DETECTION OF ANTIBIOTIC RESIDUES IN MILK USING DELVOTEST KIT AND THE DISC ASSAY METHODS IN KHARTOUM STATE, SUDAN

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.   47   734

   %90

.(%27.7)   (%61.7)   .

                                                                       .

      %72.3   %.85

                                                  .

         %33.1   Delvotest Kit

%23.2   %42.4 .

   disc assay   Delvotest Kit   .

      %75.1   %12.8

                                             .

                                 .%78.3   0.47

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Abstract

Seven hundred thirty four stratified random raw milk samples were collected from forty seven dairyfarms and sale points, to detect antibiotic residues and to compare between the Delvotest kit and Disc assay methods.

A direct interview (questionnaire) was used to collect data regarding the hygienic practices, management and antibiotic brands from dairy farms in Khartoum State.

Mastitis and respiratory diseases were found in about 90% of the farms. The most frequent antibiotic used was Penicillin in 61.7% of the farms, while tetracycline was used in 27.7% of the farms. It was also found that antibiotic treated cows were milked together with the healthy ones. Eighty five percentof the farmers lacked the necessary information about antibiotic residues and appearance in food material, and the absence of veterinary supervision was observed in 72.3% of visited farms.

From the total number of samples tested using Delvotest Kit, 33.1% of samples were positive, out of which 42.4% were from the farms milk while 23.2% were from sale points.
Milk samples were also tested using the two tests, 12.8% of the samples were positive in both tests , while 75.1% were negative in both tests. When using the Kappa statistic the agreement between the two tests was 0.47 (moderate agreement) and the percent aggrement was 78.3% .
In conclusion,the study showedthat most farms are in moderate hygienic conditions with the frequent use of antibiotics and farmers lack the knowledge about the withdrawal period leading to the presence of the antibiotics in milk.

4

Introduction

Milk can be defined as the product of milking of healthy cows under good conditions of hygiene. It must satisfy the standard quality criteria (microbiological and nutritional) so as consumers be confident that their milk supply is free from contaminates or residues of pesticides, drugs, or antibiotics (Peter *et al*, 2003).

Raw milk can be contaminated with residue of anti-microbial agents which are used to treat a variety of diseases or added intentionally to increase the usable life of the product. It was reported that 80% of conventional dairy herds use antibiotics for treatment of mastitis which was the first diseaseand remains the most common reason for administration of antibiotics in cattle (Barros *et al* 2011, Zwald *et al*., 2004).

Milk supplies containing higher concentrations of penicillin are not acceptable and the presence of beta–lactam antibiotics in milk represents a potential hazard to consumers, because of their allergenic properties and inhibitory effect on culturing processes of fermented milk products. The residues maybe also responsible for the development of resistant strains of bacteria (Peter *et al* , 2004).

Detection of beta–lactam antibiotics is becoming an important priority in the dairy industry. An easy, reliable and accurate test is necessary. Several methods are in use including the disc assay technique, immunological (Charm II test), microbial inhibition (Delvotest SP) tests, chromatographic, immunochemical, receptor and enzyme-based tests have been developed for the detection of drug residues in milk.(Peter *et al* , 2004, Seyda and Ayhan,2010).

Microbiological disc assay utilizes the genus *Bacillus* because of its high sensitivity to the majority of antibiotics. Delvotest is a multiple microbial inhibitor test usable to detect antimicrobial agents such as beta-lactam and sulpha compounds (Peter *et al*, 2003) .It is an economic, easy-to-use screening test giving results within a relatively short period (2.30 to 3.00 h).

Handling of raw milk is a big problem in Sudan as dairy farms are located in different remote areas with minimum infra-structure. In addition to the prevailing hot climate of the country with lack of cooling facilities, and the absence of general hygiene and sanitation measures in the farms, appreciable amount of milk is spoiled while in transit. Therefore the use of antibiotics and other milk additives are being widely practiced in the country (Mohammed , 2011a, Salman and El Nasri, 2011).

Accordingly, the present study was conducted toevaluate the general hygienic status of the dairy farms in Khartoum State, to detect any contaminates or residues in milk with antibiotics using Delvotest kit and disc assay and to compare between these two methods.

