

Detection of β - Lactam antibiotics (Penicillin and Amoxicillin) residues in Goat milk

Victoria Olusola Adetunji and Opeyemi Oyinda Olaoye

Veterinary Public Health Unit, Department of Veterinary
Public Health and Preventive Medicine, University of Ibadan, Ibadan Oyo State, Nigeria
vadetunji@gmail.com; vo.adetunji@mail.ui.edu.ng; +234-7040979193

Abstract: The use of antibiotics may result in drug residues and the promotion of drug resistant strain of organisms. This study evaluated penicillin and amoxicillin residues in goat milk sourced from Ibadan city in South Western Nigeria. A total of one-hundred and sixty-six goat milk samples from Red Sokoto and West African breeds of goats were analysed using the high performance liquid chromatography method. All the samples were positive for the presence of penicillin and amoxicillin residues. The mean penicillin and amoxicillin residues was 0.2823 ± 0.1227 and 0.1228 ± 0.0571 ppm for Red Sokoto (RS) and 0.2572 ± 0.0194 and 0.1076 ± 0.0058 ppm for West African dwarf (WAD) breed of goats respectively. Hence, values obtained were in line with Maximum residue limit of (4 μ g/L) set by the European Union regulations or Food and Drug Administration (U.S. FDA), of 5 μ g/L for both antibiotics. However, there was no significant difference at $p < 0.05$ in the means of the penicillin residues in both breeds, but a reverse was the case with amoxicillin residues. Thus, the goat milk are safe for consumption. None the less, it calls for close monitoring to prevent values from increasing in the near future due to indiscriminate use of these antibiotics.

[Adetunji, VO and Olaoye, OO. **Detection of β - Lactam antibiotics (Penicillin and Amoxicillin) residues in Goat milk.** *Nat Sci* 2012;10(10):60-64]. (ISSN: 1545-0740). <http://www.sciencepub.net/nature>. 9

Key words: Antibiotic residues, Penicillin, Amoxicillin, Goat milk

Introduction

Milk obtained from lactating goats is a richer source of animal protein as compared to cow milk, to mean farmers; goat is referred to as a poor man's cow. Peasant farmers that cannot afford raising a cow can rear several goats as a source of livelihood and for consumption. These goats are reared within the household, being fed majorly with wastes and exposed to various hazards the environment can afford. They in turn serve as ready supply of; raw milk for consumption, processing of other milk products and are easily slaughtered for meat. Thus, it is important to evaluate the constituent of goat milk, since most farmers in Nigeria fall into this category, in order to ensure its safety to the health of the populace.

Antibiotic residues in milk are of great concern to dairy farmers, milk processors, regulatory agencies, and consumers. They emanate from the wide use of antibiotics in dairy animal management for the treatment of disease and as dietary supplements. They may be administered orally, as feed additives or directly by injection, incorrect use of these pharmacologically active drugs causes residues to remain in edible parts. The presence of antimicrobial drug residues in milk can provoke allergic reactions in some hypersensitive individuals as reported by Dewdney et al. (1991) and Dayan (1993) and may induce resistant populations of bacteria that do not respond to treatments commonly used for human

illnesses (Nijsten et al., 1993; Van den Bogaard and Stobberingh, 1999). Drug residues also alter the processing qualities of raw milk by inhibiting starter cultures used in the preparation of cheese and other fermented dairy products as presented by Brady and Katz, (1988) or indicate that the milk may have been obtained from an animal with a serious infection (Schenck, Callery, 1998; Choma et al., 1999; Phillips et al., 2004). Pasteurization and other forms of heat treatment eliminate pathogenic microorganisms but have limited or variable effects on drug residues (Moats, 1988).

Penicillin and amoxicillin are classified among the β -lactam antimicrobials including ampicillin, cloxacillin and hetacillin. Penicillins are divided into four classes ranging from narrow to broad range of effectiveness, based on their ability to kill various types of bacteria. Amoxicillin, is a semi-synthetic broad-spectrum penicillin, it is acid-stable, but not penicillinase (or beta-lactamase)-stable.

