Assiut Vet. Med. J. Vol. 60 No. 140 January 2014

BACTERIOLOGICAL STUDIES OF *SALMONELLA Typhimurium* ISOLATED FROM COW CALVES AND LAMBS

LAMYAA, M. REDA and SAHAR R. MOHAMED
Central Lab., Vet., Hospital, Faculty of Vet., Med., Zagazig Univ. Animal Health Research Institute, Dokki, Giza.
Email: tota.16@live.com

Received at: 10/12/2013

Accepted: 30/12/2013

ABSTRACT

Salmonellae are considered one of the most important microorganisms that cause diseases in man and animals. The current research aims to obtain complete picture of *Salmonella typhimurium*. Two hundred four rectal swabs (140 cow calves and 64 lambs) were collected from apparently healthy and diarrhoiec cases in Sharkia Governorate. All rectal swabs were subjected to both bacteriological and serological examination. The total incidence of positive samples was 15 (10.7%) *Salmonella typhimurium* isolates obtained from 140 cow calves, in addition to 6 (9.37%) isolates from 64 lambs rectal swabs. Six *Salmonella typhimurium* serotypes were detected by PCR for *invA* gene, all tested isolates gave characteristic band of *invA* gene at 521 bp. Moreover, antimicrobial drug sensitivity were carried out.

Key words: *Salmonella, Calves, lambes, PCR.*

INTRODUCTION

Salmonellae serovars are one of the most frequent causes of bacterial infection in animals and human and major causes of food-borne diseases (Abu-Zeed *et al.,* 2000 and Atyabi *et al.,* 2012). Salmonella is a member of family Enterobacteriaceae that comprise a large and diverse group of Gram-negative rods (Jeffrey and Paula, 2002) animals are mainly infected through feed, drinking water or environmental sources (Diohisi *et al.,* 2009).

Salmonellae cause acute and chronic enteritis, septicemia, abortion, polyarthritis, nervous manifestation, death and agalactiae in lactating animals (Dinjus *et al.,* 1998 and Gorman and Adly, 2004).

Salmonella enterica serovar typhimurium is highly pathogenic, as it has many virulence factors such as adhesions, invasions, frimiae and endotoxin**.** *Salmonella typhimurium* strains carrying genetic information on several genes are necessary for invasion,most of these genes are located at centrisom 63 on *Salmonella typhimurium* chromosome, designated *Salmonella* pathogenicity island 1 (SPI 1). The *invA* gene one of these genes which located on (SPI 1). The *invA* gene is important in the invasion of phagocytic epithelial cells and entry into the intestinal mucosa. They adhered equally well to epithelial cells, could penetrate into these and survive and multiply inside cells (Jones *et al.,* 1994).

The use of PCR tecnnique was widely adopted during the last few years as it was used to amplify *Salmonella*-specific sequence using a new set of primers taken from *invA* gene and was predicted to amplify at 521 bp PCR product (Swamy *et al.,* 1996).

The aim of this study was to:

- record incidence of salmonellae after isolation,biotyping and serotyping.
- Determination of the genetic characteristics using PCR including presence of virulence *invA* gene.
- Antibiotics sensitivity of all *Salmonella typhimurium* isolates.

MATERIALS and METHODS

Samples

Rectal swabs

Two hundred and four rectal swabs were collected from apparently healthy and diarrhoeic cow calves and lambs,aged 15day-6months old in Sharkia Governorate as shown table (1).

Table 1: Number of examined samples and animal status.

Animal species	Number of samples	Apparently healthy animals	Diarrhoeic animals
Cow calves	140	42	95
Lambs	64	19	45
Total	204	61	140

All samples were submitted to the laboratory for bacteriological and serological examination.

Media used for isolation and identification
Selenite F broth, nutrient agar (Oxoid), Salmonella Shigella agar (S.S. agar) (Oxoid), xylose lysine deoxycholate agar (XLD), Oxidase test, sugar fermentation media, indole test and methyl red test, voges-proskauer test (V.P. test), simmon citrate agar, urea agar (urea fermentation), triple sugar agar (TSI medium), gelatin media (gelatin liquification) (Cruickshank *et al.*, 1975).

Stating for identification
Gram's stain (Cruickshank *et al.*, 1975).

