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Research Article

Identification of *Mycobacterium bovis* in Fresh Cheeses Expended at Markets in the Veracruz-Boca Del Río Metropolitan Area, Mexico

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Abstract: The aim of this study was to identify *Mycobacterium bovis* presence in fresh cheese sold at markets in the Veracruz-Boca del Rio metropolitan area, Mexico and to determine the associated risk factors, using a cross-sectional study. A sample size of 30 was calculated with the program Win Episcope Ver 2.0. Samples were collected in two occasions, during the dry and rainy seasons of 2010. Sampling was conducted in six cheese markets officially registered by the health authorities. Samples were processed by conventional bacteriological and molecular techniques (PCR). *Mycobacterium* spp. was isolated from one of the samples (1.67%), but its typing by biochemical tests was unsuccessful; however, by PCR it was identified as *M. fortuitum*. There were no differences between the variables ($p>0.05$) and no risk factors were identified. We concluded that *Mycobacterium* spp. was isolated, even though it was not identified by PCR as *M. bovis*, but as *M. fortuitum*.

Keywords: Markets, PCR, quality assurance, retailing, risk factors

INTRODUCTION

Currently, food-borne diseases show a worldwide increasing trend and represent a serious public health problem (Pietro *et al*., 2004). As a result and because of an augmented consumer interest in food quality, different international organizations such as the World Health Organization (WHO) are working to ensure food safety, defined as "foods property of being harmless to public health" (Varela and Martínez, 2006).

Food of animal origin and its derivatives are often involved in disease transmission since its contamination originates from animals that may be ill and, in turn, can contaminate meat, milk or eggs (Vásquez, 2003). Dairy products are one of the main sources of food-borne diseases, mainly those coming from artisanal production that does not meet any quality standards and that are commonly consumed unprocessed (Kopper *et al*., 2009).

The WHO has published a list identifying those pathogens transmitted by milk that can cause disease in humans. One of the most important is *Mycobacterium bovis* (Magariños, 2000). In Mexico, about 30% of the milk produced is sold raw and some of it goes to cheese production which is expended in popular markets (Mateos, 2000). Moreover, bovine tuberculosis is an important zoonosis, as people may become infected by *M. bovis*, which represents a public health problem (López de Buen *et al.*, 2007; OIE, 2008). The main routes of entry of *M. bovis* are the digestive and respiratory. Because of this, the main risk factors for people are close contact with infected animals and the consumption of contaminated raw milk or products made from it (Acha and Szyfres, 2003).

In addition, human tuberculosis is one of the most important infectious diseases worldwide. It is currently ranked as the most common cause of death in adults by an infectious agent. According to WHO, one third of the population is infected with tuberculosis, of which 20 million is due to *M. bovis* and every year there are about 8 million new cases (Montoro, 2004). It is estimated that *M. bovis* is responsible for approximately 3% of cases of human tuberculosis in the world (Mateos, 2000).

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In the state of Veracruz, Mexico there is a lack of information regarding the possible contamination of cheese by this organism, so it is important to know if it is possible to identify *M. bovis* from fresh cheese sold at markets in the Veracruz-Boca del Rio metropolitan area and its associated risk factors.

MATERIALS AND METHODS

Location: Cheeses used in this study were collected from cheese retailers officially registered by the health authorities and located in five markets of the Veracruz-Boca del Rio metropolitan area, namely Malibran, Hidalgo, Zaragoza, Unidad Veracruzana and Abastos Boticaria. Laboratory analyses were conducted in the Laboratory of Microbiology Diagnosis, School of Veterinary Medicine and Animal Science, University of Veracruz and at the Veracruz Public Health State Laboratory in the city of Veracruz.

Study design and sampling: This was an epidemiological transversal study. Win 2.0 Episcope program was used to calculate sample size using the option "disease detection" (Thrusfield *et al*., 2001). The prevalence of human tuberculosis caused by *Mycobacterium bovis* was estimated at 3% and a 95% confidence interval was used. Calculated sample size was 30 cheeses to be collected from an equal number of retailers. The total number of cheeses analyzed was 60, of which 30 samples were processed during the dry season and 30 in the rainy season.

During the dry and rainy season of 2010, fresh artisanal cheese samples were collected within five days after its preparation. About 250 g were obtained from each cheese and stored in a Ziploc bag (Ziplok ®) according to the Ministry of Health guidelines for such a purpose (SSA, 1994), including a proper identification for each sample (place, date and time of purchase). Bags were kept inside a cooler box and transported to the Microbiology Laboratory where they were kept frozen at -20°C until processed.

Persons responsible for the retail sales were also interviewed at the time of the cheese sampling procedure. Collected data included cheese´s place of origin, shelf life in display, storage type, if the milk used was pasteurized or not, amount left for self-consumption and other details related to cheese origin, processing and retailing.

