RAW MILK TRADE: PROFILE OF THE CONSUMERS AND MICROBIOLOGICAL AND PHYSICOCHEMICAL CHARACTERIZATION OF THE PRODUCT IN PALOTINA – PR REGION

Comércio de leite cru: perfil do consumidor e caracterização microbiológica e físicoquímica do produto na região de Palotina – PR

Luciano dos Santos BERSOT¹
Heitor DAGUER²
Maike Taís MAZIERO³
José Paes de Almeida Nogueira PINTO⁴
Vinicius Cunha BARCELLOS⁵
Júlia Arantes GALVÃO⁶

ABSTRACT

The aim of this study was to assess the profile of milk consumers in Palotina - PR using a standard questionnaire, and focusing on the identification of the consumer of raw milk. At the same time, microbiological and physicochemical analysis of samples of this milk were also carried out. From 235 interviews conducted, it was found that 10.6% households consumed type B milk; 17.4% consumed type C milk; 33.6% consumed UHT milk and the majority, 38.3% households, consumed raw milk. From the 30 samples of raw milk analyzed, the presence of Escherichia coli and positive coagulase Staphylococcus was observed in at 70 and 73.3% of them, respectively. Mean mesophilic microorganism count was 4.6 x 107 CFU / ml; psychrotrophs, 1.4 x 107 CFU / ml; total coliforms, 9.3 x 106 CFU / ml; thermotolerant coliforms, 5.4 x 105 CFU / ml and mean E. coli count was 6.4 x 103 CFU / ml. Addition of water was found in 20% of all samples; 23.3% of the samples showed high acidity. California Mastitis Test (CMT) was positive in 92.7% of the samples. Based on the results, we concluded that raw milk trade in Palotina is highly common and that the physicochemical and microbiological quality of the milk evaluated was inadequate. Because of all these factors, raw milk consumption is considered to be a cultural, social and technological problem, both for producers and consumers.

Index terms: raw milk; quality; contamination.

1 INTRODUCTION

Due to public health concerns, veterinary inspection of animal products before trade is mandatory, in order to ensure safety and minimum acceptable quality. However, direct trade of raw milk by the producer is a common practice in many parts of Brazil, despite the ban imposed by

the regulations (BRASIL 1969, 1970, 1975, 1997; BADINI et al.,1996). In spite of the hazard that this kind of product could pose to the health of consumers, factors like practicality, low prices and regional culture contribute to the continuous increase in raw milk trade all over Brazil.

Milk is a highly nutritious food and depending on its characteristics, it may represent a source of

Prof. Adjunto I, Curso de Medicina Veterinária, UFPR, Campus Palotina, PR. E-mail: lucianobersot@ufpr.br

² Fiscal Federal Agropecuário, MSc. Ministério da Agricultura, Pecuária e Abastecimento, Laboratório Nacional Agropecuário (LANAGRO/RS), Serviço Laboratorial Avançado em Santa Catarina. Rua João Grumiché, 117, 88102-600, São José, SC, Brasil

³ Doutoranda em Tecnologia de Alimentos – UFPR e-mail: maikemaziero@yahoo.com.br

⁴ Prof^o Doutor Assistente. Faculdade de Medicina Veterinária e Zootecnia, UNESP, Campus de Botucatu, SP, E-mail: josepaes@fmvz.unesp.br

Prof. Assistente I, Curso de Medicina Veterinária, UFPR, Campus Palotina, PR. E-mail: vcbarcellos@ufpr.br.
 Médica Veterinária. UFPR, Campus Palotina, PR

Correspondência: Luciano dos Santos Bersot. UFPR, Campus Palotina. Rua Pioneiro, 2153, Jardim Dallas, Palotina, PR, CEP 85.950-000. Fone: 44 3649-3444. FAX 44 3649-6616

nutrition for the growth of microorganisms from the environment, from the animal, from humans or from utensils and equipment used throughout the production chain, from the moment of milking to final distribution (PONSANO et al., 1990; ÁVILA & GALLO, 1996). According to Santos & Fonseca (2002). Milk temperature and period of storage are equally important, since these two factors are directly linked to the multiplication of microorganisms.

Two main groups of microorganisms contaminate and multiply in milk: deteriorating and potentially pathogenic ones. The first, besides causing economic losses to industries, uses the constituents of milk as substrates for growth, reducing the quality of the products, while the latter poses a hazard for the health of the consumer (DONELLY, 1990; BORR, 1997).