Diagnostic Salmonella antisera
Serotyping of isolates was carried out by diagnostic polyvalent and monovalent (O) and (H) Salmonella antisera obtained from Animal Health Research Institute, Dokki, Giza, Egypt.

Materials used for polymerase chain reaction (PCR):
Buffers,solutions and reagents for PCR (Beyer *et al.*,1998). The complete sequences of the *invA*gene was obtained from GenBank accession number M64295, the sequence of oligonucleotide primers are given in table(2). DNA Ladder (100 bp, pharmacia) Catalogue number 27-4001-01 (USA).

Table 2: The sequence of oligonucleotide primers specific for *Salmonella invA* gene.

Gene name	Primer Sequence	Product size	References
The *invA* gens primer	5`- TGTTACGGCTATTTTGACCA-3`	521	**Swamy et al. (1996)**
The *invA* antisense primer	5` CTGACTGCTACCTTGCTGATG-3`		

Media used for antimicrobial susceptibility testing
Muller- Hinton agar (Oxoid) and antimicrobial sensitivity discs (Oxoid) as described by Lorian (1985) for antibiogram assay for *Salmonellla typhimurium*, ampicillin 40 µg/ml, Chloramphenicol 30 µg/ml, Ciprocin 5 µg/ml, Erythromycin 15 µg/ml, Gentamicin 10 µg/ml, Nalidixic acid 30 µg/ml, Nitrofurantoin 300 µg/ml, Ofloxacin 5 µg/ml, Streptomycin 10 µg/ml and Tetracycline 30 µg/ml were used.

Barium sulphate standard turbidity solution (0.5 McFarland standard tube) (Baüer *et al.*, 1966).

Bacteriological examination
Each prepared rectal swabs was added to Selenit F broth incubated at 37ºC for 24 hours (Rengel and Mendoza, 1984), then the samples were streaked into S.S. agar and XLD agar, incubated at 37ºC for 24 hours.

Detection of salmonella suspected colonies (Mackie and McCarteny, 1990)
The growing colonies were examined morphologically (size, shape, consistency, lactose fermentation and H_2S production).

Microscopical examination: was done according to (Quinn *et al.*, 2002)

Biochemical identification
Pure colonies were picked up and preserved onto slope semisolid agar for further biochemical identification (Quinn *et al.*, 2002).

Serological typing of salmonellae:
The isolates were serologically examined according to (Edwards and Ewing, 1972).

DNA extraction and purification from *S. typhimurium* (Soumet *et al.*, 1999):
1. Put 5 ml of a 24 hrs broth culture of *Salmonella* isolates in Eppendorf tube.
2. Centrifuge the tubes for 10 min at 12000 rpm.
3. Wash the pellet twice in 1 ml of PBS (pH 7.2).
4. The washed pellet was suspended in 50 µ1 PBS and heat directly at 100ºC for 10 min in a heat block to break the cell membranes.
5. Cool in refrigerator for 5 min.
6. Finally, the cell suspension was centrifuged for 5 min at 12000 rpm and the supernatant containing chromosomal DNA was collected and stored at -20ºC until used.

Purity assessment:
The concentration of DNA in µg/ml was measured at 260 and 280 nm by ultraviolet spectrophotometer (Shimadzu, Japan) then the ratio 260/280 was calculated. Pure DNA should have ratio of >1:8 that contamination with protein resulted in a significantly lower value. The DNA solution was kept at -20ºC until used.

PCR procedure modified by Abou Zeed *et al.* (2000):
The reaction mixture (total volume of 100 µl) was 10 µl of 10X reaction mixture buffer, 8µl of 200 mM of each of the four deoxynucleotide Triphosphates(d ATP,d CTP,d GTP and d TTP), DNA was added 5 µl

(containing 50ng) and 2 µl Primer (containing 20 pmol of each oligonucleotide primer). Then 2Uof 1,5 M DNA Taq polymerase enzyme was added and the mixture was completed by ultra-pure distilled water to 100 µl. Each reaction mixture was over laid with 50 µl of sterile mineral oil. PCR was performed on PE applied biosystem (USA).

Amplification was performed by applying the following program in the thermocycler , initial heating for 5 minutes at 94^{o}C then 35 cycles (denaturation at 93 ^{o}C for 1minutes, annealing for 1 minutes at 52 ^{o}C and extension for 2minutes at 72 ^{o}C), followed by a final 10 minutes extention at 72 ^{o}C.

