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DETECTION OF ANTIBIOTIC RESIDUES IN TABLE EGGS USING DISC ASSAY AND PREMI TEST IN KHARTOUM STATE, SUDAN

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المستخلص

تم اعداد هذه الدراسة للكشف عن وجود بقايا للمضادات الحيوية فى بيض المائدة فى ولاية الخرطوم. تم جمع 157 عينة بيض من 32 مزرعة و 74 مركز بيع وتم ايضاً اعداد استبيان لجمع بيانات عن الحالة الصحية للمزارع شملت اكثر الاعراض المرضية انتشاراً وانواع المضادات الحيوية المستخدمة و مدى معرفة المزارعين عن فترة الامان. اظهرت نتائج الاستبيان ان 34.6% من المزارع توجد بها حالات اسهالات و صاحبها استعمال التتراسيكلين فى 36.4% كأكثر المضادات الحيوية استخداماً. تبين ايضا غياب الاشراف البيطرى و كانت نسبة المزارع التى يتم الاشراف عليها بواسطة المزارعين 57.6% بينما بلغت نسبة المزارعين الذين لا يعلمون عن فترة الامان للادوية 94%. تم استخدام نوعين من الاختبارات هما Disc assay و Premi kit لتحديد وجود بقايا المضادات الحيوية . كانت نسبة العينات الموجبة 49.6% و 12.7% على التوالى. تم حساب اختبار كابا لمعرفة مدى الاتفاق بين الاختبارين و كان الاتفاق بينهما ضعيف (0.30) بينما كانت نسبة الاتفاق 61.7%. اظهرت الدراسة ان معظم المزارع فى الولاية فى حالة صحية سيئة مما ادى الى استعمال المضادات الحيوية بغرض معالجة او وقاية الدواجن و بالتالى الى ظهورها فى البيض. بالاضافة الى عدم معرفة المزارعين بشان فترة الامان للادوية. بالرغم من الاستخدام المفرط فى استعمال الادوية بمزارع الدواجن، اظهر اختبار Premi test نسبة اقل من النتائج الموجبة مقارنة بـ Disc assay .

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Abstract

The aim of this study was to detect antibiotic residues in table eggs consumed in Khartoum State- Sudan. A total of 157 chicken egg samples were collected from 32 poultry farms and 74 sale points. A structured questionnaire was also used to gather information about the hygienic status of the farms, common signs of diseases, awareness of farmers and type of antibiotics used. It was found that diarrhea was present in 34.6% of the farms and the most frequently used antibiotic was tetracycline in 36.4% of the farms. The farmers were supervising their own farms in 57.6% of the farms, and 94% of the farmers lack the knowledge about withdrawal period.

The disc assay method and Premi test kit were used for antibiotic residues detection. The total number of positive reactors was 49.6% and 12.7% respectively. Using the Kappa statistic the agreement between the two tests was 0.30 (poor agreement) and the percent of the agreement was 61.7% .

The data showed the poor hygienic status of poultry farms with the concomitant use of antibiotics for treatment of diseases and the lack of knowledge regarding the use of antibiotics resulted in the high percentage of positive samples. Although antibiotics were excessively used in poultry farms the Premi test detected lower positive percentage of the positive samples while the disc assay correlated well with this excessive use of antibiotics.

Key words: antibiotic residues, table eggs , Premi test kit , Disc assay , Sudan.

Introduction

Antibiotics and other drugs are being widely used in poultry farms for different purposes: treatment, prevention and control of diseases , as growth promoters and aid in combating stress in poultry farms resulting from environmental changes (Dipeolu *et al,*2004;
Pavlov *et al,* 2008). The continuous use of the antibiotics can be a serious problem to consumers because of the possible existence of its residues in different food materials from animal sources (milk and dairy products, meat, eggs). Low-level doses of antibiotics consumed by humans for long

periods can lead to allergies, and spreading of drug-resistant microorganisms , carcinogenic effect and potential harmful effect on human intestinal micro flora (Ferrinie *et al*, 2006; Jafari *et al*, 2007; Nonga *et al*, 2010).

Detection of sulphanomides and other antibiotics is becoming an important priority to ensure food safety. Easy, reliable and accurate tests are necessary. Several methods are currently in use including the microbiological methods (Premi test Kit, Explorer Kit), ELISA, HPLC (Hussein *et al* , 2005, Gaudin *et al* ,2009).

Microbiological disc assay technique utilizes the genus Bacillus because of its high sensitivity to the majority of antibiotics. Delvotest and Premi test are a multiple microbial inhibitor test usable to detect antimicrobial agents such as beta-lactam and sulpha compounds. It is an economic, easy-to-use screening test giving results within a relatively short period (2.30 to 3.00 h).