Reference strains: For developing diagnostic tests for bacteriology and PCR, reference strains of *M. bovis*, *M. tuberculosis*, *M. avium paratuberculosis* and *M. fortuitum* were provided by the Veracruz Public Health State Laboratory.

Bacteriological methods: For bacteriological diagnosis, standard techniques established by the United States Department of Agriculture (USDA) and the World Organization for Animal Health (OIE) were used (Payeur *et al*., 1993, OIE, 2008). Sample processing was performed within a biological biosafety cabinet class II. Cheese samples were cut into pieces of approximately 2.5 cm^3. Petroff alkali-acid method was used for decontamination (Payeur *et al*., 1993).

Culture media used were those established in the NOM-031-ZOO-1995 (SAGARPA, 1995) and produced by the Productora Nacional de Biológicos Veterinarios (PRONABIVE) namely, Lowenstein-Jensen, Stonebrink, Herrold's with yolk egg and mycobactin. One, two and one tubes of each one of the previous media were inoculated, respectively and were incubated at 37°C. Tubes were weekly reviewed during an 8-week period checking for the presence of colonies with morphology compatible with mycobacteria. Suspected colonies were stained by Ziehl-Neelsen and acid-resistant colonies were identified by biochemical tests of niacin, nitrate, Tween 80, urea, catalase and growth at 42 and 45°C (Payeur *et al*., 1993).

Molecular methods: PCR was used to identify species and was made from the strains isolated by bacteriology. The techniques were those described by Devallois *et al*. (1997) and Leao *et al*. (2005) and routinely used at the Veracruz Public Health State Laboratory in Veracruz.

DNA extraction: DNA was extracted from the isolated colonies on solid media. To extract DNA TE-chloroform was used according to the technique described by Fernández *et al*. (2005).

DNA amplification for the *Mycobacterium tuberculosis* complex: For amplification of DNA the techniques described by Leão *et al.* (2005) and Fernández *et al*. (2005) were used. The goal was to amplify a segment of 123 pb of the insert fragment IS6110, which is present in all mycobacteria of the complex *M. tuberculosis*. For this, the IS1 (5 'CCTGCGAGCGTAGGCGTCGG 3') and the IS2 primers (5 'CTCGTCCAGCGCCGCTTCGG 3') were used. The amplification products were visualized on agarose gels stained with 2% ethidium bromide, as described by Leão *et al*. (2005).

DNA amplification for atypical mycobacteria: For DNA amplification, the PCR-RFLP technique described by Telenti *et al.* (1993) and Devallois *et al*. (1997) were used. The goal was to amplify a segment of 441 pb of the hsp65 gene, which is present in all species of the genus *Mycobacterium*. The amplification product is essential for enzymatic digestion, thus without it no digestion products exists. To do this, primers Tb11 (5 'ACCAACGATGGTGTGTCCAT 3') and Tb12 (5 'CTTGTCGAACCGCATACCCT 3') were used.

Digestion of restriction enzymes: Before performing the digestion with the restriction enzymes, PCR products were analyzed by electrophoresis to verify if the samples amplified a 441 bp fragment. Afterwards, digestion with the restriction enzymes Hae III and BstE

II was performed according to the technique described by Leão *et al*. (2005).

After digestion, restriction fragments analysis was carried out by electrophoresis on 3% agarose gel. Then, species of the analyzed strain were identified by comparing the RPC-RFLP patterns with the open access PRASITE database (PRASITE, 1999).

Statistical analysis: Data were stored in an Excel spreadsheet. Results were analyzed for significance by categorical data analysis using Xi^2 and the association by Odds Ratio (OR) (Thrusfield, 2005). Statistical significance was declared at 0.05.

RESULTS AND DISCUSSION

Isolation and microbiological identification: Only one strain of *Mycobacterium* spp. was isolated from the cheeses collected (1/60, 1.67%, $CI_{95\%}$ 0.09-10.14). This isolate was collected in the dry season at the Abastos Boticaria market and its origin was the nearby municipality of Alvarado. As far as we are aware of, this is the first study conducted in the state of Veracruz to demonstrate the presence of mycobacteria in fresh cheeses. The low prevalence found (1.67%) was lower than the 4.9% determined in the U.S. by Harris *et al*. (2007) in fresh cheeses from Mexican origin. Ikonomopoulos *et al*. (2005) analyzed different types of commercial cheeses from Greece and the Czech Republic by bacterial culture and found an overall prevalence of 3.6%.

There are several reports in the literature on the isolation of *Mycobacterium* spp. from dairy products, particularly milk samples. Hruska *et al*. (2011), analyzed samples of powdered milk in seven European countries and isolated *Mycobacterium* spp. in one of them. In Argentina, Cirone (2004) analyzed fluid milk samples and isolated the germ from two samples, which is similar to what was found in this study.

As colonies growth in the culture media were poor, it was not possible to identify species by biochemical tests. Among other causes of such poor growth, the original concentration of contaminant bacteria or fungus may be a cause. Competition for nutrients with other germs is often crucial to keep the balance between the targeted mycobacteria and possible contamination coming from non-sterile samples (Koneman *et al*., 1997; OIE, 2008).

