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Epidemiology of *Salmonella* Species in Diarrheic Sheep and Goats

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ABSTRACT

A cross sectional survey study aimed to: 1) Identify the management factors associated with the occurrence of salmonellosis in some diarrheic sheep and goats belonging to mixed reared flocks in Giza governorate, Egypt, 2) Investigate the presence of enterotoxin (*stn*) gene in the recovered *Salmonella* spp. strains, and 3) Build a phylogenetic tree for the partial codon sequence of *stn* gene of some recovered strains in order to provide a scientific basis for the implementation of practical preventive measures. A total number of 518 diarrheic sheep and goats belonging to 7 mixed flocks of sheep and goats were enrolled and from which rectal swabs were collected and subjected to culture for *Salmonella* spp. Enterotoxin (*stn*) gene was detected using polymerase chain reaction assay (PCR), and the PCR amplicons of nine randomly selected strains were purified, sequenced and deposited in the GenBank. The obtained data about disease occurrence were statistically analyzed using Chi-square test in order to identify disease-associated factors. The overall prevalence of salmonellosis among diarrheic sheep and goats was 3.86%, and the disease prevalence per each flock ranged from 0% to 7.55%. The factors that were found to be associated with disease occurrence included, absence of isolation of newly- purchased animals and isolation pen for sick animals, over the counter use of antimicrobials, lack of disinfection of feeding utensils and water troughs, and presence of rodents in feed storage area. Enterotoxin (*stn*) gene was detected in all recovered salmonellae, and the phylogenic analysis of *stn* gene of the selected strains and the retrieved sequences from GenBank showed the relatedness of the isolated strains to the other strains isolated from different sources. Accordingly, the analysis of disease associated factors and the robust phylogeny findings provide valuable data that will be useful for implementation of preventive measures for salmonellosis in small ruminants.

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Key words
***Salmonella*, Sheep, Goats, *stn*, Risk, Egypt**

INTRODUCTION

Salmonellosis is concerned as one of the most prevalent diseases in small ruminants (Ferreras *et al*., 2007). *Salmonella* infection is mostly an enteric infection, which has clinical and subclinical forms; *Salmonella* infection can be established in some animals without any contribution to clinical illness, resulting in asymptomatic carrier or dissemination of infection /contamination within herds or flocks due to a latent infection where *Salmonella* remains dormant in the lymph nodes and may be shed in the feces. Therefore, *Salmonella* can be exist in a farm, but can only develop an outbreak if the animals are exposed to stressors or other causes (Acha and Szyfres, 2001; Veterinary Record, 2017). Additionally, the most common clinical manifestation of salmonellosis is enteritis, resulting in diarrhea, or as septicemia, which may be fatal. Less commonly, *Salmonella* can cause suppurative epididymo-orchitis, arthritis, respiratory disease, meningitis, abortion and stillbirth (Ferreras *et al*., 2007; Veterinary Record, 2017).

Diarrhea is one of the most serious problems which causes massive economic losses in sheep and goats farming due to poor growth rates, mortalities, and veterinary costs. *Salmonella* infection is considered one of the most common causes of diarrheal illness in sheep and goats flocks (Shabana *et al*., 2017). The severity of *Salmonella* infection depends on bacterial factors including the serovar, virulence, and the antimicrobials susceptibility. However, there are more than 2600 identified *Salmonella* serovars are identified globally, recent serovars are still existing each year (Jajere, 2019). *Salmonella* serovars were worldwide distributed with the majority of some serovars in specific geographical areas and emergence of others by the time (Molla *et al*., 2003). Furthermore, the transboundary movements of animals, manufactured products and people lead to the pan and rapid distribution and existence of *Salmonella* serovars among countries (D'Aoust 1994; Plummer *et al*., 1995). The enterotoxin (*stn*) virulence gene is involved in *Salmonella* replication, and it is

