DOI: 10.5433/1679-0359.2017v38n4p1897

Detection of *Brucella* spp., *Campylobacter* spp. and *Listeria monocytogenes* in raw milk and cheese of uninspected production in the metropolitan area of São Paulo

Detecção de *Brucella* spp., *Campylobacter* spp. e *Listeria monoytogenes* em leite cru e queijo de produção informal na Região Metropolitana de São Paulo

Patrícia de Freitas Kobayashi[1]; Aline Feola de Carvalho[2]; Rodrigo César Fredrigo[1]; Andréa Moura Costa[1]; Rosa Maria Piatti[3]; Eliana Scarcelli Pinheiro[3*]

Abstract

Foodborne diseases are a major public health issue but their overall incidence is underestimated due to insufficient report. The present study aimed to investigate the presence of *Brucella* spp., *Campylobacter* spp. and *Listeria monocytogenes* in raw milk and cheese of uninspected production obtained from cattle bred on the polluted banks of the Tietê River. Generally, milk from these animals is used to prepare fresh cheese, which is then commercialized by the producers themselves or in local markets. We analyzed 81 samples consisting of 38 samples of cheeses, 15 samples of raw milk and 28 samples of water collected from the Tietê River. These samples were evaluated for the presence of the three pathogens using bacteriological methods and the conventional polymerase chain reaction (PCR), with primers specific for each bacterial genus. In the bacteriological examination, all samples were negative for *Brucella* spp., *Campylobacter* spp. and *Listeria monocytogenes*. In the PCR test, *Brucella* spp. was detected in 5/38 (13.16%) cheese samples. *Campylobacter* spp. was present in 18/38 (47.37%) cheese samples, 1/15 (6.66%) raw milk samples and in 12/28 (42.86%) water samples. *Listeria monocytogenes* was not detected by PCR. The detection of *Brucella* spp. DNA in cheese and *Campylobacter* spp. DNA in cheese, milk and water may reflect inadequate animal sanitary management and deficiencies in good manufacturing practices. The presence of these pathogens in the food and water may pose a threat to the health of the consumer and increase the incidence of zoonosis.

Key words: *Brucella* spp. *Campylobacter* spp. *Listeria monocytogenes*. Informal cheese production. Milk.

Resumo

As doenças transmitidas por alimentos (DTA) ainda são subnotificadas, sendo difícil estimar a incidência global. O presente estudo objetivou detectar a presença de *Brucella* spp., *Campylobacter* spp. e *Listeria monocytogenes* em queijo de produção informal e leite cru, provenientes de bovinos criados às margens poluídas do Rio Tietê. O leite desses animais era utilizado na preparação de queijos tipo frescal, comercializados pelos próprios produtores ou no comércio local. No total, 81 amostras foram analisadas: 38 de queijos, 15 de leite cru e 28 alíquotas de água do Rio Tietê. Os materiais foram processados por métodos bacteriológicos e pela reação da polimerase em cadeia (PCR) convencional,

[1] Discentes, Programa de Pós-Graduação em Sanidade, Segurança Alimentar e Ambiental no Agronegócio, Instituto Biológico, IB, São Paulo, SP, Brasil. E-mail: patykobayashi7@hotmail.com; rodrigofredrigo@gmail.com; andreavet2003@yahoo.com.br
[2] Bióloga, M.e, Instituto Biológico, IB, São Paulo, SP, Brasil. E-mail: linebio@gmail.com
[3] Pesquisadoras, Instituto Biológico, IB, São Paulo, SP, Brasil. E-mail: piatti@biologico.so.gov.br; pinheiro@biologico.sp.gov.br
[*] Author for correspondence

Received: Apr. 11, 2016 - Approved: Apr. 06, 2017

específicos para cada gênero. Todas as amostras foram negativas no exame bacteriológico para *Brucella* spp., *Campylobacter* spp. e *Listeria monocytogenes*. Pela PCR, *Brucella* spp. foi detectada em 5/38 (13,16%) queijos de produção informal; *Campylobacter* spp. em 18/38 (47,37%) queijos, 1/15 (6,66%) leite cru e em 12/28 (42,86%) amostras das águas analisadas. *Listeria monocytogenes* não foi detectada pela PCR. A detecção da presença de DNA de *Brucella* spp. no queijo e de DNA de *Campylobacter* spp. no queijo, leite e água, podem indicar manejo sanitário inadequado dos animais e deficiência nas boas práticas de fabricação, com potencial risco para a saúde do consumidor e, aumentando assim, o risco da disseminação de zoonoses.
Palavras-chave: *Brucella* spp. *Campylobacter* spp. *Listeria monocytogenes*. Queijo de produção informal. Leite.

