SUST
Journal of Agricultural and Veterinary Sciences
Journal homepage:
http:// journals.sustech.edu

Prevalence of *Salmonella*, *Escherichia coli* in Meat Products in Khartoum State

N.A. M. Osman;[1] S.E. Suliman[2]; Y.Y.Alian[2]; M.A.Abdalla[2]

[1]Department of Microbiology, Sudanese Standards and Metrology Organization, Khartoum, Sudan.
[2]College of Veterinary Medicine, Sudan University of Science and Technology, Khartoum, Sudan
Corresponding author*:* Mohammed A. Abdallah, E-mail*:* salamaa2000@sustech.edu

Article history: Recieved : January 2018
Accepted:February 2018

Abstract

This study aimed to determine the prevalence of *Salmonella* spp, *E. coli* and aerobic plate count in meat products in Khartoum State. Aerobic plate count was used to identify the contamination of the product with pathogenic microbes. A total of 100 samples of meat products were collected from factories and analyzed. The samples included 20(20%) burger, 30 (30%), sausage, 25 (25%) kofta, 12 (12%) minced meat, and 13 (13%), different type of meat products. Culture and different biochemical and serological tests and aerobic plate count were used to detect *Salmonella.*spp, *Escherichia coli* and aerobic microbial contamination. The results showed that 4 (4%) of samples were positive for *Salmonella* spp. and 44 (44%) were presence for *Escherichia coli*. In addition to 71% of samples exceeded the limit 10^5 cfu/g of the Sudanese Standard for Aerobic Plate count test in meat product. The study concluded that there is contamination of *Salmonella*, *E. coli* and there is high in total aerobic plate count test in meat products in Khartoum.

Keywords: *Salmonella spp*. *Escherichia coli*. Khartoum. Meat.

Introduction

Since *Salmonella* spp. were first discovered in human tissues in 1880, and then isolated from pigs in 1885 by Salmon (Buxton and Fraser, 1977), their significance as important pathogens has been recognized. To date, more than 2,500 *Salmonella* serovars have been identified (Popoff *et al.,* 2004), approximately 2, 000 of which are capable of infecting humans. Their host specificity may vary even between variants within a serovar (Wall *et al.,* 1995). Epidemiologically, *Salmonella* bacteria can be divided into human-specific serovars, serovars with host adaptation but also able to infect humans, and serovars able to infect both humans and animals (Jay *et al.,* 2005). *Salmonella* is a Gram-negative, rod-shaped belonging to the family Enterobacteriaceae, (Marlony *et al*., 2003).

Salmonella are facultative anaerobes, flagellated rod-shaped bacteria with both respiratory and fermentative metabolic pathways. They are oxidase negative, ferment glucose and produce acid and gas. The organisms grow on citrate as a sole source of energy. They decarboxylate lysine and ornithine, generally produce hydrogen sulfide, and do not hydrolyse urea. One of the characteristics of this genus is that most

