (STEC)* outbreak traced to the southwest desert leafy green vegetable production region along the border between the United States and Mexico (Jay-Russell *et al* 2014), while in our study we isolated O157, O126, O114, O18, O26, O158, O111 and O18,01.

It is found that *salmonella* and *E. coli* infection was increased in winter and decreased in summer and autumn (Figure 4, 8, 12,16), this disagrees with the study of (Ishii *et al.* 2006) that found soil born *E. coli* was observed in higher ratio in summer to fall and lower in winter to spring. Density of it is the greatest in the summer to fall (June to October), and the lowest numbers, occurred during the winter to spring months (February to May). In the summer of 1999, an outbreak of human salmonellosis happened in Alberta (Canada). The cause of this outbreak was treats for dogs produced from processed pig ears contaminated with *S. Infantis* that was isolated in 51% treats for dogs from shops in Canada and from 41% samples of the same feed in shops in the USA (Milanov *et al.* 2019), this disagrees with our study.

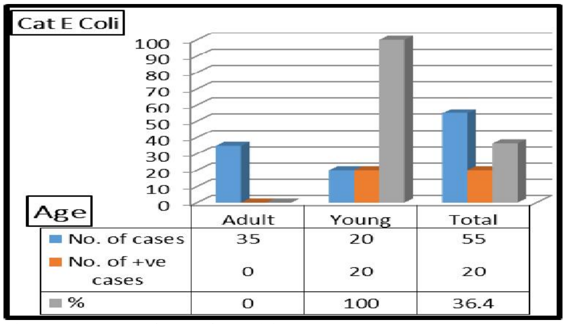

Figure13 Age prevalence of *E. coli* in cat.

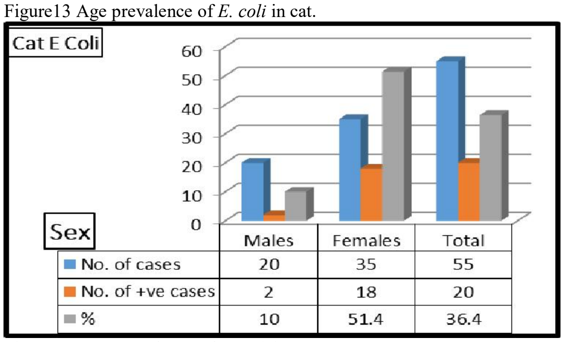

Figure 14 Sex prevalence of *E. coli* in cat.

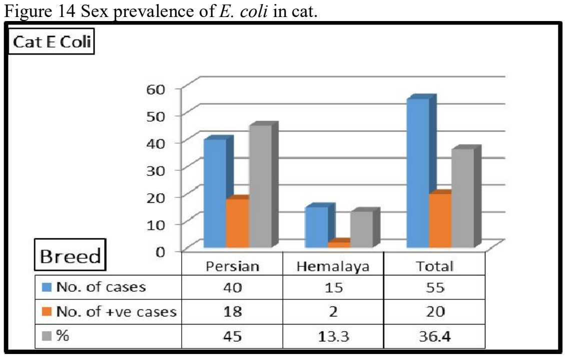

Figure 15 Breed prevalence of *E. coli* in cat.

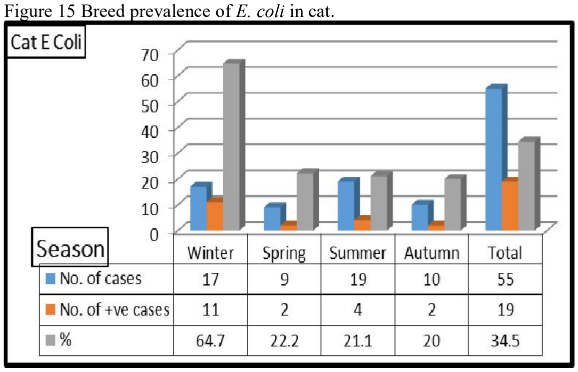

Figure 16 Seasonal prevalence of *E. coli* in cat.

Fecal shedding of *salmonella* in naturally occurring infections may continue for a period of six weeks except in case when the lymph nodes harbor the agent. In the experimental canine infections with *S. infantis* shedding of the organism lasts for 117 days so *salmonella* shedding increases in adult than newborn (Morse *et al* 1976), this study was agreed with our study as *salmonella* was 42.9% in adult dog and0% in young dogs (Figure1).

For this study we found that *salmonella* infection is higher in females (62.2%) than males (4%) Figure 2, this agreed with the study that found *Salmonella* was isolated from 4.1% to 9.1% of male and female dogs respectively (Akwuobu *et al.* 2018).

5. CONCLUSION

This study revealed that dogs or cats may be carrier for *salmonella* or *E. coli* without any signs. These diseases may be occupational for veterinarians and owners so, both of them must be aware of this. Both must take the safety precautions during treatment and dealing with a dog or a cat. *Salmonella* and *E. coli* may be detected in apparent healthy or diseased dogs or cats, of both sexes, at any age for any breed with difference in rate of infection.

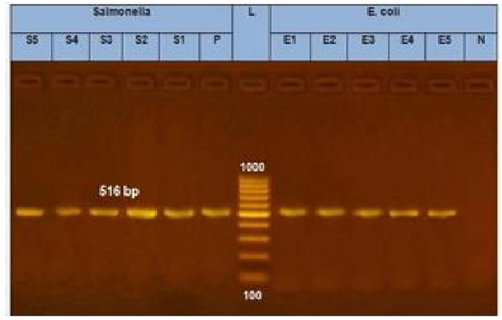

Figure 17 Agarose gel electrophoresis reveled amplification product for (blaTEM) antibiotic virulence resistant gene at 516 bp. L 100bp DNA molecular Size marker, lanes P. and N positive and negative Controls, respectively. Lanes (E1- E5) referred to the examined E. coli isolates. While Lanes (S1– S5) to positive examined *salmonella* isolates.

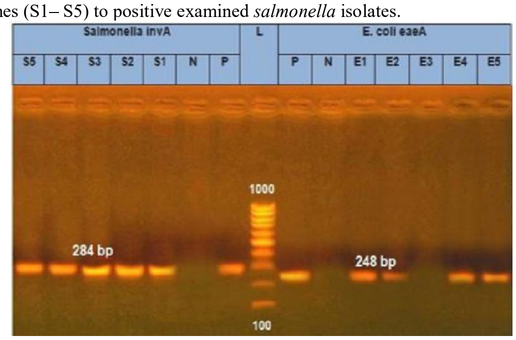

Figure 18 Agarose gel electrophoresis reveled amplification product for *Salmonella* invA genes at 284 bp and *E. coli* eaeA virulence gene at 248 bp. L 100bp DNA molecular Size marker, lanes P. and N positive and negative Controls, respectively. Lanes (E1- E5) referred to the examined E. coli isolates. While Lanes (S1– S5) to positive examined *salmonella* isolates.

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