Forensic Med. & Toxicology Dept.,
Faculty of Vet. Medicine, Assiut University
Head of Dept.: Prof. Dr. A. A. Shaaban

ESTIMATION OF ANTIBIOTICS, SULPHONAMIDES AND NITROFURANS RESIDUES IN CHICKEN MEAT

(With One Table)

By
D.A. SALEM

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قياس متبقيات المضادات الحيوية ومركبات السلفا والنيتروفيوران في لحوم الدواجن

ضيفي أحمد سالم

تم جمع ٤٠ عينة من العضلات و١٠ عينات من الكبد و١٠ عينات من الكلى بصورة عشوائية من أسواق مدينة أسيوط . وقد تم تحليل هذه العينات لمتبقيات المضادات الحيوية ومركبات السلفا والنيتروفيوران . حيث تم قياس بقايا الكلورامفينيكول بواسطة التحليل المناعي الإنزيمي المرتبط (إليزا) والتحليل المناعي الإشعاعي (RIA). وقد تواجد الكلورامفينيكول في ٤٠ و ٣٠ و٣٠% من عينات العضلات والكبد والكلى على التوالي بمتوسط قيم بلغ ٦,٤٤٣ ± ٥,٩٦٣ و ٢,٠١٥ ± ١,٥٧٣ و ٤,٠٠٢ ± ٤,٦١٠ جزء في البليون (ميكروجرام/كيلوجرام) على الترتيب . أورت عينة واحدة من العضلات وكذلك الكلية كميات بارزة من الكلورامفينيكول بلغت ٢٠,٥ و١٠,٥ جزء في البليون علي الترتيب وتفوق هذه النسب الحدود المسموح بها بواسطة المجموعة الأوربية والتي تبلغ ١٠ جزء في البليون . كما تم الكشف عن بقايا الإستربتوميسين ، داي هيدروستربتوميسين ، جنتاميسين ، سبكتينوميسين ، أوكسي تتراسيكلين ، ايرثروميسين ، لنكوميسين ، تراي ميثوبريم ، أمبيسيلين ، أوكساسيلين ، بنزيل بنسيلين (بنسيلين ج) و فينوكسي ميثيل بنسيلين (بنسيلين ف) وذلك باستخدام طرق الفصل الكهربائي ولم يتم التعرف على أي من المركبات السابقة المذكورة في العينات المفحوصة . وباستخدام كروماتوجرافيا السائل عالي الكفاءة مع مكشاف الداي أيودو الطيفي (HPLC-DAD) لم يتم الكشف عن أي من مركبات السلفا أو النيتروفيوران في هذه العينات أيضاً. وقد نوقشت المخاطر الصحية لهذه البقايا الدوائية وخاصة الكلورامفينيكول علي صحة المستهلك لهذه الأغذية ذات الأصل الحيواني ونبهت الدراسة علي أهمية قيام السلطات بمسئولياتها تجاه ضمان سلامة الغذاء من الملوثات عامة والأدوية بصفة خاصة .

SUMMARY

Fourty chicken muscle, ten liver and ten kidney samples were collected randomly from Assiut Governorate and analyzed for antibiotic, sulphonamide and nitrofuran residues. Chloramphenicol (CAP) residues were measured by enzyme immunoassays (ELISA) and confirmed with radioimmunoassay (RIA). CAP was found in 40, 30 and 30% of the analyzed muscle, liver and kidney samples with mean values of 6.443 ± 5.963, 2.015 ± 1.573 and 4.002 ± 4.610 ppb, respectively. One muscle and kidney sample were contained violative amounts of CAP residue (20.5 and 10.5 ppb, respectively) and exceeded the Maximum Residue Limit (MRL = 10 ppb) allocated by European Economic Community (EEC). Streptomycin, Dihydrostreptomycin, Gentamycin, Spectinomycin, Oxytetracyclin (OTC), Erythromycin, Lincomycin, Trimethoprim, Ampicillin, Oxacillin, Benzylpenicillin (Penicillin G) and Phenoximethyl-penicillin (penicillin V) residues that screened by using electrophoretic technique could not be detected in all investigated samples. Neither sulphonamides nor nitrofurans were detected by HPLC-DAD in all analyzed samples. Health hazards of the investigated drugs were discussed.

Key words: *Chicken Meat – Antibiotic Residues.*

INTRODUCTION

An adequate supply of safe, wholesome food is essential to the health and well-being of man. Veterinary drugs use in animal husbandry and poultry production has increased considerably during the last years. About fifty years ago the addition of certain antibiotics and synthetic antibacterial substances to the normal feed of farm livestock has been shown to exert a growth promoting effect by improving the rate of live weight gain and the efficiency of food conversion. For more than fourty years these growth promoters are now routinely added to the feed of pigs, cattle and poultry (Ungemach, 1995).

