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ISOLATION AND IDENTIFICATION OF *SALMONELLA* SPP. FROM DIARRHEIC SHEEP AND GOATS IN EGYPT

By

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ABSTRACT

Diarrhea remains one of the problems in sheep and goats causing economic losses. In the current study, a total number of 346 rectal swabs were collected at different seasons from diarrheic sheep and goats of different age groups and from different localities. Rectal swabs were bacteriologically examined for isolation of *Salmonella*. Five *Salmonella* isolates were recovered from 149 diarrheic goats and three isolates from 197 diarrheic sheep with isolation rate 3.36% and 1.52%, respectively. The isolation rate was correlated to some factors such as season, age and the localities included in the study. Where the highest seasonal rate was in Spring season followed by winter; the highest rate was in animals belonging to (1-2.5 years) age group followed by (0 up to 1 year) age group while the lowest one belongs to (>2.5 years) age group and the highest isolation rate among the localities included in the study was in El-Fayoum governorate followed by Giza governorate. All recovered isolates were biochemically and serologically characterized and confirmed by amplification of *invA* gene using PCR technique. The serotyping results revealed that the 8 isolates belonged to 6 serotypes: *Salmonella* Bonarensis, *Salmonella* Paratyphi A, *Salmonella* Ferruch ,*Salmonella* Kottbus, *Salmonella* Stanleyville and *Salmonella* Enteritidis, while 2 isolates were not typed and the PCR technique confirmed that all *Salmonella* isolates carried the *invA* gene.

Key words:

Diarrhea, sheep, goats, *Salmonella* and *invA*.

INTRODUCTION

Sheep and goats are the most important livestock that are noted for their ability to convert low opportunity cost feed into valuable products as meat, milk, fiber, manure and hides **(FAO, 1986).** Salmonellosis is worldwide infectious disease in animals causing losses in livestock and in humans, causing serious public health problems **(Montagne *et al.* 2001).** The diagnosis of salmonellosis is difficult in the living animal due to the non-specific clinical symptoms and necropsy findings. Apparent diagnosis has to be confirmed by the isolation of the organism either from tissues collected aseptically at necropsy or from feces, rectal swabs or environmental samples **(OIÉ, 2010).** PCR amplification of *invA* gene was rapid, sensitive and specific for detection of *Salmonella* in many clinical samples **(Lampel *et al.* 2000).**

MATERIAL AND METHODS

Animals:

A total number of 197 diarrheic sheep and 149 diarrheic goats presented to different Clinical Veterinary Units at different governorates, farm on Cairo- Alexandria Desert Road and farm in Damietta during the period from December 2015 till July 2016 were used in this study.

Samples:

Rectal swabs were collected from 197 diarrheic sheep and 149 diarrheic goats of different age using sterile cotton swabs and transferred to the laboratory on ice box for bacteriological examination.

Procedure for isolation and identification of *salmonellae*:

The samples were examined for *Salmonella* isolation and identification according to **ISO 6579 (2002)** method. The identification of the isolated *Salmonella* was done on the basis of colonial morphology, staining characters and biochemical reaction **(Quinn *et al*., 2002)**. The isolates were biochemically confirmed using Rapid-one kit. Typical *Salmonella* isolates were serotyped using Denka-Seiken (Tokyo, Japan) antisera following Kauffman-white scheme **(Poppof, 2001)** in the Serology Unit, Animal Health Research Institute, Dokki, Giza. After complete identification, the bacterial isolates were stored at -20°C in brain heart infusion broth containing 16% glycerol for long term preservation.

Identification of *Salmonella* isolates by Polymerase chain reaction):

DNA extraction: DNA was extracted from bacterial colonies using QIAamp DNA mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations.

Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56˚C for 10 min. After incubation, 200 µl of 100% ethanol added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide primer: Primer used was supplied from (Metabion Company, Germany) is listed in (Table 1).