Penicillin is the antimicrobial for which consultation is most frequently sought through Food Animal Residues Avoidance Databank (FARAD) and is one of the most commonly detected drug residues in tissue and milk. Allergic reactions to foods containing residue concentrations of penicillin are rare and are almost always dermatologic reactions (Sundlof, 1989). There are, however, reports of anaphylactic reactions developing after consumption of food containing

penicillin residues (Lindemayr et al., 1981). Moats (1988) reported that pasteurization only reduces penicillin residues by approximately 10% to 20%, and penicillin can persist at concentrations that can adversely affect the growth of starter cultures for fermented dairy products (Suhren, 1996).

Although many extra label penicillin products have been approved for cattle, swine and sheep, none are approved for goats. When used IM in sheep at the label dose of approximately 6,600 U/kg, an 8- or 9-day slaughter withdrawal time is required, however data regarding tissue depletion after extra label PPG administration in small ruminants do not exist. Limited plasma data regarding IV administration of penicillin to sheep and goats suggest that penicillin serum half-lives in those species are similar to or shorter than that of cattle (Bengtsson et al., 1997; Schadewinkel-Scherkl, 1991).

However, after IM administration of only 10,000 to 12,000 U of PPG/kg (4,545 to 5,454 U of PPG/lb), measurable residues in goat milk samples persisted through 72 hours (Zeng et al., 1996).

This limited and conflicting data in sheep and goats do not allow FARAD to make recommendations for extra label withdrawal intervals with confidence. Practitioners choosing to treat off-label with PPG should consider testing milk for residues of penicillin prior to marketing.

The presence of drug residues in milk supplies and products is of public health implications and are perceived by consumers as undesirable (McEwen et al., 1991; Bencini and Pulina, 1997). Today, it is the general consensus that even slight traces of antibiotics in milk and food for human consumption should not be tolerated (Jepsen, 1990). Allergic reactions in highly sensitive consumers and potential carcinogenicity, mutagenicity, teratogenicity and long-term toxic effects of the residues of all classes of antibiotics which were presented in a report by Epstein (2000) are of public health concern.

Although reports on antibiotics residues in milk and milk products did not eliminate the antibiotics especially with products that had undergone heat processing like cheese, evaporated milk and yoghurt (O'Keefe and Kennedy, 2008). The presence of residues of these antibiotics in some milk products at a level exceeding the MRLs of FAO/WHO (Joint Food and Agricultural Organisation (1998) in report presented by the Expert Committee on Food Additives Standards is probably due to injudicious use of antibiotics in the treatment of infections in animals and lack of adherence to withdrawal period before milking. Dina and Arowolo (1991) reported the widespread misuse of veterinary drugs in Nigeria due to inadequate monitoring and prescription by

untrained personnel. The objective of this study is to evaluate levels of antibiotics (penicillin and amoxicillin) residues in goat milk samples.

Materials and methods

Sampling

One hundred and sixty-six goat milk (66 West African dwarf and 100 Red Sokoto goats) samples were randomly collected from different locations in Ibadan, Oyo-State. The samples were collected using sterile universal bottles, and transported on ice to the food hygiene laboratory, Department of veterinary public health and preventive medicine, University of Ibadan, Samples were stored at -20°C until analysis.

Antibiotic residue analysis of samples

High performance liquid chromatography standard methods were used for antibiotic residue analysis. Penicillin- and amoxicillin were analyzed according to Shaikh and Moats (1993) and Wenhong Luo et al. (1997) respectively.

Results

The mean levels for penicillin and amoxicillin red Sokoto goats were 0.2823 ± 0.0123 and 0.1228 ± 0.0057 respectively, while the corresponding values for the West African dwarf goats were 0.2572 ± 0.0194 and 0.1076 ± 0.0058 . In the both breeds the amoxicillin levels were significant different ($p < 0.05$) (table 1). The values for penicillin was significantly ($p < 0.05$) higher in both breeds of goat than amoxicillin (figure 1).

