



## Detection of *Escherichia coli* and *E. coli O157* in Veal Mincemeat in Baghdad

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### Abstract

In order to investigate the prevalence of *Escherichia coli* and *E. coli O157* in veal mincemeat, thirty pooled samples (three replicates from each type, n=90) were randomly collected from different markets and butcher shops in Baghdad during April until August (2015), in which they processed and analyzed by different food microbiological procedures. The results showed isolation and identification of 27 (90%) isolates out of 30 pooled samples as 13 (86.7%) isolates from 15 locally butcher shops mincemeat, in which the serotype *O157* was detected in 5 (33.4%) samples; and 14 (93.4%) isolates from 15 imported veal meat (minced locally for human consumption), in which 7 (46.7%) isolates were identified as a serotype *O157*. The mean log count of *E. coli* to *E. coli O157* in locally produced mincemeat range from 2.845 to 5.602 log<sub>10</sub> cfu g<sup>-1</sup>, while in imported ones range from 4.778 to 8.954 log<sub>10</sub> cfu g<sup>-1</sup>. Results profile provide useful information on biosafety and hazard analyses critical control points of hygienic measurements of *E. coli* and *E. coli O157* in mincemeat marketed in Baghdad.

**Keywords:** *E. coli*, *E. coli O157*, Veal Mincemeat.

الكشف عن الإشريكية القولونية ونمطها المصلي *O157* في اللحوم المفرومة للعجول في بغداد.  
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### الخلاصة

من أجل التحري عن الإشريكية القولونية ونمطها المصلي *O157* في اللحوم المفرومة للعجول، جمعت ثلاثين أنموذج جمعي (ثلاثة مكررات من كل نوع، كليا تسعين أنموذج) عشوائيا من مختلف الأسواق ومحلات الجزارة في بغداد خلال شهر نيسان حتى آب (2015)، حيث عوملت النماذج وحللت حسب طرائق ميكروبيولوجيا الغذاء. أظهرت النتائج عزل وتشخيص 27 (90%) عزلة من 30 أنموذج جمعي: 13 (86.7%) عزلة من 15 أنموذج جمعي محلي من محلات الجزارة، كان منها 5 (33.4%) عزلات النمط المصلي *O157*؛ و 14 (93.4%) عزلة من 15 أنموذج جمعي من لحوم العجول المستوردة (مفرومة محليا للاستهلاك البشري)، كان منها 7 (46.7%) عزلات النمط المصلي *O157*. تراوحت معدلات العد اللوغاريتمي للإشريكية القولونية الى نمطها المصلي *O157* في اللحوم المحلية المفرومة من 2.845 – 5.602 مستعمرة / غرام منه، في حين كانت القيم للمستورد والمفروم محليا من 4.778 – 8.954 مستعمرة / غرام. نوعية النتائج وفرت معلومات مفيدة في مجال السلامة الأحيائية والمخاطر الصحية الناجمة من تلوث اللحوم المفرومة للعجول بالإشريكية القولونية ونمطها المصلي *O157* في أسواق بغداد. مفاتيح البحث: بكتريا القولون، النمط المصلي *O157*، اللحوم المفرومة للعجول.

## Introduction

There are four major pathogens that have frequently been associated with meat and

meat products including *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7. These organisms have been linked to a number of cases of human illness (1-3). One of the most significant food-borne pathogens that has gained increased attention in recent years is *E. coli* O157:H7. Typical illness as a result of an *E. coli* O157:H7 infection can be life threatening, and susceptible individuals show a range of symptoms including hemolytic colitis, hemolytic-uremic syndrome, and thrombotic thrombocytopenic purpura (2, 4).

*E. coli* O157 was firstly reported in the United States in 1982 and gave rise to serious outbreaks in many countries in decades (5, 7). It has been reported that most of the O157 infections in the US were raised from foods and most of these infections were resulted from consumption of contaminated foods including ground beef (6, 7).

Among the *E. coli* causing intestinal diseases, there are six well-described pathotypes: Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enterohaemorrhagic *E. coli* (EHEC), Enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (7,8). Among the six recognized diarrhegenic categories of *E. coli*, ETEC is the most common, particularly in the developing countries (7,9). EHEC is the most important recently emerged group of food-borne pathogens. It can cause severe gastrointestinal disease, including fatal infections and is being detected more frequently worldwide. EHEC strains not only produce potent cytotoxins (verotoxins) but have also acquired the ability to adhere to the intestinal mucosa

in an intimate fashion (7, 10, 11). Specific virulence factors such as enterotoxins and colonization factors differentiate ETEC from other categories of diarrhegenic *E. coli* (7, 9).

The infection is mainly food-borne, but it can also be acquired by person-to-person spread or direct contact with animals. Domestic and wild animals are the sources of *E. coli* O157, but ruminants are regarded as the main natural reservoirs particularly cattle carrying these agents in their digestive tract causing contamination of meat and meat products during slaughtering. On the other hand, contamination come up through the environmental route or while dressing (3, 7, 12). Besides this, the pathogen can enter the production chain in storehouses, butchers, markets, restaurants etc. and pose risk for public health (3, 7, 13).

Currently, there was limited information regarding the prevalence of *E. coli* and its serotype O157 in red mincemeat in Iraq. Therefore, this study was conducted to determine the contamination ratio of *E. coli* and its serotype O157, from locally and imported retail raw veal mincemeat in Baghdad markets.