PCR amplification:

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

Analysis of the PCR products:

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

Table (1): Primer sequence, target gene, amplicon size and cycling condition

Target gene	Primer sequence	Amplified Segment (bp)	Primary Denatur-ation	Amplification(35 cycle)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
invA	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	284	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec	72˚C 30 sec	72˚C 7 min.	Oliveira *et al* (2003)

RESULTS

A total number of 5 *Salmonella* isolates were recovered from 197 diarrheic sheep and 3 *Salmonella* isolates were recovered from 149 diarrheic goats with isolation rates 1.52% and 3.36% in sheep and goats, respectively. All recovered *Salmonella* isolates colonies were

bright pink with/without black center on XLD and bright pink on BGA agar medium and appeared as gram negative coccobacilli arranged singly or in pairs. Biochemical identification of all recovered *Salmonella* isolates revealed that, on oxidase strip test *Salmonella* not produce change in color; on catalase test *Salmonella* result in effervescence; on urease test produce yellow color; on TSI agar *Salmonella* produce yellow butt and red slant with /without H_2S production; on LIA *Salmonella* produce purple color with/without H_2S production; on Simmon's citrate agar *Salmonella* utilized citrate so result in change of media color into blue. Whereas, on indole and voges proskauer all isolates were negative and on methyl red all isolates were positive. All *Salmonella* isolates were further biochemically confirmed using Rapid one test. After the biochemical confirmation, all isolates were subjected to serological confirmation. The results of serological identification revealed that they were 6 different *Salmonella* strains and two not typed strains. The strains recovered from sheep were *Salmonella* Paratyphi A, *Salmonella* Ferruch and not-typed *Salmonella* isolate, while the recovered strains from goats were *Salmonella* Bonariensis, *Salmonella* Kottbus, *Salmonella* Stanleyville, *Salmonella* Enteritidis and not-typed *Salmonella* isolate. The result of the amplification of *invA* gene using PCR revealed that, all *Salmonella* strains were positive for *invA* gene (100%) and showing amplicon size (284bp) Fig. (1)

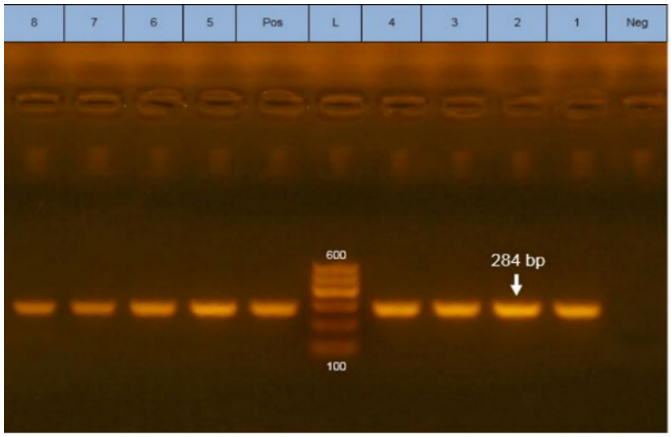


Fig. (1): Agarose gel electrophoresis of PCR for detection of *invA* virulence gene in 8 *Salmonella* isolates.

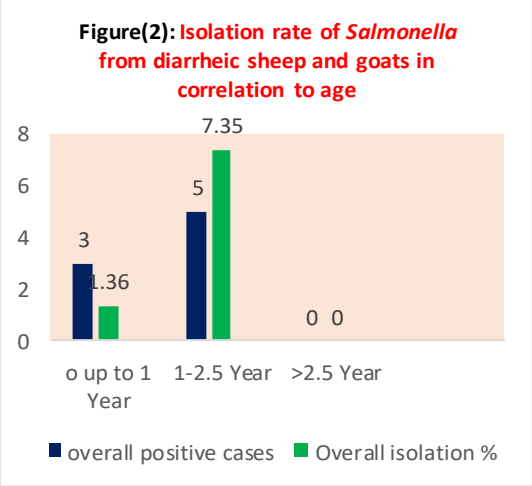
Lane L: Molecular weight marker, 100-600 bp.