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associated with the development of clinical manifestations especially diarrhea (Murugkar *et al*., 2003). Concerning the previous reports about the prevalence of salmonellosis in sheep and goats in Egypt, there were few studies. For example, the prevalence of salmonellosis was 3.6% and 3.3% in diarrheic lambs and goat kids, respectively in El-Menofiya and El-Kalubia governorates (Abd El-Twab *et al*., 2016), and 5.26% among diarrheic lambs in Behera province (Nasr *et al*., 2014). The limitations of the previous studies including, limited available data about the factors associated with occurrence and dissemination of salmonellosis especially in diarrheic sheep and goats at any age, belonging to different flocks in Giza governorate, and the molecular investigation of presence of *stn* gene in development of diarrhea. Without any doubt, providing necessary information about the disease epidemiology for stakeholders and farmers to implement the control and preventive measures to reduce the economic losses is of a great value. Therefore, the current study aimed to estimate the prevalence of salmonellosis in diarrheic sheep and goats belonging to Giza governorate in Egypt. Also, this study aimed to identify management factors associated with the disease occurrence. Furthermore, the study aimed to investigate the presence of *stn* gene in the isolated strains and its role in development of diarrhea, and to analyze the genetic relatedness between the isolated strains and other strains retrieved from GenBank based on partial codon sequence of *stn* gene.

MATERIALS AND METHODS

Study design and the characteristics of enrollment

A cross sectional survey study targeting sheep and goats suffering from diarrhea. The inclusion criteria of enrolled animals in the study including: (1) Sheep and goats suffering from diarrhea, (2) The enrolled animals housed in small-scale farms belonged to Giza governorate, (3) The enrolled animals belonged to mixed reared flocks of sheep and goats, and (4) The enrolled animals were of any age and sex. The exclusion criteria included non-diarrheic animals.

Questionnaire covering survey

The questionnaire survey covered investigated animals' individual data (age and sex), season during sampling, and flocks' management and biosecurity related factors. The management and biosecurity related factors included, applying of quarantine policy (isolation of newly purchased animals and presence of isolation pen for sick animals), flock size (>50 head), over the counter use of antimicrobials (use of antimicrobials was not under veterinary supervision), lack of disinfection (not regularly disinfect the feeding utensils and watering troughs), presence of indoor dogs, and presence of rodents in feed storage area. All data were collected in a form of a written questionnaire by the researcher through a personal interview conducted with flocks' owners. Distribution of animals in shown Table I.

Table I. Distribution of diarrheic sheep and goats per the investigated flocks.

Flocks (Farms) number	Diarrheic sheep	Diarrheic goats	Total
1	54	52	106
2	71	24	95
3	81	8	89
4	94	79	173
5	11	8	19
6	14	11	25
7	0	11	11

Sample size and collection

There were no available governmental data about the number of sheep and goats' flocks in Giza governorate, and this why we decided to take a convenient sample from seven flocks. Samples were collected from all diarrheic animals within the flocks. Therefore, no sample size was calculated.

A total number of 518 rectal swab, including 325 from diarrheic sheep and 193 from diarrheic goats were collected by a study researcher after evaluation of the clinical signs. Swabs were transported on ice to the laboratory within 1 h to be processed immediately.

Isolation and identification of Salmonella *spp.*

The current study is a part of a bigger project sub-targeting the prevalence of *Salmonella* infection in diarrheic sheep and goats. All procedures of isolation, biochemical identification, serotyping and molecular confirmation of *Salmonella* spp. were carried as previously described (Farouk *et al*., 2020).

Amplification of Salmonella enterotoxin (stn) *virulence gene*

DNA was extracted from the serotyped strains using the boiling method (Wani *et al*., 2003; Bhat *et al*., 2008). Briefly, a 1.5 mL of brain heart infusion broth (inoculated with single colony and overnight incubated at 37°C) was pelleted at 1200 xg for 10 min. The pellets were resuspended in 150 µL of sterile distilled water and

were subjected to lysis for 15min at 100°C using the heat thermo-blocker. After that, the lysate was pelleted by centrifugation at 1200 xg for 10 min. and the supernatant harboring bacterial DNA was used as a DNA template for PCR.

The DNA amplification of *stn* gene was accomplished in 25 µL mixture comprising 5 µL HotStarTaq® Master Mix (Qiagen, Germany) 3 µL DNA template 0.5 µL of each of the following F and R primer (with total 1 µL) 16 µL PCR-grade water; F: 5` TTG TGT CGC TAT CAC TGG CAA CC 3`; R: 5` ATT CGT AAC CCG CTC TCG TCC 3`. PCR grade water and the extracted nucleic acid of *Salmonella* Typhimurium (ATCC®14028™) reference strain were utilized as negative controls and positive controls for the *stn* gene of *Salmonella* spp., respectively. Primary denaturation was done at 95 °C for 3 min, followed by 25 cycles, each of denaturation at 94 °C for 1 min, annealing at 59 °C for 1min, extension at 72 °C for 1min, and final extension at 72 °C for 10 min (Murugkar *et al*., 2003). In order to detect specific bands at 617bp, the amplicons were separated through 1.5% agarose (wt/vol) gel electrophoresis, stained with 0.5 µg/mL ethidium bromide, screened under UV illumination, imaged with a GelDoc 1000 fluorescent imaging system (Bio-Rad), and analyzed by Gel-pro analyzer® version 4 (Media Cybernetics, Silver Spring, MD, USA).