Introduction

Food safety is a growing concern to public health. While several countries have been investing in the identification of risk factors, foodborne diseases are still underreported, making it difficult to estimate their overall incidence. In 2014, *FoodNet* identified 19,507 laboratory-confirmed cases of foodborne infection, 4,476 hospital admissions and 75 deaths in the United States of America (CDC, 2014; CREMONESI et al., 2016).

According to the Food and Agriculture Organization of the United Nations (FAO, 2009), better monitoring and surveillance have helped demonstrate that foodborne diseases are mainly caused by microbiological pathogens of animal origin. The prevention and control of foodborne zoonosis are therefore essential and can be achieved by preventing and controlling diseases and conditions that affect animal health.

The Minas fresh cheese is a type of cheese traditionally produced in the State of Minas Gerais, Brazil. It is prepared with good quality whole milk that has been previously cooked, pasteurized and cooled, with or without the addition of lactic acid, calcium chloride, and liquid rennet. After coagulation, the cheese-making process includes curd cutting, stirring, serum drainage and resting, followed by shaping, pressing and salting. The produced cheese lasts seven days under refrigeration (EMBRAPA, 2004). Ideally, the entire cheese-making process should follow strict hygiene standards, and milk should originate from animals bred under adequate sanitary conditions (CRESPO et al., 2009).

In municipalities located in the metropolitan area of São Paulo (also known as Greater São Paulo), dairy cows graze on the polluted banks of the Tietê River (CETESB, 2015). The cattle owners are small producers with herds ranging from 20 to 50 animals per property who profit from this activity to increase family's income. Some properties are directly connected to the Tietê River and the animals drink the river´s water. Animals from more distant farms are taken to the river banks by trucks, where they are maintained in improvised stalls.

According to Salotti et al. (2006), although the commercialization of fresh and soft cheese prepared from raw milk is prohibited in Brazil, there is an open clandestine marketing of these products. Among the major diseases that can be transmitted by raw milk, contaminated water or cheese from uninspected production, Brucellosis, Campylobacteriosis and Listeriosis are of particular importance.

The present study aimed to detect the presence of three important zoonosis agents, *Brucella* spp., *Campylobacter* spp. and *Listeria monocytogenes*, in samples of raw milk and cheese from uninspected production obtained from dairy cows bred on the banks of the Tietê River, as well as in water samples from this river, whose water was used in these properties.

Material and Methods

From March 2010 to March 2012, we collected 38 samples of fresh cheese and 15 samples of raw milk every 15 days directly from 11 small producers and two local shops. The municipalities surveyed

are located in the metropolitan area of São Paulo (Barueri, Santana do Parnaíba and Pirapora do Bom Jesus), on the banks of the Tietê River.

Milk was obtained straight from the producers before it was commercialized or used for uninspected cheese production. The rural properties to which these animals belonged marketed cheese and unpasteurized milk without any kind of municipal, state, or federal inspection, and did not meet the requirements of the Regulation for Industrial and Sanitary Inspection of Animal Products – RIISPOA (BRASIL, 1997). Milk and cheese were, therefore, considered as food from uninspected production.

We also collected 28 water samples from a total of ten locations along the Tietê River or its streams, which supply these rural properties. The samples were collected every 15 days so that each area was represented at least once. There was no information available on water treatment before its consumption by the animals or its use in cheese preparation.

Cheese samples were kept in their original commercial packages, and milk in the original 2-liter "PET" bottles. Water samples were stored in 80 mL sterile universal collectors. All materials were transported in isothermal boxes with recyclable ice, at a temperature of up to 8°C, and analyzed within 24 hours.

