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SHORT COMMUNICATION

CHLORAMPHENICOL RESIDUE IN POULTRY MEAT

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SUMMARY

A study was conducted to detect and quantify the amount of chloramphenicol residue in chicken meat purchased at various outlets. A total of 50 chicken meat samples were purchased at random from the wet markets, supermarkets and fast-food outlets for the study. All samples were initially subjected to a rapid test using Brilliant Black Reduction Test-Kit to detect the presence of antibiotic. The samples were then further analysed using high-performance liquid chromatography to detect and quantify the level of chloramphenicol. The analysis revealed that 3 out of 50 samples contained chloramphenicol at various levels, 638.8, 748.1 and 914.2 ppb respectively. The study, although involved small sample size, has clearly showed that our chicken meat were not totally free of chloramphenicol residue, thus violated the Food Act and Regulation Malaysia 1998 and may have serious health and economic implications.

Keywords: Chloramphenicol, residue, bacteria, antibiotic resistant

INTRODUCTION

Malaysia has become sufficient in chicken meat and eggs since 1960 and became a net exporter of poultry meat and eggs since 1983 on a sustained basis (Seri Masran, 1996). The farm value output and the quantity increased almost 100% in 1994 as compared with the value for 1987 (Hadi, 1995). This expansion has been attributed to the efficient and modern intensive farming system.

Excellent health status and rapid growth of the birds are two important factors in successful intensive poultry farming system and the use of antibiotics has become an integral part and catalyst of this system of farming. Antibiotics are used for disease prevention and treatment and as growth promotion for birds. Animals receiving antibiotics in their feed gain 4-5% more body weight than animals that do not receive such supplements (Witte, 1998). The antibiotics commonly used in intensive poultry farming in this country include tetracycline, streptomycin, the beta-lactam antibiotics such as penicillin and cephalosporin, chloramphenicol and sulphonamides.

Chloramphenicol is a broad-spectrum antibiotic, which is particularly effective against gram-negative bacteria such as salmonella, pasteurella and coliforms (Milhaud, 1985). Chloramphenicol has the potential for producing untoward or adverse effect and may cause serious haematological disorders such as aplastic anaemia in man (Fraunfelder and Babby, 1982; Tyczkoarska *et al.*, 1988).

It is important to note that the United States of America has banned the use of chloramphenicol in their food-producing animals due to its adverse effects (Tyczkowska *et al.*, 1988). This is o prevent the spread of bacteria resistant to chloramphenicol from animals to man, which subsequently limits the usefulness of this antibiotic in human (Levy *et al.*, 1976). However, chloramphenicol is still being prescribed in food-producing animals in this country even though our Food Regulations Act 1998 prohibits the use of this antibiotic in food. Thus, animal products must be tested for the presence of chloramphenicol. To our limited knowledge, no such studies have been done earlier in this country. Thus, the objective of this study is to determine the presence of chloramphenicol and its level in poultry meat available for our consumption.

MATERIALS AND METHODS

Sampling and rapid screening

Chicken muscle samples were purchased at random from the wet markets (25), supermarket (15) and fast-food outlets (10). All fatty tissues were removed from the samples and were labelled accordingly. Each cleaned muscle sample (150 g) was packed and stored at -20°C until analysis. All samples were subjected to the Brilliant-Black Reduction (BR) antibiotic residue-screening test on the same day of collection according to the procedure prescribed by the manufacturer (Laboratorium Enterotox, Germany). A small portion of each muscle sample was homogenised before the fluid (100µL) was added to the BR vials and incubated at 60°C for 3 h in a water bath.

Chloramphenicol analysis using high-performance liquid chromatography (HPLC)

All positive samples following rapid BR test were subjected to further analysis using fully computerised

HPLC system. Ten grams of the minced muscle sample were mixed with 4 mL water and 12 mL ethyl acetate, vortexed and sonicated for 10 min. The mixture was centrifuged at 2500 rpm for 10 min. The solid and aqueous phases were discarded. Ten mL of the organic phase was placed in 15 mL graduated tube and evaporated by a stream of nitrogen at 50°C. The oily residue (approximately 400g) was mixed with 1 mL of methanol and 7 mL of petroleum ether. The mixture was sonicated for 5 min and vortexed for 1 min before being centrifuged for 5 min at 3,000 rpm. The organic phase was discarded and 3 mL of n-pentane was added to the aqueous phase. This mixture was again vortexed for 30 seconds and centrifuged for 10 min at 3,000 rpm. The upper organic phase was discarded and the lower phase (the final extract) was injected into the HPLC column.

