Risk factors associated with buffalo mastitis in the Brazilian Northeast[1]

Elizabeth S. Medeiros[2], Manuela F. Lyra de Freitas[3], Tomoe N. Saukas[4], Sérgio S. Azevedo[5], José Wilton Pinheiro Junior[6], Daniel F. Brandespim[6], Orestes Luiz de S. Neto[4] and Rinaldo A. Mota[4*]

ABSTRACT.- Medeiros E.S., Freitas M.F.L., Saukas T.N., Azevedo S.S., Pinheiro Junior J.W., Brandespim D.F., Souza Neto O.L. & Mota R.A. 2011. **Risk factors associated with buffalo mastitis in the Brazilian Northeast.** *Pesquisa Veterinária Brasileira 31(6):499-504.* Laboratório de Doenças Infecto-Contagiosas dos Animais Domésticos, Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros s/n,Dois Irmãos, Recife, PE 52171-900, Brazil. E-mail: rinaldo.mota@hotmail.com

Risk factors for subclinical mastitis were studied in 474 buffaloes cows proceeding from four dairy farms located in the states of Pernambuco, Alagoas, Bahia and Ceará, Brazil. Milk samples (n=1896) of lactating buffaloes cows were examined for somatic cells count (SCC) and microbiologic exam, and a questionnaire composed by objective questions was applied in order to obtain animals and herd management data. Risk factors analysis was performed in two stages: univariate and mutivariate analysis. Two analysis were performed, one considering the animal classification for SCC as the dependent variable (<400,000 – negative; >400,000 – positive) and another, considering the microbiologic exam result (positive and negative). In the multivariate analysis considering SCC as dependent variable, the lack of teat washing was the only variable identified as a risk factor, so that farms without this procedure before the milking presented risk of infection of 2.68 (I.C. 1.49-4.83). In the multivariate analysis considering the microbiologic exam as dependent variable, it was observed that properties that performed the cleaning of the milking equipment manually presented risk of 1.85 (I.CI. 1.32-3.64), which was higher than those properties that performed the cleaning mechanically (p=0.019). Risk factors for the occurrence of subclinical mastitis in dairy buffaloes in Brazilian Northeast farms were related to the characteristics of improper milking management. Risk factors identified in this study must be carefully corrected in order to reduce the frequency of mastitis cases, and therefore, contribute for disease control and prevention in the herds.

INDEX TERMS: Buffaloes, mastitis, milking, epidemiology.

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Parte da Tese de Doutorado do primeiro autor, Programa de Pós-Graduação em Biociência Animal, Universidade Federal Rural de Pernambuco (UFRPE), Rua Dom Manoel de Medeiros s/n, Dois Irmãos, Recife, PE 52171-900, Brasil.

[2] Campus Arapiraca, Universidade Federal de Alagoas, UFAL, Pólo Viçosa, Fazenda de São Luís s/n, Viçosa, AL 57700-970, Brasil.

[3] Departamento de Anatomia, Universidade Federal de Pernambuco, UFPE, Centro Acadêmico de Vitória, Rua do Alto do Reservatório s/n, Bela Vista, Vitória de Santo Antão, PE 55608-680, Brasil.

[4] Departamento de Medicina Veterinária, UFRPE, Recife, PE 52171-900, Brasil. *Corresponding author: rinaldo.mota@hotmail.com

[5] Centro de Saúde e Tecnologia Rural, CSTR, Universidade Federal de Campina Grande, UFCG, Avenida Universitária s/n, Santa Cecília, Campus de Patos, Patos, PB 58700-970, Brasil.

[6] Unidade Acadêmica de Garanhuns, UAG, Universidade Federal Rural de Pernambuco, UFRPE, Av. Bom Pastor s/n, Boa Vista, Garanhuns, PE 55.296-901.

RESUMO.- [**Fatores de risco associados à mastite bubalina no Nordeste brasileiro.**] Foram estudados os fatores de risco para mastite subclínica em 474 búfalas procedentes de quatro propriedades de exploração leiteira situadas nos Estados de Pernambuco, Alagoas, Bahia e Ceará. Amostras de leite (n=1896) de búfalas em lactação foram examinadas para contagem de células somáticas (CCS) e exame microbiológico, e um questionário constituído de perguntas objetivas foi aplicado para obtenção de dados dos animais e do manejo do rebanho. A análise dos fatores de risco foi efetuada em duas eta-pas: análise univariada e multivariada. Foram realizadas duas análises, uma considerando a classificação dos animais quanto à CCS como variável dependente (<400.000 – negativo; > 400.000 – positivo) e outra considerando o resultado do exame microbiológico (positivo e negativo). Na análise multivariada consi-

