ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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International Journal of Poultry Science 11 (1): 50-54, 2012
ISSN 1682-8356

Detection of Antimicrobial Drug Residues in Commercial Eggs Using Premi® Test

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Abstract: Nigeria is one of the African nations with known reports of drug residue occurrence in tissues and matrices. Although the actual prevalence is not certain, previous studies have shown an increasing trend over time in the occurrence of antimicrobial drug residues. The current study was developed to further study the present status of occurrence of antimicrobial drug residues utilizing commercial eggs in the most centrally located state of the north central geopolitical zone, Nigeria. Other considerations investigated in the study include the possible roles of rapid expansion of unregistered small scale poultry (flock size) and management system on the occurrence of antimicrobial drug residues. Out of the total of 105 commercial farms randomly selected from 5 Local Government Areas (LGAs) of Kaduna State for the survey of antimicrobial usage, 92 (87.6%) used veterinary drugs frequently out of which 31.0% administered the drugs by self without adhering to veterinary recommendation. Further laboratory investigation showed a true occurrence of 7.6% residue out of the 1440 commercial eggs analyzed. Uncontrolled expansion of small scale/backyard farming and management system seemed to exert influence on the occurrence of antimicrobial drug residues.

Key words: Antimicrobial, residues, premi® test, commercial eggs

INTRODUCTION

Drug residues present in foods are known to have global health problems. Such health problems include the development of allergic reactions, emergence of multiple resistant strains of pathogenic bacteria (Clewell, 1981; Ogarawa, 1981; Gomes and Demoly, 2005), interference with spermatogenesis, development of cancer and mutation in humans (Ali *et al*., 1987; NAFDAC, 1996), bone marrow aplasia and leukemia (Dollery, 1999; FAO, 2003). There has also been long standing report of genetically determined renal tubular functional defect due to consumption of degraded tetracycline (Gross, 1963). As a result of reported occurrences in different parts of the world, coupled with low awareness reported in the developing world, the World Health Organization encourages effective reporting of drug residues in foods of animal origin destined for human consumption (WHO, 2004).

Antimicrobial drug residues have been demonstrated present in tissues of food animals and their respective by-products in different parts of Nigeria (Kabir *et al*., 2004; Dipeolu *et al*., 2000; Dipeolu and Ayinde, 2001). However, most reports only identified the presence of such residues as a risk factor without considering the possible determinant factors such as the rapidly expanding unregistered small scale backyard farms and management system. An increasing trend over time in the occurrences of drug residues has been observed from previous reports. Higher occurrence of 3.6% was reported in the recent time (Fagbamila *et al*., 2010) while in the earlier report, an occurrence of 1.0% was demonstrated (Kabir *et al*., 2004). This observed trend might have arisen from a relative state of unregulated expansion of poultry sector which necessitates periodic residue assessment.

In terms of the study location, Kaduna State is located in the heart of the North central geopolitical zone of Nigeria where majority of commercial farms are claimed to have been registered by the government (Avian Influenza Desk Office Report Summary, 2006). Of great interest in this study is the presumed improvement in veterinary services and biosecurity in the poultry industries, especially after the outbreaks of Avian Influenza (AI) between 2006 and 2009 in Nigeria, including the study location. It is expected that, all these improvements be translated into reduced infection rates and drug use amongst poultry farmers and residue occurrence in Nigeria. However, rapid expansion of unregistered backyard poultry farms is viewed as a threat to this expected translation. It therefore, became very imperative to execute research which investigates the current antimicrobial drug residue status at every seasoned

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time and the possible determinant factors especially in an area like this with high economic pedigree in food animal production.

MATERIALS AND METHODS

Survey for drug use among poultry farmers: Structured questionnaires were administered to 105 poultry farmers including 96 registered and 9 non-registered poultry farms. The structure was designed to obtain information relating to drug usage and awareness of legislation guiding the use of veterinary drugs. Use of human drug preparations and type of management system were also examined.

Sampling at the farms: At the farm level, systematic sampling method was used. Imaginary lines were drawn along the diagonal axes of the pen. Egg samples were selected at every 10 sequence along any of these lines drawn inside the pen. Sampling of eggs was done in every 14 days. The sampling covered a period of about 3 months, to arrive at a total of 1440 egg samples.