Preparation of chforamphenicol standard solution

Standard chloramphenicol dilution curve was established using commercial standard chloramphenicol (Sigma Chemical Co.). Known amounts of working standard chloramphenicol containing 50, 100, 200 and 400 ppb stock solution were added to residue free chicken meat samples to act as positive control. Residue free chicken meat samples were used as negative control. All samples were subjected to the same procedure of samples preparation to determine the recovery of chloramphenicol.

HPLC Procedure

A fully computerised HPLC system equipped with a pump (Beckman 126), Waters 168 detector, set at 273 nm and the column Nova-Pak C18 300 x 3.9 mm id, 4 μrn was used. The mobile phase, isocratic 0.01M sodium acetate buffer pH 4.3/acetonitrile 75:25 v/v at a flow rate 1.0 mL/min was used. A 50μL aliquot of the sample extract in mobile phase was injected into the column. The detection and quantification of chloramphenicol residues in standard and test samples were analysed by a special computer software programme.

RESULTS AND DISCUSSION

The results of the rapid screening (BR) test showed that all samples were tested positive of antibiotic residue although only 3 out of 50 samples (6%) were positive for chloramphenicol on HPLC. The BR test is a qualitative screening test for antimicrobial drugs in animal produces. It is a simple and quick biological screening test giving qualitative results. The detection level is in a range of μg/mL (ppm).

The three samples that were positive for chloramphenicol were from the wet market. It is postulated that the antibiotic chloramphenicol may have been added into either food or drinking water while waiting for slaughter in the market place.

Figs. 1 and 2 depict the chromatographs of the known standard sample and the three positive samples respectively. All chromatographs showed that the retention time of chforamphenicol in the known standard and the test samples was approximately 6-7 min. The levels of chloramphenicol residue detected in the three positive samples were 638.8, 748.1 and 914.2 ppb, clearly violating the Food Act and Regulation Malaysia 1998, which prohibited the use of this antibiotic. To our limited knowledge, there is no published report on the detection of chloramphenicol in local chicken meat. A review of local literature indicated that only a limited number of HPLC analysis have been carried out in this country to determine the presence of antibiotic in animal food products (Salam Abdullah *et al.*, 1991). This is probably due to the cost and time-consuming process in HPLC analysis.

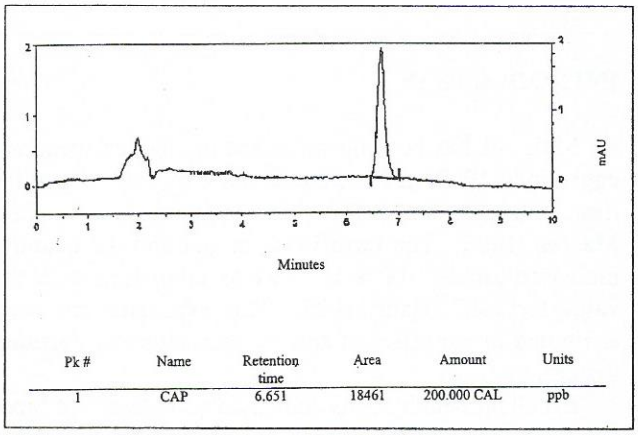

Fig. 1. Chromoatograph of a 200 μL standard solution of chloramphenicol showing a retention time of 6.5 min.

Food Act and Regulation Malaysia 1998 is correct in prohibiting the use of chloramphenicol in food products because of the risk of this antibiotics in causing cancer, especially in sensitive individuals. In order to ensure that chloramphenicol is totally absent in animal food products, it should be banned from food producing animals. Banning it will help to reduce the risk of transmitting the bacteria resistant to chloramphenicol, particularly *Salmonella*, from animals to man, ensuring continued usefulness of this antibiotic in treating infections in human patients (Levy *et al.*, 1976).