The trade network of informality is characterized by lack of veterinary inspection and technical staff responsible for the farms and industries. Thus, it is not possible to assess microbiological quality of the product, much less to detect frauds such as addition of water, removal of fat, addition of whey or substances to reconstitute density and / or acidity, and presence of potential health hazards, such as preservatives and residues of antibiotics, pesticides and heavy metals.

Ultimately, one of the major issues involving raw milk trade is the potential public health implications. Veterinary inspection and sanitary policies for milk production aim at ensuring that the food meets regulatory standards as a way to guarantee quality and safety.

According to the IBGE (2007), Palotina is a town in the countryside of Parana with an estimated population of 25,765 inhabitants; 5,031 of them are located in rural areas. As a small municipality whose main economic activity is agriculture, it is not uncommon to find milk of small producers in bottles or plastic bags being directly sold to the consumer on the streets.

Considering the importance of milk in human nutrition, the aim of this study was to assess the profile of milk consumers, to identify the consumer of raw milk and to assess their knowledge in relation to the health risks that this type of milk can pose. The physicochemical and microbiological characteristics of raw milk sold in Palotina/PR was also evaluated.

2 METHODS

2.1 Assessment of the consumers' profile

Using a standard questionnaire, we interviewed 235 randomly chosen families between February and June 2005. The questions were initially conducted to determine the type

of milk consumed by the family. Whatever the answer was, the purpose of use, the price, the practice of boiling milk before consumption and / or store it in a refrigerator were assessed, as well as the knowledge of the consumer on possible diseases transmitted by contaminated milk, which they listed. If the person interviewed declared that he or she consumed milk directly from the producer, they were also asked how the milk was delivered and the name of producer/deliverer.

2.2 Sampling

After the interview, and having identified the families who consumed raw milk, an appointment was set for the collection of milk samples in these households, in order to characterize the product as it was delivered to the consumer. After collection, samples were transported in isothermal containers to the Laboratory of Food Microbiology at UFPR/Campus Palotina, where they were examined in up to one hour after sampling. Thirty samples of raw milk were analyzed.

2.3 Microbiological analysis

The following microbiological analysis were conducted: a) total count of strictly and facultative aerobic mesophilic microorganisms; b) total count of psychrotrophic microorganisms; c) Most Probable Number of total and fecal coliforms and thermotolerant *Escherichia coli*; d) coagulase positive *Staphylococcus* counts. All analysis were in accordance with Brazilian official methods established by the Ministry of Agriculture, Livestock and Supply (BRASIL, 2003), except for psychrotrophic counts (SILVA et al., 2007).

2.4 Physicochemical analysis

The following physicochemical analysis were carried out, according to Brazilian official methods (BRASIL, 1981): a) acidity (g of lactic acid/100ml); b) density (g / ml); c) freezing point or cryoscopic index depression (°H); d) fat; e) peroxidase and alkaline phosphatase. Additionally, the CMT (California Mastits Test) was done.

3 RESULTS AND DISCUSSION

From a total of 235 interviews, it was found that 25 families consumed B type milk; 41 families consumed C type milk; UHT milk consumption occurred in 79 families and 90 families consumed raw milk (Figure 1). However, it was observed that 100% of households boiled milk before its consumption.

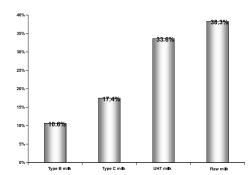


Figure 1 – Distribution of the type of milk consumed in Palotina, from a total of 235 interviews.

The main factors considered important in the choice of raw milk were: "quality", quoted by 117 families; "price", cited by 55 families; "convenience", for being delivered at home, concern mentioned by 37 families; "kinship with the deliverer", referred to by 26 families (Figure 2). A total of 21 small producers who delivered raw milk directly to consumers were identified.

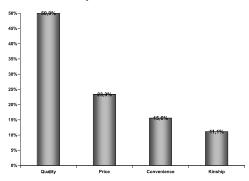


Figure 2 - Distribution of consumers of raw milk (235 families), according to the characteristics considered important for choosing the product.

Over 75% of the consumers interviewed were not aware of problems arising from the consumption of raw milk. Other 24% cited foot and mouth disease as the major disease that can be transmitted by raw milk, followed by brucellosis, tuberculosis and enteric problems.

