Vet.Med.J.,Giza. Vol.57, No.1. (2009):23-33.

STUDIES ON SOME SALMONELLA SEROVARS ISOLATED FROM SLAUGHTERED IMPORTED CAMELS

Hala I. Scharawe. Hanan M. Ibrahim. El_Sawy Safwat.
Serology Unit Animal Health Research Inst. Dokki, Giza.

Recived 15/04/ 2009.
Accepted 30/04/2009.

SUMMARY:

The present study was undertaken to determine the prevalence and distribution of salmonellae from apparently healthy slaughtered camels in Cairo abattoir. One hundered imported slaughtered camels were examined for salmonellae. The mesenteric lymph nodes were collected from all animals after slaughtering. In addition to 55 camels were examined by taking muscle swabs (diaphragmatic or abdominal). Salmonellae were found in 5% of the mesenteric lymph nodes and in 3.6% of the muscle swabs. Four different serovars were identified, namely Salmonella Typhimurium , S.Florida , *S.*Washington and *S.*Uganda. Antibiogram patteren of 7 isolated Salmonella serovars against 16 antibiotics was studied. Plasmid profiles of *other S. typhimurium* Isolates and other *S.typhimurium* From other sources were compared. Public health importance and hygienec measures were discussed

INTRODUCTION

Salmonella enterica is a significant food borne pathogen of humans transmitted via the consumption of meat, animal products and food products contaminated with animal waste. Clinical manifestations of human and animal salmonellosis range from self-limiting gastroenteritis to severe bacteremia and typhoid fever. More than 2.300 serovars of *S.*enterica have been identified and classified previously (Meithoff et al., 2008). Sandiford [1944] was the first to attract the attention to the risk of transmission of salmonellosis by camels to persons eating camel's meat in Egypt.He attributed 7 food poisoning outbreaks to S.Typhimurium. Four of them resulted after consumption of camel's meat.The used for work and meat production in Egypt. Thus infected

camels constitute a health hazard to man through consumption of meat or meat products from infected camels. On the other hand, Salmonella carriers disseminate the organisms in their surroundings and infection may spread to other animals (RE FAI. et al, 1984 and Safwat et al, 1984).

A number of authors have reported salmonellosis in camels in different countries (Ramadan and Sadek, 1971, Pegram Tareke, 1981 and Refai, 1992).Therefore, the aim of the present work was to demonstrate the distribution of salmonellae from apparently healthy slaughtered imported camels. Antimicrobial suscebility test to different antibiotics for Salmonella isolates also plasmid profile character of S.Typhimurium from differentsources were studied.

MATERIALS AND METHODS

I.Isolation and identification:

First, the mesenteric lymph nodes of 100 apparently slaughtered camels were examined for Salmonella. The isolation and identification of Salmonella was carried out according to the techniques recommended by Quinn and Colleagues (1994).About 25 g of each sample was weighed and then cut into smaller pieces with a sterile scalpel blade. The pieces were put in sterile plastic bags with 225 ml of buffered peptone water (BPW) and homogenized using astomacher (Colwarth, Stomacher 400, London). The pre-enriched samples were incubated for 16-20 h. at 37°C.Second, the muscle swab samples direct or indirect contact or as a result of were enriched in BPW- about 1 ml of the pre-enrichment broth was transferred aseptically into 10 ml selenite-F-broth and incubated for 24 h at 37°C. This was followed by streaking from selenite F. broth onto XLD (Xylose- Lysine-Dextrose) agar and S.S. (Salmonella Shigella) agar media and incubated at 37°C for 24 h. The plates were examined for the presence of Salmonella colonies. Suspect Salmonella colonies were tested biochemically using api IOS (bio.Merieux) .For the serological identification , Salmonella antisera (DENKA SEIKEN) were used. The antigenic formulae of Minor and Popoff (2000) were used to name the serovars.

II.Antibiogram patterns of isolated Salmonella serovars

Culture and sensitivity for the serotyped strains were done. Seven Salmonella Serovars were tested againsted 16 antibiotics by the disc agar diffusion method. The interpretation of the results was carried out according to National Committee for Clinical Laboratory Standards NCCLS, 2002.