Lane 1-8: Positive samples with band of amplicon size at 284bp.

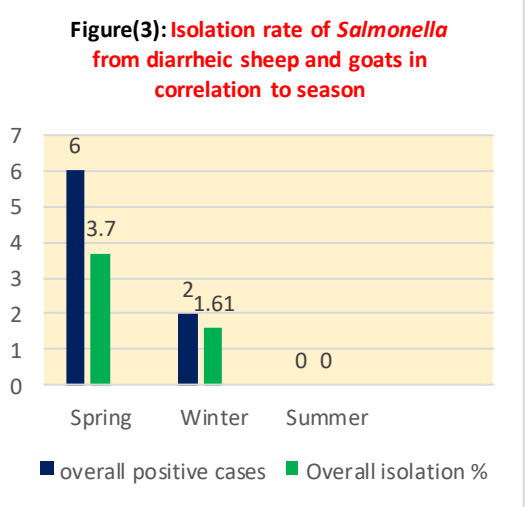
Lane Neg: Negative control.

Lane Pos: Positive control of *invA* gene with band of amplicon size at 284bp.

The *Salmonella* isolation rate was correlated to some epidemiological factors such as age, season and localities. It was found that, the highest isolation rate from the diarrheic sheep and goats belonging to (1-2.5 years) age group followed by (0 up to 1 year) age group while the lowest one belongs to (>2.5 years) age group (Table2), Fig. (2); the highest isolation rate from diarrheic sheep and goats is in spring season followed by winter season while the lowest one is in summer season (Table 3), Fig. (3) and the highest isolation rate of *Salmonella* from diarrheic sheep and goats is in El-Fayoum governorate followed by Giza governorate while the lowest one is in Menofiya, Damietta and Beni-Suef governorates where it was 0% (Table, 4) and Fig. (4).



Figure(2): Isolation rate of *Salmonella* from diarrheic sheep and goats in correlation to age



Figure(3): Isolation rate of *Salmonella* from diarrheic sheep and goats in correlation to season

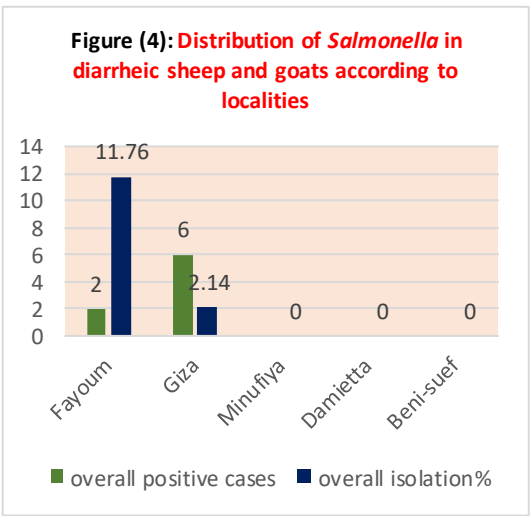


Figure (4): Distribution of *Salmonella* in diarrheic sheep and goats according to localities

Table (2): Isolation rate of *Salmonella* from diarrheic sheep and goats in correlation with age.

Age (Year)	Total number of sheep	*Salmonella* Isolation Rate		Total number of goats	*Salmonella* Isolation Rate		Overall positive cases	Overall Isolation %
		Number of positive cases	%		Number of positive cases	%		
Up to 1	105	1	0.95	115	2	1.74	3	1.36
1-2.5	47	2	4.26	21	3	14.29	5	7.35
>2.5	45	0	0	13	0	0	0	0
Total	197	3	1.52	149	5	3.36	8	2.31

Table (3): Isolation rate of *Salmonella* from diarrheic sheep and goats in correlation with seasons.

Season	Total number of sheep	*Salmonella* Isolation Rate		Total number of goats	*Salmonella* Isolation Rate		Overall positive cases	Overall Isolation %
		Number of positive cases	%		Number of positive cases	%		
Spring	95	2	2.11	67	4	5.97	6	3.70
Summer	29	0	0	31	0	0	0	0
Winter	73	1	1.36	51	1	1.96	2	1.61
Total	197	3	1.52	149	5	3.36	8	2.31

Table (4): Isolation rate of *Salmonella* from diarrheic sheep and goats in correlation with localities.