Materials and Methods

Sample collection:
 A questionnaire was designed and distributed to gather information from different dairyfarms in Khartoum State (which include 3 municipalities: Khartoum , Khartoum North and Omdurman) during the period from August to December 2011. The general hygienic status of the farms, diseases present, the extent of antibiotics usage, and the awareness of the effects of antibiotic residues by the farmers, presence of veterinary supervision were investigated.
Seven hundred thirty four stratified random milk samples were collected in sterile tubes from farms and sale points.
Antibiotic detection: it was conducted using two methods:

i**) The disc assay method** which was described by APHA, (1985).

Sterilized agar was inoculated with 1 ml of broth culture of *Bacillusstearothermophilus*. Using a clean sterile forceps, a disc was removed from the vial and immersed into the milk sample until it was soaked and then the disc was removed and placed on the agar surface. The plates were then inverted and incubated at 37 – 38 °C for 24 hours, till growth became clear. The presence of antibiotic residues in the milk sample was indicated by the presence of inhibition zone of the growth of *Bacillus S* around the disc. Absence of antibiotic residues was indicated by the absence of inhibition zone around the growth.

ii) The Delvotest SP kit was used as described by the manufacturer (DSM, Netherlands). 100 µl of milk sample was transferred to the ampoule containing nutrient agar embedded with *Bacillus stearothermophilus* spores and Bromocresol purpleindicatorand incubated for 2-3 hours. A clear color change purple to yellow indicates that the antimicrobial compounds are below the detection limits. A purple color indicates the presence of antibiotics at or above the detection limits of the test.

iii) Statistical Analysis: SPSS statistical programme was used for the analysis of the

questionnaire.Kappa statistic was used to describe the association between the two methods (Gordis, 2004)

Results

A total of 734 milk samples were collected from the three municipalities of Khartoum State either from farms or sale points as shown in Table(1)

Area	Farms	Sale points	Total
Omdurman	98 44.5%	122 55.4%	220 30%
Khartoum north	227 60.7%	147 39.3%	374 51%
Khartoum	55 39.3%	85 60.7%	140 19.1%
Total	380 51.8%	354 48.2%	734

Table (1): Total number of samples collected.

Mastitis was found to be the most common disease in about 90% of the visited farms, while 68.8% of the farms showed the presence of mastitis During collection of milk samples, direct interview of the farmers was recorded in a questionnaire. The hygienic status of the farm was classified into3 criteria:

Good : if there is daily cleaning and removal of dung,general appearance of the farms (clean feeders, drinkers, milking utensils and an acceptable personnel hygiene of the workers, presence of sheds).Moderate: general appearance and the frequency of dung removal (twice a week).Bad: general appearance and irregular dung removal is practiced

Most of the farms were in a moderate condition (45%)(Fig.1).Omdurma area showed a high percentage of moderate to bad status of farms (76% compared to other areas .
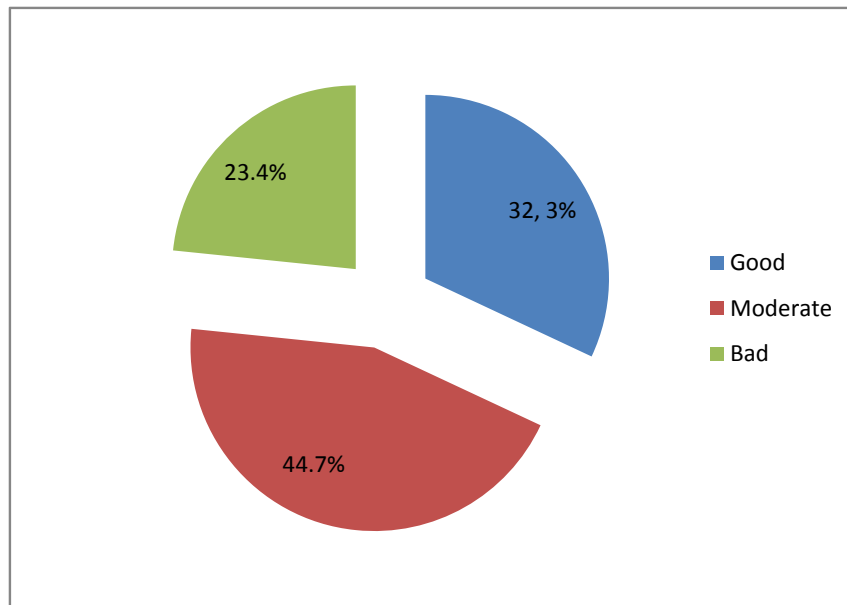
Fig.1: General Hygeinic Status of the farms

