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MICROBIOLOGICAL QUALITY OF RAW MILK OF *Bubalus bubalis* IN A BUFFALO FARM, COLÓN MUNICIPALITY, ZULIA STATE

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ABSTRACT

In order to assess the microbiological quality of raw milk of *Bubalus bubalis* on a buffalo farm located in the Colón municipality, Zulia state, ten samples of milk were evaluated and were determined: thermoduric bacteria, total coliforms, psychrophilic bacteria, thermophilic bacteria and *Staphylococcus* spp. by counting viable colony in Petri dishes according to COVENIN Standards of Venezuela. Moreover, phenotypic assays for the identification of Gram-positive cocci that grow in the milk-salt agar were performed. Through this strategy we identified an average of 23 CFU/mL of thermoduric bacteria, and in 3 of the 10 raw milk samples were identified *Staphylococcus aureus* (3/3: 100%). Samples of raw milk of buffalos are unsuitable for human consumption and necessary assessment of mastitis in the herd of this farm.

KEYWORDS: *Bubalus bubalis*, raw milk, *Staphylococcus aureus*, microbiological quality.

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CALIDAD MICROBIOLÓGICA DE LECHE CRUDA DE *Bubalus bubalis* EN UNA BUFALERA COMERCIAL DEL MUNICIPIO COLÓN, ESTADO ZULIA

RESUMEN

Con el fin de evaluar la calidad microbiológica de la leche cruda de *Bubalus bubalis* en una bufalera comercial ubicada en el municipio Colón, estado Zulia se evaluaron 10 muestras de leche y se determinaron: bacterias termodúricas, coliformes totales, bacterias termófilas, bacterias psicrófilas, y *Staphylococcus* spp. contando colonias viables en placas de Petri de acuerdo a las Normas COVENIN de Venezuela. Por otra parte, se realizaron ensayos fenotípicos para la identificación de cocos Gram positivos que crecieron en el agar de leche-sal. Mediante ésta estrategia se identificaron un promedio de 23 UFC/mL de bacterias termodúricas, y en 3 de las 10 muestras de leche cruda se identificó *Staphylococcus aureus* (3/3: 100%). Las muestras de leche cruda de búfalos no son aptas para el consumo humano y es necesaria la evaluación de la mastitis en el rebaño de esta granja.

PALABRAS CLAVE: *Bubalus bubalis*, leche cruda, *Staphylococcus aureus*, calidad microbiológica.

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INTRODUCTION

The buffalo is a domesticated animal; it has slower movements than cattle (1, 2). Despite being more resistant to infectious diseases compared to cattle, they are also susceptible to them, and therefore require vaccination plans (1). The domestic buffaloes can be classified into two types: river buffalo and swamp buffalo. Both belong to the same family, but have different habitats. The swamp buffalo has long, slightly bulky horns and used as a producer of animal flesh and to work in the field, is located in eastern South Asia. River buffalo generally has small curved horns and is located in the western part of Asia, Eastern Europe, Italy and America (3). Both Buffalo and cattle are classified zoologically in the family *Bovidae* and subfamily, *bovinae*. The cattle belong to the species *Bos taurus* and buffalo to *Bubalus*. Within it is three subspecies turn, *Bubalis*, *Karebau* or Carabao and *Fulvus*. *Bubalus* species is also classified as water buffalo (*Bubalus bubalis bubalis*) and swamp (*Bubalus bubalis karebau*). In *Bubalus* genus five species are known: *B. bubalis bubalis* (water buffalo), *B. depressicornis*, *B. mephistopheles*, *B. mindorensis*, and *B. quarlesi* (3). Milk is one of the most complete foods of nature and of vital importance to the growth and strengthening of bones in human. The cow is, par excellence; the largest milk producer in the world, but in recent years there has been a significant increase in the production and processing of buffalo milk (4-6). In health and food security,

