STUDIES ON *E.COLI AND SALMONELLAE* IN SOME EDIBLE OFFAL OF BOVINE CARCASSES

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A B S T R A C T

A grand total of one hundred fresh random samples of different edible offal of bovine carcasses represented by lungs, livers, kidneys and hearts (25 of each) were collected directly after slaughtering and evisceration from different slaughter houses and street vendors, EL-Gharbia Provience. The collected samples were subjected to bacteriological examination for detection and identification of *E.coli* and *Salmonella spp*. *Isolates of E.coli* were serotyped into O_{55} and $O111:H_4$ serovers, O_{26} and O_{128} serovers , O_{26} and $O_{119}:H_6$ serovers and $O_{111}:H_4$ from lung, liver, kidney, heart samples, respectively. Furthermore, *S.entertidis* var O:1,4,5,12 ,H: i(1,2) could be isolated from the examined liver and kidney samples ,*S.typhimurium* var O:1,9,12, H:g,m(1,7) could be isolated from examined liver and lung samples ,while, *S.virchow* var O:6,7,14,H:r (1, 2) could be isolated from the examined lung samples only. *Salmonella* failed to be isolated from all the examined samples of heart. The public health importance of isolated microorganisms and the possible sources of contamination of edible bovine offal with these organisms as well as suggestive hygienic measures to improve the quality of offal were discussed.

(BVMJ-25 [2]:276 -283, 2013)

1. INTRODUCTION

The slaughter of animals yields many edible products other than carcass meat (such as red offal), which are fit for human consumption. They are used either as prepared items (e.g. slices of liver) or used as ingredients in meat products. The market for "edible by-products" differs by country (even region) and culture. Many of these products could be used for human consumption also diverted into the pet food chain *(7)*. Edible offal such as liver, kidney and spleen are widely consumed. Although they are rich in mineral and vitamin contents they can be contaminated more frequently than animal carcasses by many types of microorganisms from the moment of animal slaughtering until consumption. *(25) and (40)*. In the abattoir itself, there are numerous sources of microorganisms such as hides of slaughtered animals, soil, feet, intestinal content and equipment used for dressing, air and water used for washing of the carcasses *(3)*. The prevalence of food borne pathogens in animals and human has caught the attention of researchers, food industry, health organization, governments and all stake holders .Such data give an idea of the possibility of the transfer of pathogenic organisms from animal food stuffs to human and subsequently cause food borne diseases, illness or food poisoning *(4)*.

Escherichia coli is a facultative anaerobic bacterium commonly found in the mammalian intestinal tract. *Escherichia coli* lives as a fecal-oral lifestyle and can comprise up to 1% of the gastrointestinal population of mammals and used as indicator of environmental fecal contamination of water supplies. Cattle have been implicated as an important reservoir for *E. coli*. Most of *E. coli* strains

are commensal; however, some *E. coli* strains can be pathogenic to human, and harboured within food animals *(42)*. Presence of enteropathogenic *E.coli* (EPEC) strains were recognized as a cause of infantile diarrhea and gastrointestinal illness of adult human .While, enterotoxigenic *E.coli* (ETEC) strains are considered as a common cause of traveler's diarrhea and sporadic summer diarrhea in children,as well as, food poisoning outbreaks. Other types are enteroinvasive *E.coli* (EIEC), enterohaemorhagic *E.coli* (EHEC) and enteroaggregative *E.coli* (EAggEC) *(42)*. *Salmonella* is generally divided into two categories, non-typhoidal *Salmonella*, the most common form, which is carried by both human and animals and caused by most serotypes of *Salmonella*, such as *S. typhimurium*, *S. javiana* and *S. enteritidis*. Typhoidal *Salmonella*, which causes typhoid fever, is rare, and is caused by *S. typhi*, *(30)*. Considering all these hazards, the present research was planned out to evaluate the microbial profile status of bovine edible offal through identification of *E.coli* and *Salmonella* isolated from bovine edible offal samples.

2. MATERIALS AND METHODS

2.1. Collection of samples:

A grand total of one hundred fresh random samples of different edible offal of bovine carcasses represented by lungs, livers, kidneys and hearts (25 of each) were collected directly after slaughtering and evisceration from different slaughter houses and street vendors, EL-Gharbia Provience. Each sample was kept in a separated sterile plastic bag and preserved in an ice box then transferred to the laboratory under complete aseptic conditions without undue delay and examined as quickly as possible. The collected samples were subjected to bacteriological examination to detect *E.coli* and *Salmonella*.

2.2. 2.2. Preparation of samples (17):

Twenty five gms were taken aseptically from the examined liver, heart, kidney and lung samples and transferred aseptically to a sterile homogenizer bag containing 225 mls of sterile peptone water (1%) and homogenized for 2.5 minute at 3000 r.p.m. to provide a diluation 10^1, then decimal serial dilutions were prepared.