III.Plasmid analysis

Plasmid analysis of 3 *S.*Typhimurium recovered from camels and 4 *S.*Typhimurium (cattle, standard strain, reptile and birds) from other sources was performed according to the following. Preparation and purification of plasmid DNA (MINIPREP)

Extraction of miniprep performed according to Ausubel et al. (1987):

1) One colony of Salmonella was picked and inoculated in 10 ml **LB** broth containing 12 µg/ml tetracycline, 50 µg / ml ampicillin and 50 µg / ml chloramphenicol then incubated at 37°C overnight with shaking.

2) Centrifuged at 3000 rpm/ 5 min .

3) The supernatant was discarded, then 250 µl tris EDTA buffer solution (TES) pH 7.8/1.5 ml culture was added, and then shaken well.

4) Solution I 100 µl/1.5 ml cultures was added and put on ice for 10 min.

5) Freshly prepared solution II µl/1.5 ml culture was added and put on ice for 10 min

6) 150 µl/1.5 ml cultures from cold solution III was added and mixed gently then put on ice for 10 min.

7) volume of ethanol to the 9)above separated aqueous layer and kept for 30 min. on ice, then centrifuged at 14000 rpm/30 min/4°C.

8) and centrifuged at 14000 rpm/10 min/ 4°C.

9) The pellet was dried, and then dissolved in 10µl TE pH 8.To perform agarose gel electrophoresis.

10) The pellet was washed with 5 ml 70% ethanol

11) Centrifuged at 14000 rpm /10 min/4°C.

12) Proteins and genomic DNA were extracted from the supernatant with phenol: chloroform: isoamyl alcohol mixture.

Solution I	Solution II	Solution III
50 mM glucose	4 N NaOH 0.4 ml	5 M CH_3 cook 60.0 ml
25 mM tris pH 8	10 % SDS 0.8 ml	Acetic acid 11.5 ml
10 mM EDTA	Deionized water 6.8 ml	Deionized water 8.5 ml
kept in refrigerator	Must be freshly mixed	

Table I: Prevalence of Salmonella in apparently healthy slaughtered camels at Cairo abattoir.

Type of samples	No. of samples	Salmonella serovars	Number isolated	%
Mesentric lymph node	100	S.Typhimurium	3	3%
		S.Washington	1	1%
		S.Florida	1	1%
Muscle swabs	55	S.Typhimurium	1	1.8%
		S.Uganda	1	1.8%

RESULTS

I.Isolation and Identification:

Seven Salmonella positive samples were detected from a total 155 samples- Salmonellae were isolated from five of hundred apparently healthy camels (5%) at slaughter from the mesenteric lymph node samples, other two Salmonellae of fifty-five muscle swab (diaphragmatic or abdominal) samples were identified (3.6%)- Table I. These results were confirmed by api S10 as shown in photo 1.The Salmonella Serovars were distributed as shown in Table II.

II.Antibiogram results:

Zone diameter of the antibiotic discs was interpreted according to (NCCLS, 2002). Table III.

III.Plasmid analysis:

The plasmid analysis of the 3 isolated S. Typhimurium from the mesenteric lymph node were compared by other S.Typhimurium from other sources (as shown in table IV and photo 2).

Table II : Salmonella serovars isolated from examined camel samples

Type of sample	No. of samples		Prevalence
	Examined	Positive	
Mesenteric lymph node	100	5	5%
Muscle swab (Abdominal or Diaphragmatic)	55	2	3.6%
Total	155	7	4.5%

Table III: Previous reports of Salmonella Serovars isolated from camel in Egypt(1978-2004)

Author	Year	Incidence of Salmonella %
El_Monella	1978	44
Elias	1982	15
El-Nawawy et al.,	1982	33.3
Refai et al.,	1984	15
Safwat et al.,	1985	32
Yassien et al.,	1989	1.26
Selim et al.,	1990	17(apparently healthy) with enteritis
Refai	1992	review
Yousif et al.,	1993	9.33
Mohamed	2002	Experimental decontamination of Salmonella in meat camel
Rehab	2004	8.5

TableV: Plasmid analysis of *S.*Typhimurium isolates fromcamels in comparison with different sources.

No.	Source	Plasmid MW/kbp
1	camel	17.940
2		19.210
3		17.942
4	cattle	18.287
5	standard strain	Low copy number
6	reptile	15.822
7	bird	15.822

- a visible DNA plasmid bands being sized by DNA molecular marker (Amers Co.).
- MW: Molecular weight.
- kbp: Kilobase pair.

