

Risk Factors Associated with antimicrobial Residues in the Milk Consumed in Nyala, South Darfur State, Sudan

Fathalrhman E. Addoma¹; Atif E. Abdelgadir²; Ayman El Nahas³ and Ibtisam E. El Zubeir⁴

1. Department of Preventive Medicine and Public Health, Faculty of Veterinary Science, University of Nyala, P.O Box: 155 Nyala, Sudan, e-mail: fathalrhman14@vet.nyalau.edu.sd
2. Department of Preventive Medicine & Veterinary Public Health, Faculty of Veterinary Medicine, University of Khartoum, P. O. Box 32, Sudan, e-mail: atifvet@yahoo.com
3. Department of Surgery and Anesthesia, Faculty of Veterinary Medicine, University of Khartoum, P.O. 321, Khartoum, Sudan, e-mail: ayman.elnahas@dalgroup.com
4. Department of Dairy Production, Faculty of Animal Production, University of Khartoum, P.O. 321, Khartoum, Sudan, e-mail: Ibtisammohamed@hotmail.com

Abstract

This study aimed to assess the contamination of milk by antimicrobial residues consumed in Nyala, South Darfur State, Sudan. A questionnaire and direct interviews with veterinarians at the veterinary pharmacies ($n=30$) as well as with dairy farmers ($n=30$) were carried out to determine the various risk factors associated with the presence of antimicrobial residues in milk. In addition, hundred milk samples were collected from dairy farms and sale points ($n=50$, each) and were analyzed for antimicrobial residues by using Trisensor kit. The results revealed that 73.3% of the veterinarians believe that prescriptions are not necessary for drug dispensing, and that 43.3% of the veterinarians practice whole sale of antibiotics to dairy farm owners ($P<0.001$). However, 60% of the veterinarians guide the dairy farmers to be strict to antibiotic dose and 73.3% advise them about the way of administration. Moreover, only 56.7% of the veterinarians advise the dairy farmers about the importance of the withdrawal period, and 93.3% stated that most of the saleable drugs are broad spectrum antibiotics. On the other hand, 16.7% of the dairy farmers add antibacterial drugs to animal feed, with significant difference ($P>0.01$) between south and north Nyala. Fifty percent of dairy farmers administer antibiotics to healthy animal as prophylactic therapy, only 26.7% of them consult the veterinarians, 70% keep drug in the cattle yard and treat the animals by themselves, 73.3% do not apply the withdrawal period and none of them have balances to measure animal weight for suitable drug dosage. Furthermore, one hundred raw and heated milk samples (50 from dairy farms and 50 from sale points) collected by non-probability sampling method were screening for contamination with drugs residues using the Trisensor kit (detects simultaneously beta-lactams, tetracyclines and sulfonamides, in addition to the Triaminosensor kit (detects simultaneously gentamicin, streptomycin and neomycin). In both raw and heated milk, high contamination rates with tetracyclines (34% and 31%, respectively) and sulfonamides (31% and 29%, respectively) were detected, but with no significant difference ($P>0.05$). Contamination with beta-lactams was higher in milk samples collected from the sale points (37%) than in the samples from dairy farms (32%), but also the difference was not significant ($P>0.05$). While, neomycin was not detected, gentamicin was detected in 25% and 32% and streptomycin was found in 19% and 26% of raw and heated milk, respectively.

In conclusion, contamination of milk by antimicrobial in Nyala indicates lack of proper and direct veterinary supervision in implementation of the legislations on the presence of veterinary drugs residues in milk in addition to the ignorance of withdrawal period of drugs.

المستخلص

هدفت هذه الدراسة إلى تقييم تلوث اللبن ببقايا المضادات الميكروبية في اللبن المستهلك في نyla (السودان). صمم استبيان واجريت مقابلات مع الأطباء البيطريين بالصيدليات البيطرية ($n=30$) ومالك مزارع الألبان ($n=30$), بغرض تحديد عوامل الخطأ المختلفة المتعلقة بوجود بقايا المضادات الميكروبية في اللبن. بالإضافة إلى ذلك، جُمعت مائة عينة لبن من مزارع الألبان ونقطات البيع (خمسون عينة لكل) وحللت ببقايا المضادات الحيوية باستعمال test Trisensor antibiotic. أظهرت النتائج أن 73.3% من الأطباء البيطريين يعتقدون ان الرشوة ليست ضرورية لصرف الدواء وان 43.3% ($P<0.001$) يمارسون بيع المضادات الحيوية بالجملة لمالك مزارع الألبان. بالرغم من ان 60% منهم يرشدون مربى ابقار اللبن عن جرعة المضاد الحيوي وان 73.3% ينصحون ملاك مزارع الألبان بطرق اعطاء الأدوية. علاوة على ذلك فإن 56.7% فقط من الأطباء البيطريين ينصحون ملاك مزارع الألبان بأهمية التقييد بفترات الامتناع وذكر 93.3% ان معظم الادوية المباعة هي مضادات حيوية واسعة الطيف. دلت النتائج على ان 16.7% من ملاك مزارع الألبان يُضيّقون المضادات الحيوية إلى العلائق والذى اظهر فرقاً معنوباً ($P<0.01$) بين شمال وجنوب نyla. كما ان 50% من ملاك مزارع الألبان يعطون المضادات الحيوية للحيوانات السليمة بغرض الوقاية من الامراض. فقط 26.7% من ملاك مزارع الألبان يستشرون الأطباء البيطريين و 70% لديهم مضادات حيوية بالمزارع يعطونها للحيوانات

