Quantitative and Qualitative Studies on Enterobacteriaceae in Ground Beef

Abdelrahman H.; Meawad, A and Shaheen, H
Faculty of veterinary Medicine-Food Hygiene Department -Suez Canal University

Abstract:
120 packages of ground beef (40 each of fresh, frozen and frozen with Soya bean) were examined for the incidence of Enterobacteriaceae, Salmonella and *E. coli*. The incidence of Enterobacteriaceae was 100%, 75% and 87% in fresh, frozen and frozen with Soya ground beef respectively; and the mean counts were $6.3x10^4$cfu/g, $1.4x10^2$cfu/g and $1.6x10^3$cfu/g respectively. *E. coli, Klebsiella oxytoca, Serratia marcescens* and *Serratia liquefaiens* could be isolated from fresh ground beef; *Proteus vulgaries, E. coli* and *Enterobacter sakazakii* was isolated from frozen ground beef, while in case of frozen ground beef with Soya bean *Klebsiella oxytoca, Proteus vulgaries, Citrobacter freundii, E. coli* and *Pantoea a gglomerans* was isolated. The incidence *of E. coli* was 60%, 35%, 48% in fresh, frozen and frozen ground beef with Soya bean respectively. The incidence of Salmonella was 0%, 10%, 15% in fresh, frozen and frozen ground beef with Soya bean respectively. *E. coli* strains were serologically identified into 20 O-typable strains and 37 O- untypable strains. The 20 O-typable strains were identified as O_{86}, O_{119}, O_{158} ,O_{145} and O_{115} .
Key words: Ground beef, Enterobacteriaceae, *E. coli*, Salmonella

Introduction:

Meat and meat products are considered as an ideal culture medium for growth of many organisms ***(Gracey, 1986)***. Contamination of raw meat is one of the main sources of foodborne illnesses ***(Bhandare et al, 2007; Podpecan et al, 2007).*** Changes in eating habits, mass catering, unsafe food storage conditions and poor hygiene practices are major contributing factors to food associated illnesses ***(Hedberg et al, 1992).***Ground beef is either pure ground beef with or without any additives or with Soya bean protein ***(EOS, 2005).*** Enterobacteriaceae group has an epidemiological interest and importance as some of them are pathogenic and may cause serious infections and/or food poisoning. It is the most challenging bacterial contaminant to raw and processed meat products worldwide. Salmonella, *E. coli*, Proteus, and *Klebsiella species* are the most predominant species in all

food poisoning cases associated with some meat products. ***(Mercuri and Cox, 1979; Ternstro¨m and Molin, 1987)*** Although more attention is generally paid to the pathogenic properties of particular genera of Enterobacteriaceae, some members of the family constitute an important spoilage group when conditions favour their growth ***(Stanbridge and Davies, 1998; Nychas et al, 2008).*** Foodborne pathogens are the leading causes of illness and death in developing countries costing billions of dollars in medical care, medical and social costs ***(Fratmico et al, 2005)*** A number of high-profile outbreaks of foodborne illnesses have been associated with meat products. Wider recognition of the importance of emerging pathogens such as *E. coli* O157:H7 have increased consumer and public health concerns about the possible contamination of such products, with such undesirable pathogens. Most people are aware of the existence of *Escherichia coli* in ground beef. It is a very common form of bacteria that causes people to get sick. The bacteria live in the intestines of both animals and humans, and can transfer easily between them when proper food preparation methods are not employed. It can cause problems with the functioning of the digestive system and can severely affect bowel movements ***(Witherspoon, 2011)*** *E. coli* can get into meat during processing. If the contaminated ground beef is cooked to a degree less than 71°C, the bacteria can survive and cause several health problems and even death. It leads to a severe diarrhea in infants and travelers, minor discomfort to sever cholera like disease, as well as food poisoning manifestations among adults ***(Frazier and Westhoff 1988; Mackie and Mecartney, 1989).*** Salmonella is one of the most important pathogenic genera implicated in foodborne bacterial outbreaks and diseases ***(Gouws, Visser and Bro¨zel, 1998).*** There are several transmission routes for Salmonellosis, but the majority of human infections are derived from the consumption of contaminated meat and meat products ***(Hernandez et al, 2005) (Chittick et al, 2006)*** .Therefore the aim of the present study was carried out to evaluate the load of Enterobacteriaceae and the incidence of *E. coli* and salmonella in ground beef.

Materials and Methods:

Collection of samples: A total of 120 packages of ground beef (40 each of fresh, frozen and frozen with Soya bean) were collected from different local retailers in Ismailia province.