Sample processing: At the end of every sampling, egg samples collected were immediately transported on clean crates to the laboratory where they were preserved at refrigeration temperature (+4°C) till the next day before processing. A 75% solution of ethanol was used to clean the egg surfaces before breaking for separation of yolk from the albumen. The albumen was gently drained through a small opening created at the taper end of each egg sample and the portion of interest (egg yolk) was separated into a sterile polythene bag for homogenization. About 10 ml of the yolk homogenate was transferred aseptically into universal bottles and stored at -20°C till analysis (after proper label inscription).

Analysis of samples using Premi® Test: The Premi® Test has the tendency of false negative results of up to 5.0%. The procedure was conducted as recommended by manufacturer (DSM, Geleen, The Netherlands). The process is outlined as follows: A 100 µl of the yolk homogenate was aspirated using the already packaged 100 µl syringe, on to the surface of the test ampoules. The ampoules were then incubated at 80°C for 10 min. Thereafter, the ampoules were transferred to already pre-heated automated incubator at 64.3°C for a period of 2 h 45 min. (Manufacturer's recommendation is 2.45-3 h). Then both negative and positive ampoules were read and interpreted accordingly when compared with the negative controls.

Statistical analysis: Fisher's Exact Test was used to analyze the data collected. P values less than 0.05 was taken to be statistically significant. Statistically significant difference exists between the indicated alphabets, a and b.

RESULTS

Veterinary drugs usage amongst poultry farmers: The result showed that up to 90.0% of the interviewed poultry farmers used veterinary drugs frequently. Out of this proportion, 31.0% used the drugs without following due veterinary recommendation. Most of the study farms holding $\leq$ 500 birds (62.5%) were incriminated positive by the applied Premi® Test. These farmers faulted their inability to operate biosecurity, to be due to either complete lack of knowledge (58.2%) or financial constraint (41.8%).

Determination of antimicrobial drug residues amongst the selected LGAs: One hundred and ten (110) egg samples were positive out of the total of 1440 egg samples tested for the presence of antimicrobial drug residues. These samples were obtained from 8 (33.3%) poultry farms out of the total of 24 farms used for sampling. The positive samples from various LGAs include 10 were positive amongst the 120 samples (0.7%) collected from Giwa LGA; none (0.0%) was positive amongst the 180 samples collected from Zaria LGA; 10 were positive amongst the 360 samples (0.7%) collected from Kaduna North LGA; 60 were positive amongst the 360 samples (4.2%) collected from Kaduna South LGA and 30 were positive amongst the 420 samples (2.1%) collected from Sabon Gari LGA. The spread of the positive samples showed a pattern which crowded more around the middle of the sampling period than seen in the beginning and the end (Table 1).

Possible roles of management system, flock size and sampling location in the occurrence of antimicrobial residues: Table 2 showed the result of relationship between management system and antimicrobial drug residue occurrence. Out of the total of 24 poultry farms sampled to obtain information on the occurrence of antimicrobial drug residues, 8 (33.3%) were positive. All these positive farms came from the farms which admitted the use of deep litter system. The remaining 16 (66.7%) farms which tested negative were found to be spread amongst those farms using battery cages mostly (45.8%) and deep litter system (20.8%). Similarly, the occurrence of antimicrobial drug residues was largely dependent on the flock size holding capacity of a farm. Seven (29.1%) out of the 8 (33.3%) antimicrobial drug residue positive farms, had flock capacity of less than 2000 birds per farm. It was only 1 (4.2%) of the positive farms that had a flock capacity of 2000 birds and above (Table 3). There was no statistically significant difference ($p>0.05$) between the two main sampling locations (Kaduna and Zaria towns).

DISCUSSION

All the samples used for this study were obtained from areas of Kaduna State known to be centrally located amongst the northern states of Nigeria and

Table 1: Antimicrobial drug residues in commercial eggs amongst the selected LGAs

