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Original Article

Safety of frozen liver for human consumption

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ABSTRACT

The objective of this study was to ensure and evaluate the safety of imported frozen beef liver traded in supermarkets of Kafr El-Sheikh Governorate, Egypt, through detection of *Salmonella typhimurium*, *Salmonella enteritidies*, *Escherichia coli* O157:H7, antibiotic residues, and aflatoxin B1 residue. Fifty samples of imported frozen liver were randomly collected from different shops at Kafr El-Sheikh Governorate for isolation of *S. typhimurium*, *S. enteritidies*, and *E. coli* O157:H7. The results revealed that for both microorganisms 4% of the examined samples presumed to contain *Salmonella* and *E. coli* O157:H7 organisms, according to the colonial character on Harlequin *Salmonella* ABC agar media and Harlequin SMAC-BCIG agar media. According to biochemical and serological identifications, both organisms could not be detected in the examined samples. A total of 29 (58%) samples were positive for antibiotic residues, using the Premi test (a broad-spectrum screening test for the detection of antibiotic residues in meat) at or below the maximum residue limits. In addition, aflatoxin B1 was detected in one (2%) samples with a concentration of 1.1 μg/kg. The results reflect that there was good hygiene practice for handling and preparation of frozen liver while selling to consumers. However, a high percentage of antibiotic residues reflect ignorance of withdrawal time before slaughtering of animals as well as misuse of antibiotics in veterinary fields. Furthermore, aflatoxin B1 residue was detected in examined frozen liver samples at a concentration below the maximum residual level, which is not enough to cause threat to humans, but it is enough to cause problem if it is eaten regularly reflect contamination of animal feed with aflatoxins.

1. Introduction

Beef liver provides us with significant amounts of protein, vitamins, and fat that keep our body healthy [1]; however, liver products are considered a high-risk food as these are highly nutritious and serve as an ideal medium for bacterial growth. Contamination due to poor hygienic practices by food handlers and instruments such as cutting boards, machines, and all other related materials used for preparation of liver to sell to consumers. *Salmonella typhimurium*, *Salmonella enteritidies*, and *Escherichia coli* O157:H7 are potentially pathogenic to humans and animals, and are capable of producing serious infections and food-borne zoonosis [2,3]. Salmonellosis in humans is associated with the consumption of contaminated

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food products such as beef, poultry, vegetables, and other meat byproducts [4]. Possible transmission can be from humans to animals and from animals to humans, whereas consumption of raw meat and cross-contamination of ready-to-eat meat can cause *Salmonella* infection [5]. In addition, inadequately cooked or lightly roasted meat is the source of *Salmonella* food poisoning. *E. coli* O157:H7 is an enterohemorrhagic strain of bacterium *E. coli* and one of the most common agents of food-borne illness in humans; it has been isolated from beef at all stages of production [2]. *E. coli* O157:H7 is mainly transmitted to humans through consumption of contaminated food and water. Outbreaks are often caused by eating undercooked meat, particularly meat byproducts [6].

Antibiotics used in feed animals can affect public health because of their secretion in edible animal tissues in trace amounts (residues), which may be found above the maximum residual level (MRL) in tissues [7]. Antibiotic residues in food are currently a problem across the world, particularly due to public health problems that include hypersensitivity reaction, antibiotic resistance, toxicity, teratogenicity, and carcinogenicity [8].

Animal feed contaminated with aflatoxins may lead to residues of aflatoxin and its metabolites in meat and meat products, and could subsequently create health problems in humans [9,10]. AFB1 is most acutely toxic to various species which produced by *Aspergillus flavus* and *Aspergillus paraciticus* [11] and the highest consumption of toxin is from liver [12]. The regulatory level for aflatoxin B1 in food in many countries is 5 ppb. The World Health Organizations for Cancer Research Institutions designated aflatoxin as a Class 1 carcinogen that is harmful to human and animal liver as it can cause liver cancer or even death [13]. Therefore, this study focused on the determination of the prevalence of *S. typhimurium*, *S. enteritidies*. and *E. coli* O157:H7 as well as screening of antibiotic and aflatoxin B1 residues in frozen beef liver, in order to ensure safety of this product for human consumption.

2. Materials and methods

2.1. Collection of samples

Fifty samples of imported frozen beef liver were randomly collected from different supermarkets at Kafr El-Sheikh Governorate, Egypt. Each sample weighed 250 g and was received in a sterile plastic bag in frozen state. The collected samples were transferred to the laboratory in frozen state, and immediately prepared and examined, within 1 hour of collection, for *S. typhimurium*, *S. enteritidies*, and *E. coli* O157:H7 as well as screened for antibiotic residues and aflatoxin B1.

2.2. Isolation and identification of salmonellae

2.2.1. Pre-enrichment

Examined samples (25 g) were weighed aseptically into a sterile container and homogenized with 225 mL of sterile buffered peptone water, and then incubated at 37°C for 24 hours [14].

2.2.2. Selective enrichment

Incubated pre-enrichment homogenate (1 mL) was transferred to 10 mL Muller–Kauffman tetrathionate novobiocin broth and incubated at 37°C for 24 hours.

2.2.3. Selective plating

A loop full of the selective enrichment both was streaked into the Harelquin *Salmonella* ABC media and incubated at 37°C for 18–24 hours; suspected colonies appear green in color.

2.2.4. Biochemical confirmation

The suspected *Salmonella* colonies were purified and identified using Indol, Methyle red, Vogus proskauer, Citrate utilization (IMVIC) pattern [15].

2.2.5. Serological identification

The suspected salmonellae were serologically confirmed in the Ministry of Health, Cairo, Arab Republic of Egypt.

2.3. Isolation and identification of E. coli O157:H7

2.3.1. Selective enrichment

Examined samples (25 g) were weighed aseptically into a sterile container and thoroughly homogenized with 225 mL of supplemented modified tryptone-soya broth for 2 minutes, and then incubated at 42°C for 24 hours [16].

2.3.2. Selective plating

A loop full of the selective enrichment broth was streaked into the Harlequin SMAC-BCIG media (Sorbitol McConkey Agar with BCIG) and incubated at 37°C for 18–24 hours. The plates were examined for sorbitol- and β-glucuronide-negative colonies that appear translucent.

2.3.3. Biochemical confirmation

The suspected colonies were purified and identified using IMVIC pattern [15].

2.3.4. Serological identification

The suspected *E. coli* O157:H7 isolates were serologically confirmed using immunoglobulin M (IgM) antibodies to *E. coli* O157:H7 (*E. coli* O157:H7 test kit; Oxid, England) according to the instructions of the manufacturer.

2.4. Detection of antibiotic residues

The Premi Test (a broad-spectrum screening test) at or below the MRL was used for the detection of antibiotic and sulfonamide residues in meat, according to the instructions of the manufacturer.

2.4.1. Preparation of samples

The examined frozen liver samples were cut into small pieces 2 cm^3 approximately 250 μL of sample fluid were extracted using meat press. Sample fluid (100 μL) was pipetted into the agar ampoule and kept at room temperature for 20 minutes, and then the sample juice was flushed away gently by demineralized water twice and the ampoule was washed with demineralized water. The test ampoule was incubated in a