Table 1 shows data of the physicochemical analysis. Data in bold indicates results outside the range of regulatory standards, and the last column indicates the final result based on the data obtained.

Seven samples (23.3%) showed acidity of 0.18, which is above the limit determined by law. The occurrence of high acidity milk samples denotes

hygiene and conservation problems from the moment of milking to the final consumer. Increased acidity is mainly due to increases in counts of milk bacteria, which down lactose in glucose and galactose, which are then converted in acids, mostly lactic acid (SANTOS & FONSECA, 2007).

Four samples (13.3%) were suspected (but not confirmed) of having added water and six samples (20%) had added water confirmed by the FPD and/or SNF and density. Villa & Pinto (2007) evaluated samples of raw milk in Brotas, Brazil, observing that 6.8% of them showed high acidity and 16% had added water. The physicochemical and microbiological quality of raw milk has been discussed by other authors (PONSANO et al., 1990; ÁVILA et al., 1996; BADINI et al., 1996; NERO et al., 2004; MELVILLE et al., 2006).

All samples showed typical results for raw milk enzyme profile (positive for phosphatase) indicating that the milk was not submitted to heat treatment (data not shown).

The samples that showed water added confirmed that this kind of fraud is quite usual in Brazil. As it may not be detected visually, it increases the profitability of producers by increasing the volume of the product.

CMT was conducted on 27 samples, from which 25 (92.6%) showed positive results (scores 1 or 2) (Table 2). These results show that the control of mastitis, mainly subclinical mastitis that has no obvious signs, is ignored by small milk producers. The occurrence of subclinical mastitis reduces productivity besides bringing to the consumer a product of questionable quality (SANTOS & FONSECA, 2007).

Mean mesophilic aerobic count was 4.6 x 107 CFU / ml; psychrotrophs count was 1.4 x 107 CFU / ml; total coliform count was 9.3 x 106 CFU / ml; thermotolerant coliform count was 5.4 x 10⁶ CFU / ml, and mean Escherichia coli count was 6.4 x 103 CFU / ml (the presence of this group of microorganisms was detected in 73.3% of the samples). Similar hygiene and conservation problems make mean mesophilic aerobic and / or psychrotrophic counts higher, as observed by other authors (BADINI et al., 1996; ZOCCHE et al., 2002; NERO et al., 2004; VILLA & PINTO, 2008). These counts are often higher than the limit set by law (BRASIL, 2002). Thus, if these samples were received in inspected facilities, the product would not be able to be processed.

Currently, the ANVISA (BRASIL, 2001) standard establishes a limit of 4 thermotolerant coliforms / ml for pasteurized milk. Although samples analyzed were not obtained from products submitted to any heat treatment, all samples showed levels of contamination greater than this limit. Nonetheless, the presence of these organisms

is a sign of unsatisfactory hygienic conditions or potential health hazards to the consumer.

The presence of positive coagulase *Staphylococcus* was detected in 21 samples, and in two samples (6.7%) counts were higher than 10⁵ CFU / ml, as seen in Table 3. This result is important since staphylococcal toxin can reach dangerous levels if counts of enterotoxigenic strains exceed 10⁵ (JAY, 2000). High counts of other microrganisms such as

E. coli and / or positive coagulase Staphylococcus, also found by other authors (BADINI et al., 1996; NERO et al., 2004), may be a strong indication of milk serving as a vehicle for pathogens, showing a clear public health problem.

According to Normative Instruction n° 51 (BRASIL, 2002), the limit for mesophilic counts is 1.0 x 106 CFU / ml. Means found in this study were 10 times greater than this regulatory limit.