		No. of sampling series within three month							
LGA	Code	1st	2nd	3rd	4th	5th	6th	Total	Remarks
Giwa (GW)	GW01	-	-	-	-	-	-	0	Negative
	GW02	-	trace ±	+(10)	trace ±	-	-	10	Positive
Zaria (ZR)	ZR01	-	-	trace ±	-	-	-	0	Negative
	ZR02	-	trace ±	-	-	-	-	0	Negative
	ZR03	-	-	-	-	-	-	0	Negative
Kaduna North (KN)	KN01	-	-	-	-	-	-	0	Negative
	KN02	-	-	trace ±	-	-	-	0	Negative
	KN03	-	-	-	-	-	-	0	Negative
	KN04	-	-	-	trace ±	+(10)	-	10	Positive
	KN05	-	-	-	-	-	-	0	Negative
	KN06	-	-	-	-	-	-	0	Negative
Kaduna South (KS)	KS01	-	-	-	-	-	-	0	Negative
	KS02	-	-	-	-	+(10)	-	10	Positive
	KS03	-	-	+(10)	-	-	-	10	Positive
	KS04	-	+(10)	-	-	-	-	10	Positive
	KS05	-	-	-	-	-	-	0	Negative
	KS06	-	+(10)	-	+(10)	+(10)	-	30	Positive
Sabon Gari (SG)	SG01	-	+(10)	-	+(10)	-	-	20	Positive
	SG02	-	-	-	-	-	-	0	Negative
	SG03	-	-	-	-	-	-	0	Negative
	SG04	-	-	-	-	-	-	0	Negative
	SG05	-	-	-	-	-	-	0	Negative
	SG06	-	-	-	-	-	-	0	Negative
	SG07	-	+(10)	-	-	-	-	10	Positive

Table 2: Possible role of management system on the occurrence of antimicrobial residues

	Premi® test screening result		
Farm management practices	Positive (%)	Negative (%)	Total (%)
Large scale deep litter (LSDL)	2 (8.3)[a]	3 (12.5)	5 (20.8)
Backyard deep litter (BYDL)	6 (25.0)[a]	11 (45.8)	17 (70.8)
Battery cage (BC)	0 (0.0)[b]	2 (8.3)	2 (8.3)
Total	8 (33.3)	16 (66.7)	24 (100.0)

*Statistically significant difference exist between a, b

Table 3: Possible role of flock size (scale of production) on the occurrence of antimicrobial residues

	Premi® test screening result		
Flock size (Scale of production)	Positive (%)	Negative (%)	Total (%)
≤500 birds	5 (20.8)[a]	2 (8.3)	7 (29.2)
501-1999 birds	2 (8.3)[a]	4 (16.7)	6 (25.0)
≥2000 birds	1 (4.2)[b]	10 (41.7)	11 (45.8)
Total	8 (33.3)	16 (66.7)	24 (100.0)

*Statistically significant difference exists between a, b; *Scale of production of up to 1999 birds is considered to be small scale/backyard poultry; *Scale of production of up to 2000 birds and above is considered to be large scale production

highly involved in poultry production, marketing and supplies of veterinary drugs with recognized facts of fast growing poultry industries. The use of egg yolk as a representative sample for this analysis was due to its low level of lysozymes and high drug residue concentration (Yoshimura *et al*., 1991; Kan and Petz, 2000).

Data collected and analyzed in this study showed that, 90.0% of the study farms employed the use of veterinary drugs frequently in the management of poultry diseases, out of which 31.0% used these drugs without following veterinary recommendations. This finding agrees with part of the findings of Fagbamila *et al*. (2010) who concluded on similar situation even though in a different part of Nigeria that 33.3% of farms were not adhering to the recommendations on veterinary drug use. This attitude was mostly observed amongst small scale/backyard poultry farmers; in this part of the world such may not be completely termed as intentional as many of the farmers were observed to lack awareness. The result of the analysis of antimicrobial residues in this study showed that 62.5% of the Premi® Test positive farms lacks the understanding to operate basic bio-security measures in their farms (holding ≤ 500 birds). This gives a strong indication that small scale peasant farmers are more prone to frequent drug use due to frequent experiences of poultry infections as a result of poor bio-security measures. The current study showed

only a small fragment of farms (8.6%) with no elligible registration all of which were small scale farms. The low proportion of farms in this category was however not considered reliable due to temporary folding-up immediately after the incidences of bird flu. This situation could be explained to be caused by sudden high impact of losses which was observed to be more pronounced specially amongst the small scale farms. Pattison *et al.* (2008) and FAO (2009) made similar observations and agreed that, "Impact of losses incured from poor bio-security measures in large scale poultry production may not be too obvious in large farms where farmers have means of immediate restocking to recover incured losses during the periods of scarcity of poultry products". This explains the noticed folding-up syndrome amongst the registered small scale farms.