Discussion

Both breeds presents higher values of penicillin when compared to amoxicillin, this is expected, due to the fact that penicillin is more accessible in Ibadan, South -Western Nigeria where the study was conducted. Penicillin is the first drug of choice in the treatment of bacterial infections in these animals, it is most often used as an extra labelled drug. This could also account for the closeness in the values observed in both breeds, thus, the mean residue of penicillin was not significantly different at $P < 0.05$ level in both breeds. However, the reverse was the case in the mean residue of amoxicillin.

The antibiotics quantification of the goat milk samples was limited to Penicillin and Amoxicillin due to unavailability of other β - lactam antibiotic standards at the time of study.

The result reveals that all samples analysed was contaminated with these antibiotics to an extent. Reports on the antibiotic residue levels of the breeds in this study is scarce, the findings in this study was therefore compared with reports from cow milk. The

values of penicillin in this study were remarkably lower than maximum residues level of Penicillin G (up to $6240 \pm 550 \mu\text{g/L}$) reported in bovine raw milk (Ghidini et al., 2003). These values were within maximum limits of $4 \mu\text{g/L}$ set by the European Union regulations for both antibiotics as presented by Ghidini (2002), $5 \mu\text{g/L}$ as reported by FAO/WHO (1998), $10 \mu\text{g/L}$ as reported by Holstage et al. (2002) and $3.0 \mu\text{g/L}$ as presented by Junqueira and Brito (2006).

Mean Amoxicillin residues in the raw goat milk in the two breeds of goat is also below the ranged values of (8.5 ± 1 to $53.7 \pm 2.3 \mu\text{g/L}$) reported by Cozzani et al. (2005). However, the result of this present study is in line with the MRL's of $4.0 \mu\text{g/L}$ by Ghidini et al. (2003) and $10.0 \mu\text{g/L}$ by Holstage et al. (2002).

Table 1: Showing Mean antibiotics residues in $\mu\text{g/L}$

Breed of goat	Antibiotics	Mean \pm standard error of mean ($\mu\text{g/L}$)	MRL($\mu\text{g/L}$)	Level of significance at $P < 0.05$ with equal variance assumed
Red Sokoto (RS)	Penicillin	0.2823 ± 0.0123	$4 \mu\text{g/L}$	0.430
	Amoxicillin	0.1228 ± 0.0057	$4 \mu\text{g/L}$	0.005
West African Dwarf (WAD)	Penicillin	0.2572 ± 0.0194	$4 \mu\text{g/L}$	0.430
	Amoxicillin	0.1076 ± 0.0058	$4 \mu\text{g/L}$	0.005

MRL (Maximum Residue Limit) are as defined by the European Union Regulations (EU)