derando-se a CCS como variável dependente a única variável identificada como fator de risco foi não lavar os tetos antes da ordenha, onde aquelas propriedades que não lavavam os tetos antes da ordenha apresentaram o risco de infecção de 2,68 (I.C. 1,49-4,83). Na análise multivariada considerando o microbiológico como variável dependente constatou-se que aquelas propriedades que realizavam limpeza manual do equipamento de ordenha apresentavam um risco 1,85 (I.C. 1,32-3,64) maior do que aquelas propriedades que realizavam esta limpeza de forma mecânica (p=0,019). Os fatores de risco para ocorrência de mastite subclínica em báfalas leiteiras em propriedades na região nordeste do Brasil foram relacionados às características de manejo inadequado da ordenha. Os fatores de risco identificados nesse estudo devem ser cuidadosamente corrigidos para reduzir a frequência de casos de mastite e assim contribuir para o controle e prevenção da doença nos rebanhos.

TERMOS DE INDEXAÇÃO: Búfalas, mastite, ordenha, epidemiologia.

INTRODUCTION

Buffaloes present the same sanitary problems as bovine, stressing mastitis, which is considered the disease that affects the most the profitability of dairy farms, from production losses as much as to the annual costs in prevention and treatment. The disease interferes directly in the milk quality and countless efforts have been made for its control and prevention (Pedrini & Margatho 2003, Freitas et al. 2005, Carvalho et al. 2007). It is an illness of multi-etiologic and multifactorial origin that assaults the majority of the world's dairy herd and causes problem in the whole productive chain, even for the consumer, which may receive a final product of low quality (Costa 1999).

Factors associated to the handling and characteristics such as herd size and type of milking (manual or mechanic) and procedures during the milking (the lack of disinfection before and after the milking), the improper functioning of the milking machine and the lack of milker's training and motivation were associated to the occurrence of new intramammary infection in bovine cattle, and consequently, the increase of Somatic Cells Count in the milk (Oliver et al. 1993, Brito et al. 1997, Spencer 2002).

Souza et al. (2005) developed a study on risk factors for bovine mastitis and verified that the methodology of analysis allowed the identification of likely risk factors for high Somatic Cell Count (SCC) of the herd and for specific mastitis pathogens likely risk factors identified were the lack of adoption of the milking line, feeding animals during the milking procedure and the lack of teat antisepsis after milking.

Coentrão et al. (2008) found as risk factors for subclinical mastitis, animals with the base of the udder along or below the hocks, cracks or fissures in the rubber parts of the milking machine, unsuitability of the teat holders, cleaning deficiency of the pulsators, lack of the milkers' training, not using the microbiologic diagnostic for mastitis, immersion of the teat holders set in disinfectant solution between the milking of distinct animals and total insertion of the antibiotic cannula into the teats at the cow drying.

In Brazil, published papers on risk factors in ruminants are still scarce. For the bubaline species, no references were found on this subject in the consulted literature. Hence, the aim of this work was to identify the risk factors associated to subclinical mastitis in bubaline herds in Northeastern states of Brazil.

MATERIALS AND METHODS

One thousand eight hundred and ninety six milk samples from 474 buffaloes proceeding from four dairy farms located in the states of Pernambuco, Alagoas, Bahia and Ceará, Brazil, were analyzed. Herds were composed of Murrah crossbred animals at different ages and at different lactation stages. Animals were raised under intensive or semi-intensive management and submitted to canalized mechanic milking and bucket milking.

Milk samples were submitted to California Mastitis Test (CMT) (Schalm & Noorlander 1957) and positive samples were collected for somatic cells count (SCC) in appropriate flasks containing the preservative Bronopol®, and immediately cooled. SCC was performed in electronic equipment SomaCount 300, by the flow cytometry method (Milk 1995).

Sample collection for the microbiologic exam was performed after previous teat washing with water and soap, drying with towel paper and ostium antisepsis with alcohol 70ºGL. Five mL of milk were collected in sterilized flasks with threaded caps, previously identified with the name or number of the animal and mammary quarter, properly sent to the laboratory in isothermal boxes containing recyclable ice for processing.

Aliquots of 10ìL of milk were seeded in agar base enriched with 5% of shep blood and incubated in bacteriological oven at 37ºC, performing reading after 24, 48 and 72 h. Further, the morphologic characteristics of colonies as size, type, color and hemolysis were observed. Cell arrangement and morphotinctorial characteristics to the Gram technique were observed at the microscopy (Carter 1988).