members do not ferment lactose or sucrose (Yousef and Carlstrom, 2003) . Diseases caused most frequently by *Salmonella* enterica are collectively known as salmonellosis (Yousef and Carlstrom, 2003). The disease in humans is generally contracted through the consumption of contaminated food of animal origin (mainly meat, poultry, eggs and milk), although many other foods, including green vegetables contaminated from manure, have been implicated in its transmission. The majority of pathogens causing food borne illnesses are considered to be zoonotic (Käferstein and Abdussalam, 1999). The increase in international trade in agricultural, aqua cultural and manufactured food products has facilitated the spread of *Salmonella* (D'Aoust, 1994). *Salmonella* has been the subject of public health concern as an agent causing foodborne diseases for over a century (Hardy, 2004). *Salmonella* has been estimated to be responsible for 30% of the food borne outbreaks in the United States (Mead *et al*., 1999). Isolation and identification of strains involved is an important step in controlling *Salmonella* outbreaks or sporadic clinical cases (Threlfall and Frost, 1990; Gonzalez and Mendoza, 1995). *E. coli* is a gram-negative, facultative anaerobic, non-spore-forming rod, which belongs to the Enterbacteriaceae family. The odor *Escherich* first cultured 'Bacterium coli' in 1885 from the feces of a healthy individual. It was renamed *Escherichia coli* in 1919 in a revision of bacteriological nomenclature (Law, 2000). Many benefits have been found from *E. coli* in human medicine, food industry, and the water industry. Some studies suggest that *E. coli* can serve as a benefit to the human body by synthesizing vitamin K and by using competitive inhibition to out compete other bacteria that might enter the intestinal tract. Differences between strains of *E. coli* lie in the combination of different antigens they possess. There are three types of antigens: the somatic lipopolysaccharideantigen (Law, 2000), the flagellar antigens (H), and the capsular antigens (K). There areapprox-imately 174 O antigens, 56 H antigens, and 103 antigens that have beenidentified. There are several stains of *E. coli* that have been isolated. The enteric *E. coli* are divided on the basis of virulence properties into enterotoxigenic (ETEC),enteropathogenic (EPEC), enteroinvasive (EIEC), verotoxigenic (VTEC), enterohemorrhagic (EHEC), and enteroaggregative (EaggEC). ETEC can be found in humans, pigs, sheep, goats, cattle, dogs, and horses; EPEC is found in humans ,rabbits, dogs, cats, and horses; EIEC and EAgg EC are only found in humans; VTEC is found in pigs, cattle, dogs, and cats; while EHEC is found in humans, cattle, and goats and attack porcine strains that colonize the gut in a manner similar to human EPEC strains (Fratamico *et al.,*2002). Pathogenic *Escherichia* coli, specifically *E. coli* O157:H7 has emerged as a foodborne pathogen of great concern in beef products. While generic *E. coli* is considered a part of the normal microflora in the intestinal tract of most warm-blooded animals, including humans, many pathogenic strains can cause diarrheal disease and have been associated with food-borne illness (Doyle, 1990). The aerobic plate count is designed to provide an estimate of the total number of aerobic organisms in a particular food. A series of dilutions of the food homogenate is mixed with an agar medium and incubated at 35°C for 48 hr. It is assumed that each visible colony is the result of multiplications of a single cell on the surface of the agar (Andrews, 1992).The total aerobic plate count is useful for indicating the overall microbiological quality of a product and, thus, is useful for indicating potential spoilage in perishable products. The aerobic plate count is also useful for indicating the sanitary conditions under which the food was produced and/or processed (Andrews, 1992).

This study aimed to determine the prevalence of *Salmonella* spp, *E. coli* and aerobic plate count in meat products in Khartoum State.

Materials and Methods

This is a descriptive Cross –sectional aimed to detect *Salmonella spp*, *E. coli* and aerobic plate count from meat products in Khartoum State.

Sampling and Sample size

One hundred meat products samples were randomly selected. Samples were collect from markets, factories of meat in Khartoum State. Samples were taken and analyzed in accordance with the International Organization for Standardization (ISO 17604:2003/ Amd.1:2009 (E)), (ISO 6579:2002/Amd.1:2007(E)).

The media and reagent were used from Oxoid and Mast, UK. All tests were done according to the International Organization for Standardization (ISO 6579:2002/Amd.1: 2007(E)). Twenty five gram of representative portions of poultry and poultry products was used and kept into a sterile stomacher bag. Then 225 ml buffer peptone water was added into the bag and was homogenized, then sealed and incubated at 37 °C for 18-24 hours. Portions of 1.0 and 0.1 ml of BPW pre enrichment culture were transferred to 10 ml of enrichment in selective broth medium Muller Kauffmann TetraThionate-novobiocin broth (MKTTn), and 10 ml of Rappaport-Vassiliadis medium with soya (RVS broth), respectively. MKTTn broth enrichment cultures were incubated at 37 °C for 24 h. The RV broth enrichment cultures were incubated at 42°C for 24 h. A loop ful of each Enrichment broth were streaked on selective media plates of Xylose Lysine Deoxycholate Agar (XLD) and Bismuth Sulfite (BS) agar. Then plates were incubated for 24 hours ± 2 hours at 37°C. After that plates were examined for *Salmonella*. five colonies suspected were taken for confirmatory test; selected colonies were streaked on to the surface of pre-dried nutrient agar plates and incubated at 37°C ± 1°C for 24 h ± 3 h. Isolated colonies were examined biochemically on Triple Sugar Iron agar, Urea Agar, Voges-Proskauer medium, tryptone/tryptophan medium and 1-Lysine decarboxylation medium. The isolates were further confirmed by slide agglutination test using polyclonal O-, Vi- and H-antiserum specific for genus *Salmonella* (procured from MAST, England).