بأنفسهم و 73.3 % لا يتقيدون بفترة الامتناع 100% و جمبيهم ليست لديهم موازین بالمزارع لقياس اوزان الحيوانات لإعطاء الجرعات المناسبة من الأدوية. جمعت مائة عينة لبن خام ولبن معامل حراريا (خمسون من مزارع الالبان وخمسون من نقاط البيع) باستخدام الطريقة الاحتمالية لأخذ العينات، وفحصت للتلوث باستخدام ترايسنر للكشف عن البتالاكتام والتراسيكلين والسلفانومايد، وأستخدم ترايمينوسنر للكشف عن الجنتاميسين و استربتوميسين والنويومايسين. ترايسنر و ترايمينوسنر هما مقاييس العمق متعدد الارسال والتي تحتوي على مستقلات متخصصة واجسام مضادة عامة احادية النسيلة في كل من عينات اللبن الخام والبن المعامل حراريا. اظهرت النتائج انتشار عاليًا للتراسيكلين 34 % و 31 % على التوالي) والسلفانومايد (31 % و 29 % على التوالي) ولكن الفرق لم يكن معنويًا ($P>0.05$). وكان انتشار البتالاكتام عاليًا في نقاط البيع (37%) مما هو عليه في مزارع الالبان (32%) ولكن الفرق لم يكن معنويًا. لم تحتوي كل عينات اللبن التي فُحصت على النويومايسين، بينما بلغ انتشار الجنتاميسين 25% و32% والاستربتوميسين 19 % و26% في كل من عينات اللبن الخام وعينات اللبن المعامل حراريا، على التوالي. خلصت الدراسة إلى أن اللبن الملوث بقايا المضادات الحيوية في نبلا قد يدل الافتقار إلى الاشراف البيطري الصحيح و المباشر بتطبيق القوانين على تواجد بقايا الأدوية البيطريه في اللبن و عدم التقيد بفترة الامتناع سحب الأدوية.

Introduction

Veterinary drugs have been used in livestock farming for several decades for the purpose of therapy and prophylaxis against infectious diseases and are used as growth promoters administered at low doses for extended periods (Movassagh, 2011). Antibiotic residues in foodstuffs are currently a problem of some magnitude in different parts of the world, particularly due to associated public health concerns that include hypersensitivity reactions, antibiotic resistance, toxicity, teratogenicity, and carcinogenicity (Darwish *et al.*, 2013). The extensive use of these antimicrobials, insufficient withdrawal period and lack of records are the most common reasons of residues in milk above the acceptable Maximum Residue Limits (Kaneene and Ahl, 1987). As infections caused by antibiotic resistant pathogens do not respond well to therapy by ordinary antibiotics and hence new antibiotics must be developed (Stolker and Brinkman, 2005). Many of the antimicrobials used in animals are also used in human medicine; the use of antimicrobials in animals is part of the global problem of antimicrobial resistance (Aryal, 2001). Improper application of the veterinary medicinal products (VMP) for disease therapy and growth promotion may result in presence of antimicrobial residues in milk and dairy products, and can contribute to the development and spread of drug resistant bacteria (Kirbis, 2006 and Stolker *et al.*, 2007). Komolafe, 2003 and Katakweba, *et al.* (2012) reported that lack of proper application and handling lead to occurrence of residues in foods from animal origin; particularly meat, milk and eggs. Farm animals treated with antimicrobials are required to be held for specific withdrawal periods until all residues are depleted to safe levels before the animal products can be used as food for human consumption (Kukanich *et al.*, 2005).

The abuse of antimicrobials has led to development of multi-drug resistant animal and human pathogens (Alanis, 2005 and El Zubeir *et al.*, 2012). Methods for determination of antimicrobial residues include high performance liquid chromatography (Abbasi *et al.*, 2011) and competitive immunoassay format (Adrian *et al.*, 2009). Most of the studies carried out were based on microbiological screening techniques that do

not specifically, classify and quantify the antimicrobial. Hence, the degree of risks to the consumers could not be ascertained due to low specificity and sensitivity of techniques (Barrow and Feltham, 1993).

In the Sudan, the milk samples from sales points had the highest percent of the antibiotic residues compared to those detected in the farms (Said Ahmad *et al.*, 2008). The detection of higher contamination with antimicrobials reported in the milk samples collected from venders might suggest the addition of antibiotics to the milk in order to prolong its shelf life (Said Ahmad *et al.*, 2008 and El Zubeir and El Owni, 2009). The objectives of the present study were to investigate the occurrence of drug residues in milk (raw and heated) sold in Nyala, South Darfur State, Sudan. It was also aimed to correlate the application and practices of using veterinary drugs with the occurrences of residues in milk.

Materials and Methods

Study area

This study was carried out in Nyala, South Darfur State, Sudan ($8^{\circ} 30$ and $13^{\circ} 30$ North and 22° and 28° East), Nyala is the capital city and Jabal Mara Street divided the city to South and North. It is densely populated and is an active trading center of livestock in the region. Dairy farms are located in South and North parts of the city, with dairy farms in the South more than in the North. Milk and milk products play an important role in the livelihood and in some of the social aspects in the town.