In Sudan, poultry products are among the major sources of animal protein. Now-adays there is an increase in the number of poultry farms as they are considered an important economic source to increase individual income. The owners tend to relay on using antibiotics to avoid any economic losses.

Albeit that, the routine screening of poultry products to detect antibiotic residues is not practiced and there is no official standard method for detection of antibiotic residues.

The main objectives of this study are to detect the presence of antibiotics in table egg samples, to compare between Premi® test and Disc assay method and to point out the commonly used antibiotics and factors associated with their usage.

Materials and Methods

Sample collection:

A questionnaire was designed and distributed to gather information from different poultry farms in Khartoum State during June 2012. A total of 157 eggs were collected from 32 open system farms and 74 sale points (markets) in Khartoum State (Table 1)

The general hygienic status of the farms, signs of diseases, the frequency of antibiotics usage, and the awareness of the effects of antibiotic residues by the farmers were investigated.

Table 1: Total Number of egg samples collected.

Source of collection	**Number of egg samples (%)**
Farms	83 (52.9%)
Sale points	74 (47.1%)
Total	157

Antibiotic detection: it was conducted using two different methods:

Disc assay method: Sterilized nutrient agar plates were inoculated with 1ml of broth culture of *Bacillus stearothermophilus.* Using a clean sterile forceps, a disc was immersed into the egg sample until it was soaked and then the disc was transferred and placed on the agar surface. The plates were then inverted and incubated at 37 – 38 °C for 24 hours. The presence of antibiotic residues in the sample was indicated by the presence of inhibition zone of the growth of *Bacillus stearothermophilus* around the disc. Absence of antibiotic residues was indicated by the absence of inhibition zone around the growth. (Fagbamila *et al* , 2010)

Premi®Test Kit, was used as described by the manufacturer (DSM, Netherlands). 100 µl of homogenized egg fluid was transferred to the ampoule containing nutrient agar embedded with *Bacillus stearothermophilus* spores and Bromocresol purple indicator and incubated for 2-3 hours. A clear color change purple to yellow indicates that the antimicrobial compounds are below the Premi®Test detection limits. A purple color indicates the presence of antibiotics at or above the detection limits of the Premi®Test.
(Fig. 1)

Statistical Analysis: SPSS statistical program (version 14) was used for the analysis of the questionnaire. Kappa statistic was used to describe the association between the disc assay and the Premi test (Gordis, 2004).

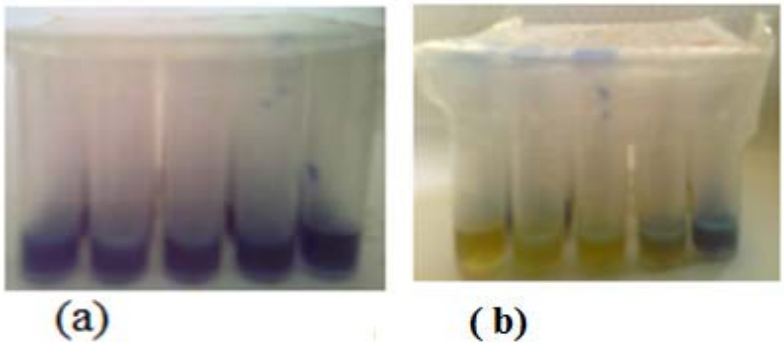

Fig.1: Premi test. (a) before incubation , (b) after 3 hours incubation

- Purple color indicates positive results.
- Yellow color indicated negative results.

Results

During collection of samples, direct interview of the farmers was recorded in a questionnaire. The interview focused on the hygienic status of the farm. Two levels of criteria were used:

Good: regular removal of litter, clean drinkers and feeders,

Bad: irregular removal of litter, muddy, drinkers and feeders are contaminated with dropping. The majority of the farms were in a bad condition (75%).

The most common signs of diseases were diarrhea in combination with respiratory signs.

This was observed in (37.5%) of the farms, followed by diarrhea alone in (34.4%) of the farms (Fig .2).

