

Animal Health Research Institute
Assiut Regional Laboratory

STUDIES ON *SALMONELLA* AND *E. COLI* IN SOME MEAT PRODUCTS (BEEF BURGERS AND LUNCHEON) SOLD IN ASSIUT CITY.

(With 3 Tables)

By

**H.H. ESSA; A.M. MANAA; N.H. MAKAR
and S.M. SAYED.**

(Received at 4/3/2009)

دراسات على ميكروبي السالمونيلا والايثيرشيا كولاي في بعض منتجات اللحوم (البيف بيجر واللانسون) المباعة في مدينة أسيوط.

حمدى حسين عيسى ، أحمد مدوح مناع ، نبيل حبيب مقار ، سيد محمد سيد .

نظرا لأهمية و خطورة بعض الميكروبات على صحة الإنسان وكيفية وصول هذه الميكروبات إلى المستهلك عن طريق تناول الغذاء فقد تناولت هذه الدراسة فحص 60 عينة من البيف بيجر واللانسون (30 عينة من كل نوع) تم جمعها من محلات متنوعة في مدينة أسيوط وعلى فترات مختلفة وتحت ظروف صحية مناسبة وذلك لمعرفة مدى تواجد ميكروبي السالمونيلا والايثيرشيا كولاي في هذه المنتجات. تم عزل ميكروب السالمونيلا في 12 عينة (20%) بواقع 7 عينات من البيف بيجر بنسبة (23.3%) وعدد 5 عينات من اللانسون بنسبة(16.7%) وقد تم تصنيف العترات المعزولة من السالمونيلا إلى *S.typhimurium* (4 عترات) و 3 عترات *S.enteritidis* تم عزلهم من عينات البيف بيجر. وكذلك 3 عترات *S.paratyphi-B* وأيضا عترتين *S.newport* تم عزلهم من عينات اللانسون. كما تم عزل ميكروب الايثيرشيا كولاي من 8 عينات بنسبة (13.3%) بواقع عدد 5 عينات من البيف بيجر بنسبة (16.7%) وعدد 3 عينات من اللانسون بنسبة(10%) وقد تم تصنيف كل العترات المعزولة من البيف بيجر، *E.coli O111k58*. أما العترات المعزولة من اللانسون فقد تم تصنيفها إلى عترتين. *E .coli O128 K67* أما العترة الأخرى تم تصنيفها إلى *Coli . E O126 K7*. هذا وقد تمت مناقشة الاشتراطات الصحية التي يجب توافرها أثناء تجهيز هذه العينات حتى لا تشكل خطراً على صحة المستهلك.

SUMMARY

Recovery of *Salmonella* and *Escherichia coli* from a total number of 60 random samples of different types of packed meat products was evaluated. The collected samples were 30 from each beef burger and luncheon samples. Out of the analyzed 60 samples. *Salmonella* could be

detected only in 12 samples (20%), where 7 (23.3%) isolates were recovered from beef burger, and another 5 (16.7%) isolates from luncheon samples. The isolated *Salmonella* serotypes were 4 strains of *Salmonella typhimurium* and 3 strains of *Salmonella enteritidis* which were detected in the examined beef burger samples, while 3 *Salmonella paratyphi- B* and 2 *Salmonella newport* strains were recovered from luncheon samples. Regarding *E.coli*, they were detected in only 8 (13.3%) samples; 5(16.7%) strains from beef burger and 3(10%) from luncheon. The isolated *E.coli* strains from beef burger were identified serologically into 5 strains *E.coli O₁₁₁ K₅₈*, while the strains isolated from luncheon were two strains *E.coli O₁₂₈K₆₇* and only one strain *E.coli O₁₂₆ K₇*. Source of contamination, precautions during preparation and manufacturing of such meat products, as well as the public health hazards of the presence of *Salmonella* and *E.coli* in meat products were discussed.

Key words: *Salmonella*, *E. Coli*, *beef burgers*, *luncheon*

INTRODUCTION

Meat and meat products are considered as a major vehicle of most reported out breaks of food borne diseases. Epidemiologic data have identified improperly handled meat products as important vehicles for infection (*ICMSF, 1980*).

Changes in the behavior of food consumption by humans, the processing and distribution of food, globalization as well as adaptations of microorganisms, themselves affect the development of new pathogens and the increase in the occurrence of bacteria admitted to be agents of diseases transmitted by food (*Jure, et al. 2006*).