together with other infectious diseases (Fig.2). All visited farms were found to use one or more type of antibiotics (Table 2). Penicillin was the drug of choice in 57.4% of the farms.
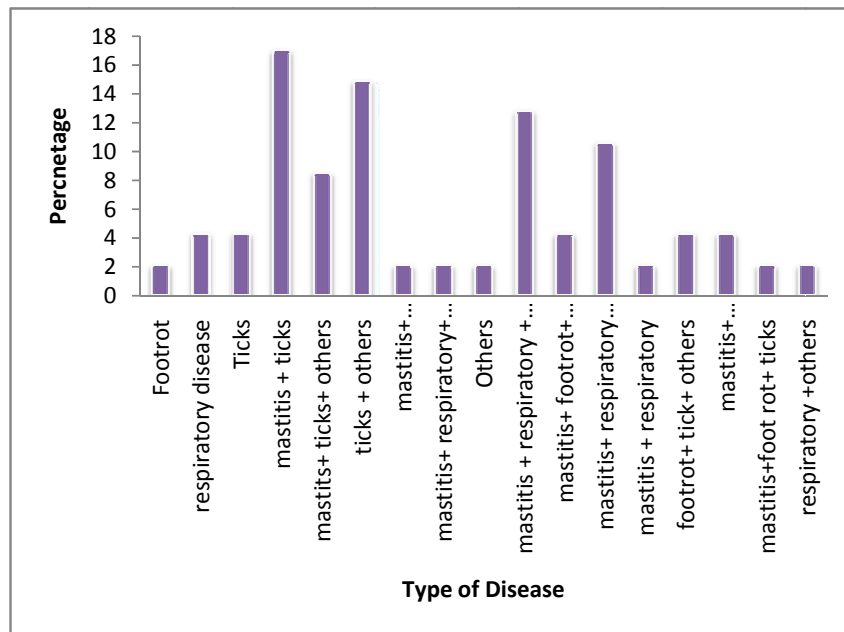
**Fig 2:** Different Type of diseases in Dairy farms

Table 2: Area * antibiotics cross tabulation (%)

Area	Antibiotics				
	Penicillin	Tetracycline	others	penicillin + tetracycline	tetracycline+ others
Omdurman	81	4.8	14.3	0	0
Khartoum North	12.5	68.8	0	12.5	6.3
Khartoum	80	10	0	0	10

This study showed that 85.1% of the farmers know nothing about the residues of antibiotic in milk, compared to 15%.who knew there was a relationship.

The trend of isolating animals that are under treatment was observed only in 30% of the farms. Lack of veterinary supervision was also observed and 81% of the farmers were responsible for the treatment of their animals alone.

When antibiotics residues were determined using the Delvotest Kit (Fig. 3), 31.8% ofthe total number of samples tested positive for presence of antibiotics. Regarding the samples obtained from the farms, 42.4% of the samples showed the presence of antibiotics
(Table 3a), compared to 23.2% from sale points (Table 3b).
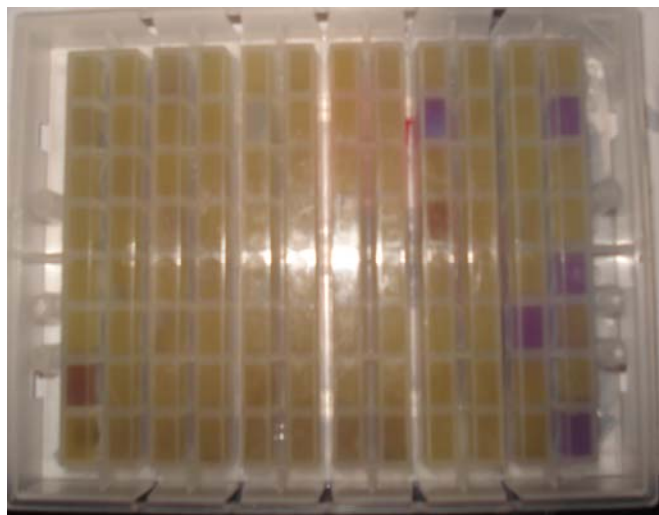
Fig.3: Delvotest result after 3 hrs. incubation period (yellow color indicates negative result and purple color indicates positive result)

