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Microbiological Quality of Raw Milk Processed from Cows Raised under Extensive System in the Republic of Benin

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ABSTRACT

Raw milk is untreated food consumed in most African countries in the south of Sahara. In order to assess the microbiological risks associated with the consumption of this food, a study was conducted from January through March 2010, in two large ranching areas of the Republic of Benin. The sanitary quality of raw milk was assessed on 42 random samples from twelve farms and six markets in the municipality of Gogounou in the Borgou Province and in the municipality of Dassa-Zoume in the Collines Province. The samples analyzed were unsatisfactory in regard to the total mesophilic aerobic flora (1.1×10^8 CFU mL^{-1}), fecal Coliforms (9.2×10^2 CFU mL^{-1}) and *Escherichia coli* (0.4×10^1 CFU mL^{-1}). Total Coliforms (9.2×10^2 CFU mL^{-1}), *Staphylococcus aureus* (4.0×10^1 CFU mL^{-1}), lactic flora (2.1×10^6 CFU mL^{-1}) and sulphite-reducing anaerobic bacteria (0.4×10^1 CFU mL^{-1}) were conformed to the standards. Yeasts (2×10^3 CFU mL^{-1}), moulds (5.1×10^1 CFU mL^{-1}) and lactic flora were also counted. *Salmonella* sp. was absent in 25 mL of all raw milk samples analyzed. Though all milk samples were larger than the standard sizes in the two municipalities under study, those of Dassa-Zoume were the more contaminated. As clearly stated, the analysis of milk samples, water samples used for washing utensils of milking collected in each ranching prospected, revealed that samples of Dassa-Zoume were the more contaminated by fecal and anaerobic sulphite-reducing bacteria. These results indicate the non-compliance with rules of good hygiene at milking, storing, transporting and selling milk. The consumption of raw cow milk presents dire health risk to the population of the study areas.

Key words: Raw milk, hygiene, microbiological quality, risk, Republic of Benin

INTRODUCTION

The recovery strategy of Benin economy gives preference to the chain approach. That is why within the sectorial policy of Livestock Development, a renewed interest in the production of milk is increasingly noted in the Republic of Benin. The qualitative development of the dairy sub-sector requires serious consideration of the control of health risks on the one hand, to ensure consumer's health, quality and hygiene products and on the other hand, to conquer foreign markets. It is up to the manufacturer to ensure the safety and wholesomeness of food products (FAO, 2011). However, an ongoing effort or effective control on the part of other stakeholders, including dairy

producers, is necessary to ensure the safety and wholesomeness of dairy products. Given that many pathogens can be transmitted by raw milk, which is a vulnerable product (Siousarran, 2003), quality control must be established even at the level of traditional producers. In fact, raw milk can be contaminated with foodborne illness agents such as *Salmonella* (D'Aoust, 1991; Steele *et al*., 1997; Headrick *et al*., 1998; Farhan and Salik, 2007) and enteropathogenic *E. coli* such as verotoxin producers, which can cause life-threatening diarrhea (Lechevallier *et al*., 1996; Jacques *et al*., 1998; Stark, 2000; Mennane *et al*., 2007). Similarly, other agents responsible for severe intoxication such as Staphylococci's disease and mycotoxicosis (Adesiyun *et al*., 1995; Farhan and Salik, 2007) can be found in milk. Few studies have been done on the microbiological quality of milk in the Republic of Benin (Kora, 2005). The guide for good hygiene practices relating to the collection of milk production and Fulani cheese wagashi preservation (Dossou *et al*., 2006) and the statement on microbiological characteristics of milk from three different breeds are the only data currently available. Therefore, information on the microbiological quality of milk in the Republic of Benin is limited to scanty data on total aerobic bacteria, lactobacilli, Enterobacteriaceae, yeasts and moulds. This study purposed to highlight the microorganisms present in the raw milk and water used for cleaning and rinsing utensils harvesting and storing milk on the one hand and on the other, to compare microbiological analyses within the two municipalities surveyed.