Data collection

A questionnaire was designed in order to obtain basic information about the manner of using antimicrobials in dairy farms. Information was collected from thirty veterinary pharmacies and thirty dairy farms owners (fifteen from each part) using Non Probability Sampling Method and closed questions as described by Thrusfield (2007).

Milk samples collection

A total of 100 milk samples were collected in sterile plain tubes from dairy farms (raw) and sales points (heated) (fifty samples for each). Milk samples collected were kept in insulated ice box at $2-8^{\circ}\text{C}$ and transferred to the lab.

Detection of the antimicrobial residues

Two antimicrobial detection kits from Unisensor (Ougrée, Liège, Belgium): (1) the trisensor kit, which detects betalactams, tetracyclines and sulphonamides simultaneously and (2) the unisensor kit, which detects the aminoglycosides group (specifically, gentamicin, streptomycin and neomycin). The test procedures and interpretation of the results were carried out according the manufacturer's protocol as described by Reybroeck and Ooghe (2012),

Data analysis

The data collected through questionnaire survey and that obtained for the milk samples examination were analyzed using Statistical Package for Social Science (SPSS version 20, 2011) programme. Descriptive statistic was used for variables. Differences between the groups were assessed by Chi-square (χ^2) test.

Results and discussion

A survey questionnaire include direct interviews with veterinarians at veterinary pharmacies and dairy farm owners to identify various risks factors associated with drugs residues in milk between South and North Nyala was conducted to determine the occurrence of antibiotic residue in the milk. The result showed that 73.3% from all interviewed veterinarians believe that prescriptions are not necessary for drugs dispensing and the difference was not significant ($P>0.05$) between South and North Nyala (Table 1). This might create some potential risks factors to the development of antimicrobials residues and resistance. In Tanzania, it was found that only 54 % of respondents obtained their antibiotics through prescription by veterinarians (Katakweba *et al.*, 2012).

As seen from the results, 43.3% of the veterinarians at veterinary pharmacies practice whole sale of antibiotics to the dairy farms' owners, 60% guide them to restrict dose and 73.3% advice the owners about the routes of administration (Table 1). However, only 56.7% from all interviewed veterinarians advice the dairy farms' owners for the withdrawal period and 93.3% stated that the most saleable drug were broad spectrum antibiotics. Komolafe (2003) and El Zuber *et al.* (2012) reported that he antibiotic abuse is one or perhaps the most important cause of the high prevalence of drugs residues and resistance among bacteria.

Regarding the knowledge of the dairy farm owners on the antimicrobials application, It was found that 16.7% from the interviewed dairy farmers add antimicrobial agents to the animal feed as prophylactic, with significant differences ($P<0.05$), and 50% administrated antibiotics to the healthy animal for prophylactic measures (Table

2). Similarly Sawant *et al.* (2005) reported that antibiotics are used extensively on dairy herds for both therapeutic and prophylactic purposes. In the present study, from all interviewed dairy farm owners, only 26.7% consult the veterinarians to advice them about antimicrobial application. El Zubeir and Mahala, (2011) reported that dairy farmers themselves always decide the drugs they administer according to their experience or to the advices of their colleagues whose cows have shown the same symptoms. They attributed that to the high financial cost of the veterinarian personnel as well as the easiness in dispensing veterinary medicinal products. Moreover, high degree of antimicrobial abuse by livestock keepers through failure in observing the recommended therapeutic doses, use of wrong routes of administration, and non-observance of withdrawal periods, were known as causes of developing and promoting spread of resistance to antibiotics (Okeke and Ojo, 2010). The results confirmed the previous report in Khartoum State that found 58% of the farmers lack to the necessary information about antibiotic residues appearance in food material, and the absence of veterinary supervision (72.3%) in the visited farms (Salman *et al.*, 2012). Furthermore, in Khartoum State, it was found that the disease control was not satisfactory as most of the labors give the treatment without consultation of veterinarians (El Zubeir and Mahala, 2011).

About 70% of dairy farm owners give the drugs to their animal. Sawant *et al* (2005) reported that in 93% of the farms, antibiotics were administered by the owners and untrained personnel (Katakweba, *et al.*, 2012). In the present study, only 23.3% from all interviewed dairy farmers were found to consider the withdrawal period (Table 2). Therefore, this might lead to presence of drugs residues in food derived from animal origin. The dairy farmers do not consider proper drug dosage, duration of treatment as well as the withdrawal period. Also Salman *et al.* (2012) found that antibiotic treated cows were milked together with the healthy ones. In Cameroon, regularly monitoring of cow health by the veterinarians is not common and the waiting time between cow treatment with antibiotic and milking is rarely respected (Edima *et al.*, 2012). The most common cause of the presence of antibiotic residues in milk is non-compliance with the withdrawal period of antimicrobial excretion from animal body (Kirbis, 2006). It was found in Tanzania that the very high proportion of milk containing antibiotic residues was due to the lack of adherence to the withdrawal period of veterinary antimicrobial which poses a risk to public health and the dairy industry (Ryoba *et al.*, 2007). Moreover, in Kenya, a questionnaire was constructed and the results suggested that lack of understanding of risks related to antibiotic

contamination of food, poor or no treatment records and lack of a monitoring system are major risks for drug residues in food derived from animal origin (Shitandi and Sternesjö, 2004). The results also confirm previous report from a rural district in China on lack of knowledge on prudent use of antibiotics and antibiotic resistance in developing countries (Chenggang *et al.*, 2011).

