

Full Length Research Paper

Bacteriological evaluation of the drinking water quality in dairy farms in Khartoum state, Sudan

Amna Mustafa Musa¹ and Atif Elamin Abdelgadir^{2*}¹Ministry of Animal Resources and Fisheries, Khartoum, Sudan.²Department of Preventive Medicine and Public Health, Faculty of Veterinary Medicine, University of Khartoum, Sudan.

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This study was conducted in Khartoum State in order to evaluate the quality of the drinking water in dairy farms based on bacteriological examinations and viable counts. A total of 39 water samples were obtained from dairy farms (13 in Khartoum, 13 in Omdurman and 13 in Khartoum North). All samples were cultured on Blood Agar and MacConkey for bacterial isolation and on nutrient agar for viable counts. The main result revealed that 39 bacterial isolates were detected in drinking water of the dairy farms in Khartoum state. *Micrococcus* spp., *Pseudomonas* spp., and *Bacillus* spp. were dominant in Khartoum, giving a percentage of 7.69 (n=3) for each. *Staphylococcus* spp. and *Corynebacterium* spp. were also observed in the samples of dairy farms in Khartoum (5.12%) (n=2) for each. The bacteria isolated from dairy farms in Omdurman were *Micrococcus* spp. and *Aeromonas* spp. (5.12%) (n=2) for each. In the same site, *Staphylococcus* spp., *Actenobacillus* spp., *Moraxella* spp. and *Flavibacterium* spp. were also detected with percentage of 2.56% and frequency of one for each. The most frequent isolate in Khartoum North was *Micrococcus* spp. (17.95%) (n=7) followed by *Aeromonas* spp. (10.26%) (n=4) and *Staphylococcs* spp. (5.12%) (n=2). Regarding bacterial counts, the results have shown high level of contamination of drinking water for all dairy farms in Khartoum State. The results were inter-operated, depending on international critical level (cut-off point) (100 CFU ml⁻¹). For instance, high mean of bacterial counts 6.44×10^8 was observed in dairy farm in Omdurman, followed by Khartoum and Khartoum North, with mean bacterial counts of 4.93×10^8 and 3.81×10^8 , respectively. Application of analytical statistic using one way analysis of variance (ANOVA) revealed that there was no statistical significance (F-value = 0.198, p-value > 0.05) for bacterial counts of the drinking water of dairy farms in different sites of Khartoum state.

Key words: Bacterial counts, bacterial isolation, drinking water, Khartoum State, Sudan.

INTRODUCTION

Water is the most important nutrient for dairy cattle but its importance has been commonly forgotten in dairy systems. It is required for all of life's processes; transport of nutrients and other compounds to and from cells, digestion and metabolism of nutrients, elimination of waste materials (urine, faeces, respiration, and excesses heat from the body), maintenance of a proper fluid and

ion balance in the body and provision of fluid environment for the developing fetus.

An adequate supply of clean and fresh drinking water is widely considered essential for optimal cow health and maximum milk production. Microorganisms enter into drinking water via humans and animals' intestinal secretions in areas where sanitation conditions are poor or absent.

*Corresponding author. E-mail: atifvet@yahoo.com.

When found in drinking water, microorganisms constitute a real indication that it should not be used for human consumption if these contaminants are found in excess of the maximum permissible level (1×10^2 CFU ml⁻¹) (World Health Organization (WHO), 2008). Bacterial contamination can get into groundwater by many ways; wild and domestic animals, birds and dairy farms wastes situated in a watershed area or within the hydrological catchments of groundwater. However, these have been found to be a pathogenic contamination source of drinking water (Gleeson and Gray, 1997; Obiri and Jones, 2001). Also, the presence of campylobacter in waters within agricultural areas is a real evidence of environmental contamination by sewage effluent coming from agricultural areas (Obiri and Jones, 2001). Biomass that resulted from degradable materials are deposited into drinking water distribution pipes and accumulates biofilms which accelerate the growth of microorganisms and protect them against disinfection agents (Lewis, 2001).