Locality	Total number of sheep	*Salmonella* Isolation Rate		Total number of goats	*Salmonella* Isolation Rate		Overall positive cases	Overall Isolation %
		Number of positive cases	%		Number of positive cases	%		
Giza	**163**	**2**	**1.23**	**118**	**4**	**3.39**	**6**	**2.14**
Fayoum	**14**	**1**	**7.14**	**3**	**1**	**33.33**	**2**	**11.76**
Minufiya	**1**	**0**	**0**	**4**	**0**	**0**	**0**	**0**
Damietta	**11**	**0**	**0**	**21**	**0**	**0**	**0**	**0**
Beni-Suef	**8**	**0**	**0**	**3**	**0**	**0**	**0**	**0**
Total	**197**	**3**	**1.52**	**149**	**5**	**3.36**	**8**	**2.31**

DISCUSSION

Sheep and goats are the most important livestock that are noted for their ability to convert low opportunity cost feed into valuable products such as meat, milk ,fiber, manure and hides **(FAO, 1986)**. *Salmonella* infection causes substantial economic loss resulting from mortality, morbidity and poor growth with hazard of transmitting food poisoning with gastroenteritis to human and represents a serious problem for the food industry **(Khan *et al*., 2007).** The present study was conducted to detect *Salmonella* isolates obtained from diarrheic sheep and goats, to determine the isolation rate of *Salmonella* among diarrheic sheep and goats and to correlate the isolation rate with some epidemiological factors. It was found that, the overall isolation rate of *Salmonella* from diarrheic sheep and goats from different localities in Egypt were 1.52% and 3.36% respectively. The isolation rate of *Salmonella* among diarrheic goats is higher than in diarrheic sheep and this is not compatible with the findings of **Pao *et al.* (2014)** who investigated that the sheep had a significantly higher prevalence of *Salmonella* than goats. This may be due to different time and localities at and from which diarrheic sheep and goats were examined. The recorded percentage (1.52%) among the examined diarrheic sheep is much lower than those reported by **Amira (2016)** who found that the percentage in diarrheic lambs was 3.6% and **Nasr *et al*., (2014)** who found the prevalence of *Salmonella* associated with diarrhea in lambs and sheep flocks in Behera province was 5.26%. The lower isolation

rate encountered in this study may be due to time or regional differences related to small number of cases collected as multiple fecal samples are required for higher rates of *Salmonella* isolation **(Duijkeren *et al*., 1995)**. The determined isolation rate (3.36%) among the examined diarrheic goats is nearly similar to those reported by **Amira (2016)** who found the prevalence of *Salmonella* species in sporadic cases of diarrheic goat's kids presented in Governmental Veterinary Clinics in El-Menofiya and El-Kalubia Governorates, Middle-Delta, Egypt was 3.3%. While much higher than reported by **Mahmood *et al*., (2014)** who determined the prevalence of *Salmonella* in the feces of diarrheic adult goats under field conditions and found that an overall prevalence of salmonellosis in diarrheic adult goats was 0.1%. In the present study some epidemiological factors were correlated with the isolation rate such as, localities; age and season. The findings of isolation rate of *Salmonella* from diarrheic sheep and goats in correlation with localities showed that highest rate of *Salmonella* infection among sheep and goats is in El-Fayoum governorate followed by Giza governorate while the lowest one is in Menofiya, Damietta and Beni-Suef governorates where it is 0% (Table, 4), Fig. (4). These differences of percentage among different localities may be due to time or regional differences as supported by **Duijkeren *et al*., (1995)**.The findings of seasonal isolation rate of *Salmonella* from diarrheic sheep and goats showed that the highest isolation rate is in spring season followed by winter season while the lowest one is in summer season (Table 3). These results disagreed with **Mahmoud *et al.* (2014)**; **Pao *et al.* (2014) and Bosilevac *et al.* (2015)** where, they investigated that, the prevalence of *Salmonella* did not exhibit any seasonal variation. These variations may be due to exposure to stressors in spring and winter seasons such as transport, starvation, parturition, overcrowding in communal grazing land and dips which activate latent infections and favor rapid spread of the disease **(Kusiluka and Kambarage, 1996)**. The most of *Salmonella* isolates occurred in the spring may be due to the lambing season, changing weather patterns and often the arrival of hot humid weather which all are major stressors. The age distribution of *Salmonella* in sheep and goats in this study revealed that, the highest rate is in both sheep and goats belonging to (1-2.5 years) age group followed by (0 up to 1 year) age group while the lowest one belongs to (>2.5 years) age group (Table 4), Fig. (4). these findings conclude that neonates are more susceptible to *Salmonella* infection than adults. This may be due to immune suppression in young animals which may result from parasitic infestation. Serological typing of *Salmonella* strains recovered from examined diarrheic sheep and goats, revealed presence of 6 *Salmonella*