Identification of the PCR products:
Following amplification, 5 µl of each amplicons was mixed with loading buffer and analyzed on an agarose gel consisting of 1.5% (w/v) agarose and 0.5 µg of ethidium bromide per ml inTBE buffer. The samples were electrophoresed at 80 V for 1 hour on a horizontal electrophoresis unit. A 100-bp DNA ladder was used as a molecular weight standard. The specific DNA bands using UV transilluminator compared with the molecular size marker and photographed with Polaroid camera (waalge *et al.*, 1999 and Murugkar *et al.*, 2003).

Antibiotic susceptibility test:
Determination of the susceptibility of isolated strains to antibiotic discs was adopted using the disc diffusion technique according to Finegold and Martin (1982).

RESULTS

Salmonella infections in farm animals have been recorded in most countries of the world. Diarrhoea and death in farm animals is the main cause of economic losses in the world.

Incidence of bacterial isolates from cow calves and lambs:
Salmonella, Klebsiella, Proteus species and *E.coli* were isolated with percentage ranged from 9.8 – 15.6% of examined isolates showing no wide variation in cow calves, also no great variation in isolation percentages of these species from lambs.

Isolation percentage of Salmonellae were 5.8% and 12.2% of examined isolates from cow calves and lambs respectively. There was no great variation between isolation *Salmonella, Klebsiella, Proteus species* and *E.coli.* as shown (Table 3).

Table 3: Incidence of Enterobacteria in rectal swabs.

Animal	*Salmonella* spp.	*Klepsiella* spp.	*Proteus* spp.	*E. coli*
	No. (%)	No. (%)	No. (%)	No. (%)
Cow calves	25/204 (12.2%)	20/204 (9.8%)	32/204 (15.6%)	26/204 (12.7%)
Lambs	12/204 (5.8%)	10/204 (4.9%)	13/204 (6.3%)	15/204 (7.3%)

Incidence of Salmonella species from cow calves and lambs:
The study included 204 animals (61 apparently healthy and 143 diarrheic animals).

Percentage of isolation of Salmonellae from diarrheic rectal swabs were 3 times than that of rectal swabs from apparently healthy.

Diarrhoeic cases with Salmonellae was higher in cow calves than lambs as shown (Table 4).

Table 4: Occurrence of Salmonellae examined rectal swabs from cow calves and lambs.

Animal species	Apparently healthy	Diarrhoeic	Total
Cow calves	3/42 (7.1%)	22/98 (22.4%)	25/140 (17.8%)
Lambs	1/19 (5.2%)	11/45 (24.4%)	12/64 (18.7%)

Serotyping of Salmonellae isolated from cow calves and lambs:
The results of bacterial identification revealed that 37 strains of Salmonella species were isolated from all examined samples. Four strains were identified serologically as *S.typhimurium* (21), *S.enteritidis* (9), *S.dublin* (4), *S.anatum* (3).

S.typhimurium represented the highest incidence (56.7%) of total *Salmonella* isolates (21/37) from cow calves and lambs.

S.Anatum represented the lowest incidence (8.1%) of the total *Salmonella* isolates (3/37) as shown in (table 5).

Table 5: Prevalence of salmonellae isolated from cow calves and lambs.

Serotype	Cow calves	Lambs	Total
S. typhymurium	15/25 (60%)	6/12 (50%)	21/37 (56.7%)
S. enteridis	5/25 (20%)	4/12 (33.3%)	9/37 (24.3%)
S. duplin	3/25 (12%)	1/12 (8.3%)	4/37 (10.8%)
S. anatum	2/25 (8%)	1/12 (8.3%)	3/37 (8.1%)

Susceptibility and resistance percentage of *S. typhimurium* isolates to each antimicrobial agent:
The results of drug sensitivity was studied and summarized in table (6) which indicated that the *S. typhimurium* isolates was found sensitive to ofloxacin, gentamycin, ciprocin and nitrofurantoin an incidence of 100%, 94.4%, 88.8% and 88.8% respectively. While most of isolates resistant to erythromycin in an incidence of 83.3% and showed variable degrees of sensitivity against other antimicrobial agents.

Table 6: Antimicrobial susceptibility pattern of Salmonella isolates from animals.