Table IV : Results of antibiogram patterns of isolated *Salmonella* Serovars

Antibiotic and chemotherapeutic agent	Disc potency	Mesentric L.n positive samples					Muscle swab positive samples	
		*S.*Washington	*S.*Florid a	*S.*Typhimurium 1	2	3	*S.*Typhimurium	*S.*Ugando
Aminosidin	60 mcg	R	R	R	R	R	R	R
Amoxicillin-Clavulanic acid	20 mcg+ 10 mcg	S	S	R	M	R	R	R
Ampicillin	10 mcg	R	R	R	R	R	R	R
Cefoperazone	75 mcg	S	S	R	R	R	R	S
Ceftazidine	30 mcg	S	S	R	R	R	R	R
Ciprocin (Ciprofloxacin)	5 µg	S	S	S	S	S	S	S
Flucloxacillin	5 mcg	R	R	R	R	R	R	R
Fusidic acid	10 µg	R	R	R	R	R	R	R
Gentamicin	10 mcg	R	R	R	M	M	M	S
Nolidixic acid	30 µg	R	S	R	R	R	R	R
Ofloxacin	5 mcg	S	S	R	M	S	S	S
Pefloxacin	5 mcg	S	S	M	R	M	S	M
Subactam- Ampicillin	10mcg + 10mcg	R	R	R	R	R	R	R
Sulphamethazone	25 mcg	R	R	R	R	R	R	R
Tetracycline	10 mcg	R	R	R	R	R	R	R
Tobramycin	10 mcg	R	R	R	R	S	M	M

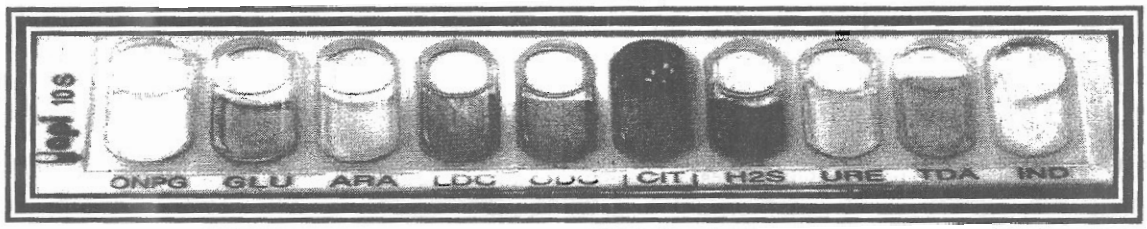
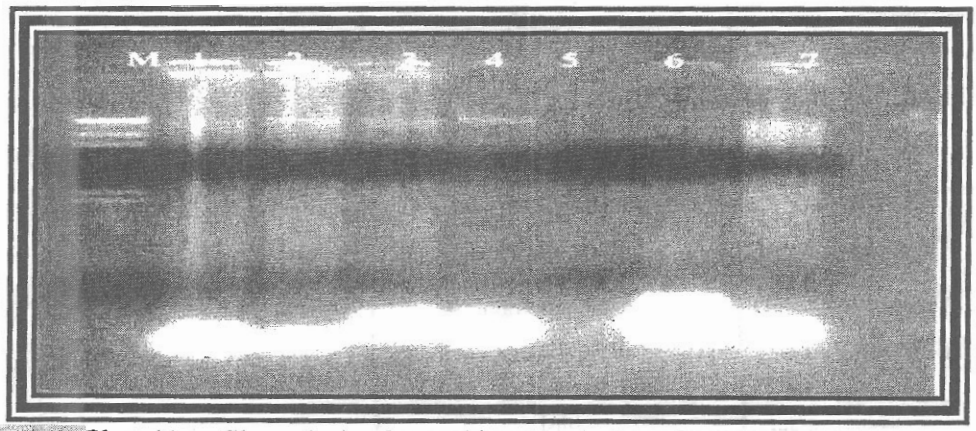


Photo (1): api 10S biochemical results Salmonella positive.



Photo(2): Plasmid profile analysis of *S.*Typhimurium isolates from camels in comparison with different sources.