*Total number of milk samples is 166

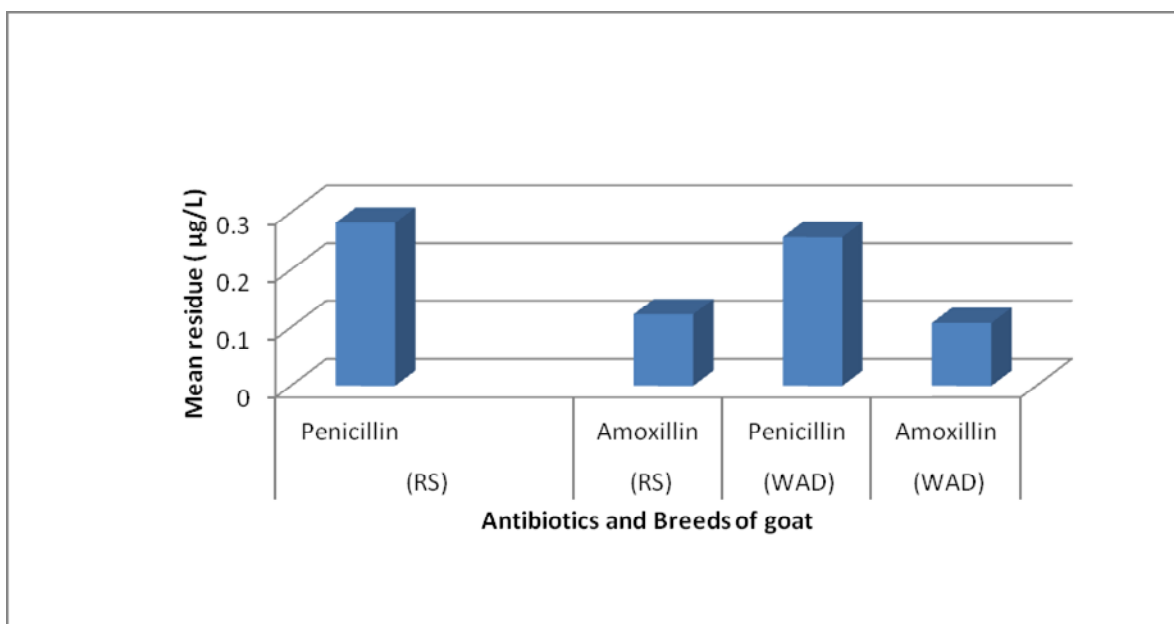


Fig 1: Chart showing mean antibiotic residues in goats

Conclusion

All the 166 samples analysed were contaminated with penicillin and amoxicillin-antibiotic residues at levels within limit required by international standards. Thus, the goat milk are regarded as safe for consumption. However, there is need for close monitoring so as to prevent values from increasing in the near future due to drug abuse (i.e. indiscriminate use of drugs).

Copyright/licence for publication

The Corresponding Author grant on behalf of all authors an exclusive licence on a worldwide basis to Nature and science to permit this article (if accepted) to be published in the journal.

Competing interests

The authors declare that they have no competing interests.

Corresponding Author:**Dr. Victoria Olusola Adetunji**Veterinary Public Health Unit, Department of
Veterinary

Public Health and Preventive Medicine,

University of Ibadan,

Ibadan Oyo State, Nigeria

vadetunji@gmail.com;vo.adetunji@mail.ui.edu.ng;

