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Antimicrobial Screening of Commercial Eggs and Determination of Tetracycline Residue Using Two Microbiological Methods

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Abstract: Thirty commercial layer farms were surveyed to obtain information on drug use and to screen eggs for antimicrobial residues. Tetracycline residue was specifically tested using a commercial test kit. The study indicated that up to a third of farmers (33.3%) were not adhering to the recommendation on drug use and thereby allowing drug residues in eggs. Of the 900 commercial eggs screened, 3.6% tested positive for antimicrobial residues but only 0.1% tested positive for tetracycline residue. The low level of tetracycline residue detected in this study is an indication of the declining use of this antibiotic in the poultry industry perhaps due to the increasing availability of cheaper alternatives. There is a need for strict regulation of veterinary drug in order to guarantee food safety and effective use. To ensure compliance with drug use in Nigeria, routine surveillance must be conducted using simple detection methods.

Key words: Antimicrobials, commercial eggs, residues, tetracycline

INTRODUCTION

Majority of food-producing animals receive medication for part or most of their lives (Lee *et al*., 2001). Antimicrobials particularly tetracycline are routinely added to animal feed at sub therapeutic levels for their growth promoting properties (Droumev, 1983; Kabir *et al*., 2004). Antimicrobials are also used for the treatment of food animals especially poultry (Johnston, 1998). The increased demand for eggs by the growing fast food industry in Nigeria and the consumption of eggs as a source of animal protein places a lot of pressure on the few poultry farms that use veterinary drugs indiscriminately to boost production with farmers failing to observe the recommended withdrawal periods.

The misuse of antimicrobial agents in food animals has become a very important public health issue. The abuse or misuse of veterinary drugs is one of the causes of drug residues in animal products (Salehzadeh *et al*., 2006; Pavlov *et al*., 2008). Information on the effects of antimicrobial residues in human in Nigeria is scanty, although NAFDAC in 1996 reported the mutagenic potentials of nitrofuran drugs used in the treatment of *Salmonella* in poultry. The human health problems that could result from the intake of sub-therapeutic exposure levels of tetracycline include gastrointestinal disturbances, poor foetal development, hypersensitivity reactions and other toxic effects (Stowe *et al*., 1980; Baker and Leyland, 1983; Blomquist and Hannigren, 1966). Tetracycline in meat may potentially stain the teeth of young children. Penicillin in chicken was reported to have caused severe anaphylactic reaction in a consumer (The and Rigg, 1992). Skin allergies in eggs containing sulfonamide residues have also been reported (WHO, 1989).

Veterinary drugs are sold in the open market in Nigeria. In a bid to reduce the cost of veterinary services, some farmers purchase drugs from the market without sound diagnostic advice leading to the abuse and misuse of drugs. An official monitoring programme on drug residues is lacking in Nigeria and consumer response toward the dangers posed by residue is passive (Kabir *et al*., 1999). In this study, a survey was conducted to assess drug use amongst poultry farmers, detect the presence of antimicrobials and in particular tetracycline in poultry eggs.

MATERIALS AND METHODS

Sampling commercial chicken eggs: Thirty commercial poultry farms were selected using stratified random sampling method. Information on drug use was collected using two different structured questionnaires that were distributed to both farmers and veterinary personnel. Also clinical observations on the farms were noted and recorded. All crates of eggs available at the

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selected farm were assigned numbers. The egg at the top left corner of the first crate was assigned No.1 and the last egg in the bottom right corner assigned No.30. Numbering was continued with No. 31 at the top left corner of the second crate in that order to the last egg according to the method used by Kabir *et al.* (2004). Nine hundred (900) eggs (30 eggs per farm) were selected using a simple random sampling method without replacement. The eggs were arranged in a clean container and transported to the laboratory immediately for processing or refrigerated at 4°C till the following day.

Testing of eggs for antimicrobial residues: The antimicrobial screening of eggs was carried out using the disc diffusion method where *Bacillus stearothermophilus* C-953 (DSM, Netherlands) was used as the test organism (Anakalo and Kihumbu, 2005). An 18 h culture of the test organism in 10 ml nutrient broth (Oxoid Basingstoke, Hampshire, UK) was used to inoculate Mueller Hinton agar plates. The egg surface was thoroughly cleansed using sterile cotton wool soaked in 70% alcohol. Sterile forceps were used to puncture the egg at the tip to create a small opening from where the albumen was carefully drained out leaving behind the yolk which was transferred into a sterile beaker and thoroughly homogenized. Half of the homogenized yolk was transferred into a transparent sterile bottle and properly labeled before refrigerating at 4°C.

Sterile filter paper disc 0.6 cm in diameter was dipped into the other half of the homogenized egg yolk and placed gently on the Mueller Hinton agar plate that has already been inoculated with the test organism according to the method of Shahid *et al.* (2007). This was then incubated at 37°C for 24 h after which the plates were viewed for the presence or absence of zones of inhibition of the test organisms around the test and control discs. The difference between the diameter of zone of inhibition and that of the disc were calculated. Any disc with a difference of 1 mm or more was considered positive for the presence of antimicrobial substance.