M = Marker
1-3 = S. Typhimurium (camels) 4 = S. Typhimurium (cattle)
5 = " " (standered strain) 6 = " " (reptile)
7 = " " (bird)

DISCUSSION]

The present study demonstrated a high prevalence of Salmonella infection in apparently healthy slaughtered imported camels. Camels, like other animals can become chronic carriers of Salmonella organisms and subsequently excrete the organisms in their faeces intermittently, especially at times of stress such as during parturition, concurrent diseases, starvation, overcrowding and transport. The carrier animals may not only be at threat for the remaining camels and other species of animals, but also present a human health hazard through contact with contaminated carcases or ingestion of other edible products. The present finding agreed with Wernery (1992) and Yousif et al., (1993), who found 4.3% and 9% prevalence of Salmonella from camels (mesenteric L.n.) in the United Arab Emirates and Egypt, respectively. The high prevalence of Salmonella in the mesenteric lymph nodes (5%) [as shown in Table I] of apparently healthy camels suggests that, unless appropriate hygienic measures are taken to separate the physically viscera from the rest of the carcase. The intestinal contents and the incised mesenteric lymph nodes could serve as sources of contamination of the carcass and other edible parts of slaughtered camels. The mesenteric lymph nodes are considered as the reservoirs of Salmonella in carrier animals and are the principal source material used in detecting Salmonella. The high prevalence of carcase infection (3.6%) observed in this study could be due to contamination during evisceration. Poor disinfection of knives and other equipments, poor personal hygiene of slaughterhouse personnel and the poor sanitation of the slaughterhouses have contributed to the high level of Salmonella contamination of carcases (Yousif et al., 1993 and Molla et al., 2004). The Salmonella serovars isolated from camels in different countries vary but are identical in healthy and diseased camels. The response to infection with Salmonella varies with age, the challenge dose, previous exposure to infection, the immunological status of the animal and exposure to stress in older animals (Wernery, 1992). The isolation of so many different serovars of Salmonella from apparently healthy camels in Egypt is worthy of comment. Other workers have also reported some of these serovars in camels. In our study, S.Typhimurium was the prevalent serotype (Table II), which was identified in Egypt previously by: Ramadan and Sadek (1971), Refai et al., (1984), Safwat et al, (1985), Selim et al., (1990), and Yousif et al., (1993), The detection of invasive Salmonella serotype such as S.Typhimurium and other salmonellae is of public health significance. The high-risk members of the human population, particularly infants, the elderly, the immunocompromised and malnourished persons, are highly susceptible and the presence of Salmonella even in low numbers constitute a major public health hazard (Bryan and Doyle, 1995). Antibiogram patterns [Table IV] showed that examined 7 Salmonella serovars were sensitive to Ciprofloxacin and Ofloxacin and variable results with other antibiotics were detected, while *S.*Typhimurium isolates were only sensitive to Ciprofloxacin and resistance to all antibiotics used in the experiment. This result agreed with Moore et al. (2002) and Molla et al. (2004).Isolated Salmonella serovars exhibited multiple resistances to seven different antimicrobials. The

tested serovars displayed multiple resistances mainly to Aminosidin, Ampicillin, Flucloxacillin, Fusidic acid, Subactam and ampicillin , Sulphamethazone and Tetracycline, these results also agreed with (Molla et al, 2004).Other antibiotics performed variable results with the isolated Salmonella serovars which were, Amoxicillin,Cefaperazone, Cefazidine, Clavulanic acid , Gentamicin, Nalidxic acid, Pefloxacin and Tobramycin.In this study, results showed that, increase of the resistant of the isolates may be due to the miss use of antibiotics in animal and poultry as prophylactic, Genotyping analysis of the organism by plasmid profiles has been useful in recent years. Plasmid profile analysis supplies a quick and relatively easy method for Salmonella spreading in human and veterinary. Some strains of Salmonella harbour several plasmids(Manual Standards, 2000).With regard to the Salmonella Typhimurium isolates which were isolated in this study (camel), they harboured a plasmid ranging from (17-20) kbP. So, significant variations were not detected between the M/W of plasmids of the same source. In comparison with other S.Typhimurium isolates recovered from other sources (cattle, reptile, bird) each isolate harboured a plasmid ranging from (15-19) kbP. So, significant variations were also not detected of S.Typhimurium

growth promotion factor or as therapeutic dose (Ibrahim, 2005). Also, the present study showed the importance of camels as a potential source of single and multiple resistant Salmonella strains to different antimicrobials that are also used in public health sector for the treatment of different bacterial disease in Egypt. Therefore, for controlling Salmonella infection , it is important to select the effective antibiotic according to the antibiogram of the isolates. between the M/W of plasmids