Classification of Gram-posiitve bacteria was performed according to Quinn et al. (1994). For the identification of the *Staphylococcus* spp. isolates, biochemical proofs were developed as production of free coagulase, DNase and catalase, according to Silva et al. (2001). Proofs for acetoin production, fermentation of glucose (anaerobiosis) and manitol (aerobiosis and anaerobiosis) were performed according to Mac Faddin (1980). After the tests, isolates were classifed as *Staphylococcus aureus* (*S. aureus*), if positive in every test; as *Staphylococcus* coagulase-positive (SCP), if positive for the coagulase production, glucose fermentation in anaerobiosis and catalase, but negative for any other test; as *Staphylococcus* coagulase-negative (SCN), when bacteria could not coagulate the rabbit plasma, presented staphylococcus characterstics at the Gram staining technique, fermented glucose in anaerobiosis and produced calatase (Baird-Parker 1990).

For the enterobacteria identification, the following biochemical proofs were applied: urease production, reaction in Triple Sugar Iron (TSI) Agar, test of MR/VP (MR - Methyl Red reaction; VP - Voges-Proskauer reaction), test in Agar SIM (S - H_2S production; I - Indole production; M - motility) and test in citrate agar (citrate's carbon utilization), being identified according to Carter (1988).

For the risk factors study, questionnaires were applied to the farms managers, composed by objective questions in order to obtain information about the property (breed characteristic, breeding management, water source, veterinary assistance, type of milking, buffaloes up to the third lactation); milking handling (flies control, strip-cup test, teat washing before milking, dry cow therapy, clinical mastitis treatment, antibiotics shift) and hygiene of milking equipments (time of utilization of the milking equipment, cleaner utilization, cleaning of the milking equipment, milker's hygiene habits).

Risk factors analysis was performed in two stages: univariate and multivariate analysis. In the first analysis, the animals' classification for SCC was considered as the dependent variable (<400,000 negative; >400,000 positive) and in the second analysis, the microbiologic exam result (positive and negative) was considered as the dependent variable. Variables presenting p value $\leq$ 0.15 in the chi-square or the Fisher's exact test when indicated (Zar 1998) were selected and used in the multivariate analysis, by using the multiple logistic regression (Hosmer & Lemeshow 2000). Collinearity among predictor variables was verified by correlation analysis and for those that presented strong collinearity (p<0.05), one of them was excluded from the multiple analysis according to the biologic plausibility (Dohoo et al. 1996). Significance level adopted in the multiple analyses was 5%. Analyses were developed by using the program SPSS 13.0 for Windows.

RESULTS

By analyzing the farms' productive profile, it was observed that all farmers were raising buffaloes for over than five, with the predominance of the Murrah breed, with 50% of pure animals and 50% crossbred. Predominant breeding system was semi-intensive (75%), followed by the intensive system (25%). All the farms used mechanic milking, of which 75% presented the bucket milking system and 25%, the canalized system.

In the univariate analysis, considering the somatic cell count as the dependent variable, the variables identified as risk factors associated to SCC higher than 400.000 cells/mL were: type of milking, number of lactations, teat washing, antisepsis, dry cow therapy, clinical mastitis treatment, antibiotics shift, time of equipment utilization and milker's habit (Table 1). However, in the multivariate analysis, it was observed that only the variable lack of teat washing before milking was confirmed as risk factor for the mammary gland infection with OR= 2.68 (I.C. 1.49-4.83).

Concerning the univariate analysis and considering the microbiologic exam as dependent variable, the variables pure breed, type of milking, number of lactations, flies control, lack of strip-cup test, antisepsis before and after milking, antibiotics shift, time of equipment utilization, manual cleaning of the milking equipment and milker's habit were considered as risk factors associated to the positivity in the microbiologic exam (Table 2). In the multivariate analysis, it was observed that properties that performed the manual cleaning of the milking equipment presented OR= 1.85 (I.C. 1.32-3.64), which represents greater chances of occurrence of subclinical mastitis in comparison to those properties that performed this cleaning mechanically (p=0.019).