Antimicrobial agents	Conc. (µg/ml)	Antimicrobial susceptibility		
		Sensitive	Intermediate	Resistant
Ampicillin	40 µg	9/18 (50%)	2/18 (11.11%)	7/18 (38.88%)
Chloramphenicol	30 µg	15/18 (83.33%)	1/18 (5.55%)	2/18 (11.11%)
Ciprocin	5 µg	16/18 (88.88%)	-	2/18 (11.11%)
Erythromycin	15 µg	2/18 (11.11%)	1/18 (5.55%)	15/18 (83.33%)
Gentamicin	10 µg	17/18 (94.44%)	-	1/18 (5.55%)
Nalidixic acid	30 µg	10/18 (55.55%)	3/18 (16.66%)	5/18 (77.77%)
Nitrofurantoin	30 µg	16/18 (88.88%)	-	2/l8 (11.11%)
Ofloxacin	5 µg	18/18 (100%)	-	-
Streptomycin	10 µg	9/18 (50%)	5/18 (27.27%)	4/18 (22.22%)
Tetracycline	30 µg	11/18 (5.55%)	3/18 (16.66%)	14/18 (77.77%)

Results of polymerase chain reaction (PCR):
The result of PCR test using the primer of *invA* gene clarified that all six isolates of *Salmonella typhimurium* gave characteristic band at 512 bp (Photo 1). These results confirm that all obtained isolates were pathogenic salmonellae and had the ability to invade the epithelial cells.

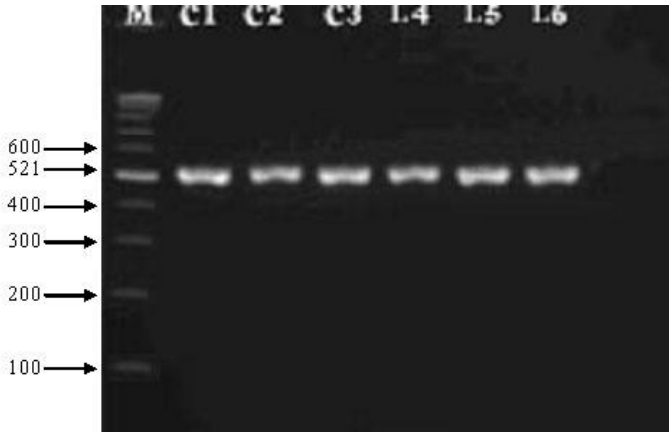

Photo (1): Electrophoretic pattern of PCR product using *invA* gene primer.
Lane C1, C2, C3 *S. typhimurium* obtained from cow calves.
Lane L4, L5, L6 *S. typhimurium* obtained from lambs

DISCUSSION

Salmonellae are thought to be major pathogens leading to serious economic losses in animal industry and cause devastating disease condition ranging from gastroenteritis to septicemia and death depending on the serovar of the bacterium and nature of the infected host (Kusters *et al.,* 1996).

Bacteriological examination is of the traditional mean to obtain accurate data about the prevalence of the infected host and carriers of salmonellae (Statutory Instraments, 1989). In addition serological tests provide a major advantage over bacteriological examination (Richandson, 1973).

The obtained result in table (3) showed that the salmonellae was isolated with an incidence (12.2%) in cow calves. These results coincide with Dargatz *et al.* (2000) and higher when compared with Ravary *et al.* (1998) who mentioned that the incidence in cattle was 1.4%. The incidence of Salmonella from lambs was (5.8%), this result agreed with Kirby (1985) who isolated salmonellae with an incidence (5.03%).

The incidence of salmonellae from apparently healthy lambs (5.2%) as shown in table (4). This result was high when compared with Ismail and El-Seedy (1989) who mentioned that the incidence of salmonellae from apparently healthy lambs was 0.32%. The difference in incidence of salmonella may be attributed to several factors including the difference in the standard of hygiene in tested farms, the presence of carriers in these farms, the rate of exposure of animals to infection, the standard of nutrition, the age of animals, the presence of stress factors as well as other factors such as technique of isolation which differs from one laboratory to another.

Concerning the serological typing of *Salmonella* isolates from cow calves and lambs (Table 5) revealed that *S. typhimurium* represented the highest percentage 60% and 50% of the total isolated serovars in comparison with other isolates. These results coincide with Al-Sanjary (1999) who stated that *S. typhimurium* is the most predominant serotypes (29.23%), in sheep and goats contrast Bernardo and Machado (1990) isolated *S. anatum* as a predominant strain and Caro *et al.* (1991) isolated *S. anatum* with highest incidence 38.2%.