from different sources (Table IV). This finding resembles that of Corre et al. (1999), who revealed that there were clonal similarity between different strains of S.Typhimurium from different origins.Many authors investigated plasmid profile of S.Typhimurium like Popa et al (1997) who isolated (1-4) plasmid of (2-9) and 132 kbP in their molecular weight. Moreover, Walt et al (1998) isolated plasmids of molecular weight ranging from 10 kb to 147 kb. Also, one Salmonella Typhimurium (standard strain) showed absence of plasmid either due to potential plasmid instability or may be of low copy number resulting in negative results during gel electrophoresis (Ibrahim, 2005).

REFERENCES

Ausubel, F.M., Brent, R., Kingston, R., Moore, D.D., Seidman, S.G., Smith, S.A. and Struhl, K. (1987): Current protocols in molecular biology. Published by Green Publishing Associates and Wiely Interscience. New YorkBryan, F.L. and Doyle, M.P. (1995): Health risk and consequences of Salmonella and Campylobacter jejuni in raw poultry. J. F. Prot., 58; 326-344

Corre, C.H.L., Donnio, P.V., Perin, M., Travet, M.F. and Avril, J.L. (1999): Increasing incidence and

comparison of nalidixic acid resistance Salmonella enterica subsp. enterica serotype Typhinurium isolates from human and animals. J.Clin.Microbiol., 37 (1): 266-269

Heithoff, D. M., Shimp, W. R., Lau, P. W., Badie, G., Enioutina E. Y., Daynes R. A., Byrne, B. A., House, J. K., and Mahan, M. J. (2008): Human Salmonella clinical isolates distinict from those of animal origin. Applied and Env. Micro., P. 1757-1766

Elias, S.S. (1982): Preliminary studies on Salmonella microorganisms in camel in Egypt. M.V.Scs Thesis (Microbiology) Fac Vet Med, Cairo Univ.

El-Monella A.A. (1978): Incidence of zoonotic disease (salmonellosis) encountered in animals slaughtered in Egypt. M.V.Sc. Thesis (Meat hygiene) Fac. of Vet.Med,Cairo Univ.

El-Nawawi, F., El-Derea, H. and Sayed, A. (1982): Salmonellosis in slaughtered animals. Arch. Lebensemittle Hyg. 33, 65.

Ibrahim, H.M. (2005): Studies on some Salmonella serovars with a special reference to their susceptibility to newly introduced antibiotics-Ph.D.Vet.Cairo Uni .Le Minor, L. and Popoff, M.Y. (2000): Antigenic formulas of the Salmonella serovars. 6th edn. (WHO Collaborating Center for Reference and Research on Salmonella Paris)

Maual of Standards for Diagnostics and Vaccines (2000): Chapter X4. Note in the code salmonellosis. Office International Des Epizooties

Mohamed, H.M. (2002): Decontamination of bacterial load of the surface of camel carcasses using 2% lactic acid. Minufya. Vet. J. 2 (1): 91-97

Molla, B., Mohamed, A., and Salah, W. (2004): Salmonella prevalence and distribution of serovars in apparently healthy slaughtered camels (camelus dromedarius) in Eastern Ethiopia. Trop. Animal Hlth. and prod., 36 (5), 451-458.

Molla, B., Salah W., Alemayehu, D. and Mohamed, A. (2004): Antimicrobial resistance pattern of Salmonella serovars isolated from apparently healthy slaughtered camels (camelus dromedarius) in eastern Ethiopia. Berl. Münch. Tieräztl. Wschr., 117 (1/2) 39-45

Moore J.E., Calmount M.Mc., Jiruxu G. Nation, Tinson A.H., Crothers L. and Harron D.W.G. (2002): Prevalence of faecal pathogens in calves of racing camels (camelus dromedarius in the United Arab Emirates. Trop. Animal Hlth. Prod., 34: 283-287

National Committee for Clinical Laboratory Standards NCCLS (2002): Performance standards for antimicrobial disk and dilutions susceptibility test for bacteria isolates from

animals "Approved standards". 2nd Ed., M 31-A2, NCCLS, Wayne, Pennsyl-vania, USA

Oxoid Limited, Basingstake Hampshire, England (1970)Pegram R.G. and Tareke F. (1981): Observation on the health of a far livestock. Ethiopian Vet. J. 5

