

Epidemiological Studies on Salmonellosis in Pet Animals and Humans in New Valley Governorate, Egypt

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ABSTRACT: Salmonellosis is one of the most important bacterial diseases that results in many public health risks. This study aims to investigate the prevalence of non-typhoidal-Salmonella (NTS) in pets and humans in New Valley Governorate, Egypt, and study their most important risk factors to help in proposing plans to prevent and control salmonellosis. A total number of 920 samples were collected from pets' feces and human's stool. The bacteriological examination of collected samples revealed that the prevalence of NTS in the New Valley Governorate was 1.7% in humans and 1.6 % in pets (0.9% in cats, and 2.2% in dogs). The locality, season, health status, sex, and age can significantly affect the prevalence of salmonellosis in pets and humans. All Salmonella isolates from dogs, cats, and humans were only obtained in the cold season and from cats, dogs, and humans with diarrhea and gastrointestinal manifestation and no Salmonella could be isolated from apparently healthy ones. Salmonella was recovered from male dogs (3.5%) more than bitches (1.1%), also male cats (1.25%) were more than female cats (0%). In addition, Salmonella isolates from human males (4.4%) were more than females (0.4%). The highest prevalence of salmonellosis was detected in the young age group, < 6 months, of dogs (2.5%) and cats (1.4%). On the other hand, the highest prevalence of human salmonellosis was found in group D (> 40: 60 years) followed by group A (<6 years) by 7.5% & 1.7%, respectively. The prevalence of salmonellosis was higher in individuals of close contact with their pets (1.7%) than those of non-contacts (1.5%). Pet sanitary practices and personal hygiene are very important measures for Salmonella prevention. The routine examination of pets against salmonellosis is recommended.

KEYWORDS: Salmonella, Risk factors, Dogs, Cats, Bacteriological examination.

1. Introduction

Salmonella are facultative anaerobic Gram-negative rod-shaped bacteria, which belong to the family Enterobacteriaceae. Salmonella is a hardy bacteria that can survive several weeks in a dry environment and several months in water, Salmonella is mesophilic with some strains being able to survive at extremely low or high temperatures (2°C to 54°C)[1]. Salmonellosis is a global health problem, affecting approximately 1.3 billion people annually, and NTS is a leading cause of 550 million diarrheal diseases worldwide each year [2]. The majority of Salmonella serovars are pathogenic to humans, animals can be asymptomatic carriers of these bacteria, infections are higher in non-developed countries and rural areas, mainly because these populations have a higher exposure to risk factors such as lack of access to good water sources, poor hygiene practices and low socioeconomic background,

Salmonella can be transmitted through water and contaminated food, posing a major problem for public health [3]. Nowadays, a wide range of animals is present in human households such as pet animals including cats and dogs. Pets play an important role in the lives of many people and are often considered to be members of families, but definitely, any contact with them is an inherent risk of transmitting zoonotic pathogens. One of the most important zoonotic pathogens is Salmonella [4]; the risk of salmonellosis in humans is increased if their pet has the infection without overt clinical signs due to their close relationship[5]. Dogs and cats play a role as asymptomatic carriers of Salmonella but the clinical symptoms of salmonellosis may include fever, loss of appetite, diarrhea, abdominal pain, and abortion these clinical pictures may appear even 12 hours after exposure to bacteria [6]. Salmonellosis in humans is divided

into minor and major diseases, minor is caused by non-typhoid *Salmonella* strains, characterized by self-limiting diarrhea, gastrointestinal infection and rarely leading to bacteremia or meningitis, but major salmonellosis is represented by typhoid fever and other severe clinical pictures such as headache, vomiting, malaise and sometimes cough [7]. This study aimed to investigate the prevalence of zoonotic *Salmonella* in pets and humans, study their most important risk factors, and propose plans to prevent and control salmonellosis.

2. Material and methods

2.1. Ethical declaration

This study was carried out according to the guidelines of the Institutional Animal Care and Use Committee of New Valley University. The collection of samples was approved with an Institutional Approval Number (IRB 04-2023-200395).