Table 1 - Results of physicochemical analysis of samples of raw milk traded in Palotina - PR.

| | physicochemical analysis | | | | | |
|---------|--------------------------|-------|-----|------|--------|---------------------------|
| Samples | A | D | G | SNF | FPD | Milk results |
| C001 | 0.26 | 1.031 | 3.4 | 8.74 | -0.562 | Acid |
| C002 | 0.17 | 1.030 | 3.3 | 8.40 | -0.540 | Normal |
| C003 | 0.18 | 1.030 | 5.3 | 8.87 | -0.542 | Normal |
| C004 | 0.17 | 1.030 | 3.0 | 8.26 | -0.543 | Suspect of water addition |
| C005 | 0.16 | 1.030 | 5.4 | 8.94 | -0.561 | Normal |
| C006 | 0.16 | 1.029 | 5.7 | 8.65 | -0.531 | Normal |
| C007 | 0.18 | 1.032 | 3.7 | 8.95 | -0.533 | Normal |
| C008 | 0.17 | 1.029 | 3.5 | 8.21 | -0.536 | Suspect of water addition |
| C009 | 0.17 | 1.031 | 4.5 | 8.81 | -0.555 | Normal |
| C010 | 0.16 | 1.031 | 3.4 | 8.64 | -0.539 | Normal |
| C011 | 0.16 | 1.029 | 4.0 | 8.40 | -0.534 | Normal |
| C012 | 0.16 | 1.027 | 3.5 | 7.71 | -0.518 | Addition of water |
| C013 | 0.19 | 1.030 | 2.1 | 8.08 | -0.528 | Addition of water / acid |
| C014 | 0.24 | 1.030 | 4.6 | 8.63 | -0.533 | Acid |
| C015 | 0.16 | 1.027 | 3.4 | 7.69 | -0.481 | Addition of water |
| C016 | 0.24 | 1.031 | 3.5 | 8.81 | -0.556 | Acid |
| C017 | 0.18 | 1.030 | 5.0 | 8.66 | -0.521 | Addition of water |
| C018 | 0.18 | 1.029 | 3.5 | 8.26 | -0.542 | Suspect of water addition |
| C019 | 0.15 | 1.033 | 4.3 | 9.27 | -0.520 | Addition of water |
| C020 | 0.16 | 1.031 | 3.0 | 8.61 | -0.536 | Normal |
| C021 | 0.16 | 1.030 | 3.5 | 8.71 | -0.541 | Normal |
| C022 | 0.24 | 1.029 | 3.2 | 8.4 | -0.539 | Acid |
| C023 | 0.17 | 1.030 | 3.5 | 8.71 | -0.543 | Normal |
| C024 | 0.14 | 1.031 | 3.7 | 9.0 | -0.541 | Normal |
| C025 | 0.18 | 1.031 | 3.6 | 8.98 | -0.547 | Normal |
| C026 | 0.21 | 1.031 | 3.5 | 8.96 | -0.547 | Acid |
| C027 | 0.17 | 1.026 | 4.0 | 7.56 | -0.546 | Suspect of water addition |
| C028 | 0.16 | 1.028 | 3.3 | 7.92 | -0.520 | Addition of water |
| C029 | 0.17 | 1.030 | 3.0 | 8.34 | -0.537 | Normal |
| C030 | 0.30 | 1.034 | 3.3 | 9.42 | -0.664 | Acid |

A = acidity (g of lactic acid/100ml); D = density (g/ml), G = fat (%); SNF = Solids non-fat (%); FPD= Freezing Point Depression ($^{\circ}$ H)

Table 2 - Number and percentage of the samples analyzed (n = 27) according to the results for the California Mastitis Test (CMT)

| C) 17 | Analysis | | |
|------------|-------------|--|--|
| CMT scores | CMT | | |
| Negative | 02 (7.4) | | |
| (+) | 15 (55.5) | | |
| (++) | 10 (37.1) | | |
| Total | 27 (100%) * | | |

^{*} Three samples were not analyzed.

As seen in Table 3, 23 samples (76.7%) showed counts above this limit.

Although there is no limit determined by Brazilian regulations for psychrotrophs, counts were also high for this group of microorganisms. These results are certainly related to the hygienic problems occurring from the moment of milking to the delivery of the product to consumers.

4 CONCLUSION

Considering the results of this study, it can be concluded that the trade of raw milk in Palotina / PR is very common, since the product is widely accepted by the consumers. However, there is little knowledge on the problems that the consumption of raw milk can pose to public health. According to our results, it can be inferred that the quality of the milk samples analyzed was inadequate, and strongly related to factors such as fraud, poor hygiene during milking, inadequate packaging, storage and transport. Also, misinformation of the producers involved in that important rural activity is widespread.