Sequencing and phylogenetic analysis of stn *gene*

The amplified fragments (617bp) of *stn* gene of 9 *Salmonella* strains that randomly selected were representing 8 different serovars were purified using the QIAquick purification Kit (QIAGEN, Germany) according to the manufacturer's instructions and partially sequenced using Big Dye Terminator V3.1 sequencing kit (Applied Biosystems). The amplicons sequenced in the current study were blasted in Genbank and compared with the sequences available in the public domain using the NCBI BLAST server. Publicly available sequences were retrieved and downloaded from NCBI GenBank and aligned using CLUSTALW in Bioedit version 7.0.1.4. Phylogenetic analysis was performed with MEGA version 7 through neighbor-joining approach and the bootstrap was estimated from 1000 replicate.

Statistical analysis

The present study just aimed to evaluate the association between *Salmonella* infection and significance between variables. Therefore, the obtained data were analyzed by the Chi-square test due to the low sample size. The statistical analyses were done at the individual animal level and accomplished using SPSS 20.0 (IBM, USA). A *p*–value <0.05 was considered to be significant.

Ethical approval

All procedures in this study met the regulations of the Ethics of Cairo University-Institutional Animal Care and Use Committee (CU-IACUC), which received the study approval number: CU/II/F/97/18.

RESULTS

Prevalence of salmonellosis

A total number of 20 *Salmonella* spp. strains with an overall prevalence of 3.86% (20/518) were detected and identified via routine bacteriological isolation and biochemical identification. The positive *Salmonella* colonies appeared pink with a black center, and a highly transparent zone of reddish color on xylose lysine deoxycholate (XLD) media. Serotyping of the 20 strains revealed a wide range of the recovered serovars; whereas the prevalence of serovars among the investigated animals were 1.93% for *S.* Mississippi, 0.39% for each of *S.* Durham and *S.* Enteritidis, and 0.19% for each of *S.* Ferruch, *S.* Paratyphi A, *S.* Allerton, *S.* Bonariensis, *S.* Kottbus, and *S.* Stanleyville (Table II).

Table II. Prevalence of different *Salmonella* serovars among diarrheic sheep and goats.

Serovars	(Number) % of the serotyped *Salmonella* spp. strains		Total (518)
	Diarrheic sheep (325)	Diarrheic goats (193)	
S. Mississippi	(9) 2.77	(1) 0.52	(10) 1.93
S. Durham	(2) 0.62	(0) 0	(2) 0.39
S. Ferruch	(1) 0.31	(0) 0	(1) 0.19
S. Paratyphi A	(1) 0.31	(0) 0	(1) 0.19
S. Enteritidis	(0) 0	(2) 1.04	(2) 0.39
S. Allerton	(0) 0	(1) 0.52	(1) 0.19
S. Bonariensis	(0) 0	(1) 0.52	(1) 0.19
S. Kottbus	(0) 0	(1) 0.52	(1) 0.19
S. Stanleyville	(0) 0	(1) 0.52	(1) 0.19
Total	(13) 4	(7) 3.63	(20) 3.86

The prevalence of salmonellosis in different flocks (point prevalence) ranged from 0% to 7.55%. There was an evidence of existence of *S.* Mississippi serovar in between sheep and goats and in different three flocks, and *S.* Enteritidis serovar recovered from goats belonging to two different flocks. Furthermore, different serovars were recovered from different animals within the same flock, and there was no evidence of coexistence of different serovars in the same animal as shown in Table III.

Table III. Point prevalence of salmonellosis and distribution of the recovered serovars in different flocks.