Preparation of samples: All samples were prepared according to the technique recommended by ***APHA (2001).*** 25g from each sample were transferred under aseptic condition to a sterile polyethylene bag containing 225mL

of 0.1% sterile buffered peptone water. The content of the bag was then homogenized using stomacher (*Lab. Blender 400, Seward Lab, London*) to have a dilution of 10^{-1} then further serial dilutions were carried out till $10^{-7.}$

Determination of total Enterobacteriaceae count: were determined by the technique recommended by ***ISO (2004)***

Identification of isolates: was carried out by using API-20E system

Isolation of *E. coli*: was carried out according to the method recommended by ***ICMSF (1996).***

Isolation of Salmonella: was carried out according to the method recommended by ***ISO (2002b).***

Serological identification of *E. coli* and Salmonella was carried out at Animal Health Research institute in Ismailia province.

Results and Discussion:

Enterobacteriaceae are wide spread in the environment and taken as useful indicators of hygiene and post processing contamination of processed meat. Furthermore, their count can be taken as an indicator of possible enteric contamination in the absence of coliforms even in low number.

The results reported in table (1) revealed that the incidence of Enterobacteriaceae in fresh, frozen and frozen with soya ground beef samples was 100%, 75% and 87% respectively. Nearly similar results were obtained by ***Lindberg et al. (1998) and Ali et al. (2010).***Such results of high incidence of Enterobacteriaceae in ground beef were attributed due to unhygienic handling during processing, storage and distribution. Also addition of certain additives to meat products may lead to marked increase in the bacterial population ***(Sharaf, 1999).***

The results recorded in table (2) showed the mean values of Enterobacteriaceae count in ground beef were $6.3x10^4 \pm 2.8x10^4$ cfu/g for fresh ground beef, $1.4x10^2 \pm 3x10$ cfu/g for frozen ground beef and $1.6x10^3 \pm 4.15x10$ cfu/g for frozen ground beef with soya bean respectively.

The results were nearly similar to those reported by ***Lindberg et al (1998)*** also by **Crowley *et al.* (2005)** in case of packaged minced beef samples. While lower results were recorded by ***Gustavsson & Borch (1993) and Murray et al (2001).*** However higher findings were obtained by ***Crowley et al. (2005)*** in case of fresh, unpackaged, ground beef samples.

The variation in the results between different authors may be due to the differences in manufacture practices, storage conditions, handling and the effectiveness of hygienic measures applied during production.

The frequency distribution of Enterobacteriaceae among the examined samples of fresh ground beef as given in table (3) revealed that *E. coli , Klebsiella oxytoca, Serratia marcescens* and *Serratia liquefaiens* were isolated at

incidence of 12%, 50%, 25% and 13% respectively and that given in Table (4) revealed that *Proteus vulgaries, E. coli* and *Enterobacter sakazakii* were isolated at incidence of 52%, 24% and 24% respectively in frozen ground beef, while that given in table (5) revealed that *Klebsiella oxytoca, Proteus vulgaries, Citrobacter freundii, E. coli* and *Pantoea agglomerans* were isolated at incidence of 13%, 50%, 12%, 13% and 12% respectively in frozen ground beef with soya bean

The obtained results were nearly similar to those reported by ***Stiles and Ng (1981), Ali et al (2010) and Doulgeraki et al (2011).***

Serratia liquefaciens was known to proliferate in refrigerated foods ***(Drosinos and Board, 1995),*** and is frequently found to predominate in ground beef. *S. liquefaciens* and other *Serratia* spp. are considered to be opportunistic pathogens but have as yet not been implicated in diarrheal diseases and could be isolated only from fresh ground beef. *Citrobacter freundii* has been involved in a case of severe gastroenteritis and meat food has been identified as a vehicle of transmission ***(Thurm and Gericke, 1994 and Tscha¨pe et al, 1995)***

Results given in table (6) revealed that the incidence of *E. coli* in fresh, frozen, and frozen with soya bean ground beef was 60%, 35%, 48% respectively.

These results were nearly similar to those obtained by ***Hassan (1986) and Hussein & Bollinger (2005).***Lower results were reported by ***Doyle and Schaeni (1987), Mousa et al (1993), Blanco and Blanco (1996), Chapman et al (2000), Fantelli and Stephan (2001), Vernozy - Rozand et al (2002), Baran and Gulmez (2003), Zaho et al (2004), Cagney et al (2004), Crawely et al (2005), Dambrosio et al (2007) and Bernardez et al (2007)*** While higher results were reported by ***Geoff et al. (2008).*** This variation in the results was attributed to poor sanitation during ground beef processing.The presence of *E. coli* in meat and meat products is considered as an indicator of the presence of a fecal contamination in addition to the unhygienic conditions during preparation, handling and storage.