different serotypes namely: *Salmonella* Bonariensis, *Salmonella* Kottbus, *Salmonella* Stanleyville and *Salmonella* Enteritidis (from goats); *Salmonella* Paratyphi A and *Salmonella* Ferruch (from sheep) and 2 untypable strains (one from each species). *Salmonella* Bonariensis was isolated in this study from a female pregnant goat of 1.5 years old presented to Educational Veterinary Hospital, Faculty of Veterinary Medicine, and Cairo University at Giza governorate in March 2016. This goat subjected to clinical examination and the investigated data are, it suffered from watery diarrhea of offensive odour, severe dehydration, acetone mouth smell odour, subnormal body temperature (36°C) and finally died. The same serovar resulted in development of rhabdomyolysis and acute renal failure in human **(Laqarde *et al*., 1989)**. This serovar also isolated from 10 human cases around the world in 2012, however, it had not been reported before in any non-human sources according to the data of Laboratory-confirmed *Salmonella* isolates from non-clinical non-human sources the National Veterinary Services Laboratories (NVSL) for typing **(CDC, 2012)**. *Salmonella* Stanleyville was isolated from a female goat, its age about 2 months suffered presented to Educational Veterinary Hospital, Faculty of Veterinary Medicine, Cairo University at Giza governorate in May 2016. This goat subjected to clinical examination and the investigated data are, it suffered from pasty diarrhea, fever (body temperature 41.2°C), nasal discharge, high respiratory rate, and cough and by chest auscultation revealed crackling sound. *Salmonella* Kottbus was isolated from a female goat of 1-year-old presented to one of the Clinical Veterinary Units at Giza governorate in June 2016. It suffered from pasty diarrhea with normal body temperature range. *Salmonella* Enteritidis was isolated in this study from male goat of 9-month age presented to one of the Clinical Veterinary Units at Giza governorate in June 2016. It suffered from pasty diarrhea with normal body temperature range. *Salmonella* Paratyphi A was isolated in this study from a male lamb of 1-month age reared at farm at Alexandria Deseret Road in Giza governorate in April 2016. It suffered from pasty diarrhea and with normal body temperature range. *Salmonella* Ferruch was isolated in this study from a female sheep about 2 years old and presented to one of Clinical Veterinary Units in Giza governorate in May 2016. It suffered from pasty diarrhea with normal body temperature range. The *invA* is chromosomally located virulence gene known as the Salmonella pathogenicity island 1 (SPI1) and encodes a protein in the bacteria inner membrane which is necessary for invasion of deeper tissue **(Khan *et al*., 1999, Jennifer *et al*., 2003 and Singer *et al* ,.2006)**. In this study, PCR assay demonstrated that all *Salmonella* spp.

isolates carried the *invA* gene. These results are closely near to other findings of previous studies reporting that *invA* gene is present in all *Salmonella* isolates **(Rahn *et al*., 1992, Jamshidi *et al*., 2002, Skyberg *et al.* 2006, Zahraei *et al*., 2006 and Mir *et al*. 2010)**. However, are not compatible with the findings of **Barman *et al*. (2013)** who reported that *invA* gene present in 63.41% of *Salmonella* isolates.

