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Molecular Identification of *E coli* from Meat product imported from Turkey via Ibrahim Khalil border and marketed in Iraq.

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Abstract
Prevention and control of foodborne diseases is an international public health goal. Imports of food of animal origin are monitored for contamination, and alerts are reported regularly, In order to assess the level of the risk for public health from imported meat entering the Kurdistan, 358 meat samples were collected at Ibraheam al-khaleal border points between Turkey and Kurdistan Region of IRAQ. molecular tools used for identified *E coli o157* ,.out of 358 samples 29samples were positive .the positive sample confirmed by traditional and biochemical assay (Vietic 2) The *E coli* 157 is one of hundered of strains of the bacterium that causes illness in human.

Keyword: *E coli*,. RT-PCR,. Meat Product

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Introduction:
Food safety is a fundamental issue in the public health of all countries, as it is a foodborne disease Because of microbial pathogens, biotic toxins or chemical contaminants is a major threat to the health of millions of people around the world. Consumers everywhere see that the spread of foodborne diseases is an ever-growing concern, as they affect human health and the way they live. Billons of people in the world are at risk of unsafe food. Many millions become sick while hundreds of thousand die yearly. Safe food enhances individual and population health. Safe food improves economic growth of the region where food safety is practiced and enhanced (Pollari et al 2017,.Fung et al 2018)
Microbial food safety is still a major concern in many European Union (EU) member states due to repeated outbreak episodes and contamination problems. The global influence and growth of the European market affect the food supply chain that is becoming increasingly complex and requires professional risk management systems to ensure consumer protection. Legal imports are well monitored for contamination, and alerts are registered through the RASFF notification systems (Anonymous., 2008)
Red meat is the main food for humans in most countries the world, is considered to be one of the main sources of protein so meat is better for the growth of various organisms, causing problems Economic damage and speed of damage as well as damage caused by Food poisoning caused by these microorganisms due to secretion toxins.
Escherichia coli O157 is highly virulent food poising pathogen capable of causing sever gastrointestinal illness in human and is the most well-known member of the group *E Coli* could verocytotoxigenic *E coli* (vtec).VTEC ara characterized by production of verotoxines which termed verotoxine 1(VT1)verotoxine 2(VT2)
Escherichia coli O157:H7 was first identified as a foodborne pathogen when it was implicated in an outbreak of hemorrhagic colitis in 1982 associated with the consumption of undercooked ground beef patties (Riley, L. W et al 1983,. William Gossman; et al 2019).
A decade later a multistate outbreak of *E. coli* O157:H7 infections occurred in which the vehicle of transmission was again undercooked hamburger patties. (CDC. (1993).
Among all enterohemorrhagic *Escherichia coli* (EHEC), *E. coli O157:H7* and *E. coli O157:H−* are recognized as major pathogens and a cause of foodborne disease in humans worldwide. The bacteria are mostly transmitted through undercooked minced beef and meat products, which have a relatively short shelf life; therefore, rapid detection in these particular foods is required (ÇETİN, O et al 2010,. Temelli

et al 2012).
According to the EFSA report for 2016, zoonotic foodborne diseases are still of major concern for public health (Anonymous., 2017). One of the bacteria that also raises an alert is Escherichia coli. In particular Verocytotoxigenic *E. coli (VTEC) O157:H7* and Shiga toxin-producing *E. coli* (STEC) may colonize the gastrointestinal tract of different animals, and potentially contaminate the meat during processing, Enterohemorrhagic *E. coli O157: H7* induces illness secondary to its production of Shiga toxin that causes a range of gastrointestinal illnesses, from watery diarrhea to hemorrhagic colitis. *E. coli 0157: H7* induces enterohemorrhagic disease that can cause systemic illness by hemolytic uremic syndrome, which manifests as hemolytic anemia, thrombocytopenia, and acute renal failure. HUS can result in both acute, potentially life-threatening illness and lifelong, chronic illness (TAMMINEN et al., 2018; WILSON et al., 2018; William Gossman et al., 2019,. Erickson MC et al 2019,.)
To date, great efforts have been made to develop appropriate methodologies for the detection of *E. coli* O157:H7.There are several validated methods incorporating culture, Biochemical, and molecular techniques, mainly for the detection of *E. coli O157:H7*. However, the culture in these methods is time consuming (up to 3-5 days) and not suitable for routine screening of large samples while providing little accurate information about the nature of the strain or isolate itself
(Savoye, F et al 2011)
PCR has been widely used for the detection of *E. coli O157:H7* from foods and environmental samples. More recently, real-time PCR is gaining popularity for its enhanced sensitivity and specificity and its speedy turnaround time. Numerous real-time PCR-based methods have been reported for rapid and sensitive detection of *E. coli O157:H7*, Real-time PCR has been used for the rapid and reliable detection of *E. coli O157* in retail red meats (Suo, B et al 2010 ;)
At present As a result of the economic openness in Iraq and the absence of regulatory bodies, companies have started importing many kinds of products Frozen meat and different origins and these meat entered Iraq without standard controls so the study aimed The current to make an assessment on some frozen meat brands imported, to identify th*e E Coli* 157 from Meat and Meat product imported from Turkey via Ibrahim Khalil border and marketed in Iraq.