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assessing the microbiological quality of food and in particular the development of artisanal mozzarella cheese from buffalo milk in Venezuela, is a prerequisite, which requires a holistic approach, from procurement of raw materials, processing and production to final consumption (7). According to FAO global production of milk of all species in 2005 reached 629.2 million tons, of which 12.2% were buffalo. The total number of microorganisms present in milk and its derivatives per unit volume is indicative of the health conditions of production and conservation as well as the commercial life of the product, very high bacterial counts in raw milk are indicative of a strong contamination during milking, handling or storage, or conservation, insufficient in temperatures cooling to retard microbial growth (8). Briñez *et al*., in 2009 (9) reported in 393 milk samples of *Bubalus bubalis* from a cross-bred buffalo farm located in Mara municipality, Zulia state in Venezuelan western, the presence of *Staphylococcus*, *Micrococcus* and *Streptococcus*, and the subclinical mastitis in the herd. The southern zone of Maracaibo Lake in Venezuela has the right conditions for semi-intensive or intensive exploitation of Buffaloes. However, the available data on the assessment of the microbiological quality of raw milk in the Colón municipality of Zulia State are limited. Therefore, it became necessary to assess the microbiological quality of raw milk *B. bubalis* on a buffalo farm in the Colón municipality of Zulia State.

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MATERIALS AND METHODS

1. **Study Type.** The study was descriptive (10).

2. **Population and samples.** The population was represented by 46 buffalos (*B. bubalis*) with different numbers of lactations. Of these, 10 samples were taken from raw milk collected randomly into three milkings in two days.

3. **Study area**. The Chiquinquirá grange is located in the Colón municipality having an area of tropical rainforest, with geographical coordinates for North latitude: 08º 57'32.3" and west longitude: 72º01'13.8" with a 4 meters altitude. The averages climate variables for the years 2007, 2008 and 2009 with a maximum value for temperature is 32.5°C and a minimum of 23.1°C with an average of 27.8°C. Relative humidity with a maximum value of 91.7% and 52.8% having bid on average 72.2%. Solar radiation is 4512.2 Mj/m^2.h in average and wind speed at its maximum value is 5.8 m/sec having an average 1.3 m/sec. The average rainfall per year is 2331 mm.

4. **Sampling raw milk from river buffaloes.** The instruments used must be sterile prior to collection of samples of raw milk. By using running water, we proceeded to the cleaning and disinfection of the nipple, using running water and 70% ethanol, then distilled water and drying the area with sterile gauze. 500 mL of raw milk were obtained during the morning and evening milking from river buffaloes selected with the help of a lighter with

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alcohol, discarding the foremilk; The samples were stored in refrigerated cellar for transfer to Laboratorio de Microbiología de Alimentos at the Universidad Nacional Experimental Sur del Lago "Jesús María Semprum" (UNESUR) for their respective microbiological analysis (11). For raw milk as (12) must meet a set of standards established by COVENIN such as the 902-87 (13), indicating the standard method for counting aerobic bacteria colony in Petri plates, 903-93 (14) which is the standard for raw milk, the 1292-1289 (15) standard for the isolation and enumeration of *S. aureus* and, finally from 1104-1996 (16) which is the standard by which to determine the number was made more total coliform likely.

5. **Serial dilutions for samples of raw milk.** Figure 1 depicts the scheme followed for performing serial dilutions of the samples: 10 mL of raw milk samples were homogenized and mixed 25 times at an angle of 45ºC. 10 mL of these samples were transferred to a dilution bottle containing 90 mL of 0.1% sterile peptone water waving preparation 25 times to obtain $1x10^{-1}$ dilution. From the first dilution is measured and 1 mL of this was mixed with 9 mL of 0.1% sterile peptone water, stirring for 25 times dilution $1x10^{-2}$. The above step is repeated until $1x10^{-6}$ dilutions with care to use a separate sterile pipette for each dilution. Planting depth was made during the next twenty minutes to the preparation of each of the respective dilutions (12).