+234-7040979193

References

1. Bencini, R, Pulina, G. The quality of sheep milk; a review. *Australian Journal of Experimental Agriculture*. 1997; 37: 485-504.
2. Bengtsson B., Jacobsson S.O., Luthman J., et al. Pharmacokinetics of penicillin-G in ewes and cows in late pregnancy and in early lactation. *Journal of Veterinary Pharmacology and Therapeutics*. 1997; 20:258–261.
3. Brady MS, Katz SE. Antibiotic/antimicrobial residues in milk. *Journal of Food Protection*. 1988; 51(1):8–11.
4. Choma, I Grenda, D Malinowska, Z Suprynowics, J. Determination of flumequine and doxycycline in milk by a simple thin-layer chromatographic method. *Journal of chromatography B*. 1999; 734(1): 7- 14.
5. Cozzani, R, Ratanw, S, Zanardi, E Varisco, G. 2005. Residues of β - lactam antibiotics in bovine milk: Confirmatory analysis by liquid chromatography tandem mass spectrometry after microbial assay screening. *Food Additives and Contaminants*, 2005; 20: 528-534.
6. Dayan AD. Allergy to antimicrobial residues in food: assessment of the risk to man. *Veterinary Microbiology*. 1993; 35 (3-4):213–226.
7. Dewdney JM, Maes L, Raynaud JP, Blanc F, Scheid JP, Jackson T, Lens S, Verschueren C. Risk assessment of antibiotic residues of beta-lactams and macrolides in food products with regard to their immuno-allergic potential. *Food and Chemical Toxicology*. 1991; 29(7):477–483.
8. Dina, OA, Arowolo, ROA. Some considerations on veterinary drug use and supply in Nigeria. *Revue d'elevage et de medecine veterinaire des pays tropicaux*. 1991; 44: 29-31.
9. Epstein, SS. The politics of cancer. *Journal of American Medical Association*. 2000; 284 (4): 442.
10. FAO/WHO. Joint Food and Agricultural Organisation and World Health Organisation Expert Committee on Food Additives. 1998.
11. Ghidini, SM, Zanardi, E, Varisco, G, Chizzolini, R. Prevalence of Molecules of β – lactam Antibiotics in Bovine Milk. In Lombardia and Emili Romagna (Italy). *Annali Della Facolta di Medicina Veterina del studi di Parma*. 2002; 22: 245-252.
12. Ghidini, SM, Zanardi, E, Varisco, G, Chizzolini, R. Residues of β - lactam Antibiotics in Bovine Milk: Confirmatory Analysis by Liquid Chromatography Tends Mass Spectrometry after Microbial Assay Screening. *Food Additives and Contaminants*. 2003; 20: 528-534.
13. Holstage, DM, Punchner, B, Whitehead, G, Galey, FD. Screening and mass spectral confirmation of β - lactam antibiotic residues in milk using LC-MS/MS, *Journal of Agriculture and Food Chemistry*. 2002; 16(50): 406- 11.
14. Jepsen, A. Residues of disinfectants and antibiotics in milk: milk hygiene. *Nordisk Veterinaer Medicin*. 1990; 2: 447.
15. Junqueira, RG, Brito, RB. Determination of β -lactam Residues in Milk by High Performance Liquid Chromatography. *Brazilian Archives of Biology Technology*. 2006; 49: 41-46.
16. Lindemayr H, Knobler R., Kraft D., et al. Challenge of penicillin- allergic volunteers with penicillin-contaminated meat. *Allergy*. 1981; 36:471–478.
17. McEwen, SA, Alan, HM, William, DB. A dairy farm survey of antibiotics treatment practises, residues control methods and association with inhibitors in milk. *Journal of Food Protection*. 1991; 54: 454-459.
18. Moats WA. Inactivation of antibiotics by heating in foods and other substrates: a review. *Journal of Food Protection*. 1988; 51(6):491–497.
19. Nijsten R, London N, van de Bogaard A, Stobberingh E. Antibiotic resistance among *Escherichia coli* isolated from faecal samples of pig farmer and pig. *Journal of Antimicrobial Chemotherapy*. 1996; 37(6):1131–1140.
20. O'Keefe, M, Kennedy, O. Residues- A food safety problem: In Food safety the implications of change from producerism to consumerism. James J. Sheridan, Michael O' Keefe and Mark Rogers (eds.), Blackwell Publishing. 2008.
21. Phillips, I, Caswell, M, Cox, T, Groot, BD, Friis, C, Jones, Nightingale, C, Preston, R and Waddell, J: Does the use of antibiotic pose a risk to human? A critical review of published data. *Journal of Antimicrobial Chemotherapy*. 2004; 53: 28-52.
22. Schadewinkel-Scherkl AM. Passage of benzylpenicillin through the blood-milk barrier—studies on active transport, in Proceedings. European Association for

- Veterinary Pharmacology and Toxicology. 5th Congress. 1991; 129–130.
23. Schenck FJ, Callery PS. Chromatographic methods of analysis of antibiotics in milk. *Journal of Chromatography A*. 1998; 812(1-2):99–109.
 24. Shaikh, B, Moats, WA. Liquid Chromatographic Analysis of Antibacterial Drug Residues in Food Products of Animal Origin. *Journal of chromatograph*. 1993; 643: 369.
 25. Suhren G. Influence of residues of antimicrobials in milk on commercially applied starter cultures—model trials. *Kieler-Milchwirtschaftliche-Forschungsberichte*. 1996; 48:131–149.
 26. Sundlof SF. Drug and chemical residues in livestock. *Veterinary Clinics of North America: Food Animal Practice*. 1989; 5:411–449.
 27. Van den Bogaard, AE and Stobberingh, EE. Antibiotic usage in animals: Impact on bacterial resistance and public health. *Drugs*. 1999; 4: 589-607.
 28. Wenhong Luo, Eugene B Hansen, Jr., Catharina YW. Ang, Joanna Deck, James P. Freeman and Harold C. Thompson, Jr. Simultaneous Determination of Amoxicillin and Ampicillin In Bovine Milk by HPLC with Fluorescence Detection. *Journal of Agricultural and Food Chemistry*. 1997; 45 (4): 1264-1268.
 29. Zeng SS, Escobar EN, Brown-Crowder I. Evaluation of screening tests for detection of antibiotic residues in goat milk. *Small Ruminant Research*. 1996; 21:155–160.

7/10/2012