Cheese, milk and water samples were submitted to bacteriological analysis to isolate and identify *Brucella* ssp., *Campylobacter* spp. and *Listeria monocytogenes*, according to Miyashiro et al. (2007), Carvalho et al. (2010) and OIE (2008), respectively, but with slight modifications. Briefly, milk and water samples (50 mL each) were centrifuged at 14,000 x g for 15 minutes to increase the concentration of the target agent. The resulting pellet was resuspended in 2 mL of the supernatant and plated directly in culture plates. For *Brucella* spp, 100 µL of the resuspended pellet was plated in duplicate in BBA (Brucella Blood Agar) plates without antibiotics and in BBA plates supplemented with an antibiotic mixture (DUFTY, 1967) composed of Polymixin B (1.000 UI/L), Cycloheximide (20 mg/L), Novobiocin (5 mg/L) and Bacitracin (15.000 UI/L) (CARVALHO et al., 2010). For *Campylobacter* spp, the resuspended pellets were filtered with a 0.65 µm pore cellulose ester membrane (Millipore®) using a plastic support (swinex®-Millipore®) and 1 ml syringes to eliminate contaminants of larger dimension. Then, 100 µL of this filtrate were cultured in BBA plates with and without antibiotics (MODOLO, 2000; CARVALHO et al., 2010). For *Listeria* spp., the resuspended pellets were cultured in selective PALCAM® medium plates (OIE, 2008).

Cheese samples (25 g) were resuspended in 100 mL of Brucella broth in sterile plastic bags (Nasco®) and homogenized for up to four minutes in a mechanical grinder (STOMACHER® 80 – LAB SYSTEM). Then, 3 mL of the resulting supernatant were centrifuged at 14,000 x g for 15 minutes and the pellet resuspended in 2 ml of the supernatant and cultured in the same media described above (MIYASHIRO et al., 2007, OIE, 2008., CARVALHO, 2010).

The plates were incubated at 37°C in a CO_2 incubator (5%) for the detection of *Brucella* spp., *Campylobacter* spp. and *Listeria monocytogenes*, and also in an aerobic incubator at 37ºC for the detection of *Brucella* spp. for ten days. After the incubation period, colonies were identified with presumptive methods (Gram staining, mobility assessment with dark field microscopy and oxidase test). We validated the presence of *Brucella, Campylobacter, or Listeria* with biochemical tests specific for each genus: catalase, urease, H_2S formation, and sugar fermentation in TSI medium (Triple Sugar Iron – Difco®), hippurate hydrolysis, tolerance to temperatures of 25ºC and 42ºC, susceptibility to nalidixic acid and cephalothin (30 µg) (HOLT et al., 1994; OIE, 2008).

Other microbiota detected in the cultures were subjected to Gram staining in addition to tests for the identification of enterobacteria using a specific kit and following the manufacturer's specifications

(EPM, MILI and Simmons Citrate – Enterokit B – Probac®) (TOLEDO et al., 1982a; TOLEDO et al., 1982b).

We also performed PCR (polymerase chain reaction) analysis to detect DNA of the target microorganisms. DNA from all samples was extracted from the resuspended pellets prepared from cheese, water, and milk samples using the commercial DNAzol®-Invitrogen Kit (CHOMKZYNSKI, 1993) according to the manufacturer's instructions. For the detection of *Brucella* spp., the extracted DNA was subjected to PCR amplification using the protocol described by Baily et al. (1992). For the detection of *Campylobacter* spp., we used the protocol reported in Marshall et al. (1999), and for the detection of *Listeria monocytogenes*, we followed the instructions from Blais et al. (1995). The standard strains *Brucella abortus* (ATCC 544), *Campylobacter jejuni* (ATCC 33291) and *Listeria monocytogenes* (4b) (ATCC 13932) were used as positive controls, and ultra-purified water was used as a negative control. The amplified DNA products of the three pathogens were then analyzed in a 2% agarose gel electrophoresis. The gel was stained with ethidium bromide (0.5 µg/mL), photographed under ultraviolet light (300-320 nm) using the Kodak® Digital DC/120 Zoom Camera, and analyzed with the 1D Image Analysis software (Kodak Digital Science®).

Results and Discussion

In this study, we used both bacteriological and molecular approaches to detect the presence of *Brucella* spp., *Campylobacter* spp. and *Listeria monocytogenes* in samples of raw milk and cheese from uninspected production obtained from dairy cows bred on the banks of the Tietê River, and in water samples from this river.