During the cheese-making process, as well as during its manipulation and display by retailers, the products tend to be contaminated with multiple bacteria. Growth of contaminating flora in the processed samples limited proper development of mycobacteria, slow growing bacteria. Many contaminating bacteria can use substrates for their metabolism and thus prevent the development of mycobacteria; in addition, some also produce bacteriocins which antibiotic function has a negative effect on growth and development of other bacterial populations (Carter and Wise, 2004).

Payeur *et al*. (1993) mentioned that the number of biochemical tests required to properly identifying *Mycobacteria* spp. is extensive and has led to problems of interpretation. Coupled with this is the description of new species whose phenotypic profiles are similar to others already known and that has significantly limited the usefulness of biochemical tests as the sole mean for identifying isolates.

Other negative aspects of these techniques are the need for large numbers of viable bacteria and the slowness in obtaining results (OIE, 2008). These reasons precluded the performance of biochemical tests in this study. However, despite these drawbacks, bacteriological methods are considered the gold standard for diagnosis of tuberculosis (Payeur *et al*., 1993). Moreover, the results of this study support those reported by Patel *et al*. (2000), in the sense that species identification should be performed using a combination of biochemical and molecular techniques.

Species identification by PCR: From the strain of *Mycobacterium* spp. isolated by bacteriology, a processed sample corresponding to 123 bp that identifies the gene IS6110 was used to determine the presence of DNA from *M. tuberculosis*. However, it was not possible to identify any amplified fragment.

The result of the current study differs from that reported by Harris *et al*. (2007), who analyzed fresh Mexican cheeses in the United States and by PCR identified *M. bovis* in a sample; however it was not possible in the present study to isolate in the chesses from the Veracruz-Boca del Río Metropolitan area processed. Durr *et al*. (2000) mentioned that the insertion sequence IS6110 is specific to the tuberculosis complex, which was confirmed in this study, because only the positive controls were able to amplify, but not the strain from the sample of cheese, neither the negative control belonging to the *M. tuberculosis* complex.

PCR was performed from the strain isolated by bacteriology. In this regard, Fernández *et al*. (2005) mentioned that even though DNA can be extracted directly from clinical samples, this is a difficult process given the chance of contamination from the samples, so that DNA quantity and quality is not very good. These techniques have proven to work better from pure culture, thus the diagnosis of tuberculosis by PCR complements the bacteriological diagnosis, but does not replace it yet (Durr *et al*., 2000; Patel *et al*., 2000).

Since the isolate did not belong to the *M. tuberculosis* complex, identification of atypical mycobacteria was intended. In the processed sample, an amplified fragment corresponding to 441 bp was identified (Fig. 1). This fragment identifies the hsp65 gene and was employed to determine the possible presence of DNA from atypical mycobacteria obtained from the strain of *Mycobacterium* spp. isolated by bacteriology.

A study on the identification of atypical mycobacteria in Greece and the Czech Republic,

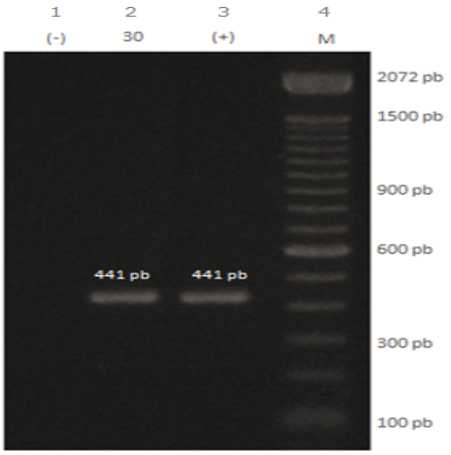

Fig. 1: Fragment amplified by PCR-RFLP hsp65
Lane 1: *M. tuberculosis* negative DNA control; Lane 2: Strain of the sample 30; Lane 3: *M. avium* positive DNA; Lane 4: Molecular marker of 100 to 1500 bp

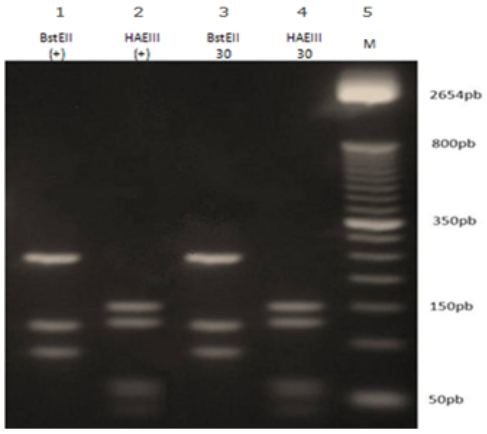

Fig. 2: Enzymatic digestion of the fragment amplified by PCR-RFLP hsp65
Lane 1: Enzymatic digestion with BstEII DNA positive control from *M. fortuitum*; Lane 2: Enzymatic digestion with HaeIII DNA positive control from *M. fortuitum*; Lane 3: Enzymatic digestion with BstEII DNA of the sample 30; Lane 4: Enzymatic digestion with HaeIII of sample DNA 30; Lane 5: Molecular marker of 50-800 bp