2.2. Study area and design:

Samples were collected from New Valley Governorate, Egypt, between July 2022 and September 2023. The New Valley Governorate is considered the biggest Governorate in the country in terms of area, which amounts to approximately 440098 km², representing approximately 43.6% of the total area of the country. Its location is between the Nile, Northern Sudan, and Southeastern Libya. It shares the borders with the Governorates of El Menia, Giza, and Marsa Matrooh on the north and Assiut, Suhag, Qena, and Aswan on the east. The study included two main oases of the New Valley Governorate, El Kharga and El Dakhla.

2.3. Sampling

A total number of 920 samples were collected from pets and humans suffering from gastrointestinal disorders such as diarrhea, vomiting, constipation, and loss of appetite. The samples included fecal and stool samples that were

collected from 310 dogs, 310 cats, and 300 humans, samples per each. The fecal and stool samples were aseptically collected in sterile containers according to [8]

2.4. Preparation of samples

One gram of each sample was thoroughly mixed with nine ml of buffered peptone water to make (1/10) dilution. In a sterile test tube, one ml of the dilution was mixed with nine ml of buffered peptone water. The contents were carefully mixed [9].

2.5. Isolation and identification of *Salmonella*:

2.5.1. Isolation of *Salmonella*

The prepared samples in buffer peptone water were incubated for 24 hours at 37 °C. 0.1 ml of the pre-enriched sample was transferred into a tube containing 9 ml of Rappaport-Vassiliadis broth (RV) and was incubated at 42 °C for 24 hours. A loop full of the broth culture was aseptically streaked on MacConkey agar plates and was incubated at 37 °C for 24 hours. The suspected colonies were colorless colonies that were subcultured on bismuth sulfite agar (BSA) and Hektoen enteric (HE) agar which are *Salmonella*-specific media and were incubated at 37 °C for 24 hours. The suspected colonies on BSA were black colonies and clear colonies with or without black centers on HE agar [10]. The suspected colonies were subcultured onto BSA agar until the pure homogenous colonies were obtained. The pure colonies were streaked onto nutrient agar plates for biochemical identification [11]

2.5.2. Identification of *Salmonella*

Morphological identification of Salmonella

The suspected colonies were identified microscopically by using a gram stain according to [12]. The gram-negative, medium size and stained evenly bacilli were suspected to be *Salmonella* [13].

Biochemical identification of Salmonella

Isolated suspected characteristic colonial morphology of *Salmonella* on BSA and HE agar were subjected to biochemical tests such as; indole test, TSI test, citrate test,

Table 1: Prevalence of salmonellosis in pets in New Valley Governorate

Species	No. of examined samples	No. of positive samples	%	Chi-square	P-value
Cat	310	3	0.9	0.333	0.564
Dog	310	7	2.2		
Total	620	10	1.6		

Table 2: Prevalence of salmonellosis in pets in relation to locality

Samples	Cat			Dog			Chi-square	P-value
	Total No.	Positive No.	%	Total No.	Positive No.	%		
El Kharga	210	2	0.9	227	5	2.2	0.333	0.564
ELDakhla	100	1	1	83	2	2.4		
Total	310	3	0.9	310	7	2.2		

oxidase test, urease test, catalase test, sugar fermentation tests, lysine decarboxylase test, ornithine decarboxylase test, Voges-Proskauer test, methyl-red test and gelatin liquefaction Standard methods were followed to conduct these tests [14]

2.6. Statistical Analysis

The statistical analyses were carried out to analyze the prevalence of Salmonella in dogs, cats, and humans about locality, season, health status, sex, and age by using Chi-square using SPSS, ver. 27 (IBM Corp. Released 2013). The significance level was set at < 0.05. Data were treated as a complete randomization design according to [15].

3. Results and discussion

Salmonellosis represents a serious health threat to both humans and animals, especially in poor countries and among people who do not maintain personal hygiene [16]. Dogs and cats can negatively affect public health, as they are an important factor in the transmission of zoonotic diseases, especially Salmonella, due to close contact with humans and other animals [17]. As shown in Tables 1 and 7, the overall prevalence of isolated NTS from the New Valley Governorate was 1.7% in humans, a higher results were recorded in many previous studies (2.9%), (23.4%) and (8.33%) [14, 18, 19]. On other hand, the

Table 3: Prevalence of salmonellosis in pets regarding season

Samples	Cat			Dog			Chi-square	P-value
	Total No.	Positive No.	%	Total No.	Positive No.	%		
Hot season	130	0	0	110	0	0	1.000	0.607
Cold season	180	3	1.6	200	7	3.5		
Total	310	3	0.9	310	7	2.2		