PCR amplification of 223 bp DNA fragments from *Brucella* spp. demonstrated the presence of this pathogen in 5/38 (13.16%) cheese samples, but not in milk and water samples. Also, we detected 1.004 bp DNA fragments of *Campylobacter* spp. in 18/38 (47.37%) cheese samples, 1/15 (6.66%) raw milk samples and 12/28 (42.86%) water samples. *Listeria monocytogenes* was not detected by PCR in any samples of cheese, milk, or Tietê River water.

In this study, *Brucella* spp. was detected by PCR in cheese samples. This result is similar to what was reported by Miyashiro et al. (2007), who detected the same pathogen in 19.27% of cheese samples from clandestine origin in the States of São Paulo and Minas Gerais. Consistent to that study, the pathogen was detected by PCR amplification but not by pathogen isolation and culture. In a study which analyzed cheese produced in Rio Grande do Sul, Zaffari et al. (2007) failed to isolate and culture *Brucella* spp., but PCR was not used as an additional detection technique. Öngor et al. (2006) also detected this pathogen using PCR in 5% of cheese samples derived from Turkey, but detection was lower than in our study. Namanda et al. (2009) found no signs of Brucella spp. in milk samples in Quenya with either method. However, Langoni et al. (2000) isolated and cultured Brucella spp. from 30,61% milk samples obtained from serologically positive animals in the States of São Paulo and Minas Gerais.

Brucella spp. and *Listeria monocytogenes* were not detected in the water samples by any of the methods used in our study. However, disease dissemination through the water cannot be ruled out, as both pathogens may be excreted in the milk, and *Brucella* spp. can also be excreted in the urine, thereby contaminating the pastures and the river itself. In addition, water pollution must be taken into consideration during sample analysis, as it may interfere with the viability of the cultures of *Brucella* and *Listeria* and impair DNA extraction and pathogen detection. Pacheco et al. (2012) showed that the strain B19 of *Brucella* spp. was excreted in the milk and urine of vaccinated cattle in the State of São Paulo. According to the authors, the excretion of the B19 vaccine strain was predominant at estrus, at 150 days of gestation and immediately

after delivery, persisting until the animals were nine years old.

The presence of *Listeria monocytogenes* was not identified in any of the samples analyzed in our work, similar to what was reported by Barancelli et al. (2011), who found no signs of these bacteria in samples of fresh cheese and raw or pasteurized milk from São Paulo, and Moraes et al. (2009), who also obtained negative results in samples of unpasteurized milk from Minas Gerais.

In our study, 18/38 (47.37%) cheese samples were positive for *Campylobacter* spp., according to PCR analysis. This number was higher than that obtained by Hussain et al. (2007), who found this pathogen in 3/26 (11.50%) samples of cheese from Pakistan, and by Medeiros et al. (2008), who did not detect *Campylobacter* in cheese samples from Canada. Of note, neither of the studies used PCR as a detection method.

Only one sample of raw milk (6.66%) was positive for *Campylobacter* spp. in the PCR analysis, which was lower than what was observed by Hussain et al. (2007), who identified this pathogen in 10,20% of the raw milk samples tested in Pakistan, and by El-Zamkan and Hameed (2016), who detected *Campylobacter* in 24,60% of samples of raw milk and derivatives in Egypt. In contrast, Medeiros *et al*. (2008) failed to isolate and culture this pathogen from any of the collected samples of raw milk in Canada.

Campylobacter spp. was also detected in Tietê River water samples, but its detection was lower than that observed by other authors investigating the presence of these bacteria in rivers, such as Jokinen et al. (2011) in Canada and Rechenburg et al. (2009) in Germany, who reported a prevalence of 26.60% and 86.00%, respectively.

It is important to note that in the present study, water samples positive for *Campylobacter* spp. were collected at different locations along the Tietê River, which surrounded the rural properties of cheese and milk production and where the animals grazed. According to Germano and Germano (2008), the surface waters of rivers, lakes and even the sea near the coast, have a high amount of this bacterium. It is of major concern that the contaminated water of the Tietê River and its marginal vegetation are used for cattle breeding. As the cattle ingest polluted water from the river and the riverside vegetation, it is possible that microorganisms can be excreted in the milk and feces (CALIL et al., 2008), further contaminating the property.

