Dept. of Animal Medicine,
Faculty of Vet. Med., Assiut University,
Head of Dept. Prof. Dr. M.F. Raghib.

A DISC ASSAY TECHNIQUE FOR THE DETERMINATION OF CHLORAMPHENICOL RESIDUES IN EGGS
(With 3 Figs.)

By
M. ABDEL-NASSER; H.A. ACET; O. ERGANIS* and B.TRAS**
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تبيان بقايا الكلورا مفينيكول في البيض
بواسطة الاقراص المنتشرة

محمود عبدالناصر، أحمد أشيت ، عثمان أرجانس ، بنيامين تراس

تم في هذا البحث اضافة الكلورامفينيكول الى علائق الدجاج البياض بالجرعة الوقائية (٢٠-٤٠مجم/كيلوجرام) والجرعة العلاجية(٤٠٠ مجم/كيلوجرام) . قيست بقايا المضاد الحيوى في البيض بواسطة استخدام الأقراص المنتشرة للكلورامفينكول. وتم استخدام السارسينيا لوتيا كميكروب اختبارى والبروسيلا اجار كوسط لزراعة الميكروب . أختبر في هذا البحث عدد ١٢٠ بيضة وقد وجد أن متبقيات الكلورامفينكول في البيض المأخوذ من الامهات التي تم تغليتها بجرعة مقدارها ٤٠مجم/كيلوجرام والجرعة ٤٠٠ مجم/كيلوجرام من العليقة هما ١٠ - ١١٥ نانوجرام و ٣٥ - ٧٥ نانوجرام لكل جرام على التوالي. أثبتت البحث أنه يمكن تشبيع القرص الواحد بكمية مقدارها ٢٥ جرام من مستخلص البيض وقد تساعد هذه الطريقة على تحديد متبقيات المضاد الحيوى بأى نسبة، ويرجع أهمية هذا التحديد لان وجود هذه المتبقيات حتى وإن كانت ضئيلة تؤثر تأثيرا بالغ الخطورة على صحة الانسان كما هو ثابت من تقارير المنظمات الدولية المعنيه بالصحة العامة.

SUMMARY

Chloramphenicol (Sigma) was added to the feeds of laying hens at prophylactic (20 and 40 mg/kg) and therapeutic (400 mg/kg) doses. Antibotic residues in eggs were determined by a disc assay method. Sarcinia lutea (ATCC 9341) was used as a test organism and brucella agar (Gibco) was used as a cultural medium. Broth cultur of Sarcinia lutea grown at 37°C for 16-18 hours, was seeded on brucella agar by disposable swab. Then, the discs absorbed chloramphenicol or egg extracts were set on the prepared plates. A total of 120 eggs were tested. Chloramphenicol residues in eggs of laying hens that have been given 40 mg/kg and 400 mg/kg doses were 10-115 ng/g and 35-75 ng/g respectively. It was shown that 6 mm in diameter could absorb an amount of 25 g homogenate of eggs.

* Dept. of Pharmacology and Microbiology, Faculty of Vet. Med., University of Selcuk, 42020 Konya, Turdey.

INTRODUCTION

In addition to the therapeutic effects of the antibiotics, they are used for increasing the animal production. Antibiotic residues in animal tissues may lead to bacterial resistance against these antibiotics. Therefore, the addition of some antibiotics to animal and poultry feeds for providing more animal production have been forbidden in some countries (ALLEN, 1985 and PETZ, 1984).

Chloramphenicol is a broad-spectrum antibiotic, and is widely used in veterinary medical practice. It is highly absorbed from intestinal tract and diffused to all tissues. It has a potent therapeutic effect, particulary against Salmonella, Escherichia coli, pasteurella, Fusiformis infection and Ricketsial infections (Infectious bovine keratitis) and secondary bacterial infections in viral diseases (BOOTH and McDONALD, 1982 and BRANDER and PUGH, 1982). ABDEL-MOTELIB and SALEM (1986) found that chloramphenicol had the highest effect among all antimicrobial agents used against pasteurellosis.

