RESIDUE IN COMMERCIAL BROILERS IN NSUKKA, SOUTHEAST, NIGERIA

Ezenduka Ekene Vivienne*, Onyeanu Chika Thankgod* and Anaga Aruh**
*Department of Veterinary Public Health and Preventive Medicine, University of Nigeria, Nsukka
**Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka

Abstract
The study aimed to determine the use of gentamicin by poultry farmers and the presence and prevalence of gentamicin residue in tissues of commercial broilers in Ikpa slaughter house Nsukka. The study adopted cross-sectional survey using questionnaire and immunological screening. A total of 108 broilers were sampled, testing 108 each of Muscle, liver and kidney tissues for gentamicin residue using Enzyme-linked immunosorbent assay (ELISA). The questionnaire survey revealed that in the management practice, 55.2% of the farms use intensive deep litter, 21% intensive battery cage, and 23.6% extensive farming system. 27 (71%) farms rear only broilers, 10.5% layers, and 18.4% both. The feeding management showed that 21.1% of farmers produce their own feed (medicated) while 78.8% use commercial feed, of which 11.5% incorporate antibiotics. hundred (100%) of the respondents use gentamicin and only 47.3% are aware of the consequences of antimicrobial residue in food with knowledge of the legislation on the prudent use of antimicrobials in food animals. The prevalence of gentamicin residue in surveyed birds was 65% with tissue distribution as follows: muscle (44.4), liver (51.9) and kidney (59.3%). 18.5% of muscle, 33.3% of liver. Poultry Farms in the study area make constant use of gentamicin without observing its withdrawal period before sale and a high percentage of the commercial birds meant for human consumption contain gentamicin residue.
Keywords: *gentamicin, residue, poultry, ELISA, farm management*

Introduction
The intensification in agricultural sector in modern times showed that increase of livestock production and crop yield have been aided using veterinary medicinal products, especially, anti-infective drugs and chemicals like herbicides and pesticides (Tatsadjieu *et al*., 2009). About 42% of all veterinary pharmaceuticals used in poultry and livestock industry are used as feed additives, 19% as anti-infectives, 13% as parasiticides, 11% as biologic, 15% as other pharmaceuticals (Biswas, *et al*. 2010). These veterinary products are used either as a chemotherapy applied individually or collectively to animals with microbial infections, or as a preventive measure against the onset of certain diseases. Preventive measures (using anti-infectives as a medicine) is a more recent development and seen as big breakthrough in medicine because the incidence of morbidity and mortality caused by many infectious diseases can be reduced by it (Sanders *et al*., 2001). Following the administration of drugs to animals and subsequent non-observance of withdrawal periods, drugs remain in the tissues of the animals and food products derived from them (Fagbamila *et al*., 2010). Amongst the most widely used antimicrobials in livestock and poultry production is gentamicin. It is an antimicrobial with bactericidal activity against most gram-negative bacteria. It has problems of toxicity and resistance when misused. Gentamicin residue in foods of animal origin is of great public health importance worldwide, mainly because of the health risk associated with it especially its nephrotoxic and ototoxic effect (Moore, *et al*, 1984).

Materials and Method
Study area
The study was carried out in Nsukka and Enugu metropolis representing 2 of the 3 agricultural zones in Enugu State, being the major cities in Enugu State, they were purposively selected. Samples for gentamicin residue detection were collected from Ikpa slaughter, which is the major slaughter house within the Nsukka local government area, serving the whole of Nsukka and environs.
Study Design

The study adopted a cross sectional survey using questionnaire to extract information on poultry farm management and gentamicin drug use, and Immunological screening for the presence of gentamicin residue in commercial birds using Enzyme linked Immunosorbent Assay (ELISA).

Sample Source, Size and Sampling Technique

Forty (40) of the listed farms from both metropolises were selected for the questionnaire survey using systematic random sampling technique, 15 from Nsukka and 25 from Enugu. Samples for creening were collected from the poultry processing unit at Ikpa slaughter house. The slaughter was visited weekly for a period of eight weeks and on the first six weeks, systematic random sampling technique was used to select 14 birds from a daily slaughter capacity of about 30 birds. 12 birds were selected on the last two weeks. A total of 108 birds were sampled. 3 major organs (liver, muscle and kidney) serving as post slaughter matrix, were harvested from each bird making a total of 324 organ specimen.