Food borne salmonellosis remains a major public health problem. Early detection of *salmonella* is important for the limitation of outbreaks (*Kawaski, et al., 2000*). Food borne diseases caused by non-typhoid *Salmonella* represent an important public health problem worldwide so nearly 104 million cases of salmonellosis occur each year in the United States (*Angulo, et al., 2000*). Intestinal salmonellosis typically resolves in five to seven days and does not require treatment with antibiotics. However, bacteraemia occur in 3 to 10% of reported, cultured confirmed cases and is particularly common among patient at the extremes of age and those who are immunocompromised. When infection spreads beyond the intestinal tract, appropriate antimicrobial therapy (e.g., ciprofloxacin in adult and ceftriaxone in children) can be lifesaving

(Glynn *et al.*, 1998; Hohamanon, 2001). Non –typhoidal salmonellosis an important enteric infection in humans, particularly in the neonates and younger children (Gupta and Vermo, 1993). Most *Salmonella* infections in humans result from the ingestion of contaminated poultry, beef, pork, eggs and milk (Gomez *et al.*, 1997). Most infections with antimicrobial resistant *Salmonella* is acquired eating contaminated food of animal origin (Angulo *et al.*, 2000). *Salmonella* do not release toxins into food in which they multiply rather, the ingested cells multiply in the small intestine of the victim, causing illness. This illness is characterized by diarrhoea, vomition and abdominal pain after an incubation period of 24-36 hours (Wyatl, 1992).

Many people enjoy beef burgers, luncheon and other meat products, especially during the summer months. However, raw and improperly handled luncheon and beef burgers can harbour harmful bacteria including *Escherichia coil*. The bacteria constituting the species *E .coli* are bacteria that normally live in the intestines of human and animals. Although most strains are harmless, several are known to produce toxins that can cause diarrhea. The pathogenic groups includes enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC). Only the first 4 groups have been implicated in food or water borne illness (Levine, 1987; Nataro and Kaper, 1998). Many researches concluded the enteropathogenic *E. coli* isolated from different meat products was incriminated in different diarrhea and gastrointestinal out breaks in adult human (Marier *et al.*, 1973; Edelman and Levine, 1983).

This study is planned to investigate the presence of *Salmonella* and other pathogenic *E. coli* serotypes among some selected meat products. The public health significant of the isolated pathogens is discussed.

MATERIALS and METHODS

I - Collection of samples:

A total number of 60 random samples of various types of packed meat products were collected from different shops and supermarkets at Assiut city. The collected samples included 30 from each beef burger and Luncheon.

The samples were obtained in their intact original package and transferred as quickly as possible to the laboratory for bacteriological

examination. Samples under investigation were prepared to be examined for the presence of *Salmonella* and *E. coli*.

II - Isolation and identification of *Salmonella* organisms:

Twenty grams of each sample were inoculated into 100 ml of peptone water and incubated at 37°C for 24 h. Then 10 ml of the incubated peptone water were inoculated into 100 ml of selenite F. broth. After incubation at 37°C for 18 – 24 h., a loopful of the incubated enrichment broth was streaked onto MacConkey's, Brilliant green and S.S agar media. Inoculated plates were incubated at 37°C for 24 h. Suspected colonies presumed to be *Salmonella* (non lactose fermenters) were further identified morphologically and biochemically according to Koneman *et al.* (1994) and Quinn *et al.* (1994).

Isolates that produced biochemical reactions simulation *Salmonella* were subjected to serological identification as described by Edward and Ewing (1972) and the instruction of the technical information of the manufacture laboratory (Anon, 1975). The final decision of typing was made according to Kauffmann white scheme (kauffmann, 1972).

III - Isolation and identification of *E.coli*

The technique was carried out according to FAO (1979).

Presumptive test: one ml from the previously prepared dilutions was inoculated into lauryl sulphate tryptose broth fermentation tubes with inverted Durham's tubes.

The tubes were incubated at 35-37 °C for 24 and 48 h. Tubes showing gas production were recorded as positive.

Confirmation test: A loopful from each positive tube in the presumptive test was transferred separately into each of three E C broth tubes with inverted Durham's tubes .The tubes were incubated at 45°C for 48h. Tubes showing gas production were considered positive. From each gas positive tube of E C broth, a loopful was streaked on Levine's Eosin Methylene Blue (EMB) agar plates. The plates were incubated at 35-37°C for 24 h. Isolated typical colonies or colonies most likely to be *E. coli* were subjected to further identification by biochemical tests including indole, Methyl red, Vogues proskauer and Citrate (IMVIC reaction). The most Probable Number (MPN) of *E. coli* per gram of the examined samples was determined (A.O.A.C., 1984).