Table 3a: Results of analysis offarm samplesusing both tests

Area	Total	Negative	Positive
Omdurman	98 (29.6%)	30 (30.8%)	68 (69.2%)
Khartoum North	227 (66.2%)	154 (67.8%)	73 (32.2%)
Khartoum	55 (4.2%)	35 (63.6)	20 (36.4%)
Total	380	219 (57.6%)	161 (42.4%)

Table 3b: Results of analysis of sale points samples using both tests

Area	Total	Negative	Positive
Omdurman	122 (34.5%)	64 (52.4%)	58 (47.5%)
KhartoumNorth	147 (41.5%)	140 (95%)	7 (4.76%)
Khartoum	85 (24%)	68 (80%)	17 (20%)
Total	354	272 (76.8%)	82 (23.2%)

Kappa test was carried out to find the association between the two methods and the obtained result was 70% suggesting a moderate agreement between the two tests (Table 5)

Table 5: Comparative results: Delvotest * disc

		Disc assay		
		Positive	**Negative**	Total
Delvotest	**Positive**	94	101	195
	Negative	58	481	539
	Total	152	582	734

% Agreement = 78.3%

 Kappa statistic (.95) = 47%

Discussion

Milk is considered an important dietary material. It must be offered to consumers free from chemical additatives and antibiotics in ordfr to avoid hazardous effects on health. This has to be accompanied by practising good hygeinic measures at all levels of milk chain.

In this study 33.8% of the samples tested were positive for antibiotic residues compared to 38.9 % reported by Said Ahmed*et al* (2008) in Khartoum State. This result is higher than what is reported in Kenya(Shitandi and Sternesjö , 2004) , Iran (Mohammed, 2011b) and

Trinidad (Adesiyun *et al,*1997), Ghana (Addo *et al*, 2011) which were 21%, 14% and 10.8% and 3.1% respectively. Which may be due to the increase level of awareness or improvement of hygienic status   of the farms.

 The hygienic status of the farms usually correlates with antibiotic usage. In this study 68.1% of visited farms were either in moderate or bad hygienic status, which may lead to excessive use of antibiotic that was observed in all these farms.

Mastitis was observed in 90% of the farms which is in agreement with (Elzubeir&Mahla, 2011) and (Mohammed, 2011a) who found that mastitis is the most prevalent disease in cattle. This was clearly observed in Omdurman area were 76% of the farms were below the acceptable level of hygiene, which lead to higher prevalence of mastitis alone or in combination with other disease (40 %) , higher use of penicillin (81%) and consequently the highest residues of antibiotic in milk (69%).

The use of penicillin and other intra mammary antibiotics, lack of information about withdrawal periods of drugs, handling of animals under treatment, workers insufficient experience, absence of veterinary supervision are among the major predisposing factors for the presence of antibiotic residues in milk. In this study, 85% of farmers lacked the necessary information about the withdrawal period and 70% of the farmers do not isolate the animals under treatment. These factors were in line with what was reported by (Shitandi and Sternesjö , 2004) and (Elzubeir and Mahla, 2011).

According to World Health Organization and Joint Expert Committee on Food Additives regarding milk hygiene, the rate of contamination of milk and dairy products with antibacterial additives in developed countries is lower than underdeveloped and developing countries, which fall behind in terms of increasing the level of awareness of stock breeders, improvement of hygienic conditions and in terms of inspection effectiveness (Seydaand Ayhan, 2010).

In this study, a higher percentage of positive samples was detected in farms (42.4%) than the salepoints (23.2%). This is contra to what is reported by Said ahmed*et al* (2008) who detected a lower rate (22.2%) of antibiotic positive raw milk samples from farms,compared to (55.6%) positive results at sale points . The higher percentage of positive samples in dairyfarms may be related to treatment of animals (70% of the farmers do not isolate the animals under treatment) and not considering the

withdrawal period rather than addition of antibiotics as a preservative by milk distributors.