When chloramphenicol was used as a feed additive in laying hens, the constructive effects on egg yield had been shown by PETZ (1984). Consumption of large dose or successive doses of chloramphenicol by human may produce toxic syndromes. The drug has hepatotoxic, retinotoxic and optic and peripheral neuritis. An increase risk to the faetus and neonates (Grey Syndrome and death) have been recorded. It also produce pancytopenia and both haemolytic and aplastic anemia (ABOU-KHALIL, et al. 1983 and BOOTH and McDONALD, 1982).

It had been proved that the residues in animal tissues and products were detected when chloramphenicol was given both orally and parenterally (SISODIA, et al. 1973; JOHNSTON, et al. 1981 and BROWN, et al. 1984). According to WHO (1969) and FAO (1988), the finding of chloramphenicol residues in animal products such as eggs, milk, and meat are to state as undesirable for human health. Also chloramphenicol shows a delayed detoxification and elimination and hence resulting in a long-time presence in the organism (HAPKE and GRAHWIT, 1987).

The addition of chloramphenicol to feeds in U.S.A. and West Germany had been forbidden (PETZ, 1984). Chloramphenicol in Turkey, and Egypt widely used for the therapy of animal disease. Determination of chloramphenicol is carried out by high pressure liquid chromatography (HPLC), gas liquid chromatography (GLC) and colour test, the first two instruments are expensive and not available in most laboratories and colour assay may not distiguish between biologically active and inactive form of chloramphinicol (TIETZ, 1983). The purpose of the present work was to determine the chloramphenicol residures in eggs of laying hens after administration in different doses by using a disc method which is a simple, practical and available method.

MATERIAL and METHODS

One hundred and twenty laying hens supplied by Konya Agricultural Research Centre were used, and divided into 4 equal groups:

Group I: Untreated control group.
Group II: Treated with chloramphenicol (20 mg/kg) in feeds for two months.
Group III: Treated with chloramphenicol (40 mg/kg) in feeds for two months.
Group IV: Treated with chloramphenicol (400 mg/kg) in feeds for 5 days.

Eggs were taken weekly to determine chloramphenicol residues in eggs of Group I, II, III. In group IV samples were taken every day at first 10 days, in the second 10 days, samples were taken day after day and once a five days till the end of the experiment, Sarcinia lutea was used as a test organism.

Extraction:

The egg homogenate (25 g) was transfered to 200 ml flask, and adjusted to PH 6 with phosphate Buffer (Citric acid 1 M/L, dipotassium hydrogen phosphate 2 M/L, PH 3.5). 50 ml acetonitril was added, and shaked for 10 minutes. Samples were centrifuged at 1200 x g for 10 minutes, and the supernatant was collected, and transfered to 250 ml separatory funnel. 50 ml dichloromethane and 3 g NaCl were added and shaken for 5 minutes. The supernatant layer was discharged. It was blended by adding 5g anhydrous $NaSO_4$, and filtred through Whatman No. 41 filter paper. The filterate was evaporated and imbibed to the disc by solving in 200 ul methanol (JACKSON, J.V., 1975).

Standard Chloramphenicol Assay:

Standard chloramphenicol discs were prepared by drying the discs embeded with various concentrations of chloramphenicol. The sensitivity of Sarcinia lutea against standard chloramphenicol was deterimined and its standard curve was drown (Fig. 1 and 2).

Preparation of Assay Plates:

The assay was done by pouring the brucella agar to petry dishes (160 x 15mm) and Sarcinia lutea was seeded by disposable swab taken from a broth culture of the organism previously incubated for 16 hours. Bioassay discs were placed onto each plate. Plates were incubated at 37°C for 16-18 hours.

Chloramphenicol residues assay:

The diameter of the inhibition zones of the tested discs which prepared from egg extracts were measured. Chloramphenicol concentrations were determined from standard curve (Fig. 2).