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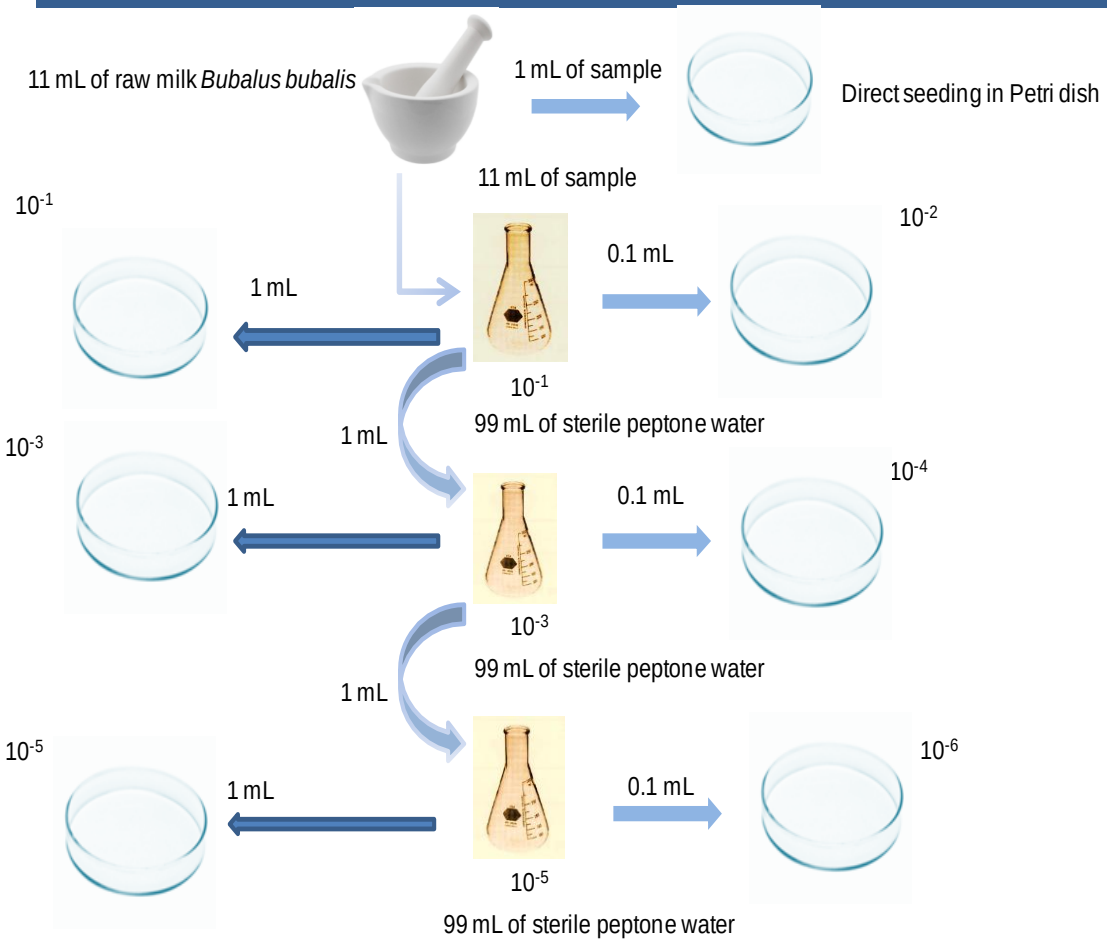

Figure 1.Scheme for performing serial dilutions and subsequent planting depth of samples of raw milk of *Bubalus bubalis*. Taken and modified Solano-Goñi (12).

6. **Microbiological quality of raw milk *Bubalus bubalis*.**

6.1 **Standard plate count.** The technique consisted of dilutions of samples of raw milk in Petri dishes with agar standard sterile after incubation for 24 to 48 hours at a temperature of 37 ± 3ºC were counted colonies observed , which allowed us to know the number of Colony Forming

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Units/mL (CFU/mL) of each of the ten samples (9, 17, 18).