The positive samples were further tested using a commercial test kit (Premi®Test Kit, DSM, Netherlands). The stored homogenized egg yolks that were positive with the disc diffusion method were allowed to thaw and 100 µl of the yolk inoculated into the Premi test ampoules and incubated at 80°C for ten minutes and later at 64°C for 3 h as described by the manufacturer. The positive samples from the test kit were tested for tetracycline residues. A modified commercial test kit (Premi®Test) protocol was carried out according to Stead *et al.* (2007). The modification is the addition of a Tetracycline (TCN) buffer to the samples. The TCN buffer was prepared using 7.88 g TRIS-HCL at 50 mM, 2.19 g Calcium chloride at 10 mM and 8.76 g Sodium chloride at 150 mM. This was then adjusted to a pH of 7.5 using Sodium hydroxide.

RESULTS

A total of 32 (3.6%) eggs from 10 (33.3%) farms tested positive for antimicrobials using the disc diffusion test. When tested with the commercial test kit, only 18 (2%) eggs samples from 10 (33.3%) gave positive results. Only one (0.1%) gave a positive result for tetracycline.

Farmers perception of dangers of drug residues: Eighty five (85) of the 110 questionnaires distributed to farmers were returned representing a total of 77.3% responses. The educational status of the farmers showed that 6 (7.1%) of the respondents have primary school education, 17 (20%) have secondary school education while 62 (72.9%) have tertiary level education. The study showed that there were no significant differences ($p>0.05$) between the frequency of drug administration and the experience of poultry farming. Twenty six (30.6%) of respondents had flock sizes less than 500 birds, 40 (47.1%) had between 500-2,000 birds while 19 (22.4%) had over 2,000 birds. Fifteen (17.7%) of the respondents kept only broilers, 59 (69.4%) kept only layers while 11 (12.9%) kept both broilers and layers. Eighty one (95.3%) of these farms were on deep litter system while 4 (4.7%) used battery cages. Seventy five (88.2%) of the farmers had veterinarians who consulted for them. Eighty two (96.5%) of respondent treated their birds with antimicrobial agents while sixty three (74.1%) were aware of drug residues in animal products. There was no significant ($p>0.05$) association between the educational status of the farmers and their awareness about antibiotic residues in poultry. Seventy four (87.1%) were concerned about antibiotic residues in poultry and poultry products treated soon before slaughter. Sixty six (77.6%) of the respondents thought antibiotic drugs were being misused or abused. This was blamed on quacks and fellow farmers. 89% of respondents were aware of withdrawal period of antimicrobial drugs but up to a third (32%) did not observe this.

Veterinary professional perception of drug residues: Thirty nine of the 55 questionnaires distributed to veterinarians, veterinary assistants and veterinary superintendents were returned. All 39 respondents agreed that there is gross misuse or abuse of veterinary drugs. Almost all respondents (97.4%) believed that the abuse of veterinary drugs is common in intensive management. All respondents believed that veterinary drug misuse or abuse could result in the presence of residues in animal tissues or their products. Most respondents (92.3%) believed that the slaughter of animals during treatment is common and could lead to the occurrence of drug residues. 25.6% of the

respondents believed that meat or eggs from birds undergoing treatment with veterinary drugs could be consumed, 20.5% believed that such meat or eggs could be sold to the public and 90% believed that such meat should be condemned. On the familiarity of respondents to local, national and international laws on drugs use, 76.9% said they were familiar with laws.

DISCUSSION

Antimicrobial use for bacterial and viral diseases is common in Nigeria (Kabir *et al*., 2004). The disease status of an animal and the way in which drugs are administered influence the potential for residues (Kaneene and Miller, 1997). Disease condition may affect the pharmacokinetics of the drug, its metabolism or the presence of infection and/or inflammation, which may cause the drug to accumulate in affected tissues (Kaneene and Miller, 1997). This study showed that 33.3% of farms were either not adhering to recommendations on drug use or were unintentionally allowing the occurrence of drug residues. This does not come as a surprise because there has not been any monitoring programme put in place by government neither has there been a deliberate effort to sensitize the populace on the dangers associated with residues in animal products (Kabir *et al*., 1999). Many farmers do not insure their farms and thus the occurrence of disease creates large financial losses. This may be the reason why farmers rarely observe withdrawal periods recommended for drugs.

The age of farmers, their experience, level of education and the frequency of administration of veterinary drugs had no significant difference with the occurrence of residues in poultry. As the flock size increased, the tendency of antimicrobial residue on the farm also increased because large farms were more likely to take measures towards minimizing financial losses resulting from mortality on the farm such as prophylactic and therapeutic use of antibiotics.