The most frequently used antibiotic was tetracycline in 37.5% of farms, while 28.1% of the farms were using penicillin (Fig.3)

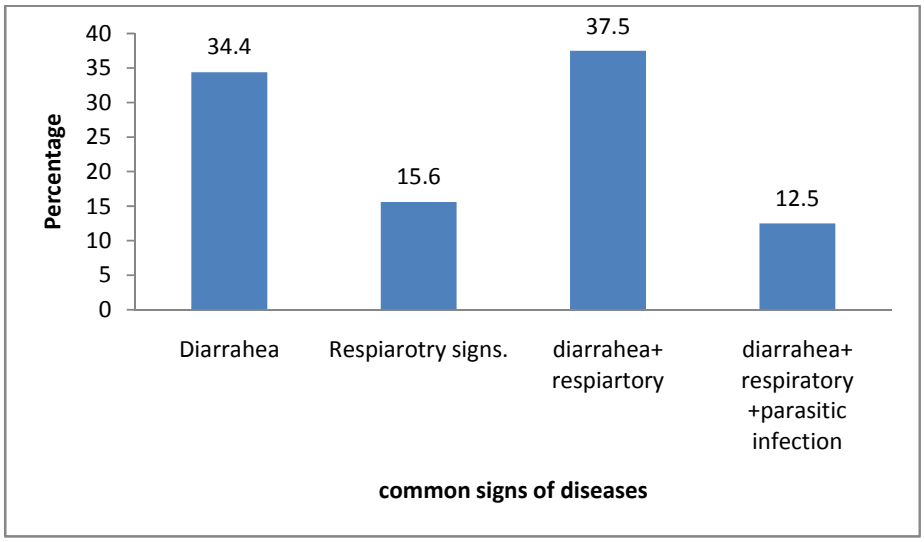

Fig. 2: Common signs of diseases in poultry farms

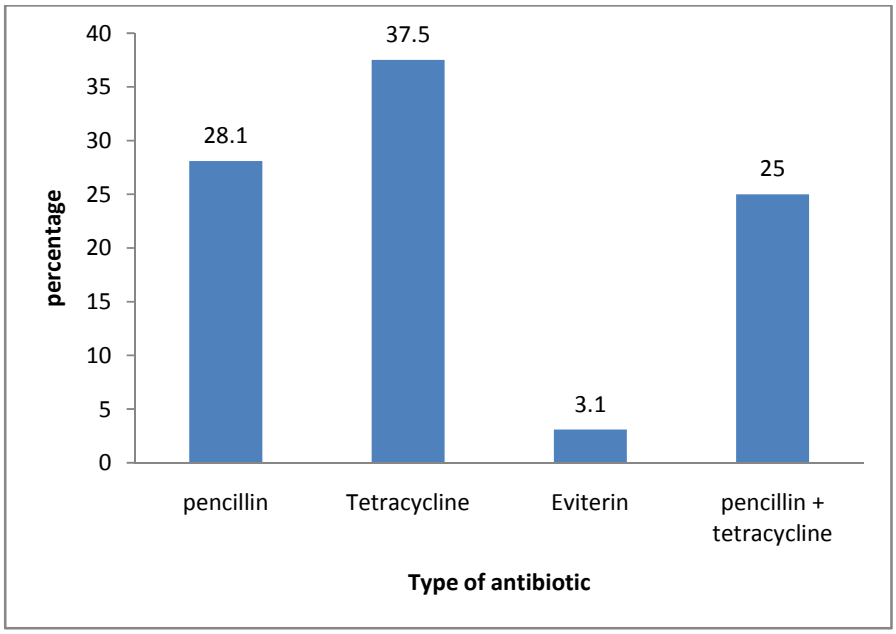

Fig.3: Type of antibiotics used in poultry farms

The data showed that 42.4% of the farms were visited by veterinarians (on call). The rest of the farms, the owners were supervising their farm completely and 75.8% of the owners were responsible for drug administration by themselves.

The majority of the bad farms were using antibiotic for treatment (37.5%), compared to (12.5%) in the good condition farms, 50% of the good farms used antibiotics for prevention , while 29% of the bad farms use antibiotics for the same purpose.

During the interview, 94% of poultry farmers believed there was no relationship between administration of antibiotics and its appearance in food material and 64% lacked the necessary information about withdrawal period of drugs and 88% of the egg samples were collected during the withdrawal period and were destined for sale.

When the presence of antibiotic residues in eggs was detected, 49.7% of the total samples were positive using disc assay method, while with the Premi kit only 12.1% of the samples were positive (Table 2).The analysis

also showed that 55.4% of farm samples were positive compared to 43.2% of market eggs (Table 3).

Table 2: Comparative results: Premi * disc assay methods

		Premi test		
		Positive	Negative	Total
Disc assay	**Positive**	19	59	78
	Negative	1	78	79
	Total	20	137	157

Kappa stastics (.95) = 30% ,
percent agreement = 61.7%

Table 3: Results of analysis of eggs from farm and sale points.