E. coli isolation and calculation

Isolation and identification of *E. coli* were done according to the International Organization for Standardization (ISO 16649-2:2001(E)).

By using of a sterile pipette1 ml of the tested sample was transferred to a sterile Petri dish. initial dilution ($10^{-}1$) inoculated two plates ,then repeated this procedure with the further decimal dilutions, after that poured into each Petri dish approximately 15 ml of the TBX medium, previously cooled at 44°C to 47°C in the water bath . Carefully the inoculums mixed with the medium and allowed the mixture to solidify with the Petri dishes. Inverted the inoculated dishes so that the bottom is uppermost and placed them in an incubator set at 44 °C for 18 h to 24 h.

Counting the colony-forming units

After the specified period of incubation counting of the typical CFU of glucuronidase-positive *Escherichia coli.*

Calculation of Aerobic Plate Count (APC)

Calculation of APC was done according to the International Organization for Standardization (ISO 4833:2003(E)). Two sterile Petri dishes transferred to each one by means of a sterile pipette 1 ml of the tested sample from initial suspension (10^{-1} dilutions). The same procedure was used for the another dilution 10^{2} then Poured 15 ml of the plate count agar into each Petri dish and allowed to solidify and incubated at 30 °C ± 1 °C for 72 h ± 3 h.

Counting of colonies

Using the colony counting bacteria isolate were counted between 15 and 300 colonies per plate.

Results

In the present study, a total of 100 samples of meat products were collected from factories and analyzed. The samples included 20(20%) burger, 30 (30%), sausage, 25 (25%) kofta, 12 (12%) minced meat, and 13 (13%), different type of meat products. Figure1. Culture and different biochemical and serological tests were used to detect *Salmonella* spp, *Escherichia coli* and aerobic plate count. The results showed that 4 (4%) of samples were positive for *Salmonella.* spp (Figure 2). Also the results showed that 44 (44%) of samples were presence for *Escherichia coli* (Figure 3). In addition to 71 (71%) of samples exceeded the limit 10^5 cfu/g of the Sudanese Standard for Aerobic Plate count test (Figure 4). Different biochemical test were used for identification of *Salmonella* spp. These including glucose fermentation test, urease test, voges-proskauer test, indole test and 1-lysine decarboxylase test. Table1. From pure colonies 4 isolated organisms were confirmed by slide agglutination method against *Salmonella* O-, Vi- and H-antisera. Table2.