Table 4: Prevalence of salmonellosis in pets regarding health status

Samples	Cat			Dog			Chi-square	P-value
	Total No.	Positive No.	%	Total No.	Positive No.	%		
Health	230	3	1.3	245	7	2.8	1.000	0.607
Diseased	80	0	0	65	0	0		
Total	310	3	1.6	310	7	2.2		

Table 5: Prevalence of salmonellosis in pets in relation to sex

Samples	Cat			Dog			Chi-square	P-value
	Total No.	Positive No.	%	Total No.	Positive No.	%		
Male	240	3	1.25	142	5	3.5	0.667	0.881
Female	70	0	0	168	2	1.1		
Total	310	3	0.9	310	7	2.2		

Table 6: Prevalence of salmonellosis in pets in relation to age

Samples	Cat			Dog			Chi-square	P-value
	Total No.	Positive No.	%	Total No.	Positive No.	%		
Age	139	2	1.4	80	2	2.5	0.667	0.881
Group A (1-6 m)	101	0	0	130	3	2.3		
Group B (7m:2y)	70	1	1.4	100	2	2.0		
Group C (3-4 y)	310	3	0.9	310	7	2.2		

Table 7: Prevalence of salmonellosis in humans at New Valley Governorate

Samples	Total No.	Positive No.	%	Negative No.	%	Chi-square	p-value
Stool samples	300	5	1.7	295	98.3	0.333	0.564

Table 8: Prevalence of salmonellosis in humans in relation to Locality

Items	Total No	Positive No	%	Chi-square	P-value
El Kharga	205	4	1.9	0.333	0.564
EL Dakhla	95	1	1.05		
Total	300	5	1.6		

Table 9: Prevalence of salmonellosis in humans in relation to season

Weather	No. of tested samples	Positive No.	%	Chi-square	P-value
Hot season	145	0	0	0.333	0.564
Cold season	155	5	3.2		
Total	300	5	1.6		

Table 10: prevalence of salmonellosis in humans in relation to health status

Health	No. of tested samples	Positive No.	%	Chi-square	P-value
Diseased	145	5	3.4	0.333	0.564
Apparent healthy	155	0	0		
Total	300	5	1.6		

Table 11: Prevalence of salmonellosis in humans in relation to gender

Gender	No. of tested samples	Positive No.	%	Chi-square	P-value
Male	90	4	4.4	0.333	0.564
Female	210	1	0.4		
Total	300	5	1.6		

Table 12: Prevalence of salmonellosis in humans in relation to age

Age	No. of tested samples	Positive No.	%	Chi-square	P-value
Group A (< 6 years)	112	2	1.7	0.333	0.564
Group B (> 6 : 18y)	65	0	0		
Group C (> 18 - 40 y)	83	0	0		
Group D (> 40 - 65 y)	40	3	7.5		
Total	300	5	1.6		

Table 13: Prevalence of salmonellosis in humans in relation to the contact status with animals

Connection with animals	Prevalence of salmonellosis in human			Chi-square	P-value
	No. of tested samples	Positive No.	%		
Contact	235	4	1.7	0.333	0.564
Non-contact	65	1	1.5		

prevalence of NTS was 1.6 % in pets (cats 0.9% and dogs 2.2%), these results are in agreement with [20] who recorded that Salmonella prevalence was 1.8 % in dogs and cats. On the other side, [21] mentioned that 19% of tested cats were found to be Salmonella-positive. In another study, the prevalence of Salmonella was 9.47% in dogs and 1.77% in cats [22]. Moreover, [23] found that Salmonella-positive samples were 31.6% in dogs and 18.2% in cats. Moreover, [24] found that 5.8% of examined pets, 9.3% of cats and 3.6% of dogs, are Salmonella positive. These different rates of Salmonella in other studies are due to several factors such as differences in pet sanitary practices, sampling strategies, and pet owners' awareness of zoonoses, also seasonal, geographical, and regional differences among studies. Many factors can affect the prevalence of salmonellosis such as the locality, season, health status, sex, and age. The obtained results in Tables 2 and 8 showed that a higher prevalence of salmonellosis in dogs and cats was recorded in EL Dakhla (2.4 % & 1%, respectively) than in EL Kharga (2.2% & .9%). In contrast, a higher prevalence of salmonellosis in humans was in EL Kharga (1.9%) than in El Dakhla (1.05%). Geographical distribution and relative population size in every region affect thoroughly Salmonella prevalence [25, 26]. As illustrated in Tables 3 and 9, all Salmonella isolates from dogs, cats, and humans were only obtained in the cold season and Salmonella couldn't be found in the hot season, this result is in agreement with [20] who recorded that the higher percentage of Salmonella was identified in low temperatures (80°F) and [27] who mentioned that the highest prevalence of Salmonella was in the winter (77.8%). On the other hand, [2] mentioned that samples which collected in winter showed the lowest Salmonella prevalence (15.86%). The

