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Original Article

Safety of frozen liver for human consumption

*Ghada A.K. Kirrella**, *Azza M.M. Deeb*, *Reda Mohamed Ibrahim Abdallah*

Food Control Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh, Egypt

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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

* *Corresponding author*. Food Control Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt.
E-mail address: Ghada.karala@vet.kfs.edu.eg (G.A.K. Kirrella).

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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

water bath (64 ± 0.5°C). A negative control ampoule was used. The results were read after 3 hours (after the negative ampoules had changed color) from the bottom two-third part of the ampoule. Presence of antibiotics and sulfonamide at or above the detection limit was indicated by no clear color change in the purple ampoule, while absence of antibiotics and sulfonamide above the detection limit was indicated by a clear color change from purple toward yellow.

2.5. *Quantitative analysis of aflatoxin B1*

Using Rida Screen aflatoxin B1 30/15 (enzyme immunoassay for the quantitative analysis of aflatoxin B1). Art. No.: R1211 according to instruction manufacturer.

2.5.1. *Preparation of samples*

Ground liver sample (5 g) was weighed; 25 mL of 70% methanol was added to it and vigorously shaken for 3 minutes, and then the extract was filtered through Whatman No. 1 filter paper. The filtrate (1 mL) was diluted with 1 mL of distilled water, then 50 μL of diluted filtrated was used per well.

2.5.2. *Test implementation*

2.5.2.1. *Test preparation*. All reagents were kept at room temperature (20–25°C) before use. The washing buffer was diluted 1:9 with distilled water before use.

2.5.2.2. *Test procedures for enzyme-linked immunosorbent assay*. Sufficient numbers of wells were inserted into the micro well holder for all standards, controls, and samples. Fifty microliters of the standard, controls, or prepared sample were added to separate wells; 50 μL of enzyme conjugate was added to the bottom of each well; and 50 μL of antiaflatoxin antibody solution was added to each well and mixed gently by shaking the plate manually. The plate was incubated for 30 minutes at room temperature (20–25°C). The liquid was poured out of the well, and the micro well holder was tapped upside down vigorously (3 times in a row) against an absorbent paper to ensure complete removal of liquid from the well. All the wells were filled with washing buffer at 250°C, and the liquid was poured out again. The washing process was repeated three times. The substrate/chromogen (100 μL) was added to each well and mixed gently by shaking the plate manually, and incubated for 15 minutes at room temperature (20–25°C) in the dark. The stop solution (100 μL) was added to each well and mixed gently by shaking the plate manually; the absorbance was measured at 450 nm, and the results were read within 15 minutes after the addition of the stop solution.

2.5.2.3. *Calculation*. The absorbance values obtained for the standards and samples were divided by the absorbance value of the first standard (zero standard) and multiplied by 100. The zero standards equaled 100% and the absorbance values were quoted in percentages. The values of the standards were entered in a system of coordination on a semilogarithmic graph paper against aflatoxin B1 concentration (μg/kg). The aflatoxin B1 concentration (μg/kg; ppb) corresponding to the absorbance of each sample was read from the calibration curve according to the test preparation record, the low detection limit in 1 ppb and the recovery rate is 100% for aflatoxin B1 (Figure 1).

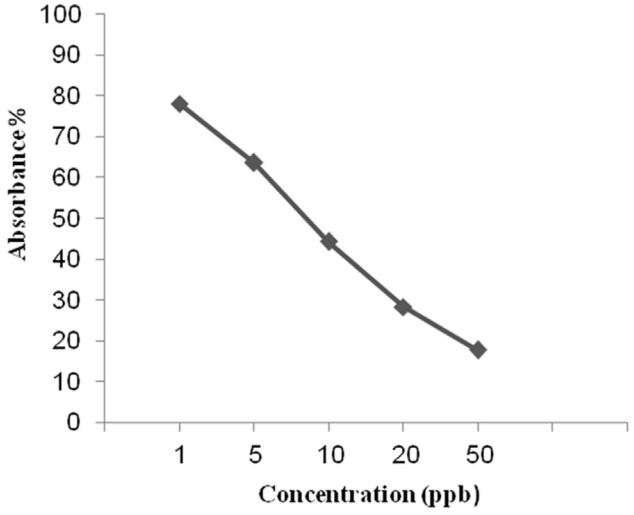

Figure 1 – Standard curve of AFB1 ($R^2 = 0.9937$). AFB1 = aflatoxin B1.

3. Results and discussion

Egypt has to import many food items to meet the demand of an increasing population, which requires a parallel rise in food production. The majority of imported food, especially of animal origin, may carry factors that are dangerous for human health [17]. Beef liver is considered a high-risk food serving as an ideal medium for bacterial growth.

Improper storage, preparation, processing, cooking, and handling of food increase the chances of cross-contamination and food-borne illness. *Salmonella* transmission occurs via fecal contamination during slaughtering and dressing of carcass due to poor hygiene during cutting or preparation of meat or meat products [18]. Table 1 reveals that two (4%) of the examined frozen liver samples were presumed to contain *Salmonella* organisms, according to colonial character on agar media, while none of the examined samples were positive for *Salmonella* organism, according to biochemical and serological

Table 1 – Incidence of *Salmonella* and *E. coli* O157:H7 in examined samples.