Flock number	Diarrheic sheep			Diarrheic Goats			Total		
	Total number of sheep at risk	Number of confirmed cases	Recovered serovars (Number)	Total number of goats at risk	Number of confirmed cases	Recovered serovars (Number)	Total number of animals at risk	Number of confirmed cases	Overall prevalence %
1	54	6	*S.* Durham (2) *S.* Mississippi (4)	52	2	*S.* Mississippi (1) *S.* Enteritidis (1)	106	8	7.55
2	71	4	*S.* ParatyphiA (1) *S.* Mississippi (3)	24	0	-	95	4	4.21
3	81	0	-	8	0	-	89	0	0
4	94	3	*S.* Ferruch (1) *S.* Mississippi (2)	79	4	*S.* Bonariensis (1) *S.* Enteritidis (1) *S.* Kottbus (1) *S.* Stanleyville (1)	173	7	4.05
5	11	0	-	8	1	*S.* Allerton (1)	19	1	5.26
6	14	0	-	11	0	-	25	0	0
7	0	0	-	11	0	-	11	0	0
Total	325	13	-	193	7	-	518	20	3.86

Table IV. Statistical analysis of bivariable association between each of putative risk factors and occurrence of salmonellosis in diarrheic sheep and goats.

Variable	Levels	*Salmonella* isolation		Pearson value	Overall P-value (≤0.05)
		Positive	Negative		
Presence of quarantine (isolation of new purchased animals, and presence of isolation pen for sick animals)	Yes=1	0	119	6.204	0.013
	No=0	20	379		
Flock size (>50 head)	Yes=1	12	283	0.079	0.779
	No=0	8	215		
Over the counter use of antimicrobials (use of antimicrobials was not under veterinary supervision)	Yes=1	20	379	6.204	0.013
	No=0	0	119		
Lack of disinfection (not regularly disinfect the feeding and watering troughs)	Yes=1	20	398	4.977	0.026
	No=0	0	100		
Presence of indoor dogs	Yes=1	9	195	0.275	0.600
	No=0	11	303		
Presence of rodents in feed storage area	Yes=1	20	398	4.977	0.026
	No=0	0	100		
Season	Winter=1	12	195	0.275	0.600
	Summer=0	8	303		
Gender	Male=1	5	145	0.158	0.691
	Female=0	15	353		
Age	< 1 year=0	7	223	1.128	0.569
	1.1-3 year=1	5	85		
	> 3 year=2	8	190		

The management of salmonellosis in diarrheic sheep and goats

The management factors which were found to be associated with *Salmonella* infection included non-applying of quarantine policy (absence of isolation of newly purchased animals, and absence of isolation pen for sick animals) and over the counter use of antimicrobials (use of antimicrobials was not under veterinary supervision) [*P-value* = 0.013] where the disease prevalence was likely higher in farms which lack quarantine policy and disregard to antimicrobials use than the applying and regarding ones (5.0% to 0.0%). In addition, lack of regular disinfection of feeding utensils and water troughs and presence of rodents in feed storage area [*P-value* = 0.026] whereas

Salmonella infection was more common in farms which lack disinfection and gain the rodents access to feed store (4.8% to 0.0%). In addition, there was no any statistical significant differences in the other tested factors as shown in Table IV.

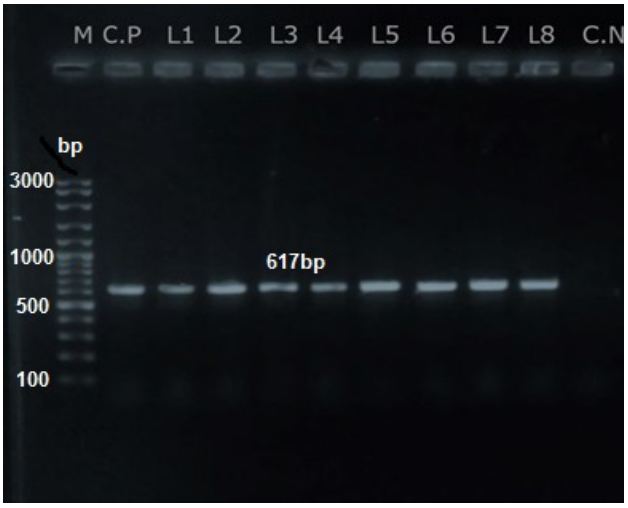

Fig. 1. Agarose gel electrophoresis of PCR products of *stn* gene. Lane M, molecular weight marker (100-3000bp); Lane C.P, control positive of *S.* Typhimurium reference strain (ATCC. 14028); Lanes 1-8, positive samples with band of amplicon size 617bp; Lane C.N, control negative (sterile nuclease free water).