RESULTS

Chloramphenicol residues weren't found at either the first group (untreated group), or the second group (20 mg/kg). It was shown that chloramphenicol residues in eggs of laying hens in group III (40 mg/kg) were 44.8 ng/g at the first and second week, 76.8 ng/g at the third and fourth week and 64.0 ng/g at the fifth week. After that no antibiotic residues were detected (Fig. 3).

The chloramphenicol residues were found in whole egg samples up to day 55 in Group IV (400 mg/kg). Antibiotic concentrations in eggs were decreased after the 20th day of chloramphenicol administration (Fig. 3).

DISCUSSION

It has been proved that the use of chloramphenicol for prophylactic and therapeutic of animal diseases lead to residues in various tissues of animals (SISODIA, et al. 1973; BOOTH and McDONALD, 1982 and BROWN, et al. 1984).

Addition of chloramphenicol in a dose of 40 mg/kg to the feeds of poultry, the residues were 0.33 ppm in egg yolks, and 0.17 ppm in egg whites at the first 80 hours (SISODIA and DUNLOP, 1972). WENZELL (1975) had found that the residue levels in eggs of laying hens given chloramphenicol via their feeds at 25, 100, 200 and 400 mg/kg doses were between 0.5-0.8, 3-6, 6-20 and 12-20 ug/kg respectively, six days after the last dose.

It had been stated that eggs from laying hens treated orally with a 10% chloramphenicol solution (50 mg/kg every 12 hours for 3 days) were 8000 ug/kg at the 5th day, 15 ug/kg at the 10th day and 3 ug/kg at the 15th day of treatment in egg whites and 1500 ug/kg at the first day, 8 ug/kg at the 5th day and less than 1 ug/kg at the 7th day (FAO, 1988).

It is known that the lowest levels of chloramphenicol residues in animal tissues and products were dangerous to humans causing aplastic anemia. Thus, the finding of chloramphenicol residues in animal products has been approved by Food and Agriculture Organization. Moreover, chloramphenicol may cause degenerative damage of liver, optic neuritis and grey syndrom in newborn (WHO, 1969; BOOTH and McDONALD, 1982; BRANDER and PUGH, 1982 and FAO, 1988). In the present study the antibiotic residues in eggs of laying hens given chloramphenicol as prophylactic (Group III) and therapeutic (Group IV) doses were found at important concentrations (Fig. 3). These results support the workers who stated that the eggs from chloramphenicol given laying hens should not be used (PETZ, 1984). According to FAO statement levels (1988),

our results showed that the eggs of these laying hens may be dangerous for humen health.

These results domonstrated that chloramphenicol residues in eggs could be detected by the disc procedure. The use of the single test organism, and disposable swabs decrease the possibility of cross contamination occuring from laboratory manipulations. It is known to be able to apply about 250 mg homogenate of eggs by agar diffusion technique. For our knowledge, this is the first report of a microbiological method in which 25 g of egg homogenate could be absorbed to one disc paper after extraction with solvents. This method has the advantage of increased sensitivity, owing to the accomodation of a greater amount of sample in one disc. This procedure can be routinely used for estimation of chloramphenicol residues in eggs.

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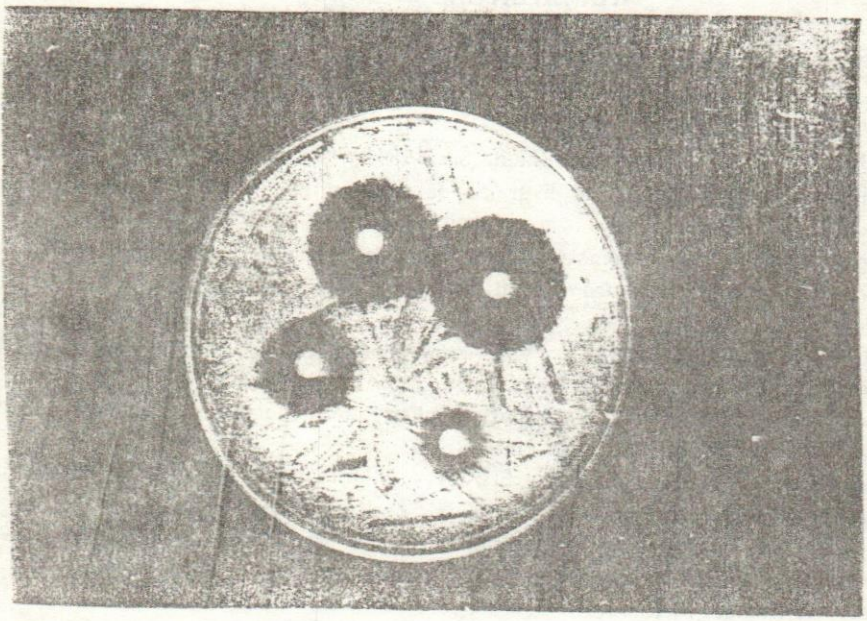