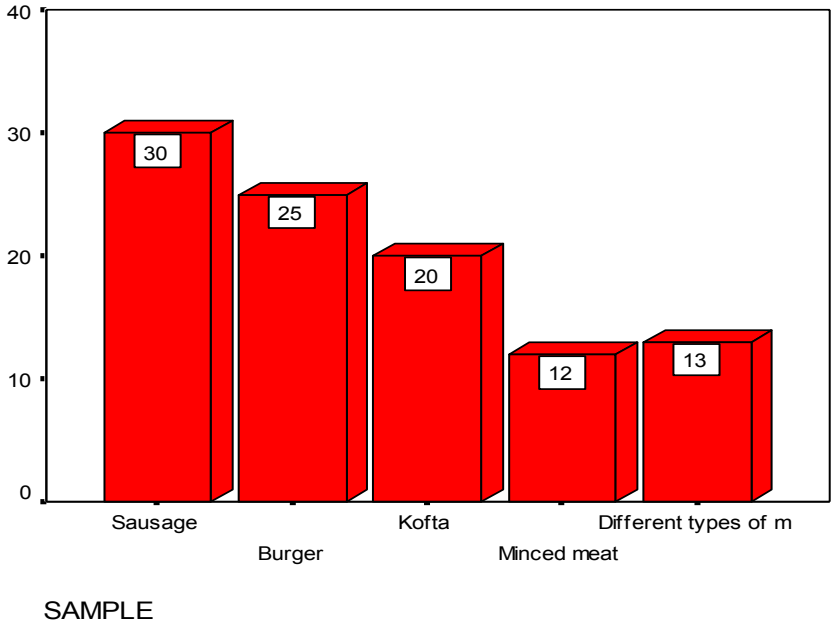


Figure1. Type and number of samples

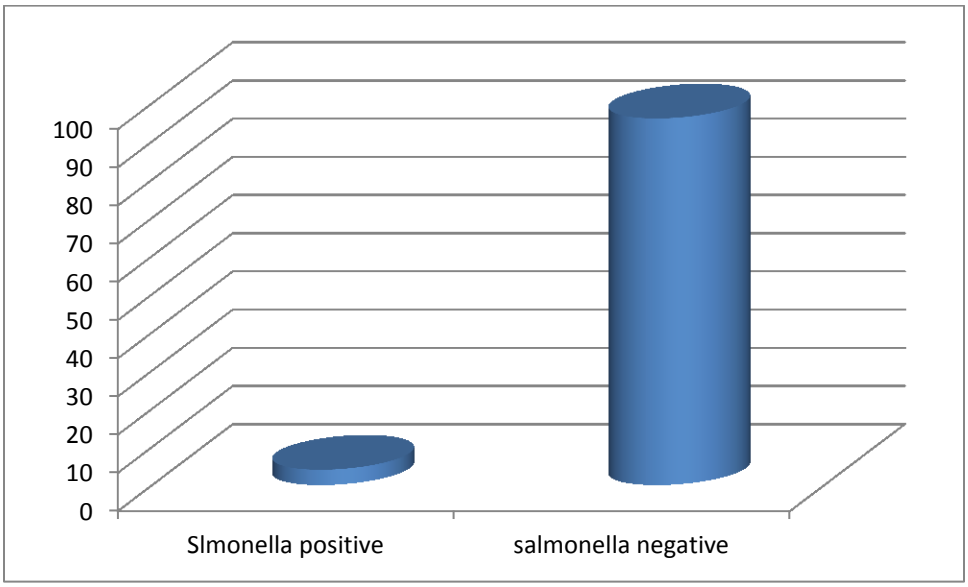


Figure 2. Positive *Salmonella* and negative in samples

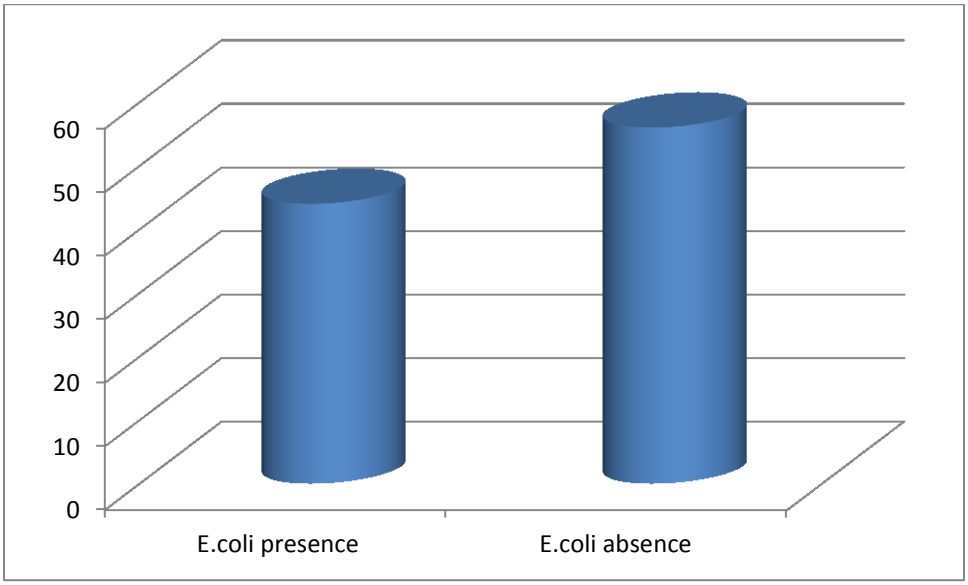
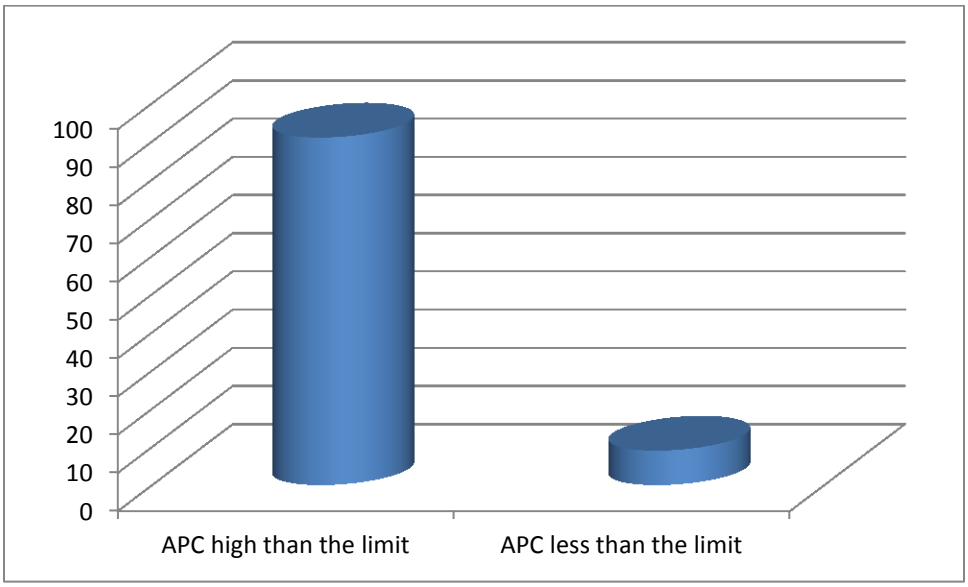


Figure 3 presences and absence *E. coli* in the samples



Fugger 4 Aerobic Plate count result according to the limit 10^5 cfu/g

Table 1. Biochemical reactions of *Salmonella* (n= 4)

Test	Result
Glucose fermentation	+
Urease test	_
Voges-Proskauer test	_
Indole test	_
1-Lysine decarboxylase test	+

Table 2. Serological reaction of *Salmonella* spp. (n= 4)

Test	Result
Polyvalent O Antisera	Agglutination (+)
Polyvalent H Antisera	Agglutination (+)
Polyvalent Vi Antisera	Agglutination (+)

Discussion

The purpose of microbial testing is to confirm that all possible avenues contamination have been identified and that these avenues are being controlled"(Kvenberg and Schwalm, 2000). Meat samples were selected for this study because they are reported to frequently harbor various enteric organisms. The present study showed that the prevalence of *Salmonella* species in meat and meat products constituted 4%. These results were in line with the results of others who obtained a relatively contamination of meat and meat products with different serotypes of *Salmonella* (Campell and Gilbert, 1995). *Salmonella.* spp. was found in minced meat, kofta and burger (Mohamed, 2013). The lowest percentage of *Salmonella* spp. In our study agree with finding of 4% by Fatin, (2004) and disagree with obtained by Essa *et*