2.3. Screening of Enteropathogenic Escherichia coli (2 ,18):

2.3.1. Pre-enrichment:

One ml from the original dilution was inoculated into MacConky broth tube supplemented with inverted Durham's tube. The inoculated and control tube were incubated at 37°C /24-48hrs. Tubes showing gas production were considered positive for coliforms.

2.3.2. Enrichment:

One ml from positive MacConkey broth was transferred into Brilliant Green Bile 2% broth tubes supplemented with inverted Durham's tube and incubated at 44± 0.5°C for 18 hours (Eijkman test).

2.3.3. Selective plating:

A loopful from a positive Brilliant Green Bile (2%) broth tube was streaked into Eosine Methylene Blue agar (EMB) incubated at37°C /24; typical colonies of E.coli appear greenish metallic with purple center.

2.3.4. Identification of Escherichia coli:

Microscopical examination (6): Gram negative coccobacilli to medium size rods.

Biochemical identification:

- Motility test: + ve result (non motile) *(29)*.
- Indol production test: + ve result (red ring) *(29)*.
- Methyl Red reaction: + ve result (red colour) *(26)*.
- Voges Proskaure test: –ve result (no change in colour) *(27)*.

- Gelatin Liquefacrion : –ve result*(5)* .
- Hydrolysis of urea: –ve result (no change in colour) *(9, 24)*.
- Hydrogen sulphide test: –ve result (no change in colour) *(28).*
- Utilization of citrate: –ve result (no change in colour) *(39).*
- Fermentation of sugars: +ve result with lactose *(28).*
- Eijkman test: *E.coli* is one of few organisms that produce gas at this temperature *(2).*

2.3.5. Serological identification:

The isolates were serologically identified according to ***(23)*** by using rapid diagnostic *E.coli* antisera sets (Denka Seiken Co., Japan) for diagnosis of the enteropathogenic types.

2.4. Screening of Salmonella (8,15 and 35):

2.4.1. Pre-enrichment :

Twenty five gms of the examined samples were homogenized in 225 ml peptone water 1%.

2.4.2. Enrichment:

One ml of the inoculated pre-enrichment broth was transferred into 9 ml Rappaport Vassiliadis enrichment broth and incubated at 43°C /24 hrs.

2.4.3. Selective plating:

Loopfuls from the inoculated tubes were separately streaked on to XLD agar medium and incubated at 37°C /24 hrs. Suspected colonies were red with or without black centers.

2.5. Identification of Salmonella:

Microscopic examination *(6, 19)*: Gram-negative non spore forming rods.
Biochemical Identification.

- Motility test: non motile *(29)*.
- Indole-production test: –ve result yellow color *(29)*.
- Methyl red test : +ve result (red color) *(26)*.
- Voges-Proskauer test: –ve result (no change in color) *(27).*
- Citrate utilization test: +ve result (blue color) *(39).*
- Hydrogen sulphide test: –ve result (no change in color) *(28).*
- Urease test: –ve urease test *(9).*
- Fermentation of sugars: +ve in dulcitol and -ve in maltose and sucrose*(28)*.

Serological identification:
Serological identification of *Salmonellae* was carried out according to *(20)* for determination of somatic (O) "slide agglutination test" and flagellar (H) antigens"tube agglutination test" using *Salmonella* antisera (Denka Seiken Co., Japan) .

3. RESULTS

Table (1): Incidence of *E. coli* isolated from bovine edible offal samples (n=25).

Offal *E.coli* Strains	Lungs		Liver		Kidneys		Heart		Strain characteristics
	No.	%	No.	%	No.	%	No.	%	
O26	-	-	2	8	1	4	-	-	EHEC
O55	1	4	-	-	-	-	-	-	EPEC
O111 : H4	1	4	-	-	-	-	1	4	EHEC
O119 : H6	-	-	-	-	1	4	-	-	EPEC
O128	-	-	1	4	-	-	-	-	ETEC
Total	2	8	3	12	2	8	1	4	

EPEC = Enteropathogenic E.coli, ETEC = Enterotoxigenic E.coli, EHEC= Enterohaemorrhagic E.coli

Table (2): Incidence of *Salmonella* isolated from bovine edible offal samples (n=25).