## Materials And Methods

### Collection and Processing of Samples:

A total of thirty pooled samples (three replicates from each type, totally: ninety replicates) were randomly collected from different markets and butcher shops in Baghdad during April till August (2015), in which they processed and analyzed by different food microbiological procedures with some modifications. Samples were collected aseptically in sterile plastic bags and containers, in which they transported to zoonotic lab as soon as possible.

Each pooled and well mixed replicates were divided in to two separate unit (direct and indirect processing): directly well mixed replicates units were cultured on freshly prepared Sorbitol – McConkey

and Eosin Methylene Blue agars (10 g well mixed sample part was added to 10 ml tryptone soya broth, then mixed well by vortex and streaked by sterile loops and swabs on agars for each pooled unit) at 37 and 42 C° for 24 hours; and indirectly replicates units were enriched by tryptone soya broth for 24 hours at 37 C°, then cultured on same agars and temperatures above. In this pathway samples units were cultured by dilution formula: 10 g pooled part of sample unit to 90ml parts of broth diluent (14-16).

Pure isolated colonies were counted by droplet technique in accordance to McFarland's opacity tubes (17), then picked up and recultured on tryptone soya broth at 37 C° for 24 hours, then cultured on tryptone soya yeast extract agar at 37 C° for 24 hours for further identification procedures. Electronic biochemical panel test system for *Enterobacteriaceae* with serotyping latex test kit for serotype O157 were used for confirmation procedure of

isolates (18). Data were statistically analyzed by Chi-square test in accordance with SPSS (19).

**Results & Discussion**

Results profile reflect contamination of mincemeat samples with *E. coli* and its serotype O157 in Baghdad. The results showed isolation and identification of 27 (90%) isolates out of 30 pooled samples as 13 (86.7%) isolates from 15 locally butcher shops mincemeat, in which the serotype O157 was detected in 5 (33.4%) samples; and 14 (93.4%) isolates from 15 imported veal meat (minced for human consumption), in which 7 (46.7%) isolates were identified as a serotype O157. The mean log count of *E. coli* to *E. coli* O157 in locally produced mincemeat range from 2.845 to 5.602 log<sub>10</sub> cfu g<sup>-1</sup>, while in imported ones range from 4.778 to 8.954 log<sub>10</sub> cfu g<sup>-1</sup>.

**Table (1):** Isolation percentages and mean log<sub>10</sub> count of *E. coli* from veal mincemeat in Baghdad.

Type of Pooled Samples	Number	Isolation %	from 30	Mean log <sub>10</sub> count cfu g <sup>-1</sup>
Locally produced mincemeat	15	8 (53.4)	26.7	2.845 <sup>a</sup>
Imported meat minced locally	15	7 (46.7)	23.4	4.778 <sup>b</sup>

**a, b:** Indicate significant differences among isolates for mean log<sub>10</sub> count vertically at level (P≤0.05).

**Table (2):** Isolation percentages and mean log<sub>10</sub> count of *E. coli*O157from veal mincemeat in Baghdad.

Type of Pooled Samples	Number	Isolation %	from 30	Mean log <sub>10</sub> count cfu g <sup>-1</sup>
Locally produced mincemeat	15	5 (33.4)	33.4	5.602 <sup>a</sup>
Imported meat minced locally	15	7 (46.7)	23.4	8.954 <sup>b</sup>

**a, b:** Indicate significant differences among isolates for mean log<sub>10</sub> count vertically at level (P≤0.05).

These findings suggest presence of complex scenarios of contamination cycle with *E. coli* and its serotype *O157* in mincemeat in Baghdad markets; contamination may occur from farm to markets, during shipment of stressed and infected animals, inside abattoirs during slaughtering and evisceration and during handling and processing of meat by infected and carrier workers, knives, butcher cutting wood surface, mincing machines, contaminated utensils, flies, unclean meat environment, etc.

As we know, most slaughtering today in Baghdad performed outside abattoirs with unhygienic monitoring system, so that repeated cycles of contamination and pollution occurs in presence of different transmitters and vehicles particularly flies leading to production of dirty, unclean and unhealthy meat and meat products as well as the unacceptable ethics in meat processing and handling with unclean environment, measurements and practices inside most butcher shops with open retailing of these mincemeats in unclean and contaminated containers in Baghdad markets.

Unrestricted hygienic monitoring systems and food policies like absence of biosafety and hazard analysis critical control points during production and handling of healthy meat, absence of risk assessments during importation of meat and meat products, all these and others result in contamination of mincemeat in Baghdad markets with different invaders.

However, while this study has focused on the detection and/or enumeration of *E. coli* and its serotype *O157* at near consumer levels, effective action to reduce or eliminate the risks posed by this organism will involve diverse and coordinated actions at a number of stages of the food chain. These include the

incorporation and consistent application of Good Agricultural practice(GAP), Good Manufacturing practice (GMP), and Hazard Analysis of Critical Control Points (HACCP) at every stage of the beef supply chain, from the farm, through the abattoir, to the retailer, and those involved with the handling and processing of such raw meat products in the home environment. In addition, suitable intervention measures may be necessary to eliminate the pathogen in food reaching the consumer (13, 20, 21).

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