The results given in table (7) showed that the incidences of Salmonella in fresh, frozen and frozen with Soya bean ground beef were 0%, 10% and 15% respectively. The results obtained are nearly similar to those obtained by ***Khalafalla (1996), Little et al (1998), Jordan et al (2006), Little et al (2008), Cetinkaya et al (2008), Duggan et al (2012) and Ahmed & Shimamato. (2014).*** While higher results were obtained by ***Mrema et al (2006).*** These variation may be attributed to the level of the hygienic procedure adopted during meat processing.

The results given in table (9) revealed that 57 strains of E. coli isolated from the examined ground

beewere serologically identified into 20 O-typable strains and 37 O-untypable strains. The 20 O-typable strains were identified in table (10) as EPEC with the following serotypes O86, O119 and O158 with incidence of 2 (10%) for each; EHEC with serotype O145 with incidence of 4 (20%) and ETEC with O115 serotype with incidence of 10 (50%).

Table 1: *Incidence of Enterobacteriaceae in examined ground beef samples*

Samples	**Positive**		**Negative**	
	No.	%	No.	%
Fresh	40	100	0	0
Frozen	30	75	10	25
Frozen with Soya	35	87	5	13
Total	105	87	15	13

Table 2: *Enterobacteriaceae count in examined ground beef samples.*

Samples	**Min.**	**Max.**	**Mean**	**S.E.**
Fresh	$13x10^2$	$5x10^5$	$6.3x10^4$	$2.8x10^4$
Frozen	<10	$4x10^2$	$1.4x10^2$	3x10
Frozen with Soya	$2x10^2$	$7x10^3$	$1.6x10^3$	4.5x10

Table 3: *Frequency distribution of Enterobacteriaceae among the examined samples of fresh ground beef*

Enterobacteriaceae species	**F.**	**%**
E. coli	7	12
Klebsiella oxytoca	30	50
Serattia marcescens	15	25
Serattia liquefaciens	8	13
Total	60	100

Table 4: *Frequency distribution of Enterobacteriaceae among the examined samples of fresh ground beef*

Enterobacteriaceae species	**F.**	**%**
Proteus vulgaries	23	52
E. coli	11	24
Enterobacter sakazakii	11	24
Total	45	100

Table 5*: Frequency distribution of Enterobacteriaceae among examined samples of frozen ground beef with Soya bean*

Enterobacteriaceae species	**F.**	**%**
Klebsiella oxytoca	7	13
Proteus vulgaris	26	50
citrobacter freundii	6	12
E. coli	7	13
Pantoea agglomerans	6	12
Total	52	100

Table 6: *Incidence of E. coli in examined ground beef samples*

Sample	**Positive**		**Negative**	
	No	%	No	%
Fresh	24	60	16	40
Frozen	14	35	26	65
Frozen with Soya	19	48	21	52
Total	57	48	63	52

Table 7: *Incidence of Salmonella examined ground beef samples*

Sample	**Positive**		**Negative**	
	No	%	No	%
Fresh	0	0	40	100
Frozen	4	10	36	90
Frozen with Soya	6	15	34	85
Total	10	8	110	92

Table 8: *Serotyping of Salmonella in the examined ground beef samples*

	Samples no.	Positive samples	%
Salmonella	120	10	8%
Serotypes	Untypable		

Table 9: *Serological identification of E. coli isolates from the examined ground beef samples*

Positive samples	O-typable	O-untypable
57	20 (35%)	37(65%)

Table 10: *Incidence of identified E. coli serotypes in the examined ground beef samples*

Strain character	Serotypes	No.	%
EPEC	O86	2	10%
	O119	2	10%
	O158	2	10%
EHEC	O145	4	20%
ETEC	O115	10	50%
Total	-	20	100%

Conclusion and Recommendations

High incidence of Enterobacteriaceae in ground beef constitute a public health hazard and has an epidemiological interest and importance as some of them are pathogenic and may cause serious infections and/or food poisoning as Salmonella, *Escherichia coli*, Enterococci, Proteus, and *Klebsiella species* which are considered as true indicator of poor sanitation during production, post processing contamination and the extent of faecal contamination. However, the greatest application of Enterobacteriaceae is the assessment of the overall quality of a food and the hygiene conditions present during the food processing.

