Food Control 33 (2013) 281–286

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Chlortetracycline and sulfanilamide residues in table eggs: Prevalence, distribution between yolk and white and effect of refrigeration and heat treatment

Akram Alaboudi [a,*], Ehab Abu Basha [b], Immadidden Musallam [c]

[a] Department of Pathology and Public Health, Faculty of Veterinary Medicine, Jordan University of Science and Technology, P.O. Box 3030, Irbid 21110, Jordan
[b] Department of Vet. Medical Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, P.O. Box 3030, Irbid 21110, Jordan
[c] Jordan Food and Drug Administration, Amman, Jordan

ARTICLE INFO

Article history:
Received 27 December 2012
Received in revised form
6 March 2013
Accepted 9 March 2013

Keywords:
Table egg
Chlortetracycline
Sulfanilamide
Antimicrobial residues
Egg processing

ABSTRACT

Poultry feed or treated improperly with antimicrobial agents can produce eggs contaminated with drug residues. The study aimed to detect the prevalence, distribution and effect of heat treatment on chlortetracycline and sulfanilamide in table egg.

A percent of 12.8 of 2500 table eggs (forming 500 composite samples) screened using Premi test were positive for presence of antimicrobial residues. Antimicrobial residues were detected in either egg white, egg yolk or both. From all 64 antimicrobial positive samples, egg white had high prevalence (47 samples) of antimicrobial residues in comparison to that of yolk (17 samples) or the 9 samples that contain the residues in both yolk and white simultaneously. The distribution of antimicrobials in between white and yolk is mainly related to antimicrobial composition and egg compartments properties. Both chlortetracycline and sulfanilamide were quantified in the 64 positive samples and were detected in 1.2 and 4% respectively at concentration exceeding recommended MRL. The antimicrobials were highly sensitive to boiling temperature or frying (160 °C), where significant reduction rates in their concentrations within white or yolk were detected. The concentrations of chlortetracycline decreased by 24 and 61% in egg white and yolk, respectively, after boiling at 100 °C for 15 min. Whereas the reduction in sulfanilamide concentration was 66% in both egg white and yolk. The two antimicrobials were also unstable at refrigeration of 10 °C and a reduction percentage of 47% and 21% for chlortetracycline and sulfanilamide, respectively, were recorded after 4 weeks storage.

1. Introduction

The intensive production of eggs has led to an extensive use of antimicrobials in layers farms for growth enhancement and feed efficiency in addition to treatment and prophylaxis of infectious diseases (Furusawa, 2003). Treatment of animals reared for food including poultry is generally directed to groups or herds, and approximately 80% of all laying flocks receive antimicrobials for part or most of their lives for enteric and pulmonary infectious diseases (Kowalski, Oledzka, & Lamparczy, 2003).

The most common cause of drug residues in food products is of management nature. It is mainly resulted from the failure to meet the withdrawal times (Donoghue & Myers, 2000; JECFA, 2004; Kabir, Umoh, Audu-okoh, Umoh, & Kwaga, 2004). The improper use of licensed substances or illegal use of unlicensed substances with extra dosages may also lead to the violative residues (Stuart, 2002). The health status of the animal, such as the presence of any infection or inflammation, may increase the potential of residues formation through affecting the pharmacokinetics and drug metabolism (Kaneene & Miller, 1997).

The use of antimicrobial drugs in animals has recently become an important public health issue. The increased use of such compounds has showed many harmful effects on consumer such as, stimulation of microbial resistance and thus increases the risk of foodborne infections when such antibiotic resistant pathogenic bacteria enters the food chain (Cerniglia & Kotarski, 2005; Donoghue, 2003). Other harms may include; hypersensitivity reactions and alteration of the intestinal microflora (Antunes, Machado, Sousa, & Peixe, 2005).

Table eggs are generally not consumed raw and are mostly refrigerated and subjected to some kinds of heat treatment before being consumed. These treatment can cause protein denaturation,

* Corresponding author. Tel.: +962 2 7201000x22069; fax: +962 2 7095117.
E-mail addresses: akrrish@just.edu.jo, akramalaboudi@yahoo.com (A. Alaboudi).

0956-7135/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodcont.2013.03.014

loss of water, pH variation that can eventually result in chemical changes in nature; concentration or alter solubility. Therefore many drugs are chemically unstable to some extent, and goes under degradation during storage and/or cooking (Fletouris & Botsoglou, 2000; Rose, Bygrave, Farrington, & Shearer, 1996). A few studies have been reported about the stability of drug residues through common food processing. However, in most of these studies only the decrease in the amount of the parent drug was measured and the cause of this decrease is rarely being described (Okerman, Van Hende, & De Zutter, 2007).

The distribution of the antimicrobial residues between egg yolk and white is one of the most important issues related to drug residues. Antimicrobial residues will appear in white or yolk or in both after drug administration to laying hens. Intestinal absorption of the drugs by chickens and transportation through blood plasma is the cause of deposition in yolk in the ovary or white (Alm El Dein & Elhearon, 2010). The physiochemical properties of the drug and the physiology of the chicken and egg formation determine the amount of deposited drug (Donoghue & Myers, 2000).