Popa, A.A.V., Danes, D., Avram, E., Sandu, L., Descauses, M.C., Setrin, S.L., Millemann, Y., Dancla, E.C. and Lafont, J.P. (1997): Characterization of antibioresistant avian Salmonella Typhimurium by ribotyping, isotyping and plasmid profiling. Stud. Res. Vet. Med., 5: 79-83

Ramadan, F.M. and Sadek, I.M. (1971): Parameters of salmonellosis in Egypt. J. Egypt Vet. Med. Ass. 31 (3-4), 193-218

Refai, M., Gad El-Said, W., Kamilia Osman, Lotfi Z., Safwat, E.E.A. and Sohier Elias (1984): Salmonella in slaughtered camels in Egypt. Zagazig Vet. J., 266-276

Refai, M., (1992): Bacterial and mycotic disease of camels in Egypt. In: Allen, W.R., Higgins A.J., Mayhew I.G., Snow D.H. and Wade J.F. (eds) Proceedings of the first international camel conference. Dubai R&W Publications, New market, UK, 59-64

Rehab Mohamed (2004): Salmonella in camel and oestriches. M.V.Sc. Thesis. Cairo Uni .

Safwat, E.E.A., Saad, M.K., EL Sawah H. and Refai, M. (1985): Occurrence of salmonellae in imported sheep, cattle and camels slaughtered at Cairo Abattoir. J.Egypt Vet.Med.Ass. 45, No. 4, 37-42

Sambrook J., Fritsch E., and Maniatis T. (1989): Molecular cloning. Alaboratory manual. Second Edition. Cold spring. Harber laboratory Press. New York.

Sandiford B (1944): Food poisoning due to Bact. Typhimurium. J.Path.Bact. 56; 254-255

Selim, A.M., Abd-El-Hamid, Y.M. and Safwat, E.E.A. (1990): Salmonellosis in camel in Egypt. Proc.Inter.Conf on camel prods and Imp. Dec. 10-13.To bruk libya 228-233

Walt, M.L., Van Der, Mare, L., Bezuidenhout, M., Barnard, A., and Nieuwoudt B. (1998): Rapid procedure for isolation and detection of Salmonella plasmids. Biotechnol. Techn. 12 (10): 769-772

Wernery U., (1992): The prevalence of Salmonella infections in camels (camelus dromedarius) in the United Arab Emirates. Brit. Vet. J., 148, 445-449

Yassien N.; Darwish A.; and Mansour N. (1989): Enterobacteriaceae among slaughtered camels with special reference to Salmonella. Vet.Med.J.Giza, 37(1): 1-7

Yousif Abdel Galil, Elsaid El-Sawi, Elsaid. Y.El-Naenaeey and Ahmed F. Abou Zied (1993): Prevalence of Salmonella micro-organisms in Drome-Daries in SHRKIA Governorate. Zag.Vet.J. 21 (4): 752-763

الملخص العربى

دراسات على أنواع من السالمونيلا المعزولة من الجمال المستوردة

هالة إسماعيل شعراوى حنان محمد إبراهيم السيد الصاوى صفوت

* وحدة السيرولوچى- معهد بحوث صحة الحيوان- الدقى

أجريت هذه الدراسة لمعرفة مدى إنتشارميكروب السالمونيلا فى الجمال المستوردة المذبوحة فى مجزر القاهرة، حيث تم فحص ١٠٠ عينة من الغدد الليمفاوية المعوية من هذه الجمال و٥٥ عينة من مسحات من عضلات الصدر والبطن، فوجد أن نسبة عزل السالمونيلا ٥% من الغدد الليمفاوية و ٣,٦ % من مسحات العضلات.

لقد تم تصنيف عترات السالمونيلا إلى ٤ عترات: سالمونيلا تيفيميوريم فلوريدا، شنجتون وأوغندا.

بالإضافة إلى ماسبق لقد تم عمل إختبار حساسية لـ ٧ عترات من السالمونيلا المختلفة ضد ١٦ نوعاً من المضادات الحيوية. وأيضاً تم عمل تحليل بلازميدات للمقارنة بين العترات المختلفة للسالمونيلا تيفيميوريم المعزولة من مصادر مختلفة.

وقد تم مناقشة الأهمية الصحية وكذلك تطبيق الشروط الصحية للسالمونيلا فى الجمال.