Table 1. Univariate analysis results for the factors associated or not to bubaline mastitis considering the somatic cells count in buffaloes milk

Variable	Total of animals	Positive		P
		N	%	
Breed				
Pure	88	47	53.4	
Crossbred	230	119	51.7	0.888
Breeding system				
Intensive	69	37	53.6	
Semi-intensive	249	129	51.8	0.896
Water source				
Still	69	37	53.6	
Still + running	249	129	51.8	0.896
Veterinary assistance				
Permanent	88	47	53.4	
Temporary	230	119	51.7	0.888
Tipe of milking				
Mechanic bucket milking	153	93	60.8	
Mechanic canalized	165	73	44.2	0.005*
Bufaloes up to 3rd lactation (%)				
0-59	165	73	44.2	
≥ 60	153	93	60.8	0.005*
Flies control				
Yes	88	47	53.4	
No	230	119	51.7	0.888
Strip-cup tes				
Yes	299	156	52.2	
No	19	10	52.6	1.000
Teat washig before milking				
Yes	253	120	47.4	
No	65	46	70.8	0.001*
Antisepsis of teats before milking				
Yes	153	93	60.8	
No	165	73	44.2	0.005*
Antisepsis of teats after milking				
Yes	230	119	51.7	
No	88	47	53.4	0.888
Dry cow therapy				
Yes	65	46	70.8	
No	253	120	47.4	0.001*
Clinical mastitis treatment				
Yes	253	120	47.4	
No	65	46	70.8	0.001*
Antimicrobian shifts				
Yes	184	83	45.1	
No	134	83	61.9	0.004*
Time of milking equipment utilization				
Up to 4 anos	165	73	44.2	
≥ 5 anos	153	93	60.8	0.004*
Cleaner utilization				
Yes	249	129	51.8	
No	69	37	53.6	0.896
Milking equipment cleaning				
Automatic	165	73	44.2	
Manual	153	93	60.8	0.005*
Milkers training				
Sim	69	37	53.6	
Não	249	129	51.8	0.896
Milkers hygiene habits				
Proper	153	93	60.8	
Improper	165	73	44.2	0.005*

* Variables selected and used in the multiple logistic regression (p < 0.15)

Table 2. Univariate analysis results for the factors associated or not to bubaline mastitis considering microbiologic exam count in buffaloes milk

Variable	Total of animals	Positive N	Positive %	P
Breed				
Pure	88	72	81.8	
Crossbred	230	156	67.8	0.019*
Breeding system				
Intensive	69	53	76.8	
Semi-intensive	249	175	70.3	0.360
Water source				
Still	69	53	76.8	
Still + running	249	175	70.3	0.360*
Veterinary assistance				
Permanent	88	72	81.8	
Temporary	230	156	67.8	0.019
Tipe of milking				
Mechanic bucket milking	153	122	79.7	
Mechanic canalized	165	106	64.2	0.003*
Bufaloes up to 3rd lactation (%)				
0 – 59	165	106	64.2	
≥ 60	153	122	79.7	0.003*
Flies control				
Yes	88	72	81.8	
No	230	156	67.8	0.019*
Strip-cup tes				
Yes	299	209	69.9	
No	19	19	100.0	0.010*
Teat washig before milking				
Yes	253	178	70.4	
No	65	50	76.9	0.371
Antisepsis of teats before milking				
Yes	153	122	79.7	
No	165	106	64.2	0.003*
Antisepsis of teats after milking				
Yes	230	156	67.8	
No	88	72	81.8	0.019*
Dry cow therapy				
Yes	65	50	76.9	
No	253	178	70.4	0.371
Clinical mastitis treatment				
Yes	253	178	70.4	
No	65	50	76.9	0.371
Antimicrobian shifts				
Yes	184	125	67.9	
No	134	103	76.9	0.105*
Time of milking equipment utilization				
Up to 4 anos	165	106	64.2	
≥5 anos	153	122	79.7	0.003*
Cleaner utilization				
Yes	249	175	70.3	
No	69	53	76.8	0.360
Milking equipment cleaning				
Automatic	165	106	64.2	
Manual	153	122	79.7	0.003*
Milkers training				
Sim	69	53	76.8	
Não	249	175	70.3	0.360
Milkers hygiene habits				
Proper	153	122	79.7	
Improper	165	106	64.2	0.003*

* Variables selected and used in the multiple logistic regression (p<0.15).

DISCUSSION

A few factors associated to the mastitis occurrence, as much in the SCC elevation, as in the microbiologic exam must be stressed in this study, because although most of them were not confirmed by the multivariate analysis, they may serve as mastitis indicators n herds and must be corrected, taking into account that they were previously reported as important factors for this disease for the bovine species.