al., (2009) which are 23.3%. *Escherichia coli* and fecal coli forms are considered to be the most important and compulsory measure of microbiological quality of food and food related products in terms of hygiene. Their presence is used as indicators of fecal pollution. Among these, *E. coli* is often preferred as a more specific indicator of fecal contamination because it is specific and most reliably reflects fecal origin (Feng and Hartman, 1982; Doyle and Erickson, 2006).Testing for generic *E. coli* is one method that is required in meat and poultry processing plants (Eisel *et al*.,1997). In this study the over-all prevalence of *E. coli* in all meat products was 44% which similar with some other studies.(Ayla and Seza, 2012) reported the results of microbiological analysis of retail meat samples relative to the contamination levels of *Escherichia coli* are 53.6%. In Australia, *E. coli* was detected on 15.4% of meat and meat products samples. In present study, 71 (71%) of samples contaminated with aerobic plate count organisms exceeded the limit allowed by the Authority of Sudanese standards and Metrology Organization 10^5 cfu/g. The higher aerobic count in meat indicated that sanitary measures during handling, manufacturing process, and packaging were neglected and also low quality of meat was used. The variations in total aerobic count in meat samples might be due to the contamination from equipment or the environment. The total aerobic plate count is useful for indicating the overall microbiological quality of a product and, thus, is useful for indicating potential spoilage in perishable products. The aerobic plate count is also useful for indicating the sanitary conditions under which the food was produced and/or processed (Doyle, 1990). The study concluded that there is contamination of *Salmonella* , *E. coli* and there is high in total aerobic plate count test on meat products in Khartoum state.

Acknowledgment:
The author is thankful to the Research Laboratory, Sudan University of Science and Technology for Technical Support.

Reference

Andrews, W. (1992). Manual of food quality control.Food and Agriculture Organization of the United Nation. VialedelleTerme di Caracalla. Rome Italy: pp. 9- 49.

Ayla, E. and Seza, A**.** (2012). Prevalence of *Escherichia coli* in retail poultry meat, ground beef and beef. *Med*. *Weter*. **68 (4).**

Buxton, A. and Fraser, G. (1977). Animal Microbiology, Vol I. Blackwell Scientific Publications, Oxford.ISBN 0 632 00690 0.

Campell, K. W. and Gilbert, S. A**.** (1995). Poultry quality assessment. Report prepared for the Public Health Commission and the Ministry of Health, Wellington, New Zealand. Available at: http://www.foodsafety. govt. Nz/elibrary /industry/risk_ profile_*Salmonella*-science_research.pdf (Last accessed on 2.12.2017).

D'Aoust, J.Y. (1994). *Salmonella* and the international food trade. *Int. J. Food Microbiol.* **24:** 11-31.

Doyle, M. P. (1990). Pathogenic *Escherichiacoli*, *Yersiniaenterocolitica*, and *Vibrioparahemolyticus*. *LANCA*. **336**:1111-1115.

Doyle, M. P. and Erickson, M. C. (2006). The fecal coliform assay, the results of which have let to numerous misinterpretations over the years, may have outlived its usefulness. *Microbe*. **4:** 162-163.

Eisel, W. G.; Linton, R. H. and Muriana, P.M. (1997). A survey of microbial levels for incoming raw beef, environmental sources, and ground beef in a red meat processing plant. *Food Microbiology*.**14**: 273 -282.

Essa, H. H.; Manaa, A. M.; Makar, N. H. and Sayed, S.M. (2009). Studies on *Salmonella* and *E .coli* in some meat products (beef burgers and luncheon) sold in Assiut city. *Assiut Vet. Med. J*. **55(121):** 211-216.

Fatin, H. S. (2004). Bacterial hazards associated with consumption of some meat products. *Benha Vet. Med. J*. 15(2): 41-54.

Feng, P. C. S. and Hartman, P. A. (1982). Fluorogenic assay for immediate confirmation of *Escherichia coli*. *Appl*. *Environ*. *Microbiol***. 43:** 1320-1329.

Fratamico, P. M.; Smith, J. L. and Buchanan, R. L. (2002). *Escherichia coli*. Foodborne Diseases (pp. 79–101). San Diego, California: Academic Press.

Gonzalez, H, M. and Mendoza, M. C. (1995). Differentiation of strains from a food-borne outbreak of *Salmonella* enterica by phenotypic and genetic typing methods: *Eur j Epidemiol*. **11:**479-482.

Hardy, A. (2004). *Salmonella*: a continuing problem. History of Medicine. *Postgraduate Medical Journal*.**80**: 541-545.

Jay, J. M.; Lessner, M. J and Golden, D. A. (2005). Modern Food Microbiology. Seventh Edition. Food Science Text Series. Springer Science + Business Media, Inc., USA. p790.