Veterinary drugs are extensively used on farm animals not only for therapeutic purpose but also for prophylactic ones. Improper use or insufficient withdrawal period may cause drug residues in edible parts of the food producing animals as for example in tissues or milk at concentration levels higher than the maximum residue levels (MRL). The presence of drug residues in food may be a health problem for the consumers as some drugs may be a carcinogen as reported in 1988 for sulphamethzine (sulphadimidine). On the other hand continuous exposure of certain microorganisms to certain drugs may result in the development of drug resistant strains (Martin De Pozuelo et al., 1996).

The list of antibiotics approved for use as growth enhancers in livestock and poultry is too long to include here, but includes penicillin, streptomycin, tetracyclines, lincomycin, bacitracines, virginiamycin, tylosin etc. Antimicrobials which are chemically synthesized and used in animal and poultry feeds include sulpha drugs (S. dimethoxine, S. methazine (dimidine), and (S. thiazole), nitrofurans (furazolidone and nitrofuranzone) and carbadox (Beerman,1995).

Chloramphenicol (CAP) is a broad-spectrum antibiotic employed in human and animal therapy. In spite of its widespread use, CAP shows various side effects, such as aplastic anemia in animals (Krishna et al., 1981) and humans (Nahata, 1987). An unusual association between aplastic anemia and leukemia made IARC (1989) conclude that "chloramphenicol is probably carcinogenic to humans". For this reason its use has been limited in human medicine. However, it has important uses in veterinary practice. The main problem arising from veterinary use is that CAP can reach the human population through the food chain (Settepani, 1984). This effect is especially important if we consider several investigations carried out during the last few years pointing out the possible carcinogenicity of chloramphenicol (Castella, 1986).

Sulphonamides are widely used as antimicrobial agents and growth promoters in veterinary practice and animal production. The safety of these drugs for consumers has been questioned because of their apparent toxicity (Larocque et al., 1990). Nitrofurans are used for more than 30 years in veterinary medicine, alone or in combination with other drugs to promote growth or improve feed efficiency. They are administered either orally or topically in prophylactic and therapeutic treatment of mainly *Escherichia coli* and *Salmonella* infections in calves, pigs, and poultry and as coccidiostats in poultry (Anonymous, 1989/90). Since the demonstration that nitrofurans are mutagenic and (pro) carcinogenic, their use has been strictly regulated or banned in many countries (Bryan, 1978).

This work was conducted to monitor the presence of antibiotics, sulphonamides and nitrofurans and their residue levels in chicken tissues to ensure its safety for human consumption. This would enabling preventive and control measures to be initiated before contamination becomes so serious or widespread.

MATERIALS and METHODS

Samples:

Forty marketable chickens were collected randomly from poultry markets in Assiut city during January-May 1996. Muscle samples were obtained, minced and weighed for extraction procedures. Liver and kidney samples (ten each) were collected at the same time and processed as mentioned above.

Methods:

A- Chloramphenicol residues were estimated by two techniques, the first was the Enzyme Immunoassays (ELISA) and the second was Radioimmunoassy (Anonymous, 1985).

B- Streptomycin, Dihydrostreptomycin, Gentamycin, Spectinomycin, Oxytetracyclin, Erythromycin, Lincomycin, Trimethoprin, Ampicillin, Oxacillin, Benzylpenicillin and Phenoximethylpenicillin residues were screened by using electrophoretic technique after Smither and Vaughan (1978) and Lott et al., (1985).

C- Sulphonamides and nitrofuran derivatives were estimated by using Hewlett-Packard 1090II liquid chromatograph after Malisch (1986a and 19986 b).

RESULTS

The obtained results revealed that chloramphenicol was found in chicken tissues with different values and frequencies. The recorded data is listed in Table (1). Other antibiotics could not be detected in any analyzed samples. Antimicrobial sulphonamides and nitrofuran derivatives were also not found in all samples.

Table 1. Levels (ppb) of chloramphenical residues in chicken tissues.

Tissues	mean ± S.E.	Range	No. of positive	Frequency%
Muscle	6.443 ± 5.963	1.5-20.5	16 out of 40	40
Liver	2.015 ± 1.573	0.70-4.6	3 out of 10	30
Kidney	4.002 ± 4.610	0.3-10.5	3 out of 10	30

DISCUSSION

Drugs used in animal production considered one of the main sources of chemical contaminants present in food that arise from agricultural practices. If any portion of the food chain becomes contaminated, the contaminants are likely to enter the human food supply, thus presenting a potential hazard for human health as well as an impediment to trade in food.