RESULTS

Table 1: Incidence of *Salmonella* and *E. coli* in examined meat products samples.

Types of examined samples	Number of examined samples	Positive samples.			
		<i>Salmonella</i>		<i>E.coli</i>	
		+Ve	%	+Ve	%
Beef burger	30	7	23.3	5	16.7
Luncheon	30	5	16.7	3	10
Total	60	12	20	8	13.3

Table 2: *Salmonella* serotypes isolated from the examined samples.

Serotypes	Frequency of isolation		Total	%
	beef burger	Luncheon		
<i>S.typhimurium</i>	4	-	12	20
<i>S.enteritidis</i>	3	-		
<i>S.paratyphi-B</i>	-	3		
S.new port	-	2		

Table 3: *E. coli* serotypes isolated from the examined samples.

Serotypes	Frequency of isolation		Total	%
	Beef burger	Luncheon		
<i>E. coli O₁₁₁K₅₈</i>	5	-	8	13.3
<i>E. coli O₁₂₈K₆₇</i>	-	2		
<i>E. coli O₁₂₆K₇</i>	-	1		

DISCUSSION

Meat and meat product play a role in most reported outbreaks of food borne diseases. Certain pathogenic microorganism in or on food of animal origin still constitute a particular hygienic risk.

Salmonellae are important pathogens in both animal and man. They are ubiquitous microorganisms that have been found in most of the animal species in most of the geographic areas of the world. Salmonellosis today is one of the most important foods borne disease which caused a significant public health problem. Foods of animal origin particularly meat and meat products are still the major source of human salmonellosis.

It is evident from the present investigation that the incidence of *Salmonella* in the examined beef burger and luncheon was 7(23.3%) and 5(16.7%), respectively (Table, 1). Table (2) shows that the *Salmonella* serotypes were identified from beef burger samples. Four strains of *S.typhimurium* and three strains of *S. enteritidis* were detected, while three strains of *S. paratyphi- B* and two strains of *S.newport* were recovered from the examined luncheon samples.

Many investigators isolated *Salmonella* from the examined beef burger. The obtained results, in the present study, were higher than the results recorded by Darwish *et al.* (1986) (5%); Ahmed and Abdel- aziz (1988) (6%); El-Mossalami. *et al.* (1989) (6%); Fathi and Thabet (2001) (16.67%) and Essa and Makar (2003) (6.6%). Whereas, other workers could detect the organism in beef burger samples similar to that obtained in the present work; as Karim (1976); Ibrahim (1981); Tolba (1986); Abdel- Aziz (1987) and Khalafalla (1988).

On the other hand *Salmonella* organisms detected in the examined luncheon were higher than that detected by Essa *et al.*, 2004 (6.6%). Concerning *Salmonella* serotypes, *S. typhimurium* were the commonest strain isolated from similar meat products by most investigators. Darwish *et al.* (1986) and Fathi and Thabet (2001) succeeded to isolate *S. typhimurium* and *S. paratyphi* from the examined beef burger. *S. typhimurum* and *S. typhi* were recovered also from luncheon by Essa *et al.* (2004).

Meat products may be contaminated with *E. coli* from food handlers, food utensils, air, soil and water under incomplete hygienic measure during manufacturing, packing and marketing of these products (Frazier and Westhoff, 1978). In the present study the incidence of *E. coli* in the examined beef burger and Luncheon samples was 5 (16.7%) and 3 (10%), respectively (Table 1). From the results achieved in Table (3) it was found that out of 8 (13.3%) isolates of *E.coli*

recovered from the examined beef burger and luncheon samples were serologically typed as Enteropathogenic *E. coli* (EPEC). *E. coli* serotypes recorded and identified from beef burger samples were 5 strains of *E. coli* O₁₁₁ K₅₈. While 2 strains of *E. coli* O₁₂₈ K₆₇ and one strain of *E. coli* O₁₂₆ K₇ were recovered from the examined luncheon samples.