Salmonella typhimurium is amongst more than 2000 serotypes that have developed the highest adaptability to a wide range of hosts including cattle, sheep, poultry and pigs (Wray *et al.,* 1991). These results reflected the importance of *S. typhimurium* as human and animal pathogen (Duvkeren *et al.,* 2002).

The PCR is a highly accurate method which makes it possible to detect nucleic acid amplification products. The results can be obtained rapidly so that they can be used not only to support bacteriological investigation but also to make the result more reliable (Galan and Curtiss, 1991).

InvA gene, is a member of a family of proteins involved in either flagellar biosynthesis or the secretion of virulence determinants by a number of plant and mammalian pathogens, which is essential for Salmonella spp. To enter cultured epithelial cells (Ginocchio and Galan, 1995).

In the present study, PCR was used to amplify Salmonella-specific sequences using a new set of primers taken from *invA* gene and was predected to amplify a 521 bp PCR product. The results obtained are in accordance with the results of Helmy and Zaki (2003), and Moganedi *et al.* (2007) who used the same set of oligonucleotides primer from *invA* gene sequence for detection and identification of *Salmonella* seovars. Other investigations using different primer of invA gene gave a characteristic band at 284 bp as those made by Singer *et al*. (2006) and Kim *et al*. (2007). The difference referred to the sequence and in the annealing sites on the nucleotide sequence of the *invA* gene.

The antibiogram of a pathogen could be variable from place to place and from case to another. This may be explained by the wide use of antibacterial agents and the variation in their use which may produce new resistant bacteria.

CONCLUSION

The results of the present study suggested that future control measures should be focused on reduction of Salmonella infection in (cow calves and lambs) and minimize infection with *Salmonellae*.

REFERENCES

Abou-Zeed, Y.M.; Hariharan, H.; Poppe, C. and Kibeno, F.S. (2000): Characterization of *Salmonella* isolates from beef cattle, broiler chickens and human sources on Prince Edward Island. Comparative Immunology, Microbiology and Infectious Diseases, 23: 253-266.

Al-Sanjary, R.A. (1999): Incidence of Salmonella in lymph nodes, spleen and feaces of sheep and goats slaughtered in Mosul abattoir Iraqi Journal of Veterinary Sciences. 12(2):359-364.

Atyabi, N.; Zahraei Salehi, T.; Ghazisaeedi, F. and Ashrafi, I. (2012): The molecular investigation of wide spread *salmonella* serovars, S.*typhimuriam* and S.*enteritidis*, involved in *salmonella* of cattle and sheep in farms around Tahran, Iran.Iranian Journal of veterinary Research, Shiraz University, Vol.13, No. 2, ser, No. 39.

Baüer, A.W.; Kirby, W.M.; Sherris, J.C. and Truck, M. (1966): antibiotic susceptibility testing by standardized single disk method. Amer. J. Clin. Pathol., 45:493-496.

Bernardo, F.M. and Machado, J.C. (1990): Prevalence of Salmonella in slaughtered animals in Portugal.Revista Portuguesa de sciencias Veterinaria. 85 (495): 94-102.

Beyer, W.; Muckendi, F.M.; Kimming, P. and Bohm, R. (1998): suitability of repetitive – DNA – sequence based PCR fingerprinting for characreterization epidemic isolates of Salmonella enteric serovar. Sainpaul J. of Clin. Microbiol., 36 (6): 1549-1554.

Caro, M.R.; Marsilla, B.A. and Salinas, J. (1991): Salmonella prevalence of different serotypes in environment of sheep abattoirs Anales de Veterinaria de Murcia. 6 (7): 31-35.

Cruikshank, R.; Duguid, J.P.; Marmion, B.P. and Swain, R.H.A. (1975): Medical Microbiology. The practice of Medical Microbiology., VII, 12th Ed., Churchill livingstone; Edinburgh, London and New York.

Dargatz, D.A.; Fedorka-Cray, P.J.; Ladely, S.R. and Ferris, K.E. (2000): Survey of Salmonella serotypes shed in feaces of beef cows and their antimicrobial susceptibility patterns. J. Food Prot. (12): 1648-1653.