Long storage of good-quality drinking water is a main factor of faecal coliforms contamination through faecal contaminated hands or utensils. In addition, coverless public reservoir contributes to pathogenic accession especially from birds feces. Contamination by microorganisms can occur through improperly installed or/and through undetected leaks in the water pipe system (Gleeson and Gray, 1997).

It has been reported that contamination with faecal coliforms may be caused by infiltration of pollutants in the recharge area of the springs (Daghray, 2009). The presence of microorganisms does not necessarily indicate that drinking water poses a health risk. The important consideration is the kind of microorganisms that are present.

Objectives

1. To evaluate the bacteriological quality of the drinking water in dairy farms in Khartoum state using colony count.
2. To isolate the most common bacteria in the drinking water in dairy farms in Khartoum state.

MATERIALS AND METHODS

The study area

The study was conducted in Khartoum State which is situated in Northern Sudan between latitude 15° 38' N and longitude 32° 26' E. The total area extends over approximately 21,000 square kilometer. The climate of Khartoum is an arid type which is characterized by a wide range in daily and seasonal temperatures. During cool season, between December and February, the weather is cool and dry, with minimum daily temperature of 24°C. The season is characterized by low humidity. A hot dry weather prevails between

March and October, where a temperature of 45°C may occur during the day. The maximum rainfall is during the period from mid July to September; in this season there is an increase in relative humidity, with a maximum of 68% in August. It is more convenient to divide the year into a cool dry season, hot dry season and hot wet season.

Sampling methods

Non-probability sampling method was employed as described by (Thrusfield, 2007). This means not all farms in Khartoum state had the same chance to be selected but only 39 farms were selected based on willingness and support of the owners.

Samples collection

A total of 39 water samples (13 from Khartoum, 13 from Omdurman, 13 from Khartoum North) were collected and all samples taken from troughs. All samples were taken by sterile 10 ml syringe and all precautions were taken in order to prevent accidental contamination of the water during its transportation in laboratory and put in sterile closed glass bottles (previously sterilized in autoclave at 120°C under 15 lb atmospheric pressure for 15 min).

Solid culture media

Nutrient agar

This media contained peptone (5 g) lab-lemco powder (1 g), yeast extract (2 g), sodium chloride (5 g) and agar No. 3 (15 g). The medium was prepared by dissolving 28 g of the dehydrated medium in one liter distilled water, and the pH adjusted to 7.4 then sterilized by autoclaving for 15 min at 121°C. The medium was allowed to cool to 55°C and poured aseptically in 15 to 20 ml amounts into sterile petri dishes (Quinn et al., 2000).

Blood agar

This is one of the enriched media that was composed of blood agar base and defibrinated sheep blood. The blood agar base contained protease peptone (15 g), liver digest (2.5 g), yeast extract (5 g), sodium chloride (5 g) and agar (12 g). It was prepared by dissolving 40 g of the basal medium in one liter of distilled water. The mixture was then boiled until the powder dissolved completely. The solution was autoclaved at 121°C for 15 min, and then cooled to 45 to 50°C. 7% of sterile blood was added with gentle rotation and then the medium was poured into petri dishes (15 to 20 ml) and left to solidify (Quinn et al., 2000).

MacConkey agar

This media contained peptone (20 g), lactose (10 g), bile Salts (1.5 g), sodium chloride (5 g), neutral red (0.03 g), crystal violet (0.001 g) and agar No. 3 (15 g). The medium was prepared by dissolving 52 g in one liter of distilled water by heating. The pH was adjusted to 7.4 and then autoclaved at 121°C for 15 min. Then it was allowed to cool to 55°C and poured gently at 15 ml amount into sterile Petri dishes (Quinn et al., 2000).

Semi- solid media

Hugh and liefsons (O/F) medium

This medium was used to test the ability of the organism to attack dextrose under aerobic and anaerobic conditions. This medium was prepared by dissolving all ingredients in one liter of distilled water by heating in water bath set at 55°C, except bromothyl blue solution which was added after adjustment of the pH to 7.1. Then sterile solution of the appropriate carbon hydrate was added aseptically to give a final concentration of 1% and the medium was sterilized at 115°C for 20 min. A volume of 10 ml of sterile glucose solution was aseptically added to 90 ml of medium, then the medium was mixed and distributed aseptically in 10 ml amounts in sterile test tube. The prepared medium was kept at 4°C until use (Quinn et al., 2000).