The comparison of the two tests showed that 12.8% of the samples detected positive in both while 65.5% were negative in both tests, and the agreement between the two test was found to be 78.8% and a kappa of 0.47 represents an intermediate agreement (Gordis, 2004). These results are different from those obtained by Said ahmed*et al* (2008) who showed 100% agreement between the two test.

Conclusion:

The data obtained showed that the badhygienic status of dairy farms, the absence of direct veterinary supervision, the misuse of antibiotics and lack of knowledge of antibiotic residues in animal products were the factors leading to the detection of the high percentage of positive samples in farm milk.

The results of the data analysis indicate that the two antibiotic residue screening methods are not sensitive enough for the examination of individual milk samples.

Due to the excessive use of antibiotics in the country, a higher percentage of positive reactors are expected. The Delvotest showed more positive reactors than the disc assay and can thus be considered to be more sensitive than the disc assay.

Acknowledgment:

References

Addo, K.K., Mensah ,G.I., Aning, K.G., Nartey, N.,Nipah, G.K., Bonsu, C., Akyeh, M.L., and Smits, H.L. **(2011)**Microbiological quality and antibiotic residues in informally marketed raw cow milk within the coastal savannah zone of Ghana. TropMedInt Health.16:227.

Adesiyun, A., Webb,L.; Balbirsingh, V**.(1997).**Prevalence of antimicrobial residues in processed cow's milk in Trindad. Journal of Food Safety.16:301.

APHA **(1985).**Standard method for the examination of dairy products. 15th ed. American public association:Washington DC. USA

Barros, L.S., Sóglia, S.L., Ferreira, M.J., Rodrigues, M.J.,and Branco, M.P. (2011). Aerobic and anaerobic bacteria and Candida species in crude milk .J Microbiology and Antimicrobials 3: 206.

El Zubeir, Ibtisam , E.M. and Ahmed, Mahala, G. **(2011)** An overview of the management practices and constrains at the dairy camps in Khartoum State, Sudan . Roavs, 1: 425.

Gordis,L .**(2004).**Epidemiology.3rd edition. Elseiver Inc. USA.

Mohammed, H.A**.(2011a).** Effect of husbandry practices on milk production in dairy farms in ShargElneel Locality.Khartoum State, M.Sc. Thesis, University of Khartoum, Sudan.

Mohammad , H.M. **(2011b).**Study of Antibiotics Residues in Cow Raw Milk by Copan Milk Test in Parsabad Region, Ardabil province, Iran . Annals of Biological Research.2:355.

Peter, P., Jozef, N., Pavel, P., Slavomir, M., Hanna, R., and Jozef, S.**(2004).**Comparison of sensitivity of various screening assays and liquid chromatography Technique for pencillin residue detection in milk.Bull Vet Inst Pulawy.48: 273.

Peter, P.,Jozef, N., Peter, P.,Jozefnagy, J.,Popelka, J., Jozef, S., Jaroslavah, C., Marcincak,S.,   and Bugarsky, A. **(2003)** Comparison of various methods for penicillin residue detection in cow milk after intrammary and parenteral treatment. Bull. Vet. Inst. Pulawy.47:203.

Said Ahmed, A.M.,Ibtisam, E.M. El Zubeir, O.A.O. El Owni   and M.K.A. Ahmed, **(2008).** Assessment of Microbial Loads and Antibiotic Residues in Milk Supply in Khartoum State, Sudan. Res. J. Dairy Sci. 2: 36.

Salman, A.and ElNasri , H. **(2011)** . Somatic cell count, total bacterial count and acidity properties of milk in Khartoum State, Sudan .Journal of Cell and Animal Biology.5: 223.

Seyda, E.And Ayhan, F **.(2010)** Determination of Antibiotic Residues in Milk Samples KafkasUniv Vet FakDerg. 16:31.

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

Zwald, A. P.,Ruegg, J. B., Kaneene, L. D., Warnick, S. J., Wells, C. F., and Halbert, L.**(2004).** Management Practices and reported antimicrobial usage on conventional and organic dairy herds. J Dairy Sci. 87:191.