The presence of *Klebsiella spp*, *Salmonella* and *Escherichia coli*, encountered in the examined samples of ground beef is alarming and give a warning signal for the possible occurrence of food borne intoxication. The following suggestive measures and recommendations should be taken in considerations:

1- Routine microbiological examination should be adopted in meat product factories, butchers shops, groceries and other food rendering outlet with a consequent certificate of nil presence food born bacteria.

2- Hygienic awareness should be applied for personnel whom involved in handling and preparing of food at factories, home or restaurants avoid fecal contamination.

3-Demands for increased food hygiene surveillance and control, with the overall objective of safeguarding the consumer against poor quality and unsafe food stuffs were recommended.

4- Application of GMP and GHP during slaughtering, processing, storage and distribution of meat.

5- Effort must be done to define a standard limit for Enterobacteriaceae count in the Egyptian Standards (E.S) for ground beef.

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الملخص العربى

دراسات كميه ونوعيه على مجموعه الامعائيات فى اللحم المفرى

حسنى عبد اللطيف عبد الرحمن، على معوض احمد، هبه محمد شاهين

أجريت هذه الدراسه لاستبيان مدى تواجد مجموعه الامعائيات فى اللحم المفرى المتداول باسواق مدينه الاسماعيليه للكشف عن مدى تواجد ميكروب السالمونيلا وميكروب الايشريشيا كولاى نظرا لما تسببه هذه الميكروبات للعديد من المشاكل الصحيه متمثلة في التسمم الغذائى. لذا فقد تم تجميع عدد ١٢٠ عينه من اللحم المفرى بالتساوى بين اللحم المفرى الطازج واللحم المفرى المجمد واللحم المفرى المجمد المضاف اليه فول الصويا. اوضحت النتائج ان نسبه العينات الايجابيه لمجموعه الامعائيات من عينات اللحم المفرى الطازج و المجمد و المجمد المضاف اليه فول الصويا هى ١٠٠%، ٧٥% و ٨٧% على التوالى، بينما كانت قيمها الدنيا والقصوى والمتوسطه هى١٣×١٠^{٢}، ٥×١٠^{٥} و ٦,٣×١٠^{٤}جرثومه لكل جرام على التوالى فى عينات اللحم المفرى الطازج، <١٠ ، ٤ ×١٠^{٢} و ١,٤×١٠^{٢}جرثومه لكل جرام على التوالى فى عينات اللحم المفرى المجمد و٢×١٠^{٢}، ٧×١٠^{٣} و ١,٦×١٠^{٣} جرثومه لكل جرام على التوالى فى عينات اللحم المفرى المجمد المضاف اليه فول الصويا.

هذا وتم تصنيف العترات المعزوله من عينات اللحم المفرى كالاتى: بالنسبه لعينات اللحم المفرى الطازج تمكنت الدراسه من عزل الايشيريشيا كولاى و الكلبسيلا اوكسيتوكا والسيرشيا مارسيسينيس بنسبه ١٢%، ٥٠%، ٢٥% و ١٣% على التوالى. وبالنسبه لعينات اللحم المفرى المجمد تمكنت الدراسه من عزل البروتيس فالجاريزو الايشيريشيا كولاى والانتيروباكتر زكازيكى بنسبه ٥٢%، ٢٤% و ٢٤% على التوالى، بينما كانت الانواع التى تم عزلها من عينات اللحم المفرى المجمد المضاف اليه فول الصويا هى و الكلبسيلا اوكسيتوكا و البروتيس فالجاريزو والسيتروباكتر فارميرى و الايشيريشيا كولاى و بانتيوا الجوميرانس بنسبه ١٣%، ٥٠%، ١٢%، ١٣% و ١٢% على التوالى.

كما تم عزل ميكروب الاشيريشيا كولاى من عينات اللحم المفرى الطازج، المجمد و المجمد المضاف اليه فول الصويا بنسب مختلفه وهى ٦٠%، ٣٥% و ٤٨% على التوالى. وبالنسبه لميكروب السالمونيلا فقد تم عزله بنسب مختلفه وهى ٠%، ١٠% و ١٥% من عينات اللحم المفرى الطازج، المجمد و المجمد المضاف اليه فول الصويا على التوالى. وتم عزل ٥٧ عترة من عترات الاشيريشيا كولاى و التى تم تصنيفها سيريولوجيا الي ٢٠ عتره مصنفه طبقا للجزئ الجسيمى و ٣٧ عتره غير مصننفه طبقا للجزئ الجسيمى والعشرون عتره بيانهم كالتالى: O_{86}, O_{119} and O_{158} (EPEC); O_{145} (EHEC) and O_{115} (ETEC).