Käferstein, F .and Abdussalam, M. (1999).Food safety in the 21st century. Bulletin of the World Health Organization, **77**: 347-351.

Kvenberg, J. E. and Schwalm, D. J. (2000). Use of Microbial Data for Hazard Analysis and Critical Control Point Verification -Food and Drug Administration Perspective. *Journal of Food Protection*. **63**(6): 810-814.

Law, D. (2000). Virulence factors of *Escherichia coli* O157 and other Shiga toxin-producing E. coli. *Journal of Applied Microbiology*. **88**: 729–745.

Marlony, B.; Hoorfar, J.; Hugas, M; Heuvelink, A.; Fach, P.; Ellerbroek, L.; Bunge, C.; Dorn, C. and Helmuth, R. (2003). Inter laboratory diagnostic accuracy of *Salmonella* specific PCR-based method. *Inter J of Food Microbiol*, **89**: 241–249.

Mead, P. S.; Slutsker, L. ; Dietz, V. ; McCraig, L. F. ; Bresee, J. S. ; Shapiro, C. ; Griffin, P. M. and Tauxe, R.V. (1999).Food-related illness and death in the United States. *Emerging Infectious Diseases***. 5**: 607-625.

Mohamed, K**.** (2013). Prevalence of *Salmonella* in Meat Products. *Global Veterinaria* **11 (5):** 685-688.

Popoff, M.Y.; Bockemuhl, J.; Gheeseling, L. L. (2004).Supplement 2002 (no. 46) to the Kaufmann-White scheme. *Research in Microbiology* **155**: 568-570.

Threlfall, E. J .and Frost, J. A**.** (1990). The identification, typing and fingerprinting of *Salmonella*: laborat-ory aspects and epidemiological applications. *J ApplBacteriol*. **68:**5-16.

Wall, P.G.; Morgan, D.; LAmden, K.; Griffin, M.; Threlwall, E.J. ; Ward, L.R. and Rowe, B. (1995). Transmission of multi resistant strains of *Salmonella* Typhimurium from cattle to man*. Veterinary Record***. 136**: 591-2.

Yousef, A. E. and Carlstrom, C. (2003). Food Microbiology: A laboratory Manual. John Wiley and Sons Inc, Hoboken, New Jersey. Available at: http: // books .google. Com /books/about/Food Microbiology. Accessed in 11.4.2017.

إنتشار السالمونيلا *Escherichia coli* في منتجات اللحوم بولاية الخرطوم

نجم الدين عبد الله محمد عثمان ، سهام الياس سليمان ، ياسر يوسف عليان ومحمد عبدالله عبدالسلام[2]

.الهيئة السودانية للمواصفات والمقاييس
.كلية الطب البيطري – جامعة السودان

المستخلص

هدفت هذه الدراسه الى تحديد مدى انتشار السالمونيلا فى منتجات اللحوم فى ولاية الخرطوم . تم جمع عدد 100 عينة منتجات اللحوم من المصانع وتم تحليلها. تضمنت العينات 0! (0 %) بيرقر و0! (0 %) سجوق و5! (5 %) كفتة و2 (2 %) لحمة مفرومة و13 (3 %) انواع مختلفة من اللحوم. تم استخدام طريقة التذريع والاختبارات الكيمياء الحيوية والاختبارات السرولوجية والاطباق الهوائية للكشف عن السالمونيلا والاشيرشيا القولونية والعدد الكلى للميكروبات الهوائية الملوثة . اظهرت النتائج ان ! (%) من العينات توجد بها سالمونيلا و44 (4 %) من العينات توجد بها الاشيرشيا القولونية بالاضافة الى أن 71 (1' %) من العينات تجاوز فيها عدد الميكروبات الهوائية الرقم 10^5 وهو الحد المسموح بها من الهيئة السودانية للمواصفات والمقاييس فى منتجات اللحوم. خلصت الدراسة الى أن هنالك تلوث بالسالمونيلا والأشيرشيا القولونية و ارتفاع للعدد الكلى للميكروبات الهوائية فى منتجات اللحوم بالخرطوم.