Source	**Total**	**Positive**	**Negative**
Farms	83 (52.9%)	46 (55.4%)	37(44.6%)
Market	74(47.1%)	32(43.2%)	42(56.8%)
Total	157	78(49.6%)	79(51.4%)

Kappa test was carried out to find the association between Premi test and disc assay

method, the % agreement was 61.7% and Kappa statistics (.95) = 30% indicating poor agreement between the two tests. (Table 2).

Discussion

Antibiotics are widely used in poultry farms as growth promoters, to increase feed efficiency, or to reduce prevalence of diseases. Beside their beneficial use, they have undesirable effects on human health.

The aim of this study was to detect the presence of antibiotic residues in eggs. All visited farms were using the bedding system which is composed of sawdust or straw and sand.

The hygienic status, occurrence of disease in the farms usually correlates with the use of antibiotics. All the visited farms – during the study- were using antibiotics for different purposes either prophylactic or for treatment or both. Every farm visited was found to have at least one type of disease and 75% of the farms with bad status were using antibiotics, tetracycline being the most commonly used by majority of the farms. Similar findings were reported by Al-Ghamdi *et al* (2000) and Nonga *et al* (2010) who found higher usage of tetracycline in chicken in Saudi Arabia and Tanzania, respectively. Ezenduka *et al* (2011) also reported that 100% of farms in Nigeria were using oxytetracyline. Nisha, (2008) reported that hens treated with antibiotics – even after withdrawal period – produce eggs with residues for some time.

Antibiotic residues were detected in 55.4% of the farm samples with a lower percentage of sale points sample (43.2%). The lower percentage of sale points samples may be due to variation of antibiotic use by different farms. These results are higher than those reported in different studies: 6.5% of farm samples and 15.2% of market egg samples in Trindad (Adesiyun *et al* , 2005). 34.2% of farm and retail points in Nigeria, (Ezenduka, *et al* , 2011), 21.4% of farm samples in Tanzania (Nonga *et al*, 2010).This may be due to the difference in the management systems.

The use of microbiological l methods has the benefit of their ability to detect a wide range of antimicrobial residues and also they are simple and cheap. In this study a higher number of positive samples was detected by the disc assay method than the Premi test kit (49.6% vs. 12.5%). Fagbamila *et al*, (2010) reported a higher number of positive farm sample with the diffusion test than the kit tests (3.6%) while only (2%) eggs samples gave positive result when tested with the commercial test kit. These results are contra to the results of Nonga *et al* (2010) who reported 100% positive samples with Delvo test kit and 21.4% with the agar method.

Although antibiotics were excessively used in poultry farms the Premi test detected lower positive percentage of the positive samples while the disc assay correlate well with this excessive use of antibiotics . The statistical analysis showed poor agreement between the two tests.

The data obtained from this study revealed that farmers lack the necessary information about how to deal with drugs and comply with the withdrawal period. In addition to the poor veterinary services and lack of regulations regarding the egg specification may be among many factors leading to the appearance of antibiotics in egg . Also, most of the farmers believed there is no relation between the use of antibiotics and their presence in food and eggs and were selling eggs during drug administration to avoid economic loss. A similar observation was also reported by Nonga *et al* (2010) in Tanzania. In a study carried out in Uganda, Sasanya *et al* (2005) reported that 95% of the farmers never observed withdrawal periods although 80% of them knew the importance of withdrawal periods and the eggs were sold during drug administration period.

In a similar study done in Tanzania, 80% of the farmers had knowledge on antimicrobial withdrawal period and still sold eggs before the end of the period and almost 85% were unaware of possible effects of antimicrobial residues in humans (Nonga *et al*, 2010).

Fagbamila *et al(* 2010) found that 89% of his respondents were aware of withdrawal period of antimicrobial drugs but up to a third (32%) did not observe this.

Determination of antimicrobial agents may be difficult with Premi and disc assay since they can not determine the quantity unless combined with other analytical methods, but still they are useful in giving an idea about the level of antibiotic misuse.

Conclusion and Recommendations:

The data obtained showed the poor hygienic status of poultry farms with the concomitant use of antibiotics for treatment of diseases resulting in the high percentage of positive samples in egg samples.

The absence of direct veterinary supervision, the misuse of antibiotics and lack of knowledge of antibiotic residues in animal products were the factors leading to the detection of the high percentage of positive samples observed in this study.

A need to increase the awareness of farmers to the disadvantages of antibiotics and to maintain good hygienic farms through extension programs is necessary.
Enforcement of veterinary supervision in all farms and routine assessment visits to poultry farms are to be set by the Veterinary authorities.

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