Peptone water sugars

The method of preparation depends on the indicator. A total of 900 ml peptone water was added to 10 ml indicator solution (bromocresol purple) and sterilized at 115°C for 20 min. A total of 5 to 10 g of the appropriate sugar was dissolved in 90 ml water and sterilized by filtration, then added to sterile peptone water indicator and distributed into sterile tubes with inverted inner (Durham) tubes and finally steamed for 30 min (Quinn et al., 2000).

Primary culture

Primary culture for all water samples was done onto blood agar and MacConky agar media. Each water sample was centrifuged at 8000 rpm for 5 min and the sediment was cultured, then all Petri dishes were incubated at 37°C for 24 h.

Staining

Smears were prepared by emulsifying part of typical and well isolated colony in a drop of sterile normal saline and spread in a clean slide. The smears were then allowed to dry by air then fixed by gentle flaming. All smears were examined by gram stain.

Microscopic examination

A smear was made from culture and purified colonies, fixed by heating and stained by Gram's method. Then the stained smears were examined microscopically under oil immersion lens. The smears were examined for cell morphology and staining reaction.

Biochemical tests

Oxidase test

The oxidase test was performed by removing a portion of freshly grown colonies with a sterile glass rod and rubbing it on a strip of filter paper which have been impregnated with 1% solution of oxidase reagent . The immediate development of a dark purple colour with 10 s indicated appositve reaction (Quinn et al., 2000).

Catalase test

This test detects the enzyme catalase that converts hydrogen peroxide to water and gaseous oxygen. A loopful of grown bacteria was taken from the top of colonies to avoid the nutrient agar medium, and were put in a clean slide and dropped with 3% hydrogen peroxide. Presence of oxygen gas within a few seconds indicated appositve reaction (Quinn et al., 2000).

O/F test

Duplicate tubes were cultured by stabbing with straight wire to one of the tubes. A layer of melted soft paraffin (petrolatum) was added in a depth of about 1 cm, then incubated at 37°C for 24 h and examined (Quinn et al., 2000).

Identification of isolates

Purified isolates from the primary or from sub cultured plates were identified to the genus level according to Barrow and feltham (1993). The identification was based mainly on colony characteristics, staining, motility and biochemical reactions

Bacterial viable count

The bacterial count was done according to Milles and Misra (1938).

Preparation of the dilution

The serial dilution was prepared according to Harrigan and Maccance (1976). A micropipette with sterile tip was held vertically and introduced not more than 3 cm below the surface of the water sample and then 1 ml was taken to the first tube of the dilution (which contain 9 ml sterile normal saline) series without touching the diluting fluid, the tip was discarded and the tube was labeled as the first dilution tube 1/10. A fresh sterile tip was used to mix the content of the first dilution and 1 ml of the first tube was transferred to the second tube of dilution series (which contain 9 ml normal saline) also without touching the diluting fluid. Then the tip was discarded and the tube was labeled as the second dilution tube 1/100. Further dilutions of 1/1000, 1/10000 or 1/100000 were prepared similarly.

Plate count agar

Method of preparation

Twenty three gram medium of plate count agar were dissolved in 1000 ml cold d/w and heated to boiling to dissolve the medium completely. The pH was adjusted to 7.0 ± 0.2 and sterilized in an autoclave at 15 lbs (121 c) pressure for 15 min, which was very hygroscopic. The medium was then stored in a refrigerator.

Colony count

Colonies were counted according to surface colony count method (Milles and Misra, 1938). An average colony count from at least 5 drops of each dilution was obtained, the conversion factor was 50

Table 1. Bacteria isolated from drinking water of dairy farms in Khartoum state.