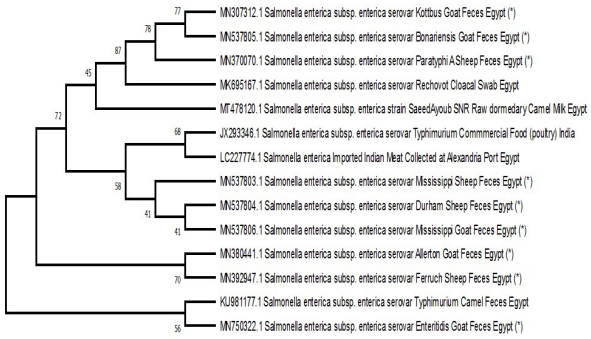

Fig. 2. Neighbor-joining tree showing the relationship between the nucleotide sequences of the partial coding regions of *stn* gene. The investigated sequences were remarked by Astros (*). The evolutionary analysis was performed with MEGA7 software. Accession number, serovar name, isolation source, country,(*): under study.

stn *gene*

The *Salmonella* enterotoxin *stn* gene was detected in the 20 recovered strains (Fig. 1). The nine *stn* gene partial codon sequences were deposited in the Genbank. The accession numbers of deposited sequences were MN307312, MN370070, MN380441, MN392947, MN537803, MN537804, MN537805, MN537806, and MN750322. Sequencing and phylogenic analysis of *stn* gene revealed the relatedness of the isolated strains to the other *Salmonella* spp. strains, which were retrieved from GenBank (Fig. 2).

DISCUSSION

Clinical diagnosis of salmonellosis is difficult in sheep and goats due to the non-specific clinical symptoms (OIÉ, 2000). Clinical diagnosis of salmonellosis in the investigated cases was confirmed by *Salmonella* spp. isolation through laboratory diagnosis (routine bacteriological isolation and identification of *Salmonella* spp.). The present study revealed that the prevalence of *Salmonella* infection in diarrheic sheep and goats belonging to Giza governorate in Egypt with an overall proportion 3.86% (20/518) and a wide range of prevalence percentages between the investigated flocks, which ranged from 0 to 7.55 (Table III). In addition to the obtained results, the disease prevalence is almost nearly similar to the findings of the previous study where the overall isolation rate was 3.49% among both diarrheic lambs and goat kids (Abd El-Twab *et al*., 2016). The variation in prevalence between different flocks as shown in Table III and the differences between the present study and the other previous studies may be attributed to the differences of the geographic variation, time encountered of the study, management factors, and hygiene practices (Alam *et al*., 2009; Jones, 2011; Vanselow *et al*., 2017), and the overall low recovery rate and non-recovery from some investigated flocks may be correlated to other pathogens as several agents may be involved in etiology of enteritis and diarrhea (Nasr *et al*., 2014).

The isolated *Salmonella* strains showed a broad range of serovars, encompassing Mississippi, Durham, Ferruch, Paratyphi A, Enteritidis, Allerton, Bonariensis, Kottbus, and Stanleyville. According to Table II, *S.* Mississippi, *S.* Enteritidis, and *S.* Durham were the most common serovars among the recovered *Salmonella* strains. Our findings are almost different from the previous studies that reported the strains isolated from sheep and goats in Egypt where the recovery percentages of Erhoma 2007 study (Erhoma, 2007) were 1.1% and 0.79% for *S.* Heidelberg and *S.* Paratyphi A, respectively, *S.* Bardo and *S.* Kentucky recovery rates were 0.53%, and 0.26% for *S.* Paratyphi B, *S.* Agoma, *S.* Braenderup, *S.* Enteritidis, *S.* Typhi and *S.* Typhimurium. Moreover, *S.* Typhimurium, *S.* Bardo and *S.* Enteritidis were the only identified and isolated serovars from diarrheic lambs and goat kids (Abd El-Twab *et al*., 2016), and serotying of *Salmonella*