Microbiological methods retain a vital role in antimicrobial residue analysis because of their broad-spectrum characteristics, which make them the most suitable option for screening. They are also simple and inexpensive to perform. Although the microbiological method has its attendant problems, for instance the zone of inhibition in the disc method that was used is dose dependent on inoculums taken by the disc. Therefore if the volume of the inoculums is small, there is likelihood that samples with little concentration of inhibitory substance may be missed. The test organism used may be resistant to a particular class of antimicrobial agents that may be present in the sample thereby resulting in underestimation of the prevalence.

The commercial test kit provides a quick and efficient screening test for drug residues and can be used in samples containing natural inhibitors (e.g. lysosomes) such as in eggs, which may give a false positive result with the disc method. In addition, the kit can be used in samples such as eggs where higher temperature may cause the egg to cake thereby preventing the disc to soak and absorb any antimicrobial agent that may be present. Determining the antimicrobial agents present in the sample is difficult with both the Premi®Test kit and the disc method. Both methods can not determine the quantity of the antimicrobial agent present in the sample except when combined with other tests. However, the use of these simple secondary screening assays provided an insight into the level of abuse of veterinary drugs in the poultry industry in Nigeria and allows a suspect positive sample to be reliably directed to the appropriate chemical method for confirmatory analysis.

Conclusion: In order to minimize the occurrence of drug residues in poultry and poultry products in Nigeria, measures need to be taken. These include regulating the sales of veterinary drugs, restricting the manufacture and marketing of medicated poultry feed, encouraging farmers to form cooperatives in order to access bank loans and to insure their farms. The enforcement of laws for every farm with up to 200 birds to register with the state veterinary services and public education on the hazards of residues in animals are also required.

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REFERENCES

Anakalo, S. and G. Kihumbu, 2005. Evaluation of the Bacillus calidolactis plate for post screening assay of beta-lactam antimicrobial residues in Kenyan dairies. Food Control, 16: 227-230.

Baker, B. and D. Leyland, 1983. The chemistry of tetracycline antibiotics. J. Chromatography, 24: 30-35.

Blomquist, L. and A. Hannigren, 1966. Fluorescent technique for distribution studies of tetracyclines. Biochem. Pharmacy, 188: 215-219.

Droumev, D.R., 1983. Review of antimicrobial growth promoting agents available. Vet. Res. Commun., 7: 85-99.

Johnston, A., 1998. Use of antimicrobial drugs in veterinary practice. Br. Med. J., 317: 665-667.

Kabir, J., J.U. Umoh and V.J. Umoh, 1999. Public health awareness and health concern for veterinary drug residues in meat in Nigeria. Health and Hygiene, 20: 20-24.

Kabir, J., V.J. Umoh, E. Audu-okoh, J.U. Umoh and J.K.P. Kwaga, 2004. Veterinary drug used in poultry farms and determination of antimicrobial drug residues in commercial eggs and slaughtered chicken in Kaduna State, Nigeria. Food Control, 15: 99-105.

Kaneene, J.B. and R. Miller, 1997. Problems associated with drug residues in beef from feeds and therapy. Revue Scientifique et Technique et Office Internationale du Epizootic, 16: 694-708.

Lee, H.J., M.H. Lee and P.D. Ruy, 2001. Public health risks: Chemical and antibiotic residues. Asian-Austr. J. Anim. Sci., 14: 402-413.

National Agency for Food and Drug Administration and Control (NAFDAC), 1996. Ban on the use of nitrofuran in livestock and poultry feeds. National Agency for Food and Drug Administration and Control, Alert No. 10, Lagos.

Pavlov, A.I., L. Lashev, I. Vachin and V. Rusev, 2008. Residues of antimicrobial drugs in chicken meat and offals. Trakia J. Sci., 6: 23-25.

Salehzadeh, F., R. Madani, A. Salehzadeh, N. Rokni and F. Golchinefar, 2006. Oxytetracycline residue in chicken tissues from Tehran slaughterhouses in Iran. Pak. J. Nutr., 5: 377-381.

Shahid, M.A., M. Siddique, S. Rehman, S. Hameed and A. Hussain, 2007. Evaluation of a microbiological growth inhibition assay as a screening test for the presence of antibiotic residues in poultry meat. Am. J. Food Technol., 2: 457-461.

Stead, S.L., M. Caldow, A. Sharma, H.M. Ashwin, M. Sharman, A. De-Rijk and J. Stark, 2007. New method for the rapid identification of tetracycline residues in foods of animal origin-using the Premi® Test in combination with a metal ion chelation assay. Food Additives Contam., 24: 583-589.

Stowe, M., D. Terhune and P. Wilmore, 1980. A survey of use and misuse of tetracyclines. USA Anim. Health Meeting, 81: 331-338.

Teh, W.L. and A.S. Rigg, 1992. Possible penicillin allergy after eating chicken, Lancet, ii, 1632.

World Health Organization (WHO), 1989. Evaluation of certain veterinary drug residues in food: Thirty fourth Report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report series, No. 788, Geneva.