Offal	Lungs		Liver		Kidneys		Antigenic structure	
Salmonella Strains	No.	%	No.	%	No.	%	O	H
S. enteritidis	-	-	1	4	1	4	1,4,5,12	i : 1,2
S. typhimurium	1	4	1	4	-	-	1,9,12	g,m : 1,7
S. virchow	1	4	-	-	-	-	6,7,14	r : 1,2
Total	2	8	2	8	1	4		

4. DISCUSSION

Edible offals such as lungs, liver, kidneys and heart, contain various nutritional components as high in vitamin content, high quality protein and energy to human beings. For example, livers are high in vitamin A, iron, zinc, vitamin B, vitamin C, vitamin D, copper and fatty acids. Hearts contain large amounts of iron selenium, zinc, phosphorous, niacin and riboflavin, but they are very low in sodium. So offal particularly, liver is consumed in large number of dishes or as common ingredients in many foods in many countries (25). In Egypt, the continuous increase in meat price lead people to search for another cheaper source of protein, so people find that the suitable source is edible offal as heart and kidney (33). The results achieved in table (1) showed that E.coli strains could be isolated from some edible offal samples and serotyped as lung: O_{55} (4%) (EPEC) and O111:H_4 (4%) (EHEC); liver: O_{26} (8%) (EHEC) and O_{128} (4%) (ETEC); kidneys: O_{26} (4%) (EHEC) and O_{119}:H_6 (4%) (EPEC) ; heart: O_{111} :H_4 (4%) (EHEC). Nearly similar E. coli serotypes were isolated from edible offal samples by Khalafalla et al. (22) who isolated E.coli from 25 samples of cattle livers. The isolated E.coli is serotyped as O_{111},O_{128} and O_{26}, while EL-Zeini and Shalab (12) isolated EPEC from lung . The isolated EPEC is serotyped as O_{55} and O_{128}. Moreover, Hassan and Osaman (16) who examined bacteriological fifty fresh bovine lung samples and found that incidence of EPEC is 20%. The isolated EPEC were belonged to serotypes O_{111} (4 strains), O_{55} (2 strains) and O_{128} (2 strains) and Mohamed- Amany (31) serologically identified *E.coli* as O_{26} O_{111}, O_{127} and O_{128} from40 bovine liver samples (20 each from cattle and buffaloes). Higher results were obtained by Roushdy et al. (36) who examined 50 liver samples obtained from healthy slaughtered cattle and isolated E.coli (42%) and by Salem- Ghada(37) who collected offal samples from butcher's shop and street cars and isolated E. coli at (40%) and(60%) in heart samples, (40%) and(60%) in liver samples and(40%) and(50%) in lung samples, respectively. Lower results were obtained by Surkiweicz et al.(43) who examined chopped liver and found that the frequency of isolated E.coli was 1%. Additionally, E.coli is the most common microorganism implicated in infants, children diarrheal cases, buffalo, cows and sheep represented the highest reservoir of E.coli infection to man Taha (44). The enteropathogenic *E.coli* induced watery diarrhea, vomiting and fever in infants and young children. The clinical illness was ranged from self-limited diarrhea to highly protracted syndrome of chronic enteritis accompanied by failure to thrive and wasting. The authors summarized the serogroups implicated as O55, O86, O111, O114, O119, O125, O126 and O142 (32). On the other hand, the

presence of E.coli on carcasses were a reliable index for other enteric zoonotic agents such as Salmonella (13). Results reported in table (2) revealed that Salmonella strains could be isolated from some edible offal samples and serotyped as lung: S.typhimurium (4%) which are O:1,9,12 ,H: g , m(1,7) and S.virchow (4%) which are O:6,7,14,H:r (1,2) ; liver: *S.entertidis* (4%) which are O:1,4,5,12 ,H: i(1 ,2) and *S.typhimurium* (4%) which are O:1,9,12 ,H:g,m(1,7) and kidneys: S.entertidis (4%) which are O:1,4,5,12 ,H: i(1 , 2). Nearly similar results were obtained by Khalafalla et al (22) who examined bacteriologicaly 25 samples of cattle livers and isolated *S. typhimurium* (4%) and *S. typhi* (4%), while Akkaya et al. (1) recorded the prevalence of Salmonella spp. using a total of 205 edible bovine offal samples collected from different abattoirs and butcheries. The isolation rate of Salmonella was found to be 8.57% and 5.71% for the liver and kidney collected from the abattoir, respectively. Concerning the offal samples obtained from the butcheries, the detection rate of Salmonella sp. was 16% in the liver and 4% in the kidneys. Higher results were obtained by Sinell et al.(41) who reported that the level of Salmonella contamination was 68. 9% in bovine lung. While Popovic et al. (34) who isolated *S. entertidis* (7%) from 30 bovine liver samples. Lower results were obtained by EL-Eidy and Diab (11) who examined 50 samples of cattle liver and hearts (25 for each) subjected to bacteriological examination ,the incidence of Salmonella recovered from liver was one strain (1.0%) and failed to be isolated from the examined heart sample ,and Keven and Ay(21) who reported that there is 2.2% of the examined liver samples obtained from markets were positive and 3.3% of the examined samples obtained from local butcheries were positive for Salmonella spp. , however no bovine liver samples from abattoirs were positive for Salmonella spp. The source of Salmonella spp. was probably gastrointestinal tract and mesenteric lymph node, both of which may show high prevalence of infection in cattle which have been held before slaughter. Therefore, edible offal should be separated from viscera at evisceration by personnel who was not involved with the alimentary tract (38). Salmonella is widely recognized as one of the most principal causes of food poisoning outbreaks occurring as a result of consumption of contaminated meat and offal. *Salmonella typhimurium* and *S.enteritidis* were the most frequent serotypes implicating in cases of human salmonellosis (14). Additionally, Salmonella is the second most common cause of food borne illness. It is responsible for millions cases of food borne illness every year. Infection with Salmonella may or may not lead to sometimes-fetal salmonellosis, a disease that can remain localized in the gastrointestinal tract as gastroenteritis, or become generalized as septicemia and affect several organs and systems. Infected animals that do not develop the disease become carriers for Salmonella and serve as a source of infection to human and other animals (10). Furthermore, it has been shown that edible offal are cross contaminated by Salmonella spp. at the abattoirs and retail sale points until they reach to the consumer. Therefore, it is recommended that adequate hygienic and sanitary measures should be taken in these places in order to protect public health (1). Farm to table control measures of E. coli and Salmonella include: managing farms to reduce fecal shedding in cattle; implementing HACCP procedures in slaughter operations and beef processing, proper handling during transport and by retail outlets and proper cooking and handling by consumers (45).