data illustrated in Tables 4 and 10 indicated that health status of tested pets and human being can harbor Salmonella prevalence as Salmonella was only isolated from cats (1.3 %), dogs (2.8%) and humans (3.4%) with diarrhea and gastrointestinal manifestation and no Salmonella could be isolated from apparently healthy ones, similar results were documented by [28] who isolated NTS from feces of dogs with significantly higher occurrence in diarrheic (15.2%) than non-diarrheic (5.5%) animals. On the other side, [22] reported that the prevalence of Salmonella in sick and healthy cats was 2.08% & 1.54% respectively, and in dogs was 10% & 8.85%, respectively. Moreover, [29] showed that the prevalence of Salmonella was 1.8% in apparently healthy dogs. The data presented in Table 5 showed that there were significant differences in Salmonella prevalence about sex as Salmonella was recovered from male dogs (3.5%) more than bitches (1.1%) also male cats (1.25%) were more than female cats (0%), these results incompatible with [28] who found that Salmonella prevalence was significantly higher in bitches (2.5%) than male dogs (0.027%) and [17] who explained that the prevalence of salmonellosis in male cats was 7.05% and in female cats was 11.77%. According to Table 11, Salmonella isolates from human males (4.4%) were more than females (0.4%), these results are in agreement with [30, 31, 32]. The results in Tables 5 and 12 revealed that age had a significant effect on the rate of salmonellosis in pets and humans, as the highest prevalence of salmonellosis was detected in younger age groups, < 6 months age groups, of dogs (2.5%) and cats (1.4%), these results agree with [33] and [3]. According to human age groups shown in Table 12, the highest prevalence of salmonellosis was found in group D (>40: 60 years) followed by group A (<6 years) by 7.5% & 1.7%, respectively. These results are compatible with [34] who showed that the highest rate of salmonellosis was in neonates (2.57%), and adults of 65 years old (3.56%). In contrast, [35] revealed that the incidence of NTS was consistently higher in a median

age of 49 years old (52%) who exposed to health, immune, and stress conditions, also people with weakened immune systems are at risk of serious NTS infection. The results obtained in Table 13 revealed that the prevalence of salmonellosis was higher in individuals in close contact with their pets (1.7%) than those in non-contacts (1.5%), these results in agreement with a study performed by [36]. In contrast, [37] showed that Salmonella prevalence relating to animal contact was 13%. On the other hand, only 0.2 % of Salmonella positive results was reported as pet owner patients in close contact with their animals and their raw foods [38].

Conclusion

This study reveals the presence of a low prevalence of salmonellosis in dogs, cats, and humans in the New Valley Governorate, but it still represents a serious public health hazard. The most important risk factors that influence the prevalence of NTS are age, sex, season, and health status of pets and humans.

Recommendation

Pet sanitary practices and personal hygiene are very important measures for the prevention of salmonellosis. The routine examination of pets against Salmonella is recommended. Collaboration between public health officials and veterinary services is required to increase pet owners' awareness about salmonellosis.

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Competing interests

All authors have no conflict of interest

Authors' contribution

All authors cooperate with each other in work design, experimental plan, sample collection, carrying out the practical part, and lettering of the manuscript. SAS, MEA, NKA, EMH and MSD conceived and designed the experiments. MSD, NKA and EMH measured the parameters. SAS and EMH statistically analyzed the data. MSD, NKA and EMH wrote the manuscript. All authors accepted and permitted this manuscript.

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