Sample Preparation, extraction

Gentamicin ELISA kit used for screening was procured from Shenzhen Lvshiyuan Biotechnology company limited. Juices from the organs were extracted following the instruction of the ELISA kit manufacturer. One gram (1±0.5g) of each organ was macerated using sterile pestle and mortar, emulsified with 5mls of diluted extraction solution and after shaking for 3 mins, centrifuged at 4000 rpm at room temperature for 10 mins, 50µl of the supernatant was decanted into Eppendorf tubes and stored for analysis.

The test kit is based on competitive enzyme immune-assay for the detection of gentamicin in the sample. The coupling antigen is pre-coated on the micro-well strips. The gentamicin in the sample and pre-coated coupling antigen on the micro-well strips compete for the anti-gentamicin antibody. After the addition of the enzyme conjugate, the TMB (3,3',5,5'-tetramethylbenzsidine) substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with the gentamicin in it. The value is compared to the standard curve and the gentamicin concentration is subsequently obtained (manfacturer's guide).

Statistical analysis

Data from the study are presented in tables, frequencies, and proportions and analyzed in GraphPad Prism Statistical Software Version 5.02 (www.graphpad.com). Chi square test (p value) was used for statistical significance and inference. The alpha value of significance was set at the probability level of <0.05

Result and Discussion

From the Questionnaire survey, out of the 40 farms evaluated, 38 (95%) responded while 2 (5%) did not. In the management practice, 55.2% of the farms use intensive deep litter, 21% intensive battery cage, and 23.6% extensive farming system. 27(71%) farms rear only broilers, 10.5% layers, and 18.4% both. The feeding management showed that 21.1% of farmers produce their own feed with inclusion of antibiotics while 78.8% use commercial feed, of which 11.5% incorporate antibiotics. almost half of the farms surveyed use intensive deep litter which allows for cross infection among birds within and across pens, this management system may make the use of antibiotics for prevention and treatment inevitable. Some farmers that produce their feeds as well as some commercial feeds incorporate antibiotics not only for prophylactic purposes but also for growth promotion, as maximum growth for the shortest amount of time is desirable for economic gains in poultry business (Oluwasile, 2014).

Table I: Gentamicin drug usage by farms in Nsukka and Enugu metropolis, Enugu, South East Nigeria.

Drug	Frequency (n=38)	Proportion (%)
Doxygen 20/20 WSP	38	100
Gentaryl D	34	90
Bio-doxygen	34	90

Inter-gendox WS	30	80
Intergen- 200WS	30	80
Gentreat genta	29	75
Centre-gentatylo	19	50

Doxy-gen, bio-doxygen, intergendox=doxycycline & gentamicin; Gentaryl D = gentamicin, doxycycline & multiviatmins, intergen and Gentreat genta = gentamicin sulphate; Centre-gentatylo = gentamycin & tylosin

Table I shows that all (100%) the farms in the last 6 months have used gentamicin either as single formulation (Intergen and gentreat genta) or in combination with other antibiotics (Doxy-gen, bio-doxygen, intergendox, Gentaryl D & Centre-gentatylo). This is at par with the findings of Adebowale et al, 2015, who recorded that Gentamicin is the mostly widely used drug in poultry production in Ogun State, Nigeria. The easy availability of these drugs over the counter makes them easy reach for farmers.

Table 2: Awareness to prudent use of antimicrobial in poultry and livestock production and observance of withdrawal period

Awareness	Yes (%)	No (%)
Awareness to antimicrobial residues	20 (54.6))	18 (45.4)
Awareness to legislation on the prudent use of antimicrobials	12 (31.5)	26 (68.5)
Awareness to antimicrobial withdrawal periods	25 (65.8)	13 (34.2)
Observance of withdrawal period	0 (0)	100 (38)

Although more than half of the respondents (54.6%) are aware of antimicrobial residues and know about withdrawal period (65.8%), yet none of them (0%) observes withdrawal periods when drugs are used for treatment, before selling the birds or eggs for human consumption. Non-observance of drug withdrawal periods (which are boldly stated as part of a drugs manufacturer's instructions for use), is the major cause of antimicrobial residues in foods of animal origin (Doyle, 2006). This is largely due to non-implementation of available legislation on the prudent use of antimicrobials in food animals and may also be a possible cause of low (31.5%) awareness to prudent use of antimicrobials.