According to the Environmental Protection Agency in São Paulo (CETESB, 2015), in the stretch of the Tietê River that crosses the metropolitan area of São Paulo, the quality of the water is classified as "Bad and Poor" and as inappropriate for human and animal consumption. Water pollution is caused by the presence of biodegradable and inorganic and organic pollutants (fertilizers, clandestine sewage, feces, among others). In the present study, we report an unprecedented PCR detection of *Campylobacter* spp. in river water samples and the presence of other contaminating microorganisms (mainly coliforms and Gram-positive cocci), which led us to consider that this water has potentially been used in critical steps of the cheese-making process, including the use of contaminated utensils and/or inadequate handling by manipulators (VARNAM, EVANS, 1991).

All tested samples were negative in the bacteriological tests for *Brucella* spp., *Campylobacter* spp. and *Listeria monocytogenes*. We observed the presence of colonizing microbiota in all culture media, mainly coliforms (*Escherichia coli*, *Enterobacter* spp. and *Klebsiella* spp.) and Gram-positive cocci, which were not inhibited by the filtration technique or the use of selective media.

A possible explanation for the negative results obtained after isolation and culture of the three pathogens is lactic antagonism (GUEDES NETO et al., 2005; MARQUES et al., 2017). The antagonistic action of lactic acid bacterial species (LAB) against undesirable microorganisms in

food products has been described in the literature. Among LAB species, the genera *Carnobacterium, Enterococcus, Lactococcus, Lactobacillus, Lactosphaera, Leuconostoc, Oenococcus, Pediococcus, Streptococcus Vagococcus* e *Weissella* are of particular importance (GUEDES NETO et al., 2005; MARQUES et al., 2017). Lactid-acid bacteria isolated from milk and cheese, such as *Staphylococcus* spp., *Listeria* spp., *Salmonella* spp., *Bacillus* spp., *Pseudomonas* spp. and bacteria of the coliform group, exerted an inhibitory action against pathogens and spoilage (ALEXANDRE, 2002; CARIDI, 2003; ESCOBAR-ZEPEDA et al., 2016; GUEDES NETO et al., 2005; MARQUES, et al., 2017).

Of note, the method of direct culture, post-centrifugation and filtration may not have been enough to properly isolate these zoonosis pathogens and reduce the intense contamination by other microorganisms. Therefore, modifications of the method should be tested to optimize the detection of these pathogens.

Importantly, the PCR technique was more effective in detecting the three bacteria than the microbiological analyses. This finding supports the use of the PCR technique in laboratories responsible for inspecting water or food of animal origin. Due to its high sensitivity and specificity, this technique should be adopted as a complementary tool to conventional tests.

Conclusion

The detection of *Brucella* spp. in cheese samples and *Campylobacter* spp. in cheese, milk and river water samples renders these materials improper for human and animal consumption, as they can pose a serious threat to the health of consumers. Cattle breeding near the polluted banks of the Tietê River and, consequently, the marketing of raw milk and cheese derived from these animals, are associated with inadequate animal sanitary management and poor hygiene in cheese and milk production. It is therefore necessary to encourage the development of governmental programs to guide small producers to improve the quality and sanitary conditions of their cheese-making processes. In addition, inspection and monitoring of small producers in rural properties should be regulated, from the collection of raw material to the final processing of the milk derivatives, in order to reduce the risk of zoonosis dissemination.

Acknowledgments

The authors would like to thank the *Coordination for the Improvement of Higher Education Personnel (CAPES) for providing a master's degree fellowship to* Patrícia de Freitas Kobayashi.

References

ALEXANDRE, D. P.; SILVA, M. R.; SOUZA, M. R. Atividade antimicrobiana de bactérias lácticas isoladas de queijo-de-minas artesanal do Serro (MG) frente a microrganismos indicadores. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 54, n. 4, p. 424-428, 2002.

BAILY, G. G.; KRAHN, J. B.; DRASAR, B. S.; SOKER, N. G. Detection of *Brucella melitensis* and *Brucella abortus* by DNA amplification. *Journal of Tropical Medicine and Hygiene,* Oxford, v. 95, n. 4, p. 271-75, 1992.