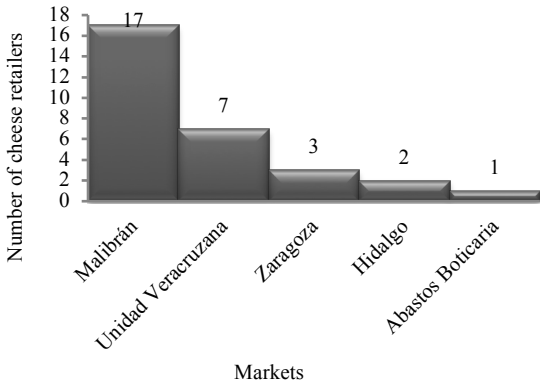

Fig. 3: Number of cheese retailers in markets of the Veracruz-Boca del Rio metropolitan area

analyzed different types of commercial cheeses and isolated *M. avium* subsp. par tuberculosis (Map) in 31.7% of the samples (Ikonomopoulos *et al*., 2005). Hruska *et al*. (2011) analyzed samples of powdered milk in seven European countries. Map was isolated in 49% of the samples, which is a high prevalence for atypical mycobacteria, compared to that obtained in this study (1.67%). In Argentina, Cirone (2004), analyzed samples of commercial fluid milk and isolated Map atypical mycobacteria in 2.8% of the samples, a figure similar to the one found in this study.

Cornejo *et al*. (1998) mentioned that with PCR, the time needed to identify species of mycobacteria is reduced and its use increases the number of positive samples. In addition, PCR solved the problem of negative results due to insufficient material growth for biochemical tests. This coincides with the results of this study, as it was not possible to identify the species by biochemical tests, while PCR identified *M. fortuitum*, which is also an atypical mycobacterium.

Several researchers claim that PCR is more effective than biochemical tests for identification of mycobacteria (Springer *et al*., 1996; Mondragón-Barreto *et al*., 2000; McNabb *et al*., 2004), because conventional methods rely on phenotypic characteristics, a parameter likely to vary in certain species and that could lead to misdiagnosis.

In order to identify species, digestion was performed with restriction enzymes Haelll and BstEll. Amplified fragments from the processed sample were compared with those in the PRASITE database and found to correspond to 235/120/85 pb for BstEll and 145/120/60/55 pb for HAElll, respectively (Fig. 2). Thus PCR identify the hsp65 gene, which in turn was used to determine the presence of DNA of *M. fortuitum* in the strain of *Mycobacterium* spp. isolated by bacteriology.

This study identified *M. fortuitum* in one out of 60 samples (1.67%, $CI_{95\%}$ 0.09-10.14), which coincides with the studies done in the U.S. by Harris *et al*. (2007) in fresh Mexican cheese, which identified *M. fortuitum* by PCR in 3.4% of the samples.

M. fortuitum belongs to the atypical or environmental mycobacteria isolated from water and soil (Leão *et al*., 2005). Hence, it is assumed that cheese contamination occurred at some point along the production or supply chains, or even during its sale in markets. Moreover, *M. fortuitum* is one of the atypical mycobacteria most frequently isolated in humans (Wong *et al*., 2003; Schulz *et al*., 2005; Yzquierdo *et al*., 2007) and is associated with skin infections and rarely with lung diseases. It is a frequent etiologic agent in postoperative infections, especially those performed in heart (Leâo *et al*., 2005).

Determination of risk factors: From the registered markets in the Veracruz-Boca del Rio metropolitan

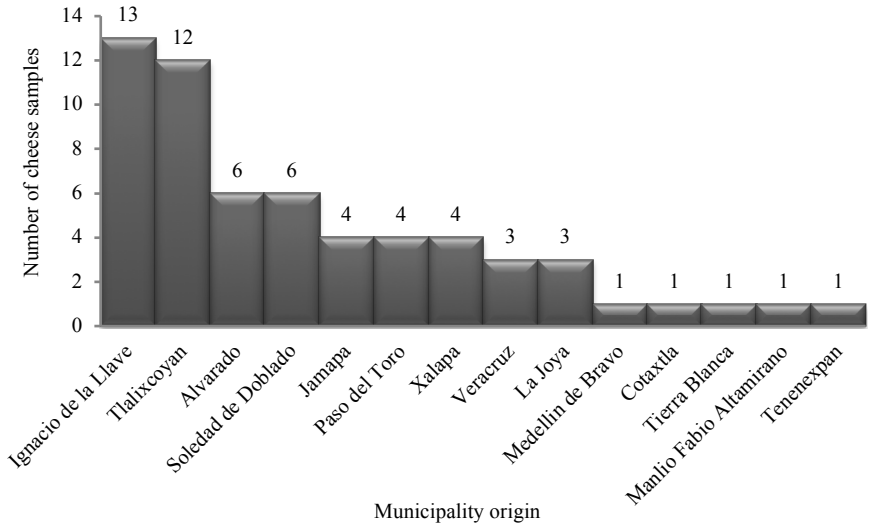

Fig. 4: Localities of origin of cheeses sold in markets of Veracruz-Boca del Rio metropolitan area