Beta-lactams were the predominant antibiotics residues in milk in south and north Nyala (35% and 34%, respectively) followed by tetracyclines (35% and 30%, respectively) and sulfonamides (26% and 34%, respectively). However, no significant difference ($P>0.05$) was found between the residues of these antimicrobial between the two parts of the city (Table 3). The presence of tetracyclines, sulfonamides and beta-lactams residues in milk in Nyala depended on the milk type. The tetracyclines residues were found in 34% of the raw milk samples and in 31% of the heated milk samples. The sulfonamides was detected in 31% of the raw milk and 29% of the heated milk, but the difference was not significant ($P>0.05$). High rate of tetracycline resistance bacteria obtained may be due to the wide spread use of tetracycline among dairy farms in Sudan (El Zubeir *et al.*, 2012). In Cameroon, it was found that 27% of the milk samples collected in farms were contaminated with antibiotic residues, with beta-lactams and/or tetracycline families may be the source of the contamination as it found in 53.85% of milk samples (Edima *et al.*, 2012). In Tanzania, 42.7% of screened milk samples were found positive for antibiotic residues (Ryoba *et al.*, 2007). Moreover it was found in Palestine that 18.7% milk samples tested for tetracyclines were above maximum residues limits (Al Zubeir, 2012). In the present study, beta-lactams were found to be high in the sale points (37%) compared to those collected from dairy farms (32%) as shown in Table 3. This might suggest the addition of antibiotics to milk as chemical preservative in order to prolong its shelf life as was stated previously by Said Ahmed *et al.* (2008). Similarly a study carried out in Khartoum State, showed that 12.55% and 6.66% of the milk samples were contaminated with antibiotic and sulphonamides respectively, with the highest prevalence in the milk samples collected from collection points compared to farms (El Zubeir and El Owni, 2009).

In Ghana, 35.5% of the milk samples collected contaminated with antimicrobial residues levels ranged from 16.6% for wholesalers or milk assemblers to 54.2% for milk processors (Aning *et al.*, 2007). In Palestine, 22.2% of milk samples that were tested for beta-lactams residues were above maximum residues limits (Al Zuheir, 2012). Gentamicin residues were the highest among aminoglycosides that contaminated milk samples in Nyala. Streptomycin (Dihydrostreptomycin) residues were higher in milk samples collected from north Nyala compared to those from south Nyala. However, all the examined milk samples without neomycin (Figure 1), which could be due to unavailability in Nyala.

Gentamicin residues were higher in the heated milk (32%) compared to the raw milk samples (25%), but the difference was not significant ($P>0.05$). The heated milk was highly contaminated by the streptomycin compared to the raw milk samples (26% and 19%, respectively) (Table 5). In Cameroon, aminoglycosides antibiotics group streptomycin as antimicrobial residues; in milk was detected in 15.38% of the samples (Edima *et al.*, 2012).

Visual results on dipsticks to compare the intensity of the line colour of the test line with the intensity of the line colour of the control line were illustrated in Figure 2 and 3

Conclusion

In the present study, higher prevalence of drugs residues in milk was obtained using trisensor and triaminosensor. In addition many risk factors associated with drugs residues in milk were identified. They include veterinarians's practices and the unawareness of dairy farms' owners mismanagement and unawareness. Hence this situation necessitate the initiation of strict legislation should be implemented in order to minimize the abuse of antibiotics, and comprehensive program should be developed through collaboration with governmental authorities, farmers, veterinarian and researchers to ensure the quality of milk in term of chemical contamination. Monitoring programs should be initiated and implemented using screening tests to ensure that safe food and wholesome milk and dairy products are available for consumption in this country.

Table 1: The opinions and practices of veterinarians in veterinary pharmacies in Nyala in dispensing antimicrobials

Unit	south Nyala	north Nyala	Total	Chi-square	P- value
Importance of prescription					
Yes	0%	0%	0%		
No	12 (40%)	10 (33.3%)	22(73.3%)	0.682	0.409
Sometimes	3 (10%)	5 (16.7%)	8 (26.7%)		
Total	15 (50%)	15 (50%)	30 (100%)		
Whole saleable antibiotics to the owners					
Yes	12 (40%)	1 (3.3%)	13(43.3%)	20.308	0.001**
No	3 (10%)	3 (10%)	6 (20%)		
Sometimes	0 (0%)	11(36.7%)	11(36.7%)		
Total	15 (50%)	15 (50%)	30 (100%)		
Dose restriction & administration to owners					
Yes	3 (10%)	3 (10%)	6 (20%)	3.556	0.169
No	11(36.7%)	7 (23.3%)	18 (60%)		
Sometimes	1 (3.3%)	5 (16.7%)	6 (20%)		
Total	15 (50%)	15 (50%)	30 (100%)		
Yes	11(36.7%)	11(36.7%)	22(73.3%)	1.143	0.565
No	4 (13.3%)	3 (10%)	7 (23.3%)		
Sometimes	0 (0%)	1 (3.3%)	1 (3.3%)		
Total	15 (50%)	15(50%)	30 (100%)		
Advice owners for withdrawal period					
Yes	8 (26.7%)	9 (30%)	17(56.7%)	0.136	0.713
No	7 (23.3%)	6 (20%)	13(43.3%)		
Sometimes	0 (0%)	0 (0%)	0 (0%)		
Total	15 (50%)	15(50%)	30 (100%)		
The most saleable broad spectrum antibiotics					
Yes	14(46.7%)	14(46.7%)	28(93.3%)	0.000	1.00
No	0 (0%)	0 (0%)	0 (0%)		
Sometimes	1 (3.3%)	1 (3.3%)	2 (6.7%)		
Total	15 (50%)	15(50%)	30 (100%)		