MATERIALS AND METHODS

Study area: The study was carried out in two municipalities in the Republic of Benin, a West African country with 112,620 km^2 land area located in the intertropical zone between parallels 6°30' and 12°30' north latitude on the one hand and meridians 1°and 3°40' east longitude on the other hand (Adam and Boko, 1993). The first municipality, Gogounou (10°50'00,00"N, 2°50'00,00"E), is located in the northern Benin cotton agro-ecological zone with abundant pastures. It is characterized by a Sudanese climate with a uni-modal climate system (900 to 1100 mm of rain) with two seasons (dry and rainy ones). The second municipality, Dassa-Zoume (7°46'15,87"N, 2°11'48,90"E) is in the central Benin cotton agro-ecological zone with abundant fodder resources and has a Sudano-Guinean climate transition, straddling on uni-modal and bimodal systems (1000 to 1200 mm of rain) (UNDP, 2007).

Sampling collection: The samples were collected in Dassa-Zoume and Gogounou, two large scale dairy production municipalities in the Republic of Benin, both located in two different agro-ecological areas. Forty-two samples of 150 mL of raw milk in dark sterile bottles were collected aseptically in twelve cattle farms and six randomly selected markets in both municipalities in accordance with standard methods (ICMSF, 1986; Bell *et al*., 1997). In order to assess the quality of the water used in cleaning utensils for milking and storing raw milk, six water samples (1 sample per farm in dark sterile bottles of 250 mL) were collected in both municipalities. The sampling and analysis of hygienic conditions were implemented in accordance with the requirements of the ICMSF (1986) and Codex Alimentarius (FAO, 2011). Milk and water samples were transported in an icebox to the laboratory at a temperature ranging between 4 and 8°C, no later than a day after the sampling. The samples were analyzed within 24 h.

Table 1: Selective media used for isolation and enumeration of colonies

	Identification's Media		Incubation	
Germs	Medium	Reference	Temperature (°C)	Times (h)
Mesophilic aerobic flora	Plate count agar	PCA Oxoid CM0463	30	72
Total coliforms	Brilliant green bile broth	BLBVB Oxoid CM0031	30	48
Fecal coliforms	Brilliant green bile broth	BLBVB Oxoid CM003	44	48
	Peptone water without indole	PW Oxoid CM0009		44
Anaerobic sulfite-reducing bacteria	Trypticase sulfite neomycin	TSN Biokar BK001HA	37	48
S. aureus	Baird parker	BP Oxoid CM0275	37	48
Fecal streptococci	Azide dextrose broth	DAB Oxoid CM0868	30	48
	Ethyl violet azide broth	EVA Biokar BK061HA	37	24
Salmonella sp.*	Rappaport-Vassiliadis	RV Oxoid CM0669	41.5	24
	Hektoen	HEA Oxoid CM0419	37	24
	Salmonella-Shigella	S-S LAB LAB52	37	24
Yeasts and moulds	Sabouraud dextrose agar	SDA Oxoid CM0041	25	120
	Chloramphenicol			

*The confirmation of *Salmonella* sp. was done using API 20 E test Kit (Biomerieux, France)

Microbiological analysis: Dilutions of milk samples studied varied between 10^{-1} and 10^{-7} . The diluent used was buffered peptone water (BPW Oxoid CM0509). The main selective media used for isolation and enumeration of colonies are described in Table 1. The isolation and enumeration of mesophilic aerobic bacteria, total Coliforms, fecal Coliforms, anaerobic sulphite-reducing bacteria, *S. aureus*, fecal streptococci, yeasts and moulds were carried out according to international standards (ICMSF, 1986; Bell *et al*., 1997).

Statistical analysis: Analyses of variance (ANOVA) for the comparison of geometric means were performed using SAS software (Statistical Analysis Systems Inc., Cary, USA) based on the results of microbiological analyses. The significance level adopted was 5%.

RESULTS

Isolation, enumeration and identification of water contaminants: The microorganisms isolated from samples collected both from Dassa-Zoume and Gogounou were mesophilic aerobic flora (6.2×10^{3} CFU mL^{-1}), total Coliforms (2.2×10^{3}CFU/100 mL), fecal Coliforms (3.0×10^{1} CFU/100 mL), *E. coli* (3.0×10^{1} CFU/100 mL), anaerobic sulphite-reducing bacteria (2.0 CFU/20 mL) and fecal streptococci (6×10^{2} CFU/50 mL). Results obtained from this analysis indicated that samples of Dassa-Zoume were the more contaminated in terms of fecal contamination (Table 2).