In Brazil, few papers were published on risk factors associated to the occurrence of mastitis in bovine. For the bubaline species, no references were found so far about this subject in the consulted literature.

According to Coentrão et al. (2008), the somatic cells count (SCC) in the milk is the most used indicator in programs for mastitis control and prevention around the world. Several factors might influence the SCC variation, being mentioned parturition order, lactation period, month and season of the year (Laevens et al. 1997, Schepers et al. 1997, Cunha et al. 2008). However, the occurrence of intramammary infection is the main factor responsible for the SCC variation (Harmon 1994).

Previous epidemiologic studies on risk factors identified characteristics related to the animal, to the environment, to the handling procedures and to the milking equipment, associated to bovine mastitis and SCC variation (Peeler et al. 2000, Ott & Novak 2001, Berry & Hillerton 2002).

According to the results obtained in the multivariate analysis considering SCC as dependent factor, it was observed that the lack of teat washing before the milking was the most important factor identified. It is known that the teat washing is of major importance for the mastitis control, since this measure removes the dirtiness and redces the infectious agents present in the teats. Another measure that must be performed associated to the washing is the teat drying with individual towel paper and the teat disinfection before and after milking. For buffaloes with aquatic habits, teat washing before milking must be considered as protection measure to prevent the mammary gland infection. Yet, the milker's training for inspection, washing and disinfection of the teats before milking is indicated, mainly in farms where animals remain in grasslands with flooded and muddy areas. This characteristic was observed in every property visited in this study. Yet on this subject, Coentrão et al. (2008) identified in a study with the bovine species that the second greater risk identified was the inexistence of training programs for milkers to perform the milking. In farms where the milkers did not received any kind of training, as explanations about proper procedures during the milking, utilization and maintenance of the milking equipment, examination of the first squirts of milk in every quarters or to proceed the "California Mastitis Test", animals presented 2.51 times more chances of presenting SCC above 200.000 cells/mL. In the farms evaluated by this study, it was observed that milkers did not receive training for the hygienic milking and mastitis prevention. Only in one farm visited, milkers received such orientation, although they did not use the teaching, since the training was punctual and the

staff responsible for the milking did not present any interest for the adoption of these measures, because, according to them, this procedure delays the time of milking.

Souza et al. (2005) also performed a study on risk factors for bovine mastitis in herds in the Zona da Mata, State of Minas Gerais. Variables used in the final models that presented risk (OR) above 2.0 and with $P \leq 0.10$ were: lack of teats antisepsis after milking, providing feed during the milking procedure, lack of adoption of milking line and interactions between lack of adoption of a milking line and lack of teat antisepsis after milking with the providing feed during milking procedure. The lack of disinfection before milking and the lack of war water utilization or the lack of training the milkers presented OR of 7.62. The authors concluded that the factors associated to high SCCLT were the lack of adoption of a milking line, the feeding during the milking and the lack of teat antisepsis after milking. Authors also discussed about the need of getting individual data of SCC or the microbiologic exam in order to identify the risk factors of a herd or an extract inside this herd. Coentrão et al. (2008) identified as risk factors for subclinical mastitis in cows: animals with the udder base along or below the hocks, cracks or fissures in the rubber parts of the milking equipment, unsuitability of the teat holders, cleaning deficiency of the pulsators, lack of the milker's training, not using the microbiologic diagnostic for mastitis, immersion of the teat holders set in disinfectant solution between the milking of distinct animals and total insertion of the antibiotic cannula into the teats at the cow drying.

In the multivariate analysis considering the microbiologic exam, it was observed that the manual cleaning of the milking equipments was the main factor identified in this study. According to literature data, farms that adopt the manual cleaning of the milking equipments must do it so carefully, in such a way that no milk residues or dirtiness remain in it. Another important subject in this cleaning is the utilization of adequate solutions and in sufficient quantities (Costa et al. 1998, Amaral 2004, Medeiros et al. 2009). Yet, it is important to emphasize the need to develop an adequate training of the workers, so they would be able to perform the post-milking activities carefully, in order to reduce the mastitis risks in the herds, and not only provide care and attention during the milking procedure.

CONCLUSIONS

Risk factors for the subclinical mastitis occurrence in dairy buffaloes in farms of the Brazilian Northeastern region are related to characteristics of improper milking handling, as the lack of teat washing and the manual washing of the milking equipments.

Although the employed methodology do not identify all factors associated to subclinical mastitis, risk factors identified in this study must be carefully corrected in order to reduce the frequency of mastitis cases and, thus, to contribute for the control and prevention of this disease in herds.

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