area, Malibran is the largest one and has more cheese retailers than the others, so the largest number of samples came from this market, as shown in Fig. 3. The isolated strain of *M. fortuitum* was obtained from the only cheese retailer in the Abastos Boticaria market, so there were no significant differences ($p>0.05$) among market sources.

In the current study, from 30 samples processed during the dry season only one strain of *M. fortuitum* was isolated (3.33%, $CI_{95\%}$ 0.17-19.05). There were no significant differences between seasons ($p>0.05$). This result differs from the study conducted by Berrang *et al*. (2002), which points to the existence of bacteria that exhibit seasonality, i.e., the possibility of concentrated seasonal isolation, but the comparison should be considered with caution because in the current study only one isolation was achieved.

Studies focused on identifying pathogenic bacteria in food, for the most part, were carried out by sampling markets or street vendors. This is because data obtained allow determining the real situation on the sanitary quality of foods and the risk that its consumption entails for the public health (Barroso *et al*., 2007; González *et al*., 2007).

Cheeses sold in the Veracruz-Boca del Rio metropolitan area came from 14 different locations (Fig. 4). Most of the cheese collected (13 out of 60 samples) was originated in the nearby town of Ignacio de la Llave (21.6%, $CI_{95\%}$ 12.4-34.5). The identification of the areas or municipalities from which the products may be contaminated with pathogens is important to properly delimitate areas of public health risk for the consumption of these products (Berrang *et al*., 2002). One of the main routes of infection for tuberculosis is by ingestion of contaminated dairy products, so it is necessary to identify the areas where the disease occurs and the provenance of contaminated food. This would allow an analysis of the population dynamics associated to the transmission and spread of the disease (Leynaud and Reati, 2009).

M. fortuitum isolated strain came from the town of Alvarado, as well as other five negative samples (16.67%, $CI_{95\%}$: 0.8-63.52), so no significant differences ($p>0.05$) for place of origin were found.

Regarding the storage type of cheese that are sold in the dairy markets of the Veracruz-Boca del Rio metropolitan area, it was found that 10 out of 60 were stored in a refrigerator (16.6%, $CI_{95\%}$ 8.7-28.9), 31 were inside a display cabinet (51.6%, $CI_{95\%}$ 38.5-64.6) and 19 were on display at tables placed outside the retailer shops (31.6%, $CI_{95\%}$ 20.6-45.1). Previous studies by Schobitz *et al*. (2001), Franco *et al*. (2001) and Baquero *et al*. (2006), from samples taken at sale points showed that non-optimal hygienic conditions during marketing were associated to product mismanagement. In this study, at the time of cheese purchase, conditions such as cheese poor appearance, deficient storage and in some cases, absence of cooling, were also observed. This situation can be particularly critical when the milk has not been previously pasteurized. Cheese in these conditions is a highly perishable food and high environmental humidity and temperature only contribute to aggravate the problem.

Most of the interviewed cheese retailers buy the products they sell (71.6%, $CI_{95\%}$ 58.3-82.1). Only 17 out of 60 cheese retailers elaborate and sell their own cheese (28.3%, $CI_{95\%}$ 17.8-41.6%). Some bacteria such as *E. coli*, *Salmonella* sp. and *Mycobacterium* sp., can be either direct milk contaminants or participate somewhere along the production chain. In this regard, Cristóbal and Maurtua (2003) identified high mesophilic aerobic bacteria in the finished product resulting from contamination at some point in the chain. Noriega *et al*. (2008) and Rossi *et al*. (2008) conclude that food contamination can occur either during

manufacturing or by the use of contaminated raw materials.

In the selected markets, the majority of the expended cheeses were processed the day before their purchase (51.6%, $CI_{95\%}$ 38.5-64.6), a lesser amount were elaborated within the two previous days (25%, $CI_{95\%}$ 15.1-38.1), or even 3 to 4 days before (23.3%, $CI_{95\%}$ 13.7-36.3). This is important since Rodríguez (2002) mentions that bacteriological studies in perishable foods must be done in the early days after the product is elaborated because as the time passes by, pollution increases and food properties are altered.

Only five out of 60 cheese samples used pasteurized milk (8.3%, $CI_{95\%}$ 3.1-19.1), meaning that a great deal of the cheeses were elaborated from unpasteurized milk (91.7%, $CI_{95\%}$ 60.1-98.5). This represents a critical situation, because one of the major risk factors for acquisition of tuberculosis is the consumption of raw milk, or of contaminated dairy products and byproducts (Acha and Szyfres, 2003).