The results in Table (1), it reveals that *E. coli* was isolated from 16.7% and 10% of the examined beef burger and luncheon, respectively. The present study indicates that the incidence of *E. coli* isolates was particularly low in beef burger which seem to be lower than the results reported by Duitschaeffer *et al.* (1977) (27.72%); Darwish *et al.* (1986) (30%); Tolba (1986) (25%) and Essa and Marker (2003) (23.33%), but the results detected in the same study was higher than the result recorded by Darwish *et al.* (1991)(12%) in the beef burger examined samples. On the other hand, very high existence of *E. coli* in beef burger has previously been reported by Abdel- Aziz (1987) (70%).

Many investigators concluded that EPEC isolated from different meat products was incriminated in different infantile diarrhea and gastrointestinal outbreaks in adult human (Dupont *et al.*, 1971; Marier *et al.*, 1973; Edelman and Levine, 1983).

In conclusion, many workers, have stated that *E. coli* as one of predominant *Enterbacteriaceae* should be taken into account when considering the sanitary standards and hygiene of food handling particularly minced meat, beef burger, luncheon and other local manufactured products either frozen or fresh (Stiles and Lai-King, 1981; Gobran, 1985; Niazi and Refai, 1988).

ACKNOWLEDGMENT

I wish to express my sincere thanks to Prof. Dr. A. El-Tamawy Professor of Bacteriology, Faculty of Medicine, Assiut University, for his help in *Salmonella* and *E. coli* stereotyping.

REFERENCES

- Abdel-Aziz, A.T. (1987): Microbiological load of some meat products as influenced by the hygienic status of the producing plant. M.V. SC., Thesis, Fac. Vet. Med., Cairo Univ.
- Ahmed, S. and Abdel-aziz, M. (1988): *Salmonellae* in locally manufactured meat products. M.V SC., Thesis, Fac. Vet. Med., Cairo Univ.

- Angulo, F.J.; Johnson, K.R.; Tawxw, R.V. and Cohen, M.L. (2000): Origin and consequences of antimicrobial resistant nontyphoidal Salmonella: Implication for the use of fluoroquinolones in food animals. *Microb Drug. Resist.* 6: 77-83.
- Anon, (1975): Serological identification of Salmonella. Difco Laboratories, Deteroit, Michigan, U. S. A., 0168.
- A.O.A.C. (Association of Official Analytical Chemistis) (1984): Bacteriological analytical Manual 6th Edition FDA, Arlington, V A, U.S.A.
- Darwish, A.; Hamdy, M. and Nouman, T.M. (1986): Quality evaluation of market meat pastes .*Vet. Med. J.*, 34, 1:37-48.
- Darwish, A.M.; Niazi, Z.M. and Zaki, E.M. (1991): *Escherichia coli* in meat products. *Vet. Med. J.*, Giza, 39, 3: 841-851.
- Duistchaever, C.L.; Bullock, D.H. and Arnott, D.R. (1977): Bacteriological evaluation of retail ground beef frozen beef patties and cooked hamburgers. *J. Food Prot.*, 40, 6: 378-381.
- Dupont, H.L; Formal , S.B.; Hornick, B.B.; Snyder, M.J.; Libonati, J.P., Sheehan, D.G.; Lobrec, E.H. and Kalas, J.P. (1971): Pathogenesis of *E coli* diarrhoea. *N. Engl. J. Med.*, 285: 1-9.
- Edelman, T. and Levine, M.M. (1983): Summary of a workshop on Entero pathogenic *Escherichia coli*. *J. Infect. Dis.*, 147: 1108-1118.
- Edwards, R.P. and Ewing, W.H. (1972): Identification of Enterobacteriaceae. Minnoapolis, Burgess, Publ. Co. Atlanta, U.S.A.
- El-Mossalmi, E.E.; Safwat, A.A.S.; Abdel-Rahim, L. and El-Sawah, H. (1989): *Salmonella* in locally produced meat products. *J. Egypt Vet. Med. Ass.*, 49, 1-2: 99-108.
- Essa, H.H. and Makar, N.H (2003): Bacteriological quality of beef burgers in Assiut City. *Assiut. Vet. Med. J.* 49, 99: 81- 88.
- Essa, H.H.; Makar, N.H. and Sohair, Z.H. (2004): Bacteriological evaluation of chicken luncheon in Assiut City. *Assiut. Vet. Med. J.* 50, 102: 64-71.
- FAO (1979): Food Agriculture Organization of the United Nations, Rome, Manuals of Food Quality Control, 4. Microbiological analysis, via delle terme dicaracalla, 00100 Rome, Italy.
- Fathi, M. Sh. and Thabet, A. El-R. (2001): Incidence of *Salmonella* and *E. coli* in packed meat products sold in Assiut City. *Assiut Vet. Med. J.* 46, 91:187-198.