6.2 **Determination of total coliforms.** Dilutions and seeding of milk samples from buffalo were made by the standard technique, to determine the most probable number (MPN) of total coliforms with the use of agar Mac Conkey as differential selective medium for Gram negative bacteria, fermenting or not the lactose. We proceeded to add the amount of samples/dilutions and respective there on agar was added, leaving it to solidify homogenized for further incubation at 32ºC for 24 hours (10, 17, 18).

6.3 **Determination of thermophilic, psychrophilic and thermoduric bacteria.** Following the same procedure given in Figure 1 for performing serial dilutions on the samples and for deep seeding described above using nutrientagar preceded incubated Petri dishes at a temperature of 55°C for 24 hours. After, this time proceeded to the reading of the results. For determination of psychrophilic bacteria and thermoduric the same protocol described for thermophilic bacteria using nutrient agar it was followed changing temperatures 7°C for 7 days, with daily check growth every 24 hours for psychrophilic bacteria. In the case of thermoduric bacteria 5 mL samples and dilutions were transferred of the refrigerator at a temperature between 0-4°C for ten minutes, and then the same was transferred to bath at a temperature of

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62.8°C for 30 minutes, later they were placed in a refrigerator at 10ºC for ten minutes. The final incubation was performed at 35ºC for 24 hours prior to reading (17, 18).

6.4 **Determination and identification of *Staphylococcus* spp. strains from samples of raw milk *Bubalus bubalis*.** Determining strains of *Staphylococcus* spp. it was performed with the use of milk salt agar. Following the same scheme for serial dilutions of the samples evidenced in Figure 1 and respecting the same planting procedure described above, the plates were incubated at 37°C for 24 hours. For morphological, tinctorial and biochemical identification *Staphylococcus* spp. performs: Gram staining, catalase, slide coagulase test, tube coagulase test, hemolysis on blood agar and agar salt milk growth (19).

RESULTS AND DISCUSSION

The microbiological analysis of samples of *Bubalus bubalis* raw milk, allowed the identification of bacteria thermoduric, with an average of 23 CFU/mL in the ten samples tested, ranging from 1 to 220 CFU/mL. The highest count obtained corresponded to the sample designated with number 3. There was no presence of psychrophilic bacteria, thermophilic bacteria or total coliforms in these samples (see Table I). In Venezuela there is no minimum or maximum value for the count bacteria in raw milk samples from buffalo. The average count of 23 CFU/mL could be due to the handling of animals in each production system and conditions of

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cleanliness and hygiene facilities and milking equipment, like humans involved in milking been a major source of contamination of raw milk. Our results differ from those reported by Acevedo, García and Vargas in 2013 (7), which obtained a count of mesophilic aerobic bacteria between $19x10^6 \pm 11x10^6$ CFU/g in samples of mozzarella cheese made from buffalo milk. Meanwhile, Briñez *et al*., in 2000 (9) determined parameters of microbiological quality of raw milk of buffalos with a standard plate count, with a $1.6x10^{-3}$ minimum value and a maximum value of $1.7x10^{-3}$. In Table I the absence of total coliforms is shown, which indicated no fecal contamination of the ten samples of raw milk tested, this group of microorganisms easily passes through the hands of workers in contact with the buffalo. The group of Granados *et al*., in 2014 reported 20 CFU/g of total coliforms in samples of "Capa" chesse made from buffalo milk (20); meanwhile the group Hernández-Palomino and Díaz-Sangama., in 2002 (21) in samples of mozzarella cheese of buffalo milk obtained a value of 10^2-10^3 CFU/g. In this sense, our results are lower than those reported by these two groups respectively. This indicates that the buffalo farm located in the Colón municipality does not own shortcomings in hygiene measures to prevent fecal contamination of food such as raw buffalo milk is obtained here

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Table I. Evaluation of the microbiological quality of raw milk of *Bubalus bubalis* on a buffalo farm in the Colón municipality, Zulia state*.