Material and Method:
A Total 358 sample of different meat and meat products were tested from period August 2017 to August 2018). Samples were analyzed immediately in the central laboratory of New Stander Company for Quality control in Ibrahim Al-Khalil Border. 25 g of the meat/product sample was aseptically placed into a sterile Stomacher Bag with a fi later that contained 225 mL of buff enrichment (MTSB 0157 Broth) which is incorporated into ISO/TS 13136:2012). The sample was incubated at 42°C aerobically for 24hrs DNA was extracted from 1 ml of the pre- nrichment broths(MTSB culture) using DNA isolation kit kogenebiotech following the manufacturers' nstructions. PCR was performed depending on amplification for detected specifisequence of VT2 gene of the *E coli* 157. The Final volume of amplification reaction was 20µl.

Table: 1 The PCR reaction consisted of 2X master mix and probed primer

Composition	Volume
Primer /probe Mix	4 µl
2x Real –Time pcr Master Mix	10 µl
Template DNA	6
Total	20

The cycling parameters consisted of an initial denaturation 10 min, 95 °C), followed by 40×3-step- cycles consisting of denaturation (15 sec at 95°C), annealing for 15 sec at 60 °C, °C. Fluorescence detection was performed at the end of the annealing stage of each cycle. The amplification done in Rotor-gene Q instrument in New –Standard company laboratories. The positive result of RT- PCR has been confirmed by microbiological and biochemical examination After incubation in MTSB 0157 culture plated onto sorbitol MacConkey agar SMAC; (LABM),. Plates were incubated at 37C for 24 h. Individual bacterial colonies were selected and used Viteck 2 according to the manufacturing procedure.

Result and discussion.

In the current study, all E. coli colonies were tested by applying PCR method in order to detect 16S rRNA gene of bacterium using *E Coli* O157 Real Time PCR kit (Kogene .korea).

Meat and meat products were imported from different countries ,the samples arrived from India ,,Brazil and isteralian countries via Turkey via Ibrahim Al-Khalil border .All samples were frozen beef meat and meet product . Out 358 samples .29 (8.1%) samples were identified coli 157 gene, Our result is O157 E. coli was similar results previously isolated from beef (HUSSEIN, 2007; NOBILI et al., 2017; Varcasia et al. 2018), this may be due to contaminated during the production processes, or by transport in bad condition, storage, manual handling and remote viewing Health conditions that may extend for long periods are factors.

At present as a result of the economic openness in Iraq and the absence of regulatory bodies, companies have started importing many kinds of products.

Frozen meat and different origins and these meat entered Iraq without standard controls so the study aimed to make an assessment on imported frozen meat.

Many brand imported via border we found all infected samples were from Indian company brand this may due to the imported meat origin and health procedures in the production processes and not following the global health conditions during production or Poor transport.

The recommended temperature or storage that used for chilled foods, such as raw meat, is 5C or less because low temperatures slow down or prevent, the growth of microorganisms that pathogens and spoilage

For the confirmation of the result the par product of positive samples was examed by using traditional microbiology test and biochemical (Vietek 2).

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