Organism	No. of examined samples	Samples showing morphological character of *Salmonella* and *E. coli* O157:H7 on media	Positive samples after biochemical and serological identification
Salmonella	50	2 (4%)	0
E. coli O157:H7	50	2 (4%)	0

Table 2 – Detection of antibiotic residues and aflatoxin B1 in examined samples.

Type of examination	No. of examined samples	Positive samples No. (%)	Negative samples No. (%)	ND samples[a]
Antibiotic residues	50	29 (58)	21 (42)	—
Aflatoxin B1	50	1 (2)[b]	2 (4)	47 (90)

ND = nondetectable.
[a] ND samples contain aflatoxin B1 concentrations lower than the detection limit of kits (1 ppb).
[b] Concentration 1.1 μg/kg.

identification. Table 1 reveals that two (4%) of examined frozen liver samples were presumed to contain *E. coli* O157:H7 organism, according to the colonial character on agar media, while none of the examined samples contained this organism, according to biochemical and serological identification. The results of this study indicated that there is no fecal contamination of carcasses and its organs in slaughterhouse which is the main source of *Salmonella* and *E. coli* contamination and there is good sanitary environment and hygienic practices under which animals are slaughtered and dressed in exported country as well as good handling and preparation of product during sale to consumers in imported country.

Table 2 reveals that 29 (58%) of the examined frozen liver samples had a detectable level of antibiotic residues. This high percentage of positive samples can pose a great health hazard to consumers. Incidence of antibiotic residues in liver samples could be due to animal taken drug of antibiotic at the time when drug was been metabolized in the liver and not clear by kidney yet; therefore, antibiotics were most often administered close to the time of slaughter.

Antibiotic residues in liver samples may be attributed to the failure to observe the preslaughter withdrawal time. Samples with highly positive antibiotic residues were not unusual, since the liver organ is the major storage for antibiotics and toxic substances in the body.

Some antibiotics undergo extensive enterohepatic circulation, which leads to prolongation of their elimination half-lives; thus, they remain in the body for a long time after cessation of drug administration.

Antibiotics may be given to treat diseased animals, but withdrawal time is required from the time of administration until it is legal to slaughter the animal to avoid residues in animal tissue [19]. However, misuse of antibiotics in different fields, including veterinary medicine and human medicine, particularly as growth-promoting agents in animal feed has created bacterial resistance in animal and food-borne human pathogens such as *Salmonella* and *E. coli* [20]. Some antibiotic drug residues have the potential to produce toxic reactions in consumers directly, some types are able to produce hypersensitivity reactions, and others cause gastrointestinal symptoms and anaphylaxis as well as microbiological effects and carcinogenicity [21].

Table 2 shows that one (2%) of the examined frozen liver samples was positive for aflatoxin B1 residue with a concentration of 1.1 μg/kg, while four (8%) of the samples were negative for aflatoxin B1. However, aflatoxin B1 is not detected in 47 (90%) of the examined samples, as aflatoxin B1 concentration was lower than the detection limit of kits (1 ppb). From a good safety point of view, the most remarkable aflatoxin is aflatoxin B1 because it is most prevalent in food and toxic for humans. Aflatoxin residues have been reported in food products including those of animal origins such as liver [22]. Aflatoxin B1 residues in human food can be dangerous for humans because aflatoxin compounds are heat stable with little degradation and so heating or cooking processes cannot be relied upon to destroy it. Pressure cooking can reduce the aflatoxin content by 83% [23]. Treatment of animal ration with ammonia reduces aflatoxin content in animal feed [24], whereas the contamination of foods of animal origin gets through the diet of the food-producing animals [25]. The European Union has set 2 μg/kg as the MRL for aflatoxin B1 [26], but 0.1 μg/kg for infants and young children in order to protect the health of this vulnerable population group.

Results from Table 2 reveal a low percentage of aflatoxin B1 residue in frozen liver, which was below the MRL; however, it was higher than the MRL stated for infants and young children, which may be life threatening for them.

Safety of frozen liver for human consumption including the absence of salmonellae and *E. coli* O157:H7 and free of liver from antibiotics and aflatoxine residues. The results reflect good sanitary environment under which animals are slaughtered and dressed, as well as good handling and preparation of products that are sold to consumers. By contrast, detection of antibiotic residues in frozen liver revealed misuse of antibiotics without observing the withdrawal time. The presence of aflatoxin B1 residues in frozen liver may be due to contamination of animal feed with aflatoxins. We, therefore, suggest that reduction of prophylactic antibiotic use in veterinary field, strict observation of antibiotic withdrawal time, and also use of alternatives to antibiotics such as probiotics and vaccination against bacterial diseases may be of great value in the near future. Regarding aflatoxin, we recommend good practice in agricultural production and food storage to prevent or limit the presence of aflatoxin in food. In addition, adequate cooking to reduce the level of residue in human food is required.

Conflict of interest

There is no conflict of interest.

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