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Residues of Tetracycline Antibiotic in Marketed Goats and Pigs in Lagos and Ogun States Nigeria

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Target Audience: Veterinarians, Livestock owners, Food policy makers

Abstract

Antibiotics used in food animals can occur as residues in their products and constitute health risks to the consumers. 360 fresh meat samples comprising of liver, kidney and muscle tissues of goats and pigs collected from open markets in Ogun and Lagos States were analyzed for residues of tetracycline antibiotics. Microbiological assay method was adopted, and Bacillus subtilis (ATCC 6633) organism used to detect residues of tetracycline on Antibiotic medium 2 agar. The study revealed that 15.6% of the meat samples from the two states were positive for residues of tetracycline antibiotic. The concentration of the residues ranged from 0.01μg/g to 0.06μg/g. The mean residue concentration recorded in goat meat samples is not significantly (P>0.05) higher than that recorded in the pig samples. The mean residue concentrations recorded between the states and across the organs were however significantly (P<0.05) different. Some of the muscle samples recorded tetracycline residue concentrations higher than the recommended tolerance levels. The health hazard associated with such high residue levels in meat is drug resistance; consumers of such meats may develop resistance to tetracycline antibiotic when the need arises.

Key words: Tetracycline, residue, commercial, goat, pig

Description of Problem

In Nigeria like most other countries of the world, antibiotics have been and are still being freely used at low and sub-therapeutic levels in livestock feeds. Antibiotics are also used for the purposes of treatment and prevention of animal diseases. When not properly used (i.e. in accordance with the recommendations), there is the risk that residues at undesirable levels might occur in the carcasses or produce of the treated animals (1). The concern about residues of antibiotics in meat is mainly on hypersensitivity reactions and the possibility of the development of microorganisms resistant to antibiotics in human (2; 3). There is therefore the need to have a system of control.

In most developed countries, close attention is being paid to sampling of animal products for residues of drugs. Much of this attention is occasioned by the increasing international pressure designed to limit the amounts of residues in food traded between different countries. It has been reported that the major meat exporting countries of the world such as the United States of America, Canada and the European Union have well developed abattoir-based programs for the surveillance and monitoring of antibiotic residues in meat (4; 5).

In Nigeria however, there is no national program for monitoring drug residues in food animals, neither is there any drug residue monitoring activities put in place in the abattoirs in the country. The quality control measures of meat and meat products available for human consumption in the country can thus be said to be

inadequate when compared to the developed countries. This study investigated marketed goats and pigs in some states in the South-Western Nigeria for residues of tetracycline antibiotic.

Materials and Methods

Meat sample collection

Fresh meat samples of liver, kidney and muscle tissues of goats and pigs were collected from open markets in fifteen Local Government Areas (LGAs) each of Ogun and Lagos States in South-Western Nigeria (Table 1). The meat samples were randomly collected from two open markets in each LGA, thus 180 samples per animal species were analyzed for residues of tetracycline antibiotic in the areas of study. The meat samples were carefully labeled and stored at 4°C prior to analysis.

Table 1.: Local Government Areas of Study

States	Local Governments
Ogun	Abeokuta North
	Abeokuta South
	Odeda
	Obafemi-Owode
	Yewa South
	Ifo
	Ado-odo/Ota
	Imeko-Afon
	Ewekoro
	Ijebu-North
	Ijebu-South
	Sagamu
	Ijebu-Igbo
	Remo
	Ikenne
Lagos	Mushin
	Oshodi-Isolo
	Somolu
	Ikeja
	Surulere
	Alimosho
	Amuwo-Odofin
	Ifako-Ijaye
	Kosofe
	Epe
	Agege
	Ibeju-Lekki
	Lagos Mainland
	Ojo

Control samples

Meat samples for the control (also comprising of liver, kidney and muscle tissues) were collected from goats and pigs raised at the Teaching and Research Farm of the University of Agriculture, Abeokuta, Ogun State. None of the animals was given antibiotics from three months before the study and throughout the study period.

Analysis of meat samples for residues of tetracycline antibiotic

The meat samples were analyzed for residues using microbiological assay (agar diffusion method). A modification of the method described by Katz and Fassbender (6) was adopted as follows:

Preparation of plate

Commercially available antibiotic medium 2 agar (Difco ®) was used for the analysis. 25.5g of the agar was dissolved in 1L of distilled water. This was thoroughly homogenized with an electromagnetic stirrer on a homogenizer (Ika-Combi, MGT RCT). The agar was then sterilized at 121°C for 30min in an autoclave (Prestige® medical series 2100). The sterilized agar was then cooled to a temperature of 60°C and the test organism *Bacillus subtilis* (ATCC 6633) was aseptically inoculated and thoroughly mixed. This was then aseptically poured on sterile petri dish (Sterilin®, with diameter of 9cm), uniformly mixed and allowed to harden on a level surface. Wells of 1.0cm were bored into the hardened agar with micropipette borer. The plate was used within 1hr of hardening.

Sample Preparation

Twenty-five gram of meat sample was homogenized with 100ml of phosphate buffer (pH 4.5) in a stomacher machine (Colworth ®). The homogenate was thereafter centrifuged at 4000rpm for 5min. The supernatant (1ml) was then aseptically applied into the well bored in the prepared seeded agar and incubated (Gallenkamp®) at 37°C overnight. Residues of tetracycline antibiotic were identified as clear zones of inhibition around the inoculated wells. This was measured with vernier calipers and the concentration of the residues in the meat samples

were determined by extrapolation from the standard curve previously drawn for the tetracycline antibiotic. Samples were analyzed as replicates.