CONCLUSION

Mycobacterium spp. was isolated by conventional bacteriology techniques in one sample of fresh cheese collected at local markets of the Veracruz-Boca del Rio metropolitan area, in Mexico.

We were unable to identify *M. bovis* by biochemical and molecular techniques. Only *M. fortuitum* was identified by PCR from a sample collected in the Abastos Boticaria market and coming from the municipality of Alvarado. No associated risk factors were identified.

REFERENCES

Acha, P. and B. Szyfres, 2003. Zoonoses and Communicable Diseases Common to Man and Animals. Volume 1: Bacterioses and Mycoses. 3rd Edn., Scientific and Technical Publication No. 580, Pan American Health Organization, Washington, D.C., pp: 266-283.

Baquero, M.A.D., A.M.G. Bernal, S. Campusano, 2006. Determination of *Listeria monocitogenes* in White Cheeses Expended in the Market Place Caqueza, Cundinamarca. Nova Scientific Publication, 4(6): 11-14 (In Spanish).

Barroso, G.P., M.M.A. Lucerna, M.M. Cortes, M.M. Toranzo, M.F.J. Escabias and C.F. Molina, 2007. Outbreak of brucellosis interprovincial for fresh cheese intake without sanitizing. Fam. Med. (And), 7(2): 27-32 (In Spanish).

Berrang, M.E., J.R. Meinersmann, K.J. Northcutt and P.D. Smith, 2002. Molecular characterization of *Listeria monocytogenes* isolated from poultry further processing facility and from fully cooked product. J. Food Protect., 65: 1574-1579.

Carter, G.R. and D.J. Wise, 2004. Essentials of Veterinary Bacteriology and Micology. 6th Edn., Iowa State Press, Ames, Iowa, pp: 107-113.

Cirone, K., 2004. Characterization and viability of *Mycobacterium avium* subsp. paratuberculosis in milk, cheese and water. B.A. Thesis, Faculty of Agricultural Sciences, National University of Mar del Plata. Balcarce, Argentina (In Spanish).

Cornejo, B.J., A. Sahagún-Ruiz, F. Suárez-Güemes, C.G. Thornton, T.A. Ficht, L.G. Adams, 1998. Comparison of C_{18}-carboxypropylbetaine and glass bead DNA extraction methods for detection of *Mycobacterium bovis* in bovine milk samples and analysis of samples by PCR. Appl. Environ. Microbiol., 64(8): 3099-3101.

Cristóbal, D.R.L. and T.D.J. Maurtua, 2003. Bacteriological assessment of fresh artisan cheeses sold in Lima, Peru and the presumed bactericidal action of *Lactobacillus* spp. Am. J. Pub. Health, 14(3): 158-164 (In Spanish).

Devallois, A., K.S. Goh and N. Rastogi, 1997. Rapid identification of mycobacteria to species level by PCR-restriction fragment length polymorphism analysis of the hsp65 gene and proposition of an algorithm to differentiate 34 mycobacterial species. J. Clinic. Microbiol., 35(29): 69-73.

Durr, P.A., R.S. Clifton-Hadley and R.G. Hewinson, 2000. Molecular epidemiology of bovine tuberculosis II. Applications of genotyping. Rev. Sci. Tech. Off. Int. Epiz., 19: 689-701.

Fernández, V.F.A, M.J. Esteban, M.J. González and G.J.J. Palacios, 2005. Procedures in Clinical Microbiology. In: Severed, E. and R. Canton (Eds.), Spanish Society of Clinical Microbiology and Infectious Diseases. Retrieved from: http://www.seimc.org/documentos/protocolos/microbiologia/cap9aindice (Accessed on: February 21, 2012) (In Spanish).

Franco, U.L., P.X. Vargas, I.A. Mendoza, R.M. Bayona and A. Plaza, 2001. Determination of *Escherichia coli* O157 from artisanal meat and dairy products using two insulation systems. Universitas Scientarum (Bogota) 6, Retrieved from: http://www.javeriana.edu.com/universitas_scientiarum/universitas_docs/ VOL6N1/ ART3.htm. (Accessed on: February 21, 2012) (In Spanish).

González, M.D., F.H. Garza and J.M. Sánchez-Yáñez, 2007. Contamination of dairy products by *Brucella* spp and other bacteria in the municipality Higuera, Nuevo Leon, Mexico. Retrieved from: http:// www. monografias. com/trabajos 33/brucelosis (Accessed on: February 21, 2012) (In Spanish).

Harris, N.B., J. Payeur, D. Bravo, R. Osorio, T. Stuber, D. Farrell, D. Palson, S. Treviso, A. Mikolon, A. Rodrígez-Lainsz, S. Cernek-Hoskins, R. Rast, M. Ginsberg and H. Kinde, 2007. Recovery of *Mycobacterium bovis* from soft fresh cheese originating in Mexico. Appl. Environ. Microbiol., 73: 1025-1028.