P-value < 0.01 highly significant while P-value >0.05 not significant

Table 2: The knowledge and of the dairy farm owners on the application of antimicrobial drugs in Nyala

Unit	South Nyala	North Nyala	Total	Chi-square	P-value
Antimicrobials add to animal feed					
Yes	1 (3.3%)	4(13.3%)	5(16.7%)	8.927	0.012*
No	13(43.3%)	5(16.7%)	18(60%)		
Sometimes	1 (3.3%)	6(20%)	7(23.3%)		
Total	15 (50%)	15 (50%)	30(100%)		
Administration of antibiotics as prophylactics					
Yes	9 (30%)	6 (20%)	15(50%)	4.671	0.097^{ns}
No	5 (16.7%)	3 (10%)	8 (26.7%)		
Sometimes	1 (3.3%)	6 (20%)	7(23.3%)		
Total	15 (50%)	15 (50%)	30(100%)		
Consultation of Veterinarians					
Yes	5 (16.7%)	3 (10%)	8 (26.7%)	0.833	0.659
No	5 (16.7%)	5(16.7%)	10(33.3%)		
Sometimes	5 (16.7%)	7(23.3%)	12 (40%)		
Total	15 (50%)	15 (50%)	30(100%)		
Availability of drugs in cattle yard					
Yes	13(43.3%)	8(26.7%)	21 (70%)		
No	2 (6.7%)	5(16.7%)	7(23.3%)		
Sometimes	0 (0.0%)	2 (6.7%)	2 (6.7%)		
Total	15 (50%)	15 (50%)	30(100)		
Consideration of withdrawal period					
Yes	4 (13.3%)	3 (10%)	7(23.3%)	1.143	0.565
No	11(36.7%)	11(36.7%)	22(73.3%)		
Sometimes	0 (0.0%)	1 (3.3%)	1 (3.3%)		
Total	15 (50%)	15 (50%)	30(100)		
Using balance for measurement of animals weight					
Yes	0 (0%)	0 (0%)	0 (0%)	No estimate	
No	15 (50%)	15 (50%)	30(100)		
Sometimes	-	-	-		
Total	15 (50%)	15 (50%)	30(100%)		

ns P-value >0.05 not significant

* P-value = significant difference (P < 0.05)

Table 3: Prevalence of beta-lactams, tetracyclines and sulfonamides residues in milk in south and north Nyala

Antibiotics	South Nyala	North Nyala	Total	Chi-square	P-value
Beta-lactams					
Positive result	35 (35%)	34 (34%)	69 (69%)	0.047	0.829
Negative result	15 (15%)	16 (16%)	31 (31%)		
Total	50 (50%)	50 (50%)	100 (100%)		
Teteracyclines					
Positive result	35 (35%)	30 (30%)	65 (6%)	1.099	0.295
Negative result	15 (15%)	20 (20%)	35 (35%)		
Total	50 (50%)	50 (50%)	100 (100%)		
Sulfonamides					
Positive result	26 (26%)	34 (34%)	60 (60%)	2.667	0.102
Negative result	24 (24%)	16 (16%)	40 (40%)		
Total	50 (50%)	50 (50%)	100 (100%)		

P-value > 0.05 not significant

Table 4: Prevalence of beta-lactams, tetracyclines and sulfonamides residues in raw and heated milk in Nyala

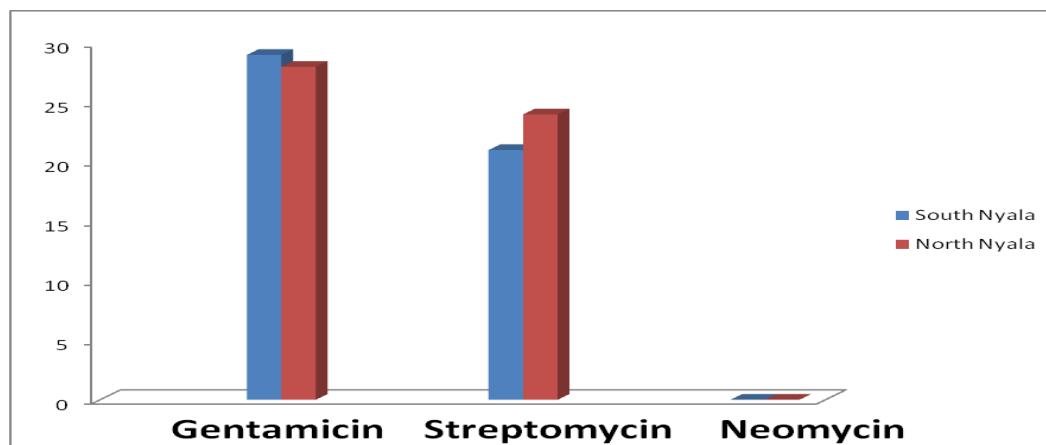
Antibiotics	Raw milk	Heated milk	Total	Chi-square	P-value
Beta-lactams					0.28
Positive	32 (32%)	37 (37%)	69	1.169	
Negative	18 (18%)	13 (13%)	31		
Total	50 (50%)	50 (50%)	100		
Teteracyclines				0.396	0.529
Positive	34 (34%)	31 (31%)	65		
Negative	16 (16%)	19 (19%)	35		
Total	50 (50%)	50 (50%)	100		
Sulfonamides				0.167	0.683
Positive	31 (31%)	29 (29%)	60		
Negative	19 (19%)	21 (21%)	40		
Total	50 (50%)	50 (50%)	100		