CONCLUSION

In conclusion salmonellosis is still a problem, where the results of this study revealed the existence of *Salmonella* serovars in sheep and goats which reflects the importance of this organism in small ruminants' diseases and the importance of small ruminants as a source of infection for human and animals. Accordingly, sheep and goats' salmonellosis needs more investigation to figure out the situation of the disease in Egypt and to establish a policy for control and eradication of this disease which has a great economic and public health importance.

REFERENCES

Amira Mohamed Ali Rizk (2016): Molecular studies on multidrug resistant *Salmonella* species isolated from newly born ruminants. A thesis submitted for the Ph.D. degree in Veterinary Medical Sciences (Bacteriology, Immunology and Mycology) - Benha University.

Barman, G.; Saikia, G. K.; Bhattacharyya, D. K. and Roychoudhury, P. (2013): Detection of virulence genes of *Salmonella* by polymerase chain reaction. Indian Journal of Animal Science. 83 (5).

Bosilevac, M. J.; Gassem, A. M..; A. Alsheddy, A. I.; Almiman, A. S. .; Al-Mohizea, S. I. ; Alowaimer, A. and Koohmaraie, M. (2015): Prevalence of *Escherichia* coliO157:H7 and *Salmonella* in Camels, Cattle, Goats, and Sheep Harvested for Meat in Riyadh. Journal of Food Protection, (78): 89-96.

Centers for Disease Control and Prevention (CDC) (2012): National Enteric Disease Surveillance, *Salmonella* Annual Report. Atlanta, Georgia: US Department of Health and Human Services. 1-63.

Duijkeren, E.; Flemming, C. and Van Oldruitenborgh Oosterbaan, M. M. (1995): Diagnosing salmonellosis in horses: culturing of multiple versus single faecal samples. Vet. Quarterly. 17:63-66.

FAO (1986): Small ruminant production in the developing countries. In: proceeding of the Expert consultation held in Sofia, Bulgaria 58: 138.

International Organization for Standardization (ISO-6579) (2002): Microbiology of food and animal feeding stuffs. Horizontal method for the detection of *Salmonella* spp. (4th ed.): International Organization for Standardization.

Jamshidi, A.; Bassami, M. R. and Afshari-Nic, S. (2002): Identification of *Salmonella* serovars typhimurium by a multiplex PCR-Based assay from Poultry carcasses in Mashhad-Iran. Int. J. Vet. Res., 3 (1): 43- 48.

Jennifer, D.; Boddicker, B.; Knosp. M. and Bradley, D. J. (2003): Transcription of the *Salmonella* Invasion Gene Activator, *hilA* requires *hilD* Activation in Absence of Negative Regulators. J. Bacteriol., Pp: 525-533.

Khan, A.A.; Melvin, C.D. and Dagdage, E.B. (2007): Identification and molecular characterization of *Salmonella* spp. from unpasteurized orange juices and identification of new serotype *Salmonella* strain *S*. enteric serovars. Food Microbiology. 24(5): 39-43.

Khan, A. A.; Nawaz, M. S.; Khan, S. and Sernigelia, C. E. (1999): Detection of multidrug resistant *Salmonella* typhimurium DT104 by Multiplex polymerase chain reaction. FEEMS Microliology. Letters, 182: 355-360.

Kusiluka, L. and Kambarage, D. (1996): Diseases of small ruminants: A Hand book. Common Diseases of Sheep and Goats in Sub- Saharan Africa. Edited and published by VETAID- Centre of Tropical Veterinary Medicine. ISBN: 0952229951.

Lampel, K. A.; Orlandi, P. A. and Kornegy, L. (2000): Improved template preparation for PCR based assay for detection of food borne bacterial pathogens. Journal of Applied Environmental Microbiology. (66): 4539-4542.