Hruska, K., I. Slana, P. Kralik and I. Pavlik, 2011. *Mycobacterium avium* subsp. *paratuberculosis* in powdered infant milk: F57 competitive real time PCR. Vet. Med., 56: 226-230.

Ikonomopoulos, J., I. Pavlik, M. Bartos, P. Svastova, W.Y. Ayele, P. Roubal, J. Lukas, N. Cook and M. Gazouli, 2005. Detection of *Mycobacterium avium* subsp. paratuberculosis in retail cheeses from Greece and Czech Republic. Appl. Environ. Microbiol., 71: 8934-8936.

Koneman, W.E., S.D. Allen, W.M. Janda, P.C. Schreckenberger and W.C. Winn, 1997. Color Atlas and Textbook of Diagnostic Microbiology. 5th Edn., Lippincott- Raven, Pub. Philadelphia, pp: 867-915.

Kopper, G., G. Calderón, S. Schneider, W. Domínguez and G. Gutiérrez, 2009. Foodborne diseases and their socioeconomic impact: Case studies in Costa Rica. Honduras and Nicaragua. United Nations Organization for Food and Agriculture, El Salvador, Guatemala, Rome, pp: 190, Retrieved from: www.fao.org/docrep/011/i0480s/i0480s00.htm (Accessed on: February 2, 2012) (In Spanish).

Leão, S.C., A. Bernardelli, A. Cataldi, M. Zumarraga, J. Robledo, T. Realpe, G.I. Mejía, M.A. da Silva Telles, E. Chimara, M. Velazco, J. Fernandez, P.A. Rodrigues, M.I. Guerrero, C.I. Leon, T.B. Porras, N. Rastogi, K.S. Goh, A. da Silva Rocha, D. dos Santos Netto, V. Ritacco, B. López, L. Barrera, J.C. Palomino, A. Martin and F. Portaels, 2005. Multicenter evaluation of mycobacteria identification by PCR restriction enzyme analysis in laboratories from Latin America and the Caribbean. J. Microbiol. Method., 61: 193-199.

Leynaud, G.C. and G.J. Reati, 2009. Identifying risk areas ophidism in Cordoba, Argentina by SIGEPI program. Am. J. Pub. Health, 26: 64-69 (In Spanish).

López de Buen, L., P.A.B. Celma, M.J. Bravo and L.H.M. Zepeda, 2007. Bovine tuberculosis: Re-emerging zoonosis? Sci. Man, 20(3): 51-56. Retrieved from: http://www.uv.mx/ cienciahombre/revistae/vol20num3/articulos/tuberculosis/index.html (Accessed on: February 21, 2012) (In Spanish).

Magariños, H., 2000. Hygienic Production of Raw Milk. Production and Services Incorporated, S.A. (Ed.), Guatemala, pp: 95, Retrieved from: http://www.science.oas.org/OEA_GTZ/LIBROS/LA_LECHE/leche_all.pdf (Accessed on: February 21, 2012) (In Spanish).

Mateos, P.A., 2000. Bovine Tuberculosis as Zoonoses. National Technical Advisory Council for Animal Health, Mexico, pp: 4, Retrieved from: http:// www. conasamexico. org/mesa 9LA% 20TUBERCULOSIS%20BOVINA%20COMO.pdf (Accessed on: February 21, 2012).

McNabb, A., D. Eisler, K. Adie, M. Amos, M. Rodríguez, G. Stephens, W.A. Black and J. Isaac-Renton, 2004. Assessment of partial sequencing of the 65 kilodalton heat shock protein gene (hsp65) for routine identification of *Mycobacterium* species isolated from clinical sources. J. Clinic. Microbiol., 42: 3000-3011.

Mondragón-Barreto, M., A. Vázquez-Chacón, C. Barrón-Rivero, P. Acosta-Blanco, K.C. Jost, S. Balandrano and H. Olivera-Díaz, 2000. Comparison among three methods for mycobacteria identification. Pub. Health Mexico, 42: 484-489 (In Spanish).

Montoro, C.E., 2004. Multidrug resistance: A threat to TB control. Am. J. Pub. Health, 16: 68-73 (In Spanish).

Noriega, R., L. Miguel, V.S. Ibáñez, A.P. González, C.M. Yamamoto, D.J. Astudillo, V.M. González, K.R. Riveros, C.F. Lira, S.A. Marcotti, G.J. Pérez, M.L. Thompson, P.M.F. Daza, I.M. Espinosa, V.C. Pinochet and C.P.A. Vial, 2008. *Listeria monocytogenes*: Report of an increase in cases in pregnant women and literature review. Chil. J. Infect. Dis., 25: 342-349 (In Spanish).

OIE, 2008. Bovine Tuberculosis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (*Terrestrial manual*). World Organisation for Animal Health, Paris, Retrieved from: http://www.oie.int/en/international-standard-setting/terrestrial-code/ (Accessed on: February 21, 2012).