P-value > 0.05 not significant

Table 5: Prevalence of aminoglycosides residues in raw and heated milk samples in Nyala

Antibiotics	Raw milk	Heated milk	Total	Chi-square	P- value
Gentamicin					
Positive	25 (25%)	32 (32%)	57	1.999	0.157
Negative	25 (23%)	18 (18%)	43		
Total	50 (50%)	50 (50%)	100		
Streptomycin					
Positive	19 (19%)	26 (26%)	45	1.980	0.159
Negative	31 (31%)	24 (24%)	55		
Total	50 (50%)	50 (50%)	100		
Neomycin					
Positive	0 (0%)	0 (0%)	0		
Negative	50 (50%)	50 (50%)	100	No estimate	
Total	50 (50%)	50 (50%)	100		

P-value > 0.05 not significant

**Figure 1: Occurrence of aminoglycoside residues in milk in both south and north Nyala**

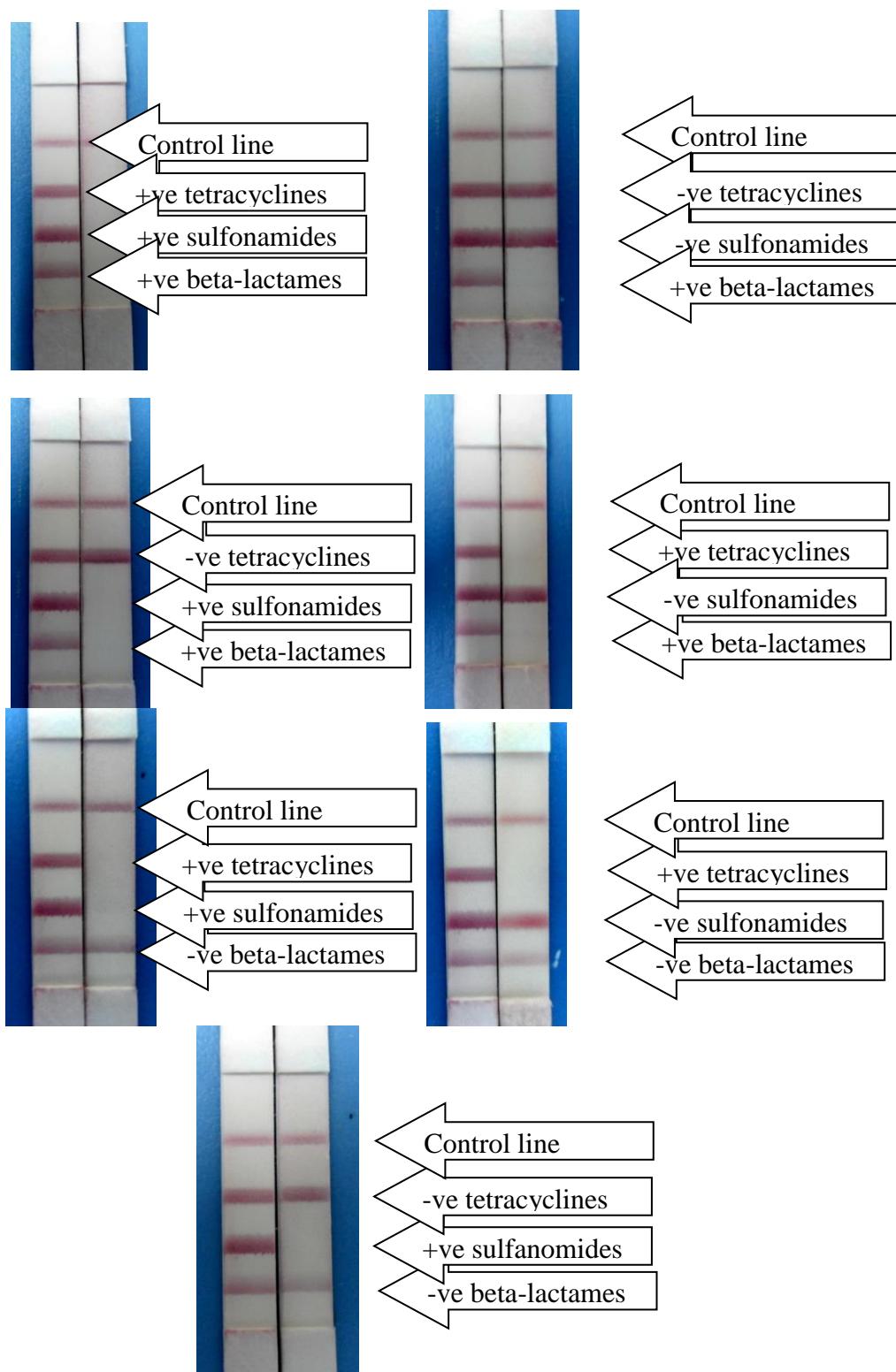


Figure 2: Comparison of the intensity of the line colour of the test line with the intensity of the line colour of the control line of the trisensor dipsticks