The occurrence of antimicrobial drug residues noticed in this study (7.6%) was considerably high when compared with the previous studies carried out in Nigeria. Kabir *et al.* (2004) and Fagbamila *et al.* (2010) previously reported the occurrences of 1.0% and 3.6% respectively. This gives a logical impression of an increasing trends in the occurrence of antimicrobial drug residues. Reasons for this trend may not be completely argued and understood in the current study. However, improved sensitivity of the Premi® Test over the one-plate test for beta-lactams, cephalosporins, lincomycin and doxycycline (Cantwell and O'keeffe, 2006) and the strong indication for uncontrolled application of drugs in most farms in an attempt to solving the emerging poultry diseases (Van Dresser and Wilcke, 1989) could explain in part the reasons for such increasing trend. Stead *et al.* (2004) also reported the effect of acetonitrile pre-treatment of matrices. He concluded that acetonitrile denatures tissues/matrices thereby releasing a cleaner extract. The study areas has centralized geographical disposition which favors high demands for poultry products including eggs. This suddenly attracted many households towards backyard poultry production with possible consequences of uncontrolled usage of drugs. All these facts pooled together may further enhance the understanding of such increased trend of antimicrobial occurrence over time.

The occurrence of Premi® Test positive results in this study revealed a seasonal pattern. The three month period of screening showed a cluster of positive samples around the period of December and January leaving the early and latter parts of the sampling period void of positive results (Table 1). The seasonality noticed in this study even though needed further clarification, was taken as a very important factor that could favor the occurrence of antimicrobial residues in Nigeria. Nwanta *et al.* (2008) demonstrated the seasonality of Newcastle disease, the most important disease of poultry in Nigeria, showing the greater occurrence during the months of December to January characterized with cold, dry and windy climate in Nigeria. This situation might present baseline information which demonstrates a logical link between a period of high incidences of infections which culminates in uncontrolled use of drugs and occurrence of antimicrobial residues.

Of importance in this study, human drug preparations, rapidly expanding small scale backyard poultry and deep litter management system were found to significantly ($p<0.05$) promote the occurrence of drug residues. The use of human drug preparation for poultry showed some considerable influence on the occurrence of antimicrobial drug residue. This current finding was indifferent from what is known and reported. Specie-specific drug preparation may show varied pharmacokinetics and metabolic profiles in different species of animals, poultry inclusive. Anadon *et al.* (1994) and Switala *et al.* (2007) had reported that certain pharmacokinetic variation exists when same drug is administered to different species of animals. In his report, he demonstrated the detection of certain residues in the tissues of chickens and not blood 12 days post treatment. Farms operating deep litter system showed higher occurrence of antimicrobial drug residue than other systems. Explanation to this finding may not be far from the reported coprophagic tendencies of birds raised on deep litter (Kan, 1991). There was no statistically significant difference ($p>0.05$) between the two locations used for sampling. This finding was in agreement with the report of Dipeolu and Dada (2005) which showed that location of purchase had no significant effect on residue deposition in tissues. The occurrence of antimicrobial drug residues in this study was higher in small scale/backyard poultry farms than in relatively well organized and established farms. This was better explained by the state of improved bio-security in the well organized poultry farms especially after the incidences of Avian flu epidemics in Nigeria.

Conclusion: This study has demonstrated wide spread use of antimicrobial drugs with the consequences of increased occurrence of antimicrobial drug residues amongst poultry farms especially during the cold, dry and windy season in Nigeria. The wide spread use of drugs and the relatively high antimicrobial drug residue were partly tagged to low level of awareness and biosecurity amongst the unregistered peasant farmers. Unregulated expansion of backyard poultry farms, use of human drugs and deep litter system may pose threats to effective control of drug residues in Nigeria. There is need for serious commitment towards targeted monitoring and surveillance especially during the period of high occurrences. Policies should be on ground which enforce and encourage small scale poultry farms to be registered by Government to enhance better scope and management through loan facilities and public health education.

ACKNOWLEDGEMENTS

We acknowledge the financial assistance of Drs. D.H.M. Du-sai and Alex Otu. The technical assistance of M.B.

Odoba and other technical staff of both the department of Veterinary Public Health and Preventive Medicine and the Microbiology section of the University Health Services, Ahmadu Bello University, Zaria are highly appreciated.

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