SUMÁRIO

O objetivo do presente estudo foi realizar um levantamento do perfil do consumidor de leite em Palotina - PR, através de um questionário padrão, com enfoque na identificação do consumidor de leite cru, e realizar análises microbiológicas e físico-químicas desse tipo de leite comercializado em Palotina, PR. Por intermédio das 235 entrevistas realizadas, foi constatado 10,6% das famílias consumiam leite tipo B; 17,4% consumiam leite tipo C; 33,6% consumiam leite UHT e a maioria, 38,3% das famílias consumiam leite cru. Das 30 amostras de leite cru analisadas, verificou-se a presença de Staphylococcus coagulase e Escherichia coli em 70 e 73,3% das amostras, respectivamente. A média das contagens de mesófilos foi de 4,6 x 107 CFU/ml; a de psicrotróficos 1.4 x 10⁷ CFU/ml; coliformes totais 9,3 x 106 CFU/ml, coliformes termotolerantes 5,4 x 105 CFU/ml e a média de E. coli foi de 6,4 x 103 CFU/ml. Foi confirmada a fraude por aguagem em 20% do total de amostras e 23,3% apresentaramse com acidez elevada. Pelo teste do CMT, 92,7% das amostras apresentaram-se positivas. Por meio dos resultados obtidos foi possível concluir que a comercialização de leite cru no município de Palotina é alta e que a qualidade físico-química e microbiológica dos leites avaliados é ruim. Devido a todos estes fatores, o consumo de leite cru deve ser considerado um problema cultural, social e tecnológico, tanto do lado dos produtores quanto dos consumidores.

Termos para indexação: leite cru; qualidade; contaminação

5 BIBLIOGRAPHICAL REFERENCES

ÁVILA, C.R.; GALLO, C.R. Pesquisa de Salmonella spp. em leite cru, leite pasteurizado tipo C e queijo "Minas frescal" comercializados no município de Piracicaba – SP. Scientia Agrícola. Piracicaba. v.53, p.159-163, 1996.

BADINI, K.; NADER FILHO, A.; AMARAL, L.A.; GERMANO, P.M.L. Risco à saúde representado pelo consumo de leite cru comercializado clandestinamente. **Revista Saúde Pública.** São Paulo. v.30, p.549-522, 1996.

BORR, K.J. Pathogenic microorganisms of concern to dairy industry. **Dairy Food and Environmental Sanitation.** Washington. v.17, p.714-717, 1997.

BRASIL. Decreto nº 30.691 de 29 de março de 1952. **Regulamento de Inspeção Industrial e Sanitária de Produtos de Origem Animal.** Alterado pelo Decreto 2.244 de 05 de junho de 1997. Brasilia. Publicado no D.O.U. de 05/06/97, seção 1, página 11.555.

BRASIL. Decreto-Lei nº 923 de 10 de outubro de 1969. **Dispõe sobre a comercialização do leite**. Brasilia. Publicado no D.O.U. em 13/10/1969, seção 1, página 8.601.

BRASIL. Decreto nº 66.183, de 05 de fevereiro de 1970. Regulamenta o Decreto-Lei nº 923, de 10 de outubro de 1969, que dispõe sobre a comercialização do leite cru. Brasilia. Publicado no D.O.U. de 06/02/1970, página 999.

BRASIL. Decreto nº 75.773, de 26 de maio de 1975. Altera o Decreto nº 66.183 de 05 de fevereiro de 1970, que dispõe sobre a comercialização do leite cru. Brasilia. Publicado no D.O.U. de 27/05/1975, página 6.321.

BRASIL. Ministério da Agricultura. Secretaria Nacional de Defesa Agropecuária. Laboratório Nacional de Defesa Animal. Portaria n.1, de 07 de outubro de 1981. O Diretor Geral Substituto... RESOLVE: Aprovar os métodos analíticos para controle de produtos de origem animal e seus ingredientes. XIV — Leite Fluido e provas para leite "in natura". Brasilia. Publicado no D.O.U. de 13/10/1981, Seção 1, Página 19.381.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Gabinete do Ministro. Instrução