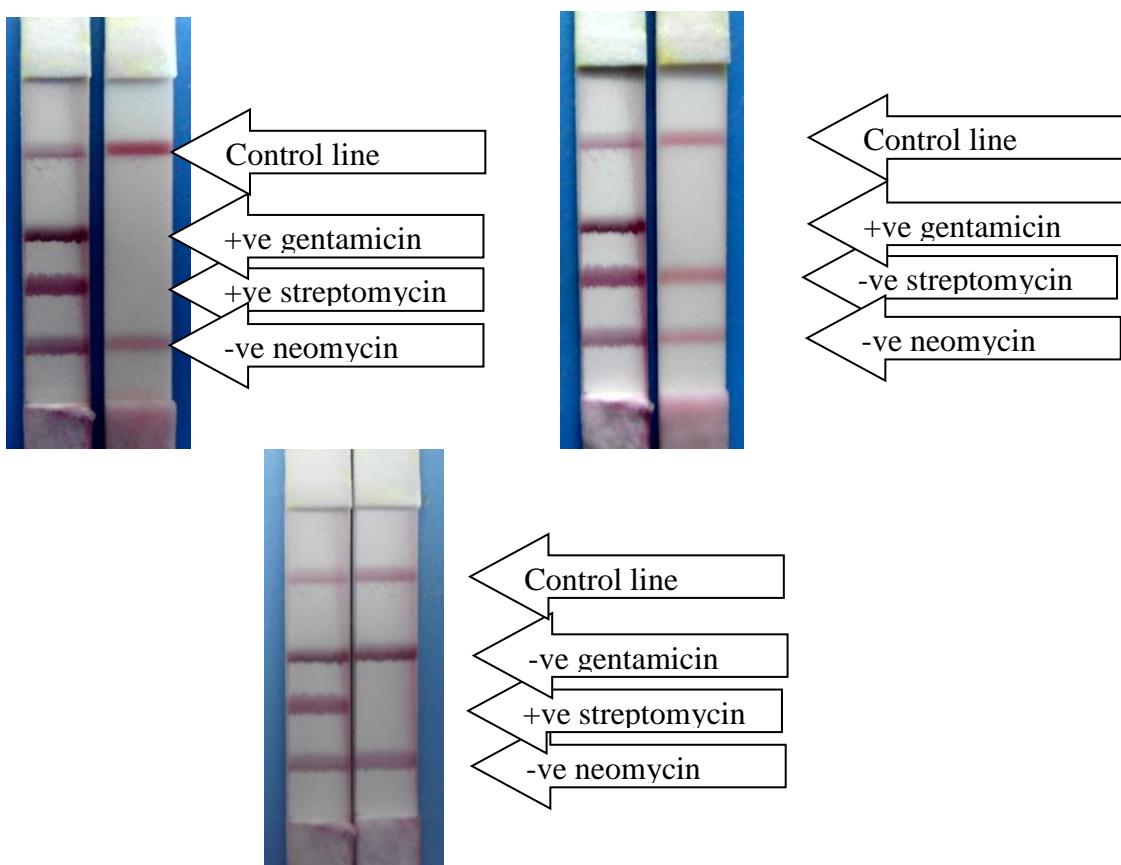


Figure 3: Comparison of the intensity of the line colour of the test line with the intensity of the line colour of the control line of triaminosensor dipsticks

References

Abbasi, M.M.; Babaei, H.; Ansarin, M.; Nourdadgar, A.O.S. and Nemati, M. (2011). Simultaneous determination of tetracyclines residues in bovine milk samples by solid phase extraction and HPLC-FL method. *Advanced Pharmaceutical Bulletin*, 1(1): 34-39.

Adrian, J.; Pasche, S.; Diserens, J.; Snchez-Baeza, F.; Gaod, H.; Marco, M.P. and Voirin, G. (2009). Waveguide interrogated optical *immunosensor* (WIOS) for detection of sulfonamide antibiotics in milk. *Biosensors and Bioelectronics*, 24: 3340-3346.

Al Zuheir, I.M. (2012). Detection of beta-lactams and tetracyclines antimicrobial residues in raw dairy milk for human consumption in Palestine. *Walailak J. Sci. and Tech.*, 9 (3): 277-279.

Alanis, A.J. (2005). Resistance to antibiotics: Are we in the post antibiotic era. *Archives of the Medical Research*, 36: 697-705.

Aning, K.G.; Donkor, E.S.; Omore, A.; Nurah, G.K.; Osafo, E.L.K. and Staal, S. (2007). Risk of exposure to marketed milk with antimicrobial drug residues in Ghana. *The Open Food Science Journal*, 1: 1-5.

Aryal, S. (2001). Antibiotic resistance: A concern to veterinary and human medicine. *Nepal Agric. Res. J.*, 5: 66-69.

Barrow, G.I. and Feltham, R.K (1993), *Cowan and Steel Manual for the Identification for Medical Bacteria*, 3rd ed., Cambridge.

Chenggang, J.; Adrian, E.; Lijie, F. and Xiaoyun, L. (2011). Framing a global health risk from the bottom-up: User perceptions and practices around antibiotics in four villages in China. *Health Risk and Society*, 13 (5): 433-449.