BARANCELLI, G. V.; CAMARGO, T. M.; REIS, C. M. F.; PORTO, E.; HOFER, E.; OLIVEIRA, C. A. F. Incidence of *Listeria monocytogenes* in cheese manufacturing plants from the Northeast Region of São Paulo, Brazil. *Journal of Food Protection,* Des Moines, v. 74, n. 5, p. 816-819, 2011.

BLAIS, B. W.; PHILLIPE, L. M.; PAGOTTO, F.; CORNEAU, N. Applicabilityof the PCR technique in the food testing laboratory: identification of *Listeria monocytogenes*. *Biotechnology Techniques,* Amsterdam, v. 9, n. 9, p. 629-632, 1995.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Regulamento da inspeção industrial e sanitária de produtos de origem animal (RIISPOA*). Brasília: RIISPOA, 1997. 241 p. Available at: <http://www3.servicos.ms.gov.br/iagro_ged/pdf/182_GED.pdf >. Accessed at: 24 feb. 2011.

CALIL, R. M.; SCARCELLI, E.; MODELLI, K. D.; CALIL, E. M. B.*Campilobacterioses*: o agente, a doença e a transmissão por alimentos. São Paulo: R. M. CALIL, 2008. 130 p.

CARIDI, A. Ripening and seasonal changes in microbial groups and in physicochemical properties of the ewes' cheese Pecorino del Poro. *International Dairy Journal*, Amsterdam, v. 13, n. 2-3, p. 191-200, 2003.

CARVALHO, F. C.; SILVA, D. M.; AZEVEDO, S. S.; PIATTI, R. M.; GENOVEZ, M. E.; SCARCELLI, E. Detecção dos genes da toxina citoletal distensiva em estirpes de *Campylobacter jejuni* isoladas de carcaças de frangos. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 62, n. 5, p. 1054-1061, 2010.

CENTERS FOR DISEASE CONTROL AND PREVENTION – CDC. *CDC Estimates of foodborne illness in the United States*. Atlanta: Department of Health and Human Services, 2014. 25 p. Available at: <http://www.cdc.gov/foodnet/reports/index.html>. Accessed at: 3 feb. 2017.

COMPANHIA AMBIENTAL DO ESTADO DE SÃO PAULO – CETESB. Síntese da qualidade das águas no Estado de São Paulo. São Paulo: Secretaria do Meio Ambiente, 2015. 406 p. Available at: <http://www.cetesb.sp.gov.br/agua/infoaguas/>. Accessed at: 03 feb. 2017.

CHOMKZYNSKI, P. A reagent for the single step simultaneous isolation of RNA, DNA and proteins from cells and tissue samples. *BioTechniques*, Orlando, v. 15, n. 3, p. 532-537, 1993.

CREMONESI, P.; CORTIMIGLIA, C.; PICOZZI, C.; MINOZZI, G.; MALVISI, M.; LUINI, M.; CASTIGLIONI, B. Development of a droplet digital polymerase chain reaction for rapid and simultaneous identification of common foodborne pathogens in soft cheese. *Frontiers in Microbiology,* Lausanne, v. 7, n. 1725, 2016.

CRESPO, L. M.; SHIMODA, E.; VIANNA, D. S.; FRANCO, I. S.; PETRUCCI, L. J. T. Olhaduras em queijo minas frescal: correlações com coliformes fecais e análise sensorial. In: ENCONTRO NACIONAL DE ENGENHARIA DE PRODUÇÃO, 29., Salvador, 2009. *Anais...* Salvador: ABEPRO, 2009, v. 1, p. 1-12.

DUFTY, J. H. Diagnosis of vibriosis in the bull. *Australian Veterinary Journal*, Sant Leonards, v. 43, n. 10, p. 433-437, 1967.

EL-ZAMKAN, M. A.; HAMEED, K. G. A. Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in raw milk and some dairy products**.** *Veterinary World*, Wankaner, v. 9, n. 10, p. 1147-1151, 2016.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA – EMBRAPA. *Tecnologia de fabricação do queijo minas frescal*. Brasília: Embrapa Informação Tecnológica, 2004. 54 p. (Comunicado técnico, 125). Available at: <http://www.cpatu.embrapa.br/publicacoes_online/comunicado-tecnico/2004/tecnologia-de-fabricacao-do-queijo-minas-frescal-com-tec-125>. Accessed at: 10 fev. 2012.