The food and Drug Administration (FDA) approved the commercial use of antibiotics as feed additives about 36 years ago. Since then, feeding of these compounds has been commonplace and, in the animal sector, sales of animal feed additives in 1983 totaled more than $ 1.1 billion, $ 270 million of which were antibacterials. Of additional interest is the observation that almost one-half of the 35 million pounds of antibiotics manufactured in the United State were fed to animals (Tindall, 1985).

Chloramphenicol remains of great therapeutic and prophylactic values in veterinary medicine on the basis of its high efficacy against a broad range of pathogenic microorganisms and animals do not seem to be susceptible to these apparently dose-independent forms of pancytopenia. In addition, it is inexpensive therapy (Arnold and Somogyi, 1985).

The chloramphenicol residue in meat, milk and eggs intended for human consumption cause particular public health concern because the bone marrow aplasia is not dose-dependent. Furthermore, CAP a known inhibitor of protein synthesis, also retards erythropoiesis, a condition that dose-dependent and may cause allergic hypersensitivity reactions (Allen, 1985).

The present data revealed that chloramphenicol residues were found in 40, 30 and 30% of the analyzed muscle, liver and kidney samples, respectively. Its values ranged from 1.5-20.5, 0.70-4.6 and 0.3-10.5 ppb in muscle, liver and kidney samples, with mean averages of 6.443 ± 5.963, 2.015 ± 1.573 and 4.002 ± 4.610 ppb, respectively. Only one muscle and kidney sample contained violative amounts of CAP residue (20.5 and 10.5 ppb, respectively) and exceeded the tolerance limit (MRL = 10 ppb) allocated by European Economic Community (EEC), while CAP tolerance level is not allocated by the Joint FAO/WHO Expert Committee on Food Additives (Heitzman, 1994).

A Joint FAO/WHO Expert Committee on Food Additives (Geneva) met in July 1969 and proclaimed that residues of CAP in the human food supply are unacceptable. They made the following recommendation: "Chloramphenicol should not be used for any purpose that might result in the presence of residues in food for human consumption" (WHO, 1969). The Food and Drug Administration of the United States has never approved the use of chloramphenicol in food producing animals (Knight, 1981 and Settepani, 1984). In the European Economic Community (EEC) CAP is not approved for laying birds and lactating cows, and its use in other large animals is restricted; a tolerance level of 10 μg/kg has been proposed for meat products (Aerts et al., 1989). Due to its toxicological risks (WHO, 1995) which may not be excluded, CAP was totally banned for food producing animals according to Council Regulations 1430/94/EEC of 22 June 1994. In spite of this ban positive results in residue control indicate that the misuse of chloramphenicol is still a problem (Wolf et al., 1996).

Prolonged topical use of CAP in the human eye at a total dose of less than 100 mg per patient produced several cases of aplastic anemia (Feder et al., 1981, Settepani, 1984 and Fraunfelder and Bagby, 1982). In one case, the lowest total dose to cause death was 82 mg administered over 40 days, i.e., about 2 mg CAP per day (Fraunfelder and Bagby, 1982). These cases are of particular public health concern because the very low levels of chloramphenicol administered may occur in edible tissues from food producing animals trated with CAP to control various veterinary diseases (Settepani, 1984 and Knight, 1981). Therefore, consumers of ckicken meat may be exposed to potentially toxic levels of CAP residues without their knowledge.

However, the continuous legal and illegal use of chloramphenicol in food producing animals should be a concern of authorities and prohibition of drug use must be existed. A continuous monitoring of chloramphenicol residues in meat, milk and eggs will be needed since its products are available that potentially exists its use in food-producing animals.

Penicillin, tetracycline and sulphonamides are the drugs used most widely at subtherapeutic levels in livestock production. They are added to feed and are used to promote weight gain and decrease disease incidence. Their presence, even at low doses, can often sensitize a person who eats meat (Galphin, 1984). In the United States, the use of antimicrobial drugs subtheraputic levels in chickens and turkeys is nearly 100% (Steele and Beran, 1984).

It is possible that low doses of antimicrobial agents, such as those found as residues in food could alter intestinal enzyme activity and have an effect on certain hormones and drugs, since in most cases the lowest doses at which the perturbations in the intestinal microflora occur have not been determined. In order to ensure human food safety, FDA's CVM considered data gathered from a large number of compounds and determined that the maximum safe concentration of antimicrobial products is 1 ppm in a total diet of 1.5 kg. This equals a maximum antibiotic dose of 1.5 mg/day from consuming residues in food (Paige et al., 1997).

Sulphonamides, nitrofurans and antibiotics rather than chloramphenicol were not be detected in any of the investigated samples in dicating no or proper use of these drugs in the investigated farms.

Residues of regulated agrochemicals administered according to standardized protocols and good veterinary practice, do not impose an appreciable risk for the consumer. However, illegal use of (mixtures) of compounds for growth promoting purposes may result in harmful residue levels for the consumer.

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