Laqarde, C.; Peyronnet, P.; Denis, F.; Benzakour, M. and Leroux-Robert, C. (1989): *Salmonella* bonariensis salmonellosis, rhabdomyolysis, and acute renal failure. Nephron Journal. 35 (2): 179-80.

Mahmood, A. K.; Khan, M. S.; Khan, M. A.; Khan, M. A. and Bila, M. (2014): Prevalence of *Salmonella* in diarrheic adult goats in field conditions. The Journal of Animal and Plant Sciences, (24): 98-102.

Mir, I.A.; Wani, S.A.; Hussain, I.; Qureshi, S.D.; Bhat, M.A. and Nishikawa, Y. (2010): Molecular epidemiology and in vitro antimicrobial susceptibility of *Salmonella* isolated from poultry in Kashmir. Rev. SciTech. 29 (3):667-86.

Montagne M., Martel A., Le Moual H. (2001): Characterization of the catalytic activities of the PhoQ histidine protein kinase of *Salmonella* enterica serovar Typhimurium. J. Bacteriol. 183.

Nasr, M.; Hammouda, H. A.; Bakeer, N. M. and Omar, A. A. (2014): Epidemiological, Clinical and Bacteriological Studies on Bacterial Lamb Enteritis at Behera Province, Egypt. Alexandria Journal of Veterinary Sciences. (43): 8-16.

Office International des Épizooties (OIÉ) (2010): OIE Terrestrial Manual 2010 - Salmonellosis, Chapter: 2.9.9.

Olivera, S.D.; Rodenbusch, C.R.; Ce, M.C.; Rocha, S.L.S. and Canal, C.W. (2003): Evaluation of selective and non-selective enrichment PCR procedures for *Salmonella* detection. Lett. Appl. Microbiol., 36: 217-221.

Pao, S.; Hagens, B.E.; Kim, C.; Wildeus, S.; Ettinger, M. R.; Wilson, M.D. ; Watts, B. D.; Whitley, N.C.; Porto-Fett, A. C. S.; Schwarz, J. G.; Kaseloo, P.; Ren, S.; Long, W.; Li, H. and Luchansky, J. B. (2014): Prevalence and molecular analyses of *Campylobacter* jejuni and *Salmonella* spp. in co-grazing small ruminants and wild-living birds. Livestock Science Journal (160): 163-171.

Popoff, M. Y.; Bockemühl, J. and Gheesling, L. L.(2001): Supplement 2001 (no. 45) to the Kauffmann-White scheme. Research in Microbiology Journal. (154):173 -174.

Quinn, P. J.; Markey, B. K.; Carter, M. E.; Donnelly, W. J. and Leonard, F. C.(2002): Veterinary Microbiology and Microbial Diseases. Great Britain by HPG, Books Ltd., Bodmin, Cornwall, UK. P.114 -118.

Rahn, K.; De Grandis, S. A.; Clarke, R. C;.McEwen, S. A.; Galan, J. E. and Ginocchio, C. (1992): Amplification of an *invA* gene sequence of *Salmonella* Typhimurium by polymerase chain reaction as a specific method of detection of *Salmonella*. Molecular and Cellular Probes, 6:271-279.

Singer, R. S.; Cooke, C. L.; Maddox, C. W.; Isaacson, R. E. and Wallace, R. L. (2006): Use of pooled samples for the detection of *Salmonella* in feces by polymerase chain reaction. J. Vet. Diagn. Invest, 18: 319-325.

Skyberg, J. A.; Logue, C. M.; Nolan, L. K. (2006): Virulence Genotyping of *Salmonella* spp. with Multiplex PCR. Avian Diseases Journal. (50) 1:77-81.

Zahraei, T.; Mahzoonae, M. R. and Ashrafi, A. (2006): Amplification of *invA* gene of *Salmonella* by polymerase chain reaction (PCR) as a specific method for detection of *Salmonella*. J. Fac. Vet. Med. Univ. Tehran, 61(2): 195-199.