Dinjus, U.; Hamel, I.; Rabsch, W. and Helmith, R. (1998): Studies the presence of the virulence factors, adhesion, invasion, intracellular multiplication and toxin formation in *Salmonella* of different origin. Zbl. Bakt, 287 (40): 387-398.

Diohisi, AM, C, Lacarelli, C. and Filetici, E. (2009): molecular characterization of *salmonella* enteric serotype typhimurium isolated from human. Food borne pathogen. 30 (6): 711-715.

Duvkeren, E.V.; Wannet, W.J.; Houwers, D.J. and Peun, W.V. (2002): Serotypes and phage type distribution of salmonella strains isolated from human, cattle, pigs and chickens in the Netherlands from 1989-2001. J.Clin.Microbiol. 40, (11): 3980-3985.

Edwards, P.R. and Ewing, G.W. (1972): Identification of Enterobactereaceae 3rd Ed.Bugers publishing company, USA.

Finegold, S.M. and Martin, E.T. (1982): Diagnostic Microbiology. 6th Ed., The C.V. Mosby Company, St. Louis, Toronto, London.

Galan, J.E. and Curtiss, R. (1991): Expression of *Salmonella typhimurium* genes required for invasion is regulated by changes in DNA supercoiling. Infect. Immun., 58 (6): 1879-1885.

Ginocchio, C.C. and Galan, J.E. (1995): Functional conservation among members of the *Salmonella typhimurium* InvA family of proteins. Infect. Immunil., 63 (2): 729-732.

Gorman, R. and Adly, C. (2004): Characterization of *Salmonella enterica* Serotype Typhimurium Isolates from Human, Food, and Animal Sources in the Republic of Ireland. J. Clin. Microbiol., 42 (5): 2314-2316.

Helmy, N.M. and Zaki, H.M. (2003): Studies on Salmonella serovars in lambs with special references to virulence and genotypic characteristics using polymerase chain reaction (PCR). J. Egypt Vet. Med. Assoc. 63,(6) 59-72.

Ismail, M. and El-Seedy, F.R. (1989): Studies on Salmonellosis among sheep and goats in Egypt. J. Egypt Vet. Med. Ass. 49 (4): 1105-1117.

Jeffrey, T.G. and Paula, J.F. (2002): Salmonella chapter 3 in food born diseases 2nd Ed. (55-68) published by Academic press London.

Jones, B.D.; Gho, N. and Falkow, S. (1994): Salmonella Typhimurium intiates murine infection by penetrating and destroying the specialized epithelial M cells of Peyer's patches. J. Exp. Med., 180: 15-23.

Kim, J.S.; Lee, G.G.; Park, J.S.; Jung, Y.H.; Kwak, H.S.; Kim, S.B.; Nam, Y.S. and Kwon, S.T. (2007): A novel multiplex PCR assay for rapid and simultaneous detection of five pathogenic bacteria: Escherichia coli O157:H7, Salmonella, Staphylococcus aureus, Listeria monocytogenes, and Vibrio parahaemolyticus. J. Food Prot., 70 (7): 1656-1662.

Kirby, F.D. (1985): Surveillance of animal Salmonella infection. Vet. Rec. 117 (18): 456-457.

Kusters, J.G.; Krameis, G.A.; Dorrnik, C.E. and Zeijst, B.A. (1993): Effect of multiplicity of infection bacterial protein synthesis and growth phase on adhesion and invasion of human cell lines by *Salmonella Typhimurium* infection. Immunity. Dec. P. 5013-5020.

Lorian, V. (1985): Antibiotics in laboratory medicine second edition, published by Lippincott Williams and Wilkins, Philadelphia, PA, U.S.A.

Mackie and McCartney (1990): Practical Medical Microbiology.International student Ed., 14th edition. Churchill Livingstone, NewYork.

Moganedi, K.L.; Goyvaerts, E.M.; Venter, S.N. and Sibara, M.M. (2007): Optimisation of the PCR-invA primers for the detection of Salmonella in drinking and surface waters following a pre-cultivation step. 33 (2): 329-340.

Murugkar, H.; Rahman, H. and Dulta, P. (2003): Distribution of virulence genes in Salmonella serovars isolated from man and animals. Indian Journal of Medical Research, 117: 66-70.