Sample number	Thermoduric bacteria CFU/mL	Thermophilic bacteria CFU/mL	Psychrophilic bacteria CFU/mL	Total coliforms CFU/mL
1	2	ND	ND	ND
2	3	ND	ND	ND
3	220	ND	ND	ND
4	1	ND	ND	ND
5	2	ND	ND	ND
6	2	ND	ND	ND
7	1	ND	ND	ND
8	1	ND	ND	ND
9	1	ND	ND	ND
10	1	ND	ND	ND

*The tests were performed in duplicate for each of the samples tested; ND = not detected

In Table II the results of samples of raw milk (1, 2 and 3), growth of suggestive colonies of *Staphylococcus spp*. were reported. In the first three samples, an average of 1 CFU/mL for dilutions of 10^{-4}, 10^{-5} and 10^{-6} was obtained. This finding is the most important of all our study because positive coagulase *Staphylococcus* is important causative agents of clinical and subclinical mastitis in cattle. For this reason we proceeded to carry out several phenotypic tests for acceded to the preliminary identification of the species involved. Table III shows the results for these assays, where 100% of *S. aureus* (samples 1, 2 and 3) was identified.

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Table II. Determination of *Staphylococcus* spp. in samples of raw milk of *Bubalus bubalis* on a buffalo farm in the Colón municipality, Zulia state*.

Sample number	Dilution $1X10^{-4}$ (CFU/mL)	Dilution $1X10^{-5}$ (CFU/mL)	Dilution $1X10^{-6}$ (CFU/mL)
1	<1	1	<1
2	<1	<1	1
3	1	1	1
4	<1	<1	<1
5	<1	<1	<1
6	<1	<1	<1
7	<1	<1	<1
8	<1	<1	<1
9	<1	<1	<1
10	<1	<1	<1

*Each of the samples was evaluated in triplicate.

Table III. Morphological and biochemical identification of strains of *Staphylococcus* spp. of raw milk of *Bubalus bubalis* on a farm buffalo in the Colón municipality, Zulia state*.

Parameter	Sample number1	Sample number 2	Sample number 3
Macroscopic characteristics			
Morphology	Round creamy colonies with a halo of bright yellow	Round creamy colonies with a halo of bright yellow	Round creamy colonies with a halo of bright yellow
Microscopic characteristics			
Gram staining	Positive	Positive	Positive
Morphology	cocci arranged in clusters of grapes	cocci arranged in clusters of grapes	cocci arranged in clusters of grapes
Hemolysis	β-hemolysis	β-hemolysis	α-hemolysis
Milksalt agar growth	Positive	Positive	Positive
Catalase	Positive	Positive	Positive
Slide coagulase test	Positive	Positive	Positive
Tube coagulase test	Positive	Positive	Positive

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***The tests were performed in triplicate for each of the samples tested.**

Acevedo, García and Vargas (7) reported $20x10^4 \pm 4x10^4$ CFU/g of *S. aureus* in samples of mozzarella cheese made from buffalo milk. Mastitis in cattle is an inflammation of the mammary gland, and secretion ducts, which generates altering milk composition, volume and flavor, with increased loading of usual microorganisms in milk. It may be able to follow an acute or chronic course and may present clinically as clinical mastitis or subclinical mastitis, which represents a challenge in terms of diagnosis for veterinary doctors. Its main cause is constituted by microorganisms such as bacteria, algae and fungi (molds and yeasts). Approximately 90 to 95% of cases are caused by four microorganisms: *S. aureus*, *Streptococcus uberis*, *Streptococcus agalactiae* and *Streptococcus dysgalactiae*. The main limitation of this study is the small number of samples tested; only 10, in other work in the municipality Mara, state Zulia has been made to evaluate 393 samples of raw milk of buffalo (9). It is necessary to evaluate the mastitis in the herd, and includes testing for antimicrobial susceptibility pathogenic strains identified.

CONCLUSION

In samples of raw milk of *Bubalus bubalis* the presence of *S. aureus* and thermoduric bacteria was found, indicating a phytosanitary improper handling flock; besides the possible presence of mastitis in these animals. This raw food is unfit for human consumption.

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