Isolation, enumeration and identification of raw milk contaminants: Total Coliforms (9.2×10^{2} CFU mL^{-1}), fecal Coliforms (9.2×10^{2} CFU mL^{-1}), *E. coli* (0.4×10^{1} CFU mL^{-1}), *S. aureus* (4.0×10^{1} CFU mL^{-1}), anaerobic sulphite-reducing bacteria (0.4×10^{1} CFU mL^{-1}), yeasts (1.2×10^{3} CFU mL^{-1}) and moulds (5.1×10^{1} CFU mL^{-1}) were isolated in all milk samples analyzed. The highest contamination was obtained with fecal Coliforms and *E. coli* (Table 3). Contamination was higher in Dassa-Zoume samples than those of Gogounou for total aerobic flora and fecal Coliforms ($p<0.05$). *Salmonella* and fecal streptococci were absent in all milk samples analyzed.

Table 2: Mean values of germs Isolated and enumerated from water samples collected from Dassa-Zoume and Gogounou

	Mean values (CFU mL^{-1})			
Germs	Gogounou	Dassa-Zoume	Mean	Standards*
Total aerobic mesophilic bacteria (CFU/mL)	4.5×10^{3}	7.9×10^{3}	6.2×10^{3}	10^{3}
Total Coliforms (CFU/100 mL)	1.0	4.5×10^{2}	2.2×10^{3}	10
Fecal Coliforms (CFU/100 mL)	1.0	6.0×10^{1}	3.0×10^{1}	-
E. coli presumed (CFU/100 mL)	0	6.0×10^{1}	3.0×10^{1}	Absence
E. coli confirmed (CFU/100 mL)	0	0	0	Absence
Anaerobic sulphite-reducing bacteria (CFU/20 mL)	0	4.0	2.0	Absence
Fecal streptococci (CFU/50 mL)	0	1.2×10^{3}	6×10^{2}	Absence

-: Not defined, *: Source:

Table 3: Mean values of germs isolated and enumerated from raw milk samples collected from Dassa-Zoume and Gogounou

	Mean value (CFU mL^{-1})			
Germs	Dassa-Zoume	Gogounou	Mean	Standards*
Mesophilic aerobic bacteria (CFU mL^{-1})	$2.3\pm47.9\times10^{8a}$	$1.6\pm41.5\times10^{7b}$	1.1×10^{8}	3×10^{4}
Total coliforms (CFU mL^{-1})	$1.3\pm1.2\times10^{3a}$	$6.2\pm1.1\times10^{2a}$	9.2×10^{2}	10^{3}
Fecal coliforms (CFU mL^{-1})	$1.3\pm1.2\times10^{3a}$	$6.2\pm1.1\times10^{2b}$	9.2×10^{2}	10^{2}
E. coli (CFU mL^{-1})	$0.4\pm0.2\times10^{1a}$	$0.4\pm0.2\times10^{1a}$	0.4×10^{1}	0
S. aureus (CFU mL^{-1})	$8.3\pm3.8\times10^{1a}$	$0.7\pm3.3\times10^{1a}$	4.0×10^{1}	10^{2}
Anaerobic sulphite-reducing germs (CFU mL^{-1})	$0.2\pm0.4\times10^{1a}$	$0.5\pm0.3\times10^{1a}$	0.4×10^{1}	50
Yeast (CFU mL^{-1})	$7.5\pm4.2\times10^{2a}$	$1.5\pm0.6\times10^{2a}$	1.2×10^{3}	-
Moulds (CFU mL^{-1})	$2.6\pm4.2\times10^{1a}$	$7.0\pm3.6\times10^{1a}$	5.1×10^{1}	-
Lactic flora (CFU mL^{-1})	$4.0\pm7.4\times10^{7a}$	$1.5\pm6.4\times10^{6b}$	2.1×10^{6}	-