Both chlortetracycline and sulfanilamide are in use in poultry industry and may lead to residues appearing in meat and eggs (AVPA, 2001). Sulfonamides as a group are synthetic compounds derived from sulfanilamide that share a common mode of action in their ability to inhibit parts of microbes folic acid pathway. They are effective against both G^- and G^+ bacteria and in poultry the most common route of administration is oral in feed or water. Tetracyclines are either naturally occurring product of fungi or semi-synthetic derivatives of such products. Tetracyclines are also effective against G^- and G^+ bacteria, widely use in diseases or growth promoters and usually given to poultry via feed or water (Goetting, Lee, & Tell, 2011).

The aims of present study were to investigate: (i) the residues prevalence of chlortetracycline and sulfanilamide, commonly used antimicrobial drugs, in locally produced and marketed table eggs in Jordan, (ii) the distribution of drugs in egg white and yolk and (iii) the effect of refrigeration and heat treatment on persistence of these antimicrobial residues in egg white and yolk.

2. Materials and methods

2.1. Samples collection

The samples were collected from the twelve Jordan governorates and the number of samples from each governorate was proportional to the number of table eggs produced in that governorate. Multi stage random sampling technique was used to select 140 layers farms and 80 retail markets distributed in Jordan. In the second stage, a cross-sectional study using simple and systemic random sampling method, where eggs from each selected farm ($n = 30$ eggs per farm) and retail market ($n = 10$ eggs per market) were collected. All collected eggs were not washed and except for refrigeration were not processed in any forms. Egg's age was ranged between 3 and 5 days as was determined through documentation offered by the producer or trader. Samples were placed in a clean bag and transported in an ice box to the laboratory, kept chilled at 0–4 °C for 3–4 days before screening for antimicrobial residues.

Fifteen eggs from each farm sample were chosen and divided into 3 pooled samples each of 5 eggs. From retail samples 5 eggs were selected and were pooled to form one composite sample. Therefore a total of 500 composite samples (2500 eggs) were screened for the presence of antimicrobial residues. Positive samples in screening test were kept frozen at −80 °C (Memort, Germany) until analyzed quantitatively and qualitatively by chromatographic technique (high performance liquid chromatography HPLC).

2.2. Antimicrobial residues screening assay using Premi test

Screening assay using Premi ampoules (DSM, Netherlands) was used. Sample preparation and working protocol were performed according to manufacturer's instruction.

2.3. Quantification and identification of antimicrobial residues using HPLC

2.3.1. Sample preparation

Chlortetracycline and sulfanilamide extraction, purification and HPLC running conditions were conducted according to the AOAC official method 995, 09, (AOAC, 1995) and Furusawa, 2003, respectively.

2.3.2. Preparation of standard calibration curves

To prepare standard calibration curves of chlortetracycline and sulfanilamide, volumes 50, 100, 250, 500, 1000 and 2000 μL of 100 μg/ml of chlortetracycline or sulfanilamide standard solution were added to 100 g of egg white or yolk samples to obtain final concentrations of 0.05, 0.1, 0.25, 0.5, 1 and 2 μg/g.

2.3.3. HPLC running conditions

The analyses were carried out on a Shimadzu Class VPHPLC system (Tokyo, Japan) equipped with SCL 10 A *VP* system controller, SPD-10 AD *VP* UV–Visible detector and LC 10 AD *VP* solvent delivery with SIL-10 AD *VP* auto injector. Chlortetracycline was detected at wave length 350 nm using C_8: 250 mm × 4.6 mm i.d., 5 μm column. The mobile phase consists of 0.01 M oxalic acid/acetonitrile/Me OH (65/15/20, v/v/v). The injection volume and the flow rate were 50 μL and 1.5 ml/min respectively.

Sulfanilamide was detected at wave length 267 nm using C4: 250 mm × 4.6 mm i.d., 5 μm columns .The mobile phase was 10.8 g citric acid/L water. The injection volume and the flow rate were 20 μL and 1 ml/min, respectively.

2.3.4. HPLC method validation

HPLC methods were validated by assessing the specificity, accuracy, precision, linearity, sensitivity (lower limit of detection) and recovery. The accuracy values for the used HPLC methods ranged (from 86.7 to 96%). The specificity of all methods were assured since there was no interfering traces (peaks) present in chromatographs corresponding to the retention times of the two antimicrobial drugs in both egg white and yolk.

The intra-day and inter-day coefficients of variation for chlortetracycline and sulfanilamide ranged between 0.3 and 8.9% at concentrations of 0.2, 0.8 and 1.6 mg/g. The coefficients of variation were below 15% in all cases. The recoveries of the two antimicrobial drugs from both egg white and egg yolk ranged from 78.3% to 84.6%.

The area under the curve was proportionally related to the antimicrobial drugs concentrations with correlation coefficients (r^2) values ranging from 0.996 to 0.998 indicating the linearity of the HPLC method. The measured quantitative lower limit of detection for the two drugs was (0.05 μg/g).

2.4. Heat treatment

Three different positive composite samples (from 5 eggs) of either white or yolk were chosen. Each of these samples of approximately 150 g was divided into approximately 3 equal portions (50 g). Each portion was subjected to either boiling, frying or refrigeration as described below.

2.4.1. Boiling

Egg white and yolk samples were boiled in a controlled temperature water bath adjusted at 100 °C for 15 min. Samples were