Patel, J.B., D.G.B. Leonard, X. Pan, J.M. Musser, R.E. Berman and I. Nachamkin, 2000. Sequence-based identification of *Mycobacterium* species using the microseq 500 16S rDNA bacterial identification system. J. Clinic. Microbiol., 38: 246-251.

Payeur, J.B., J.L. Jarnagin, J.G. Marquardt, L.A. Schaper and B.M. Martin, 1993. Manual of laboratory methods in veterinary mycobacteriology for isolation and identification of mycobacteria. National Veterinary Services Laboratories, Veterinary Services, Animal Plant Health Inspection Service, United States Department of Agriculture, Ames, Iowa, pp: 14-23.

Pietro, D.S., K. Haritchabalet, G. Cantoni, L. Iglesias, S. Mancini, A. Temperoni, J.L. Labanchi, M.T. García, M. Cofre, S. Rosales, E. Herrero, R. Bigatti, O. Orellana and E. Larrieu, 2004. Surveillance of foodborne diseases in the province of Rio Black, Argentina, 1993-2001. Med. Buenos Aires, 64: 120-124 (In Spanish).

PRASITE, 1999. Identification of Mycobacteria Database. Institute Pasteur/Swiss, National Center for Mycobacteria/Adolfo Lutz Institute/Federal University of Sao Paulo/Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland, Retrieved from: http://app. chuv. ch/prasite/index.html (Accessed on: February 21, 2012).

Rodríguez, G.A., 2002. Key features and diagnosis of pathogenic groups of *Escherichia coli*. Public Health of Mexico, 44: 464-475 (In Spanish).

Rossi, M.L., A. Paiva, M. Tornese, S. Chianelli and A. Troncoso, 2008. Outbreaks of *Listeria monocytogenes* infection: A review of the pathways that lead to their appearance. Chil. J. Infect. Dis., 25(5): 328-335 (In Spanish).

SAGARPA, 1995. NOM-031-ZOO-1995: National Campaign against Bovine Tuberculosis (*Mycobacterium bovis*). Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food, Mexico, pp: 65, Retrieved from: http://www.senasica.gob.mx/includes/asp/download.asp?iddocumento=725&idurl=1251 (Accessed on: February 21, 2012).

Schobitz, R., M. Marín, M. Horzella and E. Carrasco, 2001. Presence of *Listeria monocytogenes* in raw milk and artisan cheeses. Agro. South., 29(2): 114-119 (In Spanish).

Schulz, S., A.D. Cabras, M. Kremer, G. Weirich, T. Miethke, H.C. Bosmuller, H. Höfler, M. Werner and F. Fend, 2005. Species identification of mycobacteria in paraffin embedded tissues: Frequent detection of nontuberculous mycobacteria. Mod. Pathol., 18: 274-282.

Springer, B., L. Stockman, K. Teschner, G.D. Roberts and E.C. Böttger, 1996. Two laboratory collaborative study on identification of mycobacteria: Molecular versus phenotypic methods. J. Clinic. Microbiol., 34: 296-303.

SSA, 1994. NOM-110-SSA1-1994. Preparation and dilution of food samples for microbiological analysis. Ministry of Health, Published in the Official Journal of the Federation on May 10, 1995 Mexico, Retrieved from: http://www. salud. gob. mx/unidades/cdi/nom/110ssa14.html (Accessed on: February 21, 2012) (In Spanish).

Telenti, A., F. Marchesi, M. Balz, F. Bally, E. Bottger and T. Bodmer, 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J. Clinic. Microbiol., 31: 175-178.

Thrusfield, M., 2005. Veterinary Epidemiology. 3rd Edn., Blackwell Science, Oxford, England, pp: 600.

Thrusfield, M., C. Ortega, I. de Blas, J.P. Noordhuizen and K. Frankena, 2001. Win episcope 2.0: Improved epidemiological software for veterinary medicine. Vet. Record., 148: 567-572.

Varela, F.S.E. and G.J.L. Martínez, 2006. Safety, quality and food safety for Mexico. Rev.Digi.U @ T.1 (1). Retrieved from: http://www. turevista. uat. edu. mx/ INOCUIDAD.htm (Accessed on: February 21, 2012).

Vásquez P.G., 2003. The contamination of food: An unsolved problem. UIS Health Mag. (Bucaramanga), 35: 48-57.

Wong, D.A., P.C. Yip, D.L. Tse, V.W. Tung, D.T. Cheung and K.M. Kam, 2003. Routine use of a simple low-cost genotypic assay for the identification of mycobacteria in a high throughput laboratory. Diagnos. Microbiol. Infect. Dis., 47: 421-426.

Yzquierdo, S.S.L., C.L. Mederos, G.A. Díaz, F.M. Echemendia and C.E. Montoro, 2007. Implementation of RPC-RFLP in the diagnosis of non-tuberculous mycobacteria. Chil. J. Infect. Dis., 24: 391-396.