- Frazier, W.C. and Westhoff, D.C. (1978): Food Microbiology 3rd Ed. Tata ACG row Hill Publ, Comp. Lted, Newdelhi.
- Glynn, M.K.; Bopp, C.; Dewitt, W.; Dobney, P.; Mokhtar, M. and Angulo, F.J. (1998): Emergence of multidrug. resistant *Salmonella enterica* serotype *typhimurium* of 104 infections in the United States, N Eng. J. Med., 338:1333-1338.
- Gobran, R.A. (1985): Enterobacteriaceae in meat products in Upper Egypt. M.V Sc. Thesis, Fac. Vet. Med., Assiut Univ.
- Gomez, T.H.; Motarjemi, Y.; Miyagawa, S.; Kuferstin, K.F. and Stohr, K. (1997): Food borne salmonellosis. World Health State Q., 50: 81-89.
- Gupta, B.R. and Vermo, C.J. (1993): Monograph on animal salmonellosis. Lzatnagar: National Salmonella Center (Veterinary), Div. Bacterial, Mycol., IVRI: 9-11.
- Hohamanon, E.L. (2001): Non-typhoidal Salmonellosis. Clin Infect Dis., 32: 263-269.
- Ibrahim, A.M. (1981): Sanitary condition of locally produced hamburger, M.V. Sc. Thesis, Cairo University.
- ICMSF (1980): Microbial Ecology of Food, Vol. 1, Univ. of Toronto Press Toronto. Canada.
- Jure, A.M.; Aulet De Saab, O.; Suarez, A. and De Castillo, C.M. (2006): Assessment of survival and production of Shiga toxins by enterohemorrhagic *E. coli* in stored hamburgers. J., of Food Technology, 4(3): 194-199.
- Karim, G. (1976): Bacteriological quality of raw and cooked hamburgers at the retail level in Theran. J. Food Prot. 40: 560.
- Kawaski, S.; Kimura, B. and Fuji, T. (2000): Comparison of taq manTM Salmonella amplification detection kit with standard culture procedure for detection of *Salmonella* In meat samples. Pakistan J. of Biological Sci. 10(1): 122-126.
- Khalafalla, F.A. (1988): Sanitary status of meat, meat products and fish in Beni Suf Governorate. Ph. D. Thesis, Vet. Met., Cairo Univ.
- Konemon, E.W.; Allen, S.D.; Janda, W.M.; Schrecken-Berge, P.C. and Winn, W.C. (1994): Introduction to Diagnostic Microbiology 4th ed. J. B. Lippincott Company., P. 41.
- Kouffimann, F. (1972): Serological diagnosis of *Samonella* species. Kouffinann-White Scheme. Munksgaard. Copenhagen.
- Levine, M.M. (1987): *Escherichia coli* that cause diarrhea enterozoxigenic, enteroinvasive, enterohemorrhagic, and enteroadherent. J. Infect. Dis., 155: 377-389.

- Marier, R.; Wells, J.; Swanson, R.; Collahn, S. and Mehlaman, I. (1973): An outbreak of *E. coli* food borne disease traced to imported French cheese. *Lancet*, 2:1376.
- Nataro, J.P. and Kaper, J.B. (1998): Diarrheagic *Escherichia coli*. *Clin. Microbial. Rev.*, 11:132-201
- Niazi, M.Z. and Refai, M. (1988): Isolation of enteropathogenic and enterotoxigenic *Escherichia coli* from meat and cheese. *Vet. Med. J.*, 36, 1:121-134.
- Quinn, P.J.; Carter, M.E.; Markery, B.K. and Careter, G.R. (1994): *Clinical Vet. Microbiology*. Year book Wolfe Publishing Europe Limited, P. 209-236.
- Stiles, M.E. and Lai-King, Ng, (1981): Enterobacteriaceae associated with meat and meat handling. *Appl. and Environmental Microbiology*. 41, 4: 867-872.
- Tolba, K.S. (1986): Antibiotic resistant microorganisms in some meat products. M. V. Sc., Thesis, Fac. Vet., Med., Cairo University.
- Wyatl, G.M. (1992): *Immuno Assays for Food Poisoning Bacterial and Bacterial Toxins*. Chapman and Hall. 1st Ed, Pp. 5-15.