Isolate	Khartoum Omdurman Khartoum North			Total frequency (%)
	Frequency (%)			
<i>Micrococcus spp.</i>	3 (7.69)	2 (5.12)	7 (17.95)	12 (30.76)
<i>Aeromonas spp.</i>	0	2 (5.12)	4 (10.56)	6 (15.38)
<i>Staphylococcus spp.</i>	2 (5.12)	1 (2.56)	2 (5.12)	5 (12.82)
<i>Pseudomonas spp.</i>	3 (7.69)	0	1 (2.56)	4 (10.26)
<i>Bacillus spp.</i>	3 (7.69)	0	0	3 (7.69)
<i>Corynebacterium spp.</i>	2 (5.12)	0	0	2 (5.12)
<i>Cardiobacterium spp.</i>	0	0	3 (7.69)	3 (7.69)
<i>Actenobacillus spp.</i>	0	1 (2.56)	0	1 (2.56)
<i>Moraxella spp.</i>	0	1 (2.56)	0	1 (2.56)
<i>Flavibacterium spp.</i>	0	1 (2.56)	0	1 (2.56)
<i>Enterobacterium spp.</i>	0	0	1 (2.56)	1 (2.56)
Total	13 (33.33)	8 (20.51)	18 (46.15)	39 (100)

Table 2. Gram +ve and Gram -ve isolated from drinking water of the dairy farms in Khartoum State.

Site	No. of samples examined	Gram +ve Gram -ve	
		Frequency (%)	
Khartoum	13	10 (76.92)	3 (23.07)
Omdurman	13	8 (61.53)	5 (38.46)
Khartoum North	13	3 (23.07)	10 (76.92)
Total	39	21 (53.48)	18 (46.15)

to obtain figure for the bacteria /ml in the original sample. The formula used for counting was:

The total number of bacteria = The average of colonies count × dilution factor × 50

Statistical analysis

Statistical package for social sciences (SPSS) version 17.5 was used for data analysis. Descriptive statistic such as frequency and percentage was used for bacterial isolates. While mean, standard error and 95% confidence interval were recorded for bacterial count of drinking water of dairy farms in Khartoum state. Analytical statistic using one way ANOVA was used in order to know the significant difference regarding bacterial count of drinking water of the dairy farms in different sites of Khartoum state. Determination of contamination of the drinking water was done according to the critical level (100 CFU/ml) (Enviro labs Ltd, 2011).

RESULTS

This study was conducted in Khartoum State in order to evaluate the quality of the drinking water in dairy farms based on bacteriological examinations and viable counts.

A total of 39 water samples were obtained from dairy farms (13 in Khartoum, 13 in Omdurman and 13 in Khartoum North). The main result revealed that 39 bacterial isolates were detected in drinking water of the dairy farms in Khartoum state. *Micrococcus spp.*, *Pseudomonas spp.*, and *Bacillus spp.* were dominant in Khartoum, giving a percentage of 7.69% (n=3) for each. *Staphylococcus spp.* and *Corynebacterium spp.* were also observed in the samples of dairy farms in Khartoum (5.12%) (n=2) for each. The bacteria isolated from dairy farms in Omdurman were *Micrococcus spp.* and *Aeromonas spp.* (5.12%) (n=2) for each. In the same site, *Staphylococcus spp.*, *Actenobacillus spp.*, *Moraxella spp.* and *Flavibacterium spp.* were also detected with percentage of 2.56% and frequency of one for each. The most frequent isolate in Khartoum North was *Micrococcus spp.* (17.95%) (n=7) followed by *Aeromonas spp.* (10.26%) (n=4) and *Staphylococcus spp.* (5.12%) (n=2). The rest of the results are presented in Tables 1 and 2.

Regarding bacterial count, the results have shown high level of contamination of drinking water for all dairy farms in Khartoum State. The results were inter-operated based on international critical level (cut-off point) (100 CFU/ml).

Table 3. Descriptive statistic of bacterial count for drinking water of dairy farms in Khartoum state.

Site	No. of samples examined	Mean CFU/ml	Standard error	95% Confidence interval
Khartoum	13	4.93×10^8	1.67×10^8	1.28×10^8 - 8.58×10^8
Omdurman	13	6.44×10^8	4.74×10^8	-3.88×10^8 - 1.68×10^9
Khartoum North	13	3.81×10^8	1.03×10^8	1.56×10^8 - 6.06×10^8
Total	39	5.06×10^8	1.67×10^8	1.67×10^8 - 8.45×10^8

Critical level (cutoff point) = 100 CFU/ml.