isolated from lambs suffered from enteritis in another study revealed that 2 isolates of each *S.* Typhimurium, *S.* Enteritidis and untypable serovars (Nasr *et al*., 2014). Interestingly, the existence of recovered *S.* Enteritidis and *S.* Mississippi serovars in different flocks (Table III) may be elucidated by presence of a common source of infection in Giza locality which may attributed to the contaminated animal feedstuffs and forages, as well as the recovery of different serovars within the same flock may be due to presence of different sources of infection. Concerning the previous isolation history of the recovered serotypes, *S.* Durham, *S.* Stanleyville, *S.* Mississipi, *S.* Kottbus and *S.* Paratyphi A were responsible for human infections (Ball, 1991; Gästrin *et al*., 1972; Konadu *et al*., 1996; Tennant *et al*., 2010; Wollin, 2007), and *S.* Kottbus, *S.* Ferruch and *S.* Bonariensis were recovered from chickens (Hassan *et al*., 2016; Osman, 2005). Furthermore, *S.* Enteritidis was identified as the most prevalent serovar among diarrheic sheep and goats worldwide, and it is of a great public health importance (Abd El-Twab *et al*., 2016; CFSPH, 2005; Smith-palmer *et al*., 2003), and *S.* Allerton serovar was previously isolated from kareish cheese sample and from diarrheic goat in the present study giving rise to concern that goat may be a threat to public health as a result of dissemination and shedding of microorganism in milk (Abd El-Atty and Meshref, 2007). Remarkably, the evidence of recovery of *Salmonella* spp. serovars from different sources may provide valuable data about probability of the transmission cycle and dissemination of salmonellosis between different sources, and there is an association between the prevalent salmonellae serovrars in small ruminants and the serovars implicated in human salmonellosis in different countries due to several factors that influence occurrence of pathogen in food commodities during consumption, including food processing technologies, and the final preparation of food. So that the current study spotlights on the ability of the investigated animals in maintenance of variant serovars in Egypt.

The statistical analysis of association between the putative risk factors and the overall development of salmonellosis (risk) in diarrheic sheep and goats revealed that there were potential significant associations with the following: absence of quarantine policy (absence isolation of newly purchased animals, and absence of isolation pen for sick animals), lack of regular disinfection of feeding utensils and water troughs, presence of rodents in feed storage area, and over the counter use of antimicrobials without veterinary supervision or prescription. In addition to that, there was no of significant difference with other variables, and these findings will be elucidated and discussed. Regarding quarantine, it is considered one of the risk mitigation options that can be applied to reduce *Salmonella* infection, and these findings in the line with the pervious study (Evans, 1996), which clarified that, it is important to apply the quarantine policy as the risk increase with repeated introduction of animals to the farms. The proper cleaning and the routine disinfection of feeding utensils are expected to reduce salmonellosis in calves herd (Younis *et al*., 2009; Terazi and Abo-Shehada, 2015). In the present study, lack of disinfection of feeding and watering utensils is associated with development of salmonellosis in diarrheic sheep and goats, so this emphasize the importance of disinfection in reducing *Salmonella* infection in different ruminants species. Furthermore, rodents gaining access to feed store are important source of infection; rodents' droppings can contaminate feed where the rodents can acquire their infection from various sources (Oosterom, 1991). However, there was no evidence of significance with gender, season, and age due to low sample size as supposed by Lesaffre (Lesaffre, 2008), it is clear from Table IV the distribution of the disease prevalence was higher in females than males, and this may be attributed to hormones regulating sex and hormonal changes during gestation period which may have interacted with the immunity (Smith *et al*., 2004). Moreover, the disease prevalence was higher in winter than in summer, and these findings are in the line with Rodriguez *et al*. (2006) who reported that the highest incidence of salmonellosis occurs during the late fall (October-December) instead of the summer. In correlation of the disease prevalence with the age groups, it was found that the highest rate was in the age group >3 years, followed by <1 year, and the least one was in 1 year and1 month-3 years group, and these findings can be justified by the previous study which reported that age was not associated with *Salmonella* fecal shedding (Fossler *et al*., 2005). Accordingly, the identified management factors associated with development of salmonellosis in diarrheic sheep and goats were mostly related to the poor flock management and uncontrolled hygienic and biosecurity measures, and these findings spotlight on the critical specified hygienic and biosecurity measures which should be raised and applied in order to prevent and control the disease occurrence. Furthermore, over the counter use of antimicrobials is one of the identified risk factors, which may be incriminated in the development of antimicrobial resistance pathogens.