Antibiotic use in animals also has resulted in resistance among non-typhoid *Salmonella* serovars. The resistant bacteria are transmitted to humans in food or through contact with animals. Resistance in *Salmonella* limits the therapeutic options available to physicians in the treatment of certain cases of *salmonellosis in* human. *Salmonella typhimurium* strain DT 104, which

is resistant to ampicillin, tetracycline, streptomycin, chloramphenicol and suifonamides has been identified in many places including Britain, Europe and the United States (Witte, 1998).

The present study is no doubt small, involving small sample size but the results is quite alarming. Since the study showed that only the samples from the wet market were positive of chloramphenicol, serious effort should be made to trace the source of the contamination.

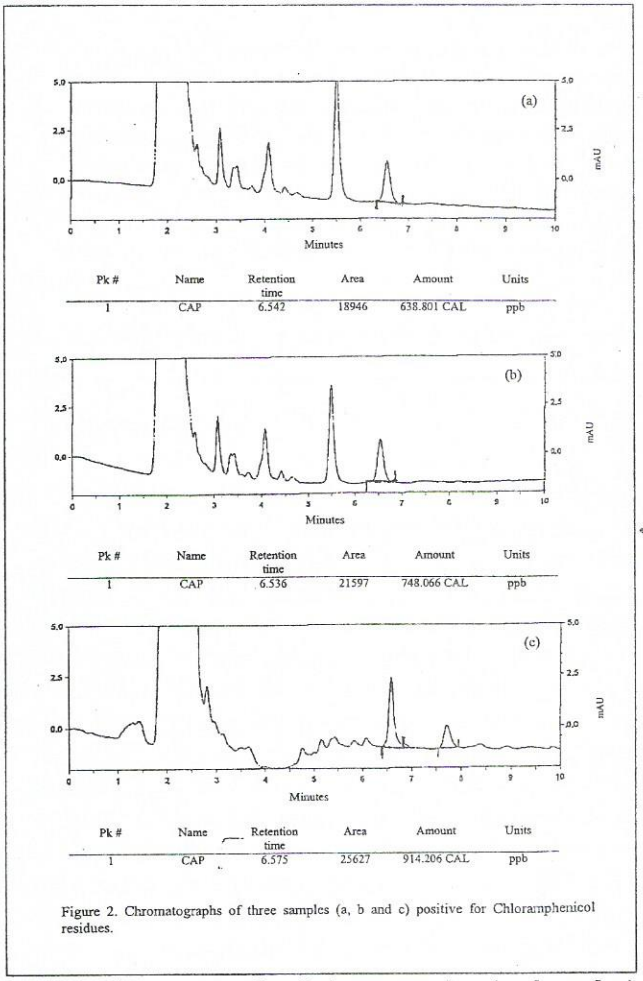

Figure 2. Chromatographs of three samples (a, b and c) positive for Chloramphenicol residues.

Fig. 2. Chromatograph of three samples (a, b and c) positive for chloramphenicol residues

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RINGKASAN

RESIDU KLORAMFENIKOL DALAM DAGING AYAM ITIK

Satu kajian telah dijalankan untuk mengesan dan menentukan kuantiti residu kloramfenikol dalam daging ayam yang dibeli daripada beberapa pembekal. Untuk kajian ini, sejumlah 50 sampel daging ayam dibeli secara rawak daripada pasar basah, pasar raya dan restoran makanan segera. Kesemua sampel pada mulanya dikenakan ujian cepat mengguna kit Ujian Penurunan Brilliant Black untuk mengesan antibiotik. Sampel ini seterusnya dianalisis mengguna kromatografi cecair tinggi-prestasi untuk mengesan dan menentukan aras kloramfenikol. Analisis ini telah menunjukkan bahawa 3 daripada 50 sampel ini mengandungi kloramfenikol pada pelbagai aras, 638.8, 748.1 dan 914.2 ppb. Kajian ini walaupun melibatkan suatu saiz sampel yang kecil, jelas telah menunjukkan yang daging ayam kita tidak sepenuhnya bebas daripada residu kloramfenikol, dan ini mencabuli Food Act and Regulation Malaysia 1998 dan mungkin juga merupakan suatu implikasi ekonomi dan kesihatan yang serius.