Table 3: Frequency of occurrence of Gentamicin residue in different organs

Sample type	**No**	**Frequency of occurrence < 0.05 (µg/kg) (undetected)**	**> 0.05 (µg/kg) (+)**	**Proportion detected (%)**
Muscle	108	60	44	44.4
Kidney	108	44	64	59.26
Liver	108	52	56	51.85
Total	**162**	**78**	**84**	**51.85**

Sixtyfive (65%) of Commercial broilers investigated were positive for gentamicin residue. They had detectable levels of gentamicin residues at the point of purchase indicating that consumers sourcing broilers from Ikpa abattoir are being exposed to violative levels of the drug residue. A similar work done in Gaza by Albayoumi *et al*. (2015) recorded 27.36% prevalence of aminoglycosides in slaughtered broilers. The disparity in the findings may be attributed to a stricter regulation for antimicrobial use in Iraq. The high prevalence of gentamicin residue detected is at par with the 100% usage of gentamicin either alone or in combination with other antibiotics from the questionnaire. This is not only due to failure to adhere to withdrawal periods (Doyle, 2006) but also, overuse, as well as the practice of self-medication by poultry farmers. Among the organs tested, liver and kidney had higher prevalence of gentamicin residue at 51.85%

and 59.26% respectively (Table 3). The high prevalence of gentamicin residue observed in liver and kidney may be attributed to the fact that the liver and kidney are organs of drug detoxification and excretion of gentamicin and other drugs (Beyene, 2016) respectively. Although the occurrence of gentamicin residue in tissues appears to be dependent on the type of tissue as presented in the result, but there is no significantl association existing between occurrence of gentamicin and the type of tissue, χ^2 (2) =1.82; (p = 0.4)

Conclusion and recommendation

There is constant use of gentamicin without observance of withdrawal period in the study area thereby causing a high prevalence of gentamicin residue in tissues of commercial birds meant for human consumption. It is then recommended that an integrated drug resistant surveillance and control program in other to protect the public from health hazards associated with drug residue should be established as well as enforcement of laws guarding the prudent use of antimicrobials in food animals.

References

Albayoumi, M.A. and Elmanama, A.A. (2015). Detection of Antibiotic Residues in Broiler Chickens in Gaza Strip. Thesis of Masters, Islamc University-Gaza.

Beyene, T. (2016). Veterinary drug residues in food animal products: Its risk factors and potential effects on public health. *J Veterinar Sci Technol.,* 7: 285. Doi: 10.4172/2157-7579.1000285.

Biswas, A.K., Kondaiah, N., Anjaneyulu, A.S.R. and Mandal, P.K. (2010). Food safety concerns of pesticides, Veterinary drug residues and mycotoxins in meat and meat products. *Asian J. Ani. Sci.* ISSN 1819-1878, volume 4(2): 46-55

Doyle, M.E (2006). Veterinary drug residues in processed meat: Potential health risk? *Food Research Institute Briefings*. University of Wisconsin-Madison. March 2006.Du W.X.,

Fagbamila, I., Kabir, J., Abdu P., Omeiza, G., Ankeli, P., Ngulukun S., Muhammad M. & Umoh, J. (2010) Antimicrobial Screening of commercial eggs and Determination of tetracycline residue using two microbiological methods. *Int. J. Poult. Sci*., 9 (10) 959-962.

Moore, R.D., Smith, C.R., Lietman P.S., (1984). Risk factors for the development of auditory toxicity in patients receiving aminoglycosides. *J. Infect. Dis.,* 149: 23-30.

Oluwasile, B. B., Agbaje, M., Ojo, O. E. and Dipeolu, M. A. (2014). Antibiotic usage pattern in selected poultry farms in Ogun state. *Sokoto Journal of Veterinary Sciences* (*SJVS)*, 12 (1): 45-50

Sanders P, Bousquet-Melou, A., Chauvin C. and Toutain, P.L. (2001) Utilisation des antibiotiques en elevage et enjuex de sante publique. INRA Productions Animales, INRA Editions 2011, 24 (2), 199-204.

Tatsadjieu Ngoume L., Tanedjieu, K.S. andMbofung C.M.F. (2009) Impact de l'utilisation des antibiotiques sur lasensibilite des bacteries pathogenes de poules dans la ville de ngaoundere. *Cameroon J. Exp. Biol, 5 (2), 52-61.*