ESCOBAR-ZEPEDA, A.; SANCHEZ-FLORES, A.; BARUCH, M. Q. Metagenomic analysis of a Mexican ripened cheese reveals a unique complex microbiota, *Food Microbiology*, Amsterdam, v. 57, n. 8, p. 116-127, 2016.

GERMANO, P. M. L.; GERMANO, M. I. S. *Higiene e vigilância sanitária de alimentos.* Barueri: Manole, 2008. 986p.

GUEDES NETO, L. G.; Souza, M. R.; NUNES, A. C.; NICOLL, J. R.; SANTOS, W. L. M. Atividade antimicrobiana de bactérias ácido-lácticas isoladas de queijos de coalho artesanal e industrial frente a microrganismos indicadores. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia,* Belo Horizonte, v. 57, p. 245-250, 2005. Suplemento 2.

HOLT, J. G.; KRIEG, N. R.; SNEATH, P. H. A.; STALEY, J. T.; WILLIAMS, S. T. *Bergey's manual of determinative bacteriology*. 9. ed. Baltimore: Williams e Wilkins, 1994. 789 p.

HUSSAIN, I.; MAHMOODA, M. S.; AKHTARB, M.; KHAN, A. Prevalence of *Campylobacter* species in meat, milk and other food commodities in Pakistan. *Food Microbiology*, Amsterdam, v. 24, n. 3, p. 219-222, 2007.

JOKINEN, C.; EDGE, T. A.; HOA, S.; KONING, W.; LAING, C.; MAURO, W.; MEDEIROS, D.; MILLER, J.; ROBERTSON, W.; TABOADA, E.; THOMAS, J. E.; TOPP, E.; ZIEBELL, K.; GANNON, V. P. J. Molecular subtypes of *Campylobacter* spp., *Salmonella enterica*, and *Escherichia coli* O157:H7 isolated from faecal and surface water samples in the Oldman River watershed, Alberta, Canada. *Water Research*, Amsterdam, v. 45, n. 3, p. 1247-1257, 2011.

LANGONI, H.; ICHIHARA, S. M.; SILVA, A. V. D.; PARDO, R. B.; TONIN, F. B.; MENDONÇA, L. J. P.; MACHADO, J. A. D. Isolamento de *Brucella* spp. do leite de vacas positivas para bruceloses nos estados de São Paulo e Minas Gerais. *Brazilian Journal of Veterinary Research and Animal Science,* São Paulo, v. 37, n. 6, p. 444-448, 2000.

MARQUES, J. L.; FUNCK, G. D.; DANNENBERG, G. S.; CRUXEN, C. E. S.; EL HALAL, S. L. M.; DIAS, A. R. G.; FIORENTINI, A. M.; SILVA, W. P.

Bacteriocin-like substances of Lactobacillus curvatus P99: characterization and application in biodegradable films for control of *Listeria monocytogenes* in cheese. *Food Microbiology*, Amsterdam, v. 63, p. 159-163, maio, 2017.

MARSHALL, S. M.; MELITO, P. L.; WOODWARD, D. L.; JOHNSON, W. M.; RODGERS, F. G.; MULVEY, M. R. Rapid identification of *Campylobacter*, *Arcobacter*, and *Helicobacter* isolates by PCR-restriction fragment length polymorphism analysis of the 16S rRNA Gene. *Journal of Clinical Microbiology,* Washington, v. 37, n. 12, p. 4158-4160, 1999.

MEDEIROS, D. T.; SATTAR, S. A.; FARBER, J. M.; CARRILL, C. D. Occurrence of *Campylobacter* spp. in Raw and Ready-to-Eat Foods and in a Canadian Food Service Operation. *Journal of Food Protection*, Des Moines, v. 71, n. 10, p. 2087-2093, 2008.

MIYASHIRO, S.; SCARCELLI, E.; PIATTI, R. M.; CAMPOS, F. R.; VIALTA, A.; KEID, L. B.; DIAS, R. A.; GENOVEZ, M. E. Detection of *Brucella abortus* DNA in illegal cheese from São Paulo and Minas Gerais and differentiation of B19 vaccinal strain by means of the polymerase chain reaction (PCR). *Brazilian Journal of Microbiology*, São Paulo, v. 38, n. 1, p. 17-22, 2007.