Fig. (1): Zones of inhibition of the standard chloramphenicol

CHLORAMPHENICOL RESIDUES IN EGGS

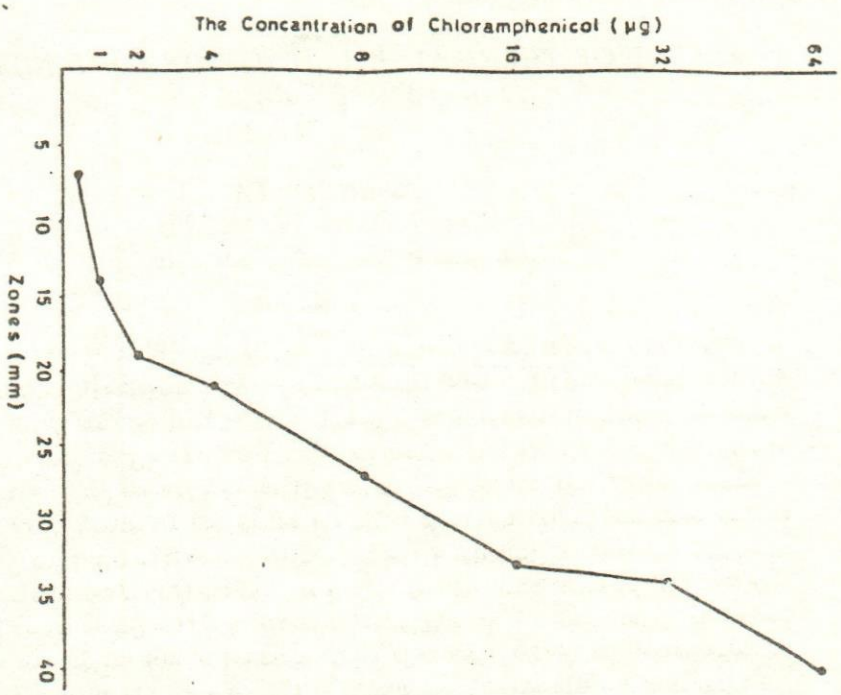

Fig. (2): Standard curve for concentratios of Chloramphenicol

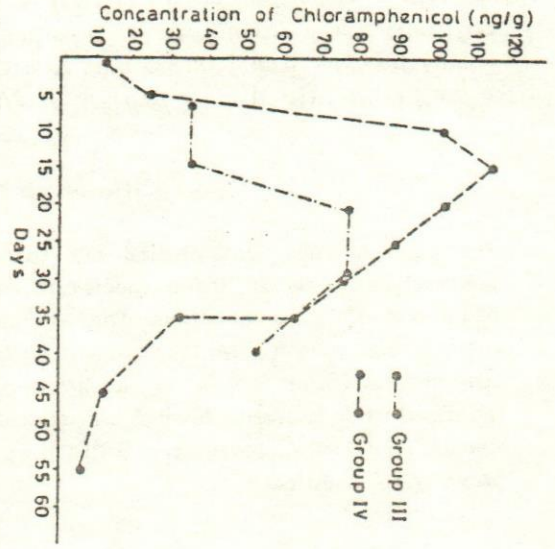

Fig. (3): Chloramphenicol concentration in eggs