Darwish, W.S.; Eldaly, E.A.; El-Abbasy, M.T.; Ikenaka, Y.; Nakayama, S. and Ishizuka, M. (2013). Antibiotic residues in food: The African scenario. *Japanese Journal of Veterinary Research*, 61: 13-22.

Edima, H.; Tofaing, J.T.; Ngoune, L.T. and Ndjouenkeu, R. (2012). Assessment of antibiotic residues in cow milk produced in Ngaoundere. *International Journal of Biology, Pharmacy and Applied Sciences*, 1 (11): 1530-1538.

El Zubeir, I.E.M. and El Owni, O.A.O. (2009). Antimicrobial resistance of bacteria associated with raw milk contaminated by chemical preservatives. *World J. Dairy and Food Sci.*, 4 (1): 65-69.

El Zubeir, I.E.M.; Kiessling, S.; Kutzer, P.; Wieler, L.H. and El Owni, O.A.O. (2012). Characterization of tetracycline resistant *Staphylococcus aureus* isolated from bovine

mastitis in Khartoum State, Sudan. University of Khart. *J. Vet. Med. and Anim. Prod.*, 3 (2): 53-64.

El Zubeir, I.E.M. and Mahala, A.G. (2011). An overview of the management practices and constraints at the dairy camps in Khartoum State, Sudan. *Research Opinions in Animal and Veterinary Sciences*, 1 (7): 425-428.

Kaneene, J. and Ahl, A. (1987). Drug residues in dairy cattle industry: epidemiological evaluation of factors influencing their occurrence. *J. Dairy Sci.*, 70: 2176- 2180.

Katakweba, A.A.S.; Mtambo, M.M.A.; Olsen, J.E. and Muhairwa, A.P. (2012). Awareness of human health risks associated with the use of antibiotics among livestock keepers and factors that contribute to selection of antibiotic resistance bacteria within livestock in Tanzania. *Livestock Research for Rural Development*, 24 (10) <http://www.lrrd.org/lrrd/kata24170.htm>

Kirbis, A. (2006). Microbiological 5-plate screening method for detection of tetracyclines, aminoglycosides, cephalosporins and macrolides in milk. *Slov. Vet. Res.*, 43 (4): 161-168.

Komolafe, O.O. (2003). Antibiotic resistance in bacteria- an emerging public health problem. *Malawi Medical Journal*, 15 (2): 63- 67.

Kukanich, B.; Gehring, R.; Webb, A.I.; Craigmill, A.L. and Riviere, J.E. (2005). Effect of formulation and route of administration on tissue residues and withdrawal times. *J. Am. Vet. Med. Assoc.*, 227: 1574-1577.

Movassagh, M.H. (2011). Study of antibiotics residues in cow raw milk by copan milk test in Parsabad region, Ardabil Province, Iran. *Annals of Biological Research*, 2 (4): 355-359.

Okeke, I.N. and Ojo, K.K. (2010). *Antimicrobial Resistance and Use in Africa*. In: Sosa, A.de J.; Byarugaba, D.K.; Ama' bile-Cuevas, C.F.; Hsueh, P.R.; Kariuki, S. and Okeke, I.N. (Editors). *Antimicrobial Resistance in Developing Countries*, US Springer Science and Business Media, pp 301-314.

Reybroeck, W. and Ooghe, S. (2012). Validation of post-screening tests applied in the procedure of the interprofessional organizations for the screening of antimicrobials in milk. In: Validation of post-screening tests applied in the procedure of the interprofessional organizations for the screening of antimicrobials in milk. Report p. 1-27.

Ryoba, R.; Kweka, L.; Kazwala, R. and Kurwijila, L.R. (2007). Assessment of antibiotic residues in milk collected at Tanga dairy cooperative union centre in Tanga municipality, Tanzania. *Tanzania Veterinary Journal*, 24 (1): 38-51.

Said Ahmad, A.M.M.; El Zubeir, I.E.M.; El Owni, O.A.O. and Ahmed, M.K.A. (2008). Assessment of microbial loads and antibiotic residues in milk supply in Khartoum State, Sudan. *Research Journal of Dairy Sciences*, 2: 57-62.

Salman, A.M.; El Nasri, H.A. and Osman, I.A.M. (2012). Detection of antibiotic residues in milk using Delvotest kit and the disc assay methods in Khartoum state, Sudan. *University of Khartoum J. Vet. Med. and Anim. Prod.*, 3(2): 3-15.

Sawant, A.A.; Sordillo, L. M. and Jayarao, B.M. (2005). A survey on antibiotic usage in dairy herds in Pennsylvania. *J. Dairy Sci.*, 88: 2991-2999.

Shitandi, A.A. and Sternesjö, A. (2004). Factors contributing to the occurrence of antimicrobial drug residues in Kenyan milk. *J. Food Prot.*, 67 (2): 399-402.

Stolker, A.A.M. and Brinkman, U.A.T.H. (2005). Analytical strategies for residue analysis of veterinary drugs and growth promoting agents in food producing animals- a review. *J. Chromatogr. A*, 1067: 15-53.

Stolker, A.A.M.; Zuidema, T. and Nielen, M.W.F. (2007). Residue analysis of veterinary drugs and growth-promoting agents. *Trends in Analytical Chemistry*, 26: 967-979

Thrusfield, M. (2007). *Veterinary Epidemiology*. 3th edition p; 189 and p: 230.