MODOLO, J. R. Comparação do meio Butzler, técnica de filtração e sua associação para o isolamento de *Campylobacter* spp. *Revista da Sociedade Brasileira de Medicina Tropical,* Uberaba**,** v. 33, n. 2, p. 1-3, 2000.

MORAES, P. M.; VIÇOSA, G. N.; YAMAZI, A. K.; ORTOLANI, M. B. T.; NERO, L. A. Foodborne pathogens and microbiological characteristics of raw milk soft cheese produced and on retail sale in Brazil. *Foodborne Pathogens and Disease*, New Rochelle, v. 6, n. 2, p. 245-249, 2009.

NAMANDA, A. T.; KAKAI, R.; OTSYULA, M. The role of unpasteurized "hawked" milk in the transmission of brucellosis in Eldoret municipality, Kenya. *Journal of Infection in Developing Countries*, Alghero, v. 3, n. 4, p. 260-266, 2009.

ORGANIZAÇÃO DAS NAÇÕES UNIDAS PARA AGRICULTURA E ALIMENTAÇÃO – FAO. The state of food and agriculture. Roma: FAO Publisching Management Service, 2009. 176 p. Available at: <http://www.fao.org/docrep/012/i0680e/i0680e00.htm>. Accessed at: 12 dez. 2009.

WORLD ORGANISATION FOR ANIMAL HEALTH – OIE. Manual of diagnostic tests and vaccines for terrestrial animals. Paris: OFFICE INTERNATIONAL DES EPIZOOTIES, 2008. 598 p. Available at: <http://www.oie.int/doc/ged/D7710.pdf>. Accessed at: 24 feb. 2010.

ÖNGOR, H.; ÇETINKAYA, B.; KARAHAN, M.; BULUT, H. Evaluation of immunomagnetic separation–polymerase chain reaction in direct detection of *Brucella abortus* and *Brucella melitensis* from cheese samples. *Foodborne Pathogens and Disease,* New Rochelle, v. 3, n. 3, p. 245-250, 2006.

PACHECO, W. A.; GENOVEZ, M. E.; POZZI, C. R.; SILVA, L. M. P.; AZEVEDO, S. S.; DID, C. C.; PIATTI, R. M.; PINHEIRO, E. S.; CASTRO, V.; MIYASHIRO, S.; GAMBARINI, M. L. Excretion of *Brucella abortus* vaccine B19 strain during a reproductive cycle in dairy cows. *Brazilian Journal of Microbiology*, São Paulo, v. 43, n. 2, p. 594-601, 2012.

RECHENBURG, A.; KIRSTEMANN, T. Sewage effluent as a source of *Campylobacter* sp. in a surface water catchment. *International Journal of Environmental Health Research*, London, v. 19, n. 4, p. 239-249, 2009.

SALOTTI, B. M.; CARVALHO, A. C. F. B.; AMARAL, L. A.; VIDAL-MARTINS, A. M. C.; CORTEZ, A. L. Qualidade microbiológica do queijo minas frescal comercializado no município de Jaboticabal, SP, Brasil. *Arquivo do Instituto Biológico,* São Paulo, v. 73, n. 2, p. 171-175, 2006.

TOLEDO, M. R. F.; FONTES, C. F.; TRABULSI, L. R. MILi – um meio para a realização dos testes de motilidade, indol e lisina descarboxilase. *Revista de Microbiologia*, São Paulo, v. 13, n. 3, p. 230-235, 1982a.

_____. EPM – uma modificação do meio de Rugai e Araújo, para a realização simultânea dos testes de produção de gás a partir de glicose, H2S, urease e triptofano desaminase. *Revista de Microbiologia*, São Paulo, v. 13, n. 4, p. 309-315, 1982b.

VARNAM, A. H.; EVANS, M. G. *Foodborne pathogens*: an illustrated text. London: Wolfe, 1991. 557 p.

ZAFFARI, C. B.; MELLO, J. F.; COSTA, M. D. Qualidade bacteriológica de queijos artesanais comercializados em estradas do litoral norte do Rio Grande do Sul, Brasil. *Ciência Rural,* Santa Maria, v. 37, n. 3, p. 862-867, 2007.