Remarkably, the *stn* gene was detected in all 20 strains, and this is in agreement with Thung *et al*. (2018), and 9 randomly selected strains were partially sequenced. *Salmonella* serovars can be introduced into sheep and goats flocks from many different sources. We illustrated in this study a robust phylogeny (Fig. 2) of *Salmonella* subspecies using the partial codon sequences of enterotoxin (*stn*) gene from 9 strains included in this study and 5 isolates retrieved

from GenBank. Analysis of these sequences showed that sequence of *S*. Kottbus, *S*. Bonariensis, *S*. Paratyphi A, *S*. Rechovot and Saeed Ayoub SNR strain were in the same clusters in which, sequence *S*. Kottbus and *S*. Bonariensis have polytomy to each other, and there were closely related to sequence *S*. Paratyphi A. Furthermore, *stn* partial codon sequences of *S*. Kottbus, *S*. Bonariensis and *S*. Paratyphi A strains were closely related to sequences of *S*. Rechovot and Saeed Ayoub SNR strains that return to the use of fecal matter of animals and poultry in fertilizing the ground leading to vegetables contamination, which reflect on hay contamination in the farm where a lot of hay growers use animals and chicken based fertilizers. In addition, sequences of *S.* Typhimurium (poultry strain), *Salmonella* enterica (LC227774), *S*. Mississippi (sheep strain), *S.* Durham and *S*. Mississippi (goat strain) were in the same clusters and closely related to each other. The sequences of *S.* Durham and *S*. Mississippi (goat strain) have polytomy to each other that explains the use of contaminated feed is one such source, and as a consequence feed companies place a high emphasis on good quality control of feed ingredients, effective feed pelleting and the biosecurity of feed mills to reduce contamination and, if possible, eliminate this route of exposure. Additionally, importation of contaminated meat represents a potential risk of introducing and maintenance of new serovars into the importing country. Moreover, sequences of *S*. Allerton and *S.* Ferruch have polytomy to each other, sequences of *S.* Typhimurium (camel strain) and *S.* Enteritidis have polytomy, and all 4 sequences were closely related to each other. This scenario emphasizes the potential role of imported camel as a source of exotic emerging *Salmonella* serovars.

There was only one sequence (MN537803) of *Salmonella* Mississippi which was recovered from diarrheic sheep that belonged to the farm had a cattle herd; however, the discussed phylogeny findings revealed a relation between sequences of different sources such as cattle, camel and birds. This may be justified by usage of untreated animal dung as manure. Therefore, serious preventive and control measures must be adopted in the treatment and disposal of animal dungs to avoid contamination of the food chain in both animals and humans (Umeh and Enwuru, 2014). Moreover, dissemination of enteric bacterial pathogens such as *Salmonella enterica subsp. diarizonae* between dogs and sheep was previously reported (Chatzopoulos *et al*., 2016), and this possessing a risk of disease transmission to the in contact persons such as shepherds, sheep breeders and veterinarians; however there was no significance between the presence of indoor dogs in flocks and occurrence of salmonellosis in the present study as shown in Table IV.

Fascinatingly, the phylogenetic analysis of *stn* gene sequence of the retrieved strains and the selected serovars from GenBank revealed a high genetic relatedness among the different sequences of different serovars retrieved from various sources, which shed the limelight on the importance of quarantine, biosecurity and hygienic measures. Therefore, the current study proved that there is a robust association between the identified risk factors and analytics of phylogeny, which clarified the prospective preventive and control measures of salmonellosis. Thus, strict quarantine should be applied on the imported living animals and the products of animal origin at country ports with bacteriological examination to detect and eliminate *Salmonella* carriers as well as adequate hygienic and biosecurity measures should be implemented at farm levels to prevent and control the occurrence of salmonellosis. Additionally, more attention should be paid to breakdown the transmission cycle of such pathogen between different ecosystems and awareness must be increased between shepherds and sheep breeders to protect themselves during handling animals. Finally, the limitations of the current study including, small sample size, the study encountered flocks of Giza governorate only, and under presentation of young aged animals among the all investigated animals.

CONCLUSION

The present study provides valuable data on the prevalence of salmonellosis among diarrheic sheep and goats in mixed reared flocks in Giza governorate, Egypt with emphasis on the robust phylogeny of partial coding sequence of *Salmonella* (*stn*) gene. Also, the study identified the management factors associated with development of salmonellosis in diarrheic animals of the investigated flocks. The disease-associated factors were absence of isolation of newly purchased animals, absence of isolation pen for sick animals, over the counter use of antimicrobials, lack of disinfection of feeding utensils and water troughs, and presence of rodents in feed storage area. The identified disease-associated factors and the illustrated phylogenetic tree will be useful for implementation of control measures in order to limit the dissemination of *Salmonella* in between different hosts and sources.

Statement of conflict of interest

The authors have declared no conflict of interest.

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