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دراسات على التقييم الميكروبي للإشيرشيا كولاي والسالمونيلا في بعض الأحشاء الداخلية لذبائح الأبقار

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الملخص العربي

تعتبر الأحشاء الداخلية للأبقار مصدرا غذائيا مهما في حياة الإنسان وذلك لقيمتها الغذائية العالية حيث أنها تمد الجسم بنسبة مرتفعة من البروتين الحيواني علاوة على احتوائها على بعض العناصر الأخرى مثل الدهون والأملاح المعدنية والفيتامينات.كما أن الارتفاع المستمر في أسعار اللحوم جعل الناس يبحثون عن مصدرا رخيصا للبروتين الحيواني مثل القلب والكلي والكبد. على الوجه الآخر تعد الأحشاء الداخلية للماشية من أكثر الأغذية عرضة للتلوث بالعديد من الميكروبات الممرضة والمسببة للفساد بدءا من أول مراحل الذبح مرورا بمراحل النقل والتخزين والتداول في الأسواق والمحلات حتى الوصول إلى المستهلك. لذا أجريت هذه الدراسة بفحص عدد (100 عينة) عشوائية من الرئة والكبد والقلب والكلى لذبائح الأبقار بواقع 25 عينة لكل نوع وذلك لفحصها ميكروبيولوجيا لتحديد نسبة الإشريشيا كولاي والسالمونيلا وقد دلت النتائج على الآتي:- تم عزل ثمانية عترات من الميكروب القولوني المعوي الإيشريكية: اثنان من الرئة، ثلاثة من الكبد، اثنان من الكلى وواحد من القلب كالآتي:- (1) عترة من O_{55} (1) عترة منH_4: O_{111} في عينات الرئة، (2) عترة من O_{26}, (1) عترة من O_{128} في عينات الكبد ، (1) عترة من O_{26} ، (1) عترة من O_{119}:H_6 في عينات الكلى، (1) عترة منH_4: O_{111} في عينات القلب. كما تم عزل ثلاثة عترات من السالمونيلا:- (2)عترة من عينات الرئة (سالمونيلا تيفيميوريم-سالمونيلا فيرشاو) ،(2) عترة من عينات الكبد (سالمونيلا تيفيميوريم-سالمونيلا انتريتيدس) و(1) عترة من عينات الكلى(سالمونيلا انتريتيدس). بينما لم يتم التمكن من عزل أي عترات للسالمونيلا من عينات القلب.
وقد خلصت هذه الدراسة إلى خطورة تلوث الأحشاء الداخلية مثل الرئة والكبد والقلب والكلى بأنواع من البكتريا والتي لها تأثير ضار على صحة الإنسان. ولقد تم دراسة ومناقشة الأهمية الصحية للميكروبات المعزولة ومصادر التلوث بالإضافة إلى اقتراح التوصيات اللازمة لضمان جودة هذه الأنواع من الأحشاء الداخلية.

(مجلة بنها للعلوم الطبية البيطرية: عدد 25(2):276–283, ديسمبر 2013)