Standard Curve

A standard solution containing 1000mg/ml of tetracycline in 0.1N HCl was prepared. Aliquots of this was dissolved in phosphate buffer solution (pH 4.5) to produce tetracycline standard solutions of 0.025μg, 0.05μg, 0.10μg, 0.20μg and 0.40mg). 1ml of each standard solution was inoculated into wells bored in seeded antibiotic medium 2 agar and incubated overnight at 37°C. Clear zones of inhibition produced around the inoculated wells were measured with vernier calipers. A linear curve was produced with the logarithm of concentration of the antibiotic against the zones of inhibition.

Statistical Analysis

Data collected were subjected to analysis of variance (7). When analyses of variance indicated a significance in the parameters, specific differences between means were detected by Duncan multiple range test (8).

Results and Discussion

Residues of tetracycline antibiotic were recorded in 15.6% of the all the meat samples analyzed from the two states of study (Table 2). This shows that the tetracycline antibiotic administered to the animals was not completely eliminated before they were slaughtered for human consumption. Dipeolu and Alonge (9) had earlier reported residues of tetracycline in marketed beef from the areas of study. It then means that tetracycline antibiotic is widely used in food animals in these areas. Ruiter (10) observed that tetracycline antibiotic belongs to the set of antibiotics most frequently used in veterinary practice.

The concentration of the residues of this antibiotic in the meat samples given in Table 3 ranged from 0.01μg/g - 0.60μg/g. The control samples whose animals were not exposed to any antibiotic had no residue of tetracycline antibiotic. It can also be seen from the Table 3 that the mean residue concentration recorded in meat samples from goat is slightly higher than that recorded from the pig samples, the increase is however not significant (P>0.05). This means that the use of tetracycline antibiotic in the production of these animals might not have been higher in one animal

Table 2: Occurrence of residues of tetracycline antibiotics in meat samples from the two states of study

Species	Total number of samples	Number of positive samples	% Positive
Goat	180	29	16.11
Pig	180	27	15.00
Total	360	56	15.56

species over the other. Usage of antibiotics in these goats will most likely be for disease prevention and treatment, since they are usually raised under the semi-intensive and extensive management systems, with little or no feed supplementation. In pig production however, the intensive system is mostly practiced so that the use of antibiotics here will likely be for growth promotion. The possibility of residue deposition due to prophylactic and therapeutic use of these drugs can however not be ruled out.

Table 3: Concentration of tetracycline residues in meat samples

	Mean Residue levels (µg/g)	± SEM	Range (µg/g)
Control	0	-	-
Species			
Goat	0.0934	0.012	0.01 – 0.28
Pig	0.0773	0.011	0.01 – 0.60
Organs			
Liver	0.0336[b]	0.014	0.01 – 0.10
Kidney	0.0731[b]	0.013	0.01 – 0.28
Muscle	0.1492[a]	0.017	0.01 – 0.60
States			
Ogun	0.0470[b]	0.009	0.01 – 0.10
Lagos	0.1227[a]	0.014	0.02 – 0.60

All values within columns in each block with different superscripts are significantly different ($P<0.05$)

The mean residue level recorded in muscle samples is significantly higher ($P<0.05$) than those recorded in liver and kidney samples. The parenteral mode of antibiotic administration usually through intramuscular injection will most likely cause higher concentration of this drug in the muscle tissue. This however depends on time interval between the administration of drug and slaughtering of the animal. The physiological state of the organs of the animals may also be an important consideration.

The mean tetracycline residue level recorded in Lagos State is also significantly higher ($P<0.05$) than that of Ogun State (Table 3). This shows that tetracycline antibiotic was used in animal production at a higher level in Lagos State than in Ogun State. This may be because Lagos State is highly cosmopolitan and the demand for these animal species would likely be more here, than in Ogun State.

In most advanced countries, tolerance levels are usually determined for antibiotics in food animals. Crosby (11) gave the Codex recommended Maximum Residue Levels (MRLs) for tetracycline antibiotic in animals as 0.1µg/g, 0.3µg/g and 0.6µg/g for muscle, liver and kidney tissues respectively. While none of the liver and kidney samples analyzed in the study recorded tetracycline residue levels higher than the recommended, some of the muscle tissue samples from the two States showed higher residue levels, which varied between 0.15µg/g and 0.60µg/g (Table 4). Since muscle tissues are usually consumed more than the visceral organs in these animal species, the implication of this result is frightening. It means that individuals that consume meat with such high residue levels (especially 0.60µg/g) may develop resistance to tetracycline antibiotic especially when such meat is consumed at regular intervals. The people in this category will likely not respond to treatment with this antibiotic when the need arises. This may be a problem if the exposed population cannot afford the alternative antibiotics, which is usually expensive.

Table 4. Areas with tetracycline residues higher than MRL*(μg/g)

MRL		Higher concentration			
		Ogun		Lagos	
Organ	Level	Concentration	Species	Concentration	Species
Muscle	0.1	0.15	Goat	0.15	Pig
		0.20	Goat	0.60	Pig
Liver	0.3	-	-	-	-
Kidney	0.6	-	-	-	-

* Codex Recommendation (Crosby, 1991)

Conclusion and Applications

1. Residues of tetracycline antibiotic were detected in marketed goats and pigs. The health hazard associated with this is drug resistance, whereby consumers of the meats with such residues would show resistance to treatment with tetracycline antibiotic
2. Antibiotics commonly used in animals and human beings should be judiciously used in food animals.
3. The National Agency for Food and Drug Administration and Control (NAFDAC) should set Maximum Residue Levels for antibiotics and other Biological agents in animal products for human consumption.
4. Testing of carcass for antibiotic residues should be made an integral part of meat inspection procedures.
5 Livestock owners should be educated on the need to abide by the withdrawal period stipulated for antibiotics before the food animals are sent into the abattoir for slaughter.

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