Quinn, P.J.; Markey, B.K.; Carter, M.E.; Donnelly, W.J. and Leonard, F.C. (2002): Veterinary Microbiology and Microbial diseases.

Blackwell Scientific Publications, Oxford, London.

Ravary, R.; Fecteau, G.; Higgins, R.; Pare, J. and Lavoi, J.P. (1998): Prevalence of infections caused by *Salmonella spp.* In cattle and horses at the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine of the University of Montreal. Can.Vet. J., 39 (9): 566-572.

Rengel, A. and Mendoza, S. (1984): Isolation of *Salmonella* from raw chicken in Venezuela. J. FoodProt. 47: 213.

Richardson, A. (1973): The transmission of *Salmonella Dublin* to calves from adult carrier cow. Vet. Rec. 92.112-115.

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 feaces by polymerase chain reaction. J. Vet. Diagn. Invest., 18 (4): 319-325.

Soumet, C.; Ermel, G.; Rose, V.; Rose, N.; Drouin, P.; Salvat, G. and Colin P. (1999): Identification by a multiplex PCR – based assay of *Salmonella typhimurium* and *Salmonella enteritidis* strains from environmental swabs of poultry houses. Lett. Appl. Microbiol., 29 (1): 1-6.

Statutory Instuments (1989): The poultry breeding flocks and hatcheries (registration and testing) order No.1963. Her Mayesty's stationary office London.

Swamy, S.C.; Barnhard, H.M.; Lee, H.D. and Dresen, D.W. (1996): Virulence determinants *invA* and *spvC* in *Salmonella* isolated from poultry products, Wastewater and human sources. App. Envir. Microbiol., 62: 3768-3771.

Waalge, A.S.; Vardund, T.; Lund, V. and Kajipcrud, G. (1999): Detection of low numbers of salmonella in environmental water, sewage and food samples by a nested PCR ASSAY. j. App. Microbiol., 87: 418-428.

Wray, C.; Beedell, Y.E. and Mclaren, I.M. (1991): a survey of antimicrobial resistance in Salmonellae isolated from animals in England and Wales during 1984-1987. BR. Vet. J. 147: 356-369.

دراسة بكتريولوجية للسالمونيلا تايفيميوريوم معزولة من صغار الأبقار والأغنام

لمياء محمد رضا محمد ، سحر رشدي محمد

Email: tota.16@live.com

تعتبر السالمونيلا من الأمراض المشتركة بين الإنسان والحيوان وإصابة الحيوانات بالسالمونيلا سجلت في كثير من بلدان العالم حيث الإصابة بالإسهال والنفوق من الأسباب الرئيسية في الخسارة الاقتصادية. الهدف من هذه الدراسة عزل وتشخيص ميكروب السالمونيلا وبخاصة السالمونيلا تايفيميوريوم. تم تجميع 204 مسحه شرجية (140 من صغار الأبقار + 64 من صغار الأغنام) من صغار الأبقار والأغنام السليمة ظاهرياً والمريضة بالإسهال من محافظة الشرقية. تم عزل 37 عترة بنسبة 18% من ميكروب السالمونيلا من 204 مسحه شرجية من صغار الأبقار والأغنام. تم التصنيف السيرولوجي لـ 37 عترة من ميكروب السالمونيلا (25 من صغار الأبقار + 12 من صغار الأغنام). وكانت نسبة السالمونيلا تايفيميوريوم 60% في صغار الأبقار ، 50% في صغار الأغنام. تم إجراء اختبار سلسلة تفاعل إنزيم البلمره PCR علي 6 عينات من السالمونيلا تايفيميوريوم تم اختيارها عشوائياً (3 من صغار الأبقار + 3 من صغار الأغنام) للكشف عن invA جين حيث أعطت جميع المعزولات نتائج إيجابية وأنتجت حزمة للحمض النووي عند الوزن الجزيئي 521 زوج من القواعد. وقد أوضحت الدراسة تأثيراً (10) من المضادات البكتيرية المختلفة علي عترات السالمونيلا تايفيميوريوم (المعزولة من صغار الأبقار والأغنام) أنها ذات حساسية عالية لكل من الافلوكساسين والجينتاميسين والسيبروسين والنيتروفيرانتين. بينما كانت معظم المعزولات مقاومة للاريثروميسين.