Table 4. Analytical statistic of bacterial counts of drinking water of dairy farms in Khartoum State.

Unit	Sum of square	df	Mean Square	F-value	p-value
Between sites	4.51×10^{17}	2	2.26×10^{17}		
Within sites	4.10×10^{19}	36	1.40×10^{18}	0.198	0.821*
Total	4.14×10^{19}	38	-		

*Statistically not significant ($P > 0.05$).

For instance, high mean of bacterial counts (6.44×10^8) was observed in dairy farm in Omdurman followed by Khartoum and Khartoum North with mean bacterial counts of 4.93×10^8 and 3.81×10^8 , respectively (Table 3). Application of analytical statistic using one way ANOVA revealed that there was no statistical significant (F-value = 0.198, p-value > 0.05) for bacterial counts of the drinking water of dairy farms in different sites of Khartoum state. The results are show in Table 4.

DISCUSSION

An adequate supply of good quality water for dairy cattle is extremely important for optimal production. The presence of high viable bacteria in drinking troughs was an indication of the contamination at these sites; this agreed with Jeffrey et al. (2001) who reported that water offered to dairy cattle is often of poor microbiological quality. The extent of bacterial contamination observed in the drinking water troughs may demonstrate animal's daily exposure to bacterial infection from water source. Water sample from direct main source of water supply are completely free from coliform bacteria (El Tom, 1997). So water can be contaminated after being poured in troughs for the following reasons:

1. Bad hygiene measures in the farms.
2. Retention of water for long time in troughs.
3. Water troughs are not cleaned regularly.
4. Disinfectants are not used for washing troughs.

The viable count technique used in this study was Miles-

Misra (1938). This method has advantages of being economical and sensitive, also it requires less laboratory equipments and glass ware compared with other techniques (Quinn, 2000). The total viable count for bacteria showed that water samples were found most loaded; this may be logical because troughs are exposed to contamination from many sources like cattle while drinking, animal faeces, air, dust and feed stuffs, similarly from bacterial contamination and bad storage of water. In contrast, the main sources of water protected from direct contact, surface water usually treated with disinfectants and ground water is expected to contain minimum bacteria unless mixed with human sewage (Alcano, 1997).

As seen from the result, a high percentage of *Micrococcus spp.* and *Aeromonas spp.* was found in Khartoum North which is similar to Shirin (2010). The result could be explained by the fact that storage places in these farms were exposed to contaminated air and dust and may rarely be cleaned. The highest isolated bacteria in all water samples were *Micrococcus spp.* (30.76%), *Aeromonas spp.* (15.38%), *Staphylococcus spp.* (12.82%), *Pseudomonas spp.* (10.26%) and *Bacillus spp.* (7.69%). These genera are pathogenic and might be of importance due to their contribution to water borne infections.

Microbiological quality of drinking water in dairy farms is of paramount concern because of the possible acute risk to health caused by bacteria in drinking water. Therefore, regular monitoring and assessment of drinking water is primarily a health-based activity which helps to protect public health through ensuring provision of quality water. Bad habits, water mishandling and lack of basic

knowledge affects clearly the quality of water in dairy farms; thus physical appearance of water had been clearly affected, and this could strongly result in bad hygiene situation, causing a high level in the incidence of water-borne disease (Al Beeli, 2006).

Conclusion

Water is the most important essential nutrient supplied to dairy cattle, however, at times and in some dairy farms, quality and provision of water may not be optimal to maximize animal performance and health. Hence, water analysis programs are needed at all drinking water sites not only in Khartoum state but all over Sudan to assess the exact magnitude of ground water pollution with faecal matters. Also, the people and animal owners should be alerted of the potential health hazards.

RECOMMENDATIONS

1. Microbiological analysis of water for total bacteria and coliform is necessary to determine sanitary quality. The possible consequence is of such severity than its control which is always very important and should never be compromised.
2. Water analysis for the detection of faecal pollution should be prompted to determine the level of faecal pollution in ground water resources whenever water is intended for animal and human use.

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