Means with different letters in a row are significantly different at $p\leq0.05$

DISCUSSION

The microbial load of milk sampled at Dassa-Zoume and Gogounou in mesophilic aerobic flora exceeded the microbiological criteria applicable to raw milk. This high contamination is probably due to poor hygiene during milking or equipment used for milking, udder infection of the cow (Villar *et al*., 1996; Yamani *et al*., 1999). Bonfoh *et al*. (2003) in Mali, Chye *et al*. (2004) in Malaysia and Farhan and Salik (2007) in Pakistan have made the same observation on milk samples analyzed. According to Aumaitre (1999), the health of the dairy herd, milking and pre-conditions of storage are also basic determinants of the quality of the milk. Bacteria can enter the milk while it is still in the udder and most microorganisms found in raw milk are contaminants from the outer surface of the udder, milking utensils and milkers (Chye *et al*., 2004). Bonfoh *et al*. (2004) reported that the poor quality of water used for washing utensils could lead to obtaining a poor microbiological quality of milk. In order to reduce the contamination of milk, utensils used during milking should be rinsed with clean water and cleaned with a detergent and a disinfectant just before and after use (Dodd and Phipps, 1994; FAO, 2011). The analysis of the water used for cleaning milking utensils and storing milk showed a strong presence of mesophilic aerobic flora, especially at Dassa-Zoume.

Total Coliforms, fecal Coliforms and *E. coli* were found in milk samples analyzed. The presence of total Coliforms does not necessarily indicate a direct fecal contamination of milk, but it is considered to be an indicator of poor hygiene and sanitation during milking and post manipulation.

The presence of these bacteria in milk can also be linked to contamination by cows' excrements, land and water used (Chye *et al.*, 2004). However, the milk contamination by fecal Coliforms necessarily indicates contamination by cows' excrements or the milker's hands. The presence of fecal indicator *E. coli* reveals the risk of the presence of other pathogenic enterobacteria in the samples (Chye *et al.*, 2004). Microbial load in *E. coli* obtained in this study is comparable to that obtained by Farhan and Salik (2007). Regarding total Coliforms and fecal Coliforms, the results in this study are comparable to those obtained by Jayarao and Wang (1999), Reuben *et al.* (2003), Hoogkamp-Korstanje (2003), Chye *et al.* (2004), Farhan and Salik (2007), Mennane *et al.* (2007), Afif *et al.* (2008) and Bille *et al.* (2009).

S. aureus was present in the milk samples analyzed, but in a consistent proportion with regard to microbiological criteria. *S. aureus* is recognized as the causative agent of clinical and subclinical mastitis in cattle. Chye *et al.* (2004) and Afif *et al.* (2008) reported that the presence of *S. aureus* in milk samples is related to environmental conditions. Indeed, these authors found that the tropical climate seriously predispose animals to infection by pathogens. Adesiyun *et al.* (1995), Bonfoh *et al.* (2005) and Mennane *et al.* (2007) found a very high microbial load in *S. aureus* in milk samples analyzed. Although *S. aureus* microbial load in the samples obtained in this study is below the accepted microbiological criteria, appropriate arrangements must be made to counteract this contamination, because the presence of *S. aureus* in food presents potential risk to consumer health due to its production of enterotoxin (Godefay and Molla, 2000; De Buyser *et al.*, 2001; Cenci-Goga *et al.*, 2003).

Salmonella sp is absent in 25 mL of all milk samples analyzed. This result is similar to those of Mennane *et al.* (2007). It indicates that *Salmonella*'s incidence in raw milk of the surveyed areas is low and hence, is not regarded as a possible danger to consumers' healths.

Significant contamination of milk by yeasts and moulds as observed in this study, is mainly the result of strong external contamination and poor hygiene utensils used for milking and storing milk (Bonfoh *et al.*, 2003).

CONCLUSION

The findings of this study highlight the poor microbiological quality of raw milk samples analyzed, which were collected from two municipalities in the Republic of Benin, viz. Dassa-Zoume and Gogounou. In fact, the quality of these samples analyzed does not meet, in general, microbiological criteria prescribed. The contaminations result, especially from the lack of hygiene on the farm and the sale point. The presence of bacteria responsible for food intoxication such as *S. aureus* may be a public health problem if no precaution is taken against this contamination. The development of the dairy producer organization with the creation of collection centers and the use of the cold chain can reduce contamination. The hygienic quality of raw milk production and sales could also be improved by heeding the rules of good manufacturing and sales practices.

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