Normativa n.51 de 18 de setembro de 2002. O Ministro de Estado da Agricultura,... RESOLVE: Aprovar os Regulamentos Técnicos de Produção, Identidade e Qualidade do Leite tipo A, do Leite tipo B, do Leite tipo C, do Leite Pasteurizado e do Leite Cru Refrigerado e o Regulamento Técnico da Coleta de Leite Cru Refrigerado e seu Transporte a Granel, em conformidade com os Anexos a esta Instrução Normativa. Brasilia. Publicado no D.O.U. de 20/09/2002, Seção 1, página 13.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Instrução Normativa Nº 62 de 26 de agosto de 2003. O Secretário de Defesa Agropecuária...RESOLVE: Oficializar os Métodos Analíticos Oficiais para Análises Microbiológicas para Controle de Produtos de Origem Animal e Água com seus respectivos capítulos e anexos... Brasilia. Publicado no D.O.U. de 18/09/2003, Seção 1, Página 14

BRASIL. Ministério da Saúde. Resolução RDC nº12, de 02 de janeiro de 2001. A Diretoria Colegiada da Agência Nacional de Vigilância Sanitária...adotou a seguinte Resolução e eu, Diretor-Presidente, determino a sua publicação: Aprovar o Regulamento Técnico sobre padrões microbiológicos para alimentos, em Anexo. Brasilia. Publicado no D.O.U. em 10/01/2001.

DONELLY, C.W. Concerns of microbial pathogens in association with daily foods. **Journal of Dairy Science.** Champaign. v.73, p.1656-1661, 1990.

IBGE – Instituto Brasileiro de Geografia e Estatística. Disponível em: http://www.ibge.gov.br/cidadesat/default.php Acesso 2007 nov 12.

JAY, J.M. Staphylococcal Gastroenteritis. In:
_____. Modern Food Microbiology. 6.ed.
Aspen Publication: Gaithersburg, 2000, cap.23,
p.441-459.

MELVILLE, P.A.; RUZ-PERES, M.; YOKOIA, E.; BENITES, N.R. Ocorrência de fungos em leite

cru proveniente de tanques de refrigeração e latões de propriedades leiteiras, bem como de leite comercializado diretamente ao consumidor. **Arquivos do Instituto Biologico.** São Paulo. [periódico on line] 2006; 73. Disponível: http://www.biologico.sp.gov.br/ARQUIVOS/v73_3/melville.pdf> Acesso em: 2007 dez 13.

NERO, L.A.; MATTOS, M.R.; BELOTI, V., NETTO, D.P.; PINTO, J.P.A.N.; ANDRADE, N.J.; SILVA, W.P.; FRANCO, B.D.G.M. Hazards in non-pasteurized milk on retail sale in Brazil: prevalence of *Salmonella* spp., *Listeria monocytogenes* and chemical redisues. **Brazilian Journal of Microbiology.** São Paulo. v.35, p.211-215, 2004.

OLIVAL, A.A.; SPEXOTO, A.A.; CAMPOS, D.F.S.; FERREIRA, F., FONSECA, L.F.L.; SANTOS, M.V.; DIAS, R.A. Hábitos de consumo do leite informal, associados ao risco de transmissão de doenças, no município de Pirassununga, SP. **Higiene Alimentar**. São Paulo. v.16, p.35-40, 2002.

PONSANO, E.H.G.; PINTO, F.F.; DELBEM, A.C.B.; DE LARA, J.A.F.; PERRI, S.H.V. Avaliação da qualidade de amostras de leite cru comercializado no município de Araçatuba e potenciais riscos decorrentes de seu consumo. **Higiene Alimentar.** São Paulo. v.11, p.89-91, 1990.

SANTOS, M.V.; FONSECA, L.F.L. Estratégias para controle de mastite e melhoria da qualidade do leite. Ed. Manole: Barueri, 2007, 328p.

SILVA, N.D.; JUNQUEIRA, V.C.A.; SILVEIRA, N.F.A. Manual de Métodos de Análise Microbiológica de Alimentos. 3.ed. Ed. Varela: São Paulo, 2007, 544p.

VILLA, F.B.; PINTO, J.P.A.N. Qualidade físicoquímica, microbiológica e presença de resíduos de antimicrobianos no leite in natura comercializado informalmente em Brotas, SP. **Higiene Alimentar**. São Paulo. v.22, p.98-103, 2008.

ZOCCHE, F.; BERSOT, L.S.; BARCELLOS, V.C.; PARANHOS, J.K.; ROSA, S.T.M.; RAYMUNDO, N.K. Qualidade Microbiológica e Físico-Química do Leite Pasteurizado Produzido na Região Oeste do Paraná. **Archives of Veterinary Science.** Curitiba. 2002, v.7, p.59-67, 2002.