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DETECTION AND SURVIVAL OF *YERSINIA ENTEROCOLITICA* IN GOAT CHEESE PRODUCED IN SAN LUIS, ARGENTINA

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ABSTRACT

Detection limits and the survival of Yersinia enterocolitica *in goat cheese were determined by culture and by nested polymerase chain reaction (PCR). Thirty goat cheese samples inoculated with* 10^4 *to* 10^1 *cfu/g* Y. enterocolitica *O:9 or O:3 strains were enriched for 0, 3 and 18h in trypticase soy broth (TSB), modified Rappaport broth and a formulated in our laboratory broth (FLB). The lowest detection limits were* 1×10^3 *cfu/g by culture on Mac Conkey agar after 3 h TSB and FLB enrichments, and* 1×10^2 *cfu/g by nested PCR at 3 h from all enrichment broths.* Y. enterocolitica *survival was studied in 20 goat cheese samples contaminated at levels of* 1×10^6 *cfu/g and stored at 4° and 22C for 120 days.* Y. enterocolitica *was detected during 7 and 30 days at 22C and 4C, respectively. Total and fecal coliforms were recovered from microflora of goat cheese, but indigenous* Y. enterocolitica *was not detected.*

PRACTICAL APPLICATIONS

In San Luis, Argentina, *Yersinia enterocolitica* strains have been isolated from several foods, samples of animal origin and stool specimens from symptomatic patients. This is the first survey of this microorganism in goat cheese manufactured in our region. The performance of two detection methods, culture and nested polymerase chain reaction, was evaluated in the assessment of the detection limits and the survival of this pathogen in goat cheese. Results might contribute to the knowledge of the behavior of *Y. enterocolitica* in this

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food and reinforce the increasing concern about the microbiological quality of this dairy product. Survival of *Y. enterocolitica* in goat cheese represents a hazard for the consumer health.

INTRODUCTION

Goat's milk cheese represents an alternative food for children with intolerance to cow milk and its subproducts and patients with gastrointestinal diseases (Pellerin 2001; Zeng *et al*. 2007). In our country, approximately 1.5 million liters of goat milk are processed and 150,000 kg of goat cheese are elaborated per year (Federal Council of Science and Technology, Bureau of Science, Technology and Productive Innovation, Argentina, http://www.cofecyt.mincyt.gov.ar/pcias_pdfs/cordoba/UIA_lacteo_caprino_08.pdf, accessed on June 26, 2009). In our region, producers use pasteurized goat milk and traditional manufacturing procedures. These practices imply cheese production on farm, no preservative addition and use of minimal equipment. Even though the inhibitory effect of organic acids and bacteriocins produced by fermenting bacteria in dairy foods on pathogenic and spoilage microorganisms is well established (Gulmez and Guven 2003; Soares Pinto *et al*. 2009), goat cheese is susceptible to be contaminated by different pathogens from elaboration to consumption. The Argentinean Food Code (AFC, http://www.anmat.gov.ar/CODIGOA/CAPITULO_VIII_Lacteos (actualiz10-06).pdf, accessed on March 29 2009) stipulates limited values of coliforms and *Staphylococcus* spp., absence of *Salmonella* spp. and *Listeria monocytogenes*, but no requirements are stated in relation to *Yersinia enterocolitica* in cheeses.

Y. enterocolitica is a human enteropathogen causing enterocolitis, extraintestinal infections and immunologic sequelae depending on the host age and its immunological status, and the serogroup and pathogenic potential of the invading strain. Virulent *Y. enterocolitica* strains belonging to serogroups O:3, O:8, O:9 and O:5,27 harbor both choromosomal and plasmid-associated pathogenic determinants. *Y. enterocolitica* is widely distributed in the environment and gastrointestinal tract of animal reservoirs and its usual route of transmission is thought to be contaminated water or food. Pigs are the major reservoirs of pathogenic *Y. enterocolitica* (Fredriksson-Ahomaa *et al*. 2006). Among other farm animals, goats are considered natural reservoirs of nonpathogenic *Y. enterocolitica* (Escudero *et al*. 1996; Bottone 1999). However, pathogenic and nonpathogenic strains have been isolated from feces of asymptomatic goats in New Zealand (Lanada *et al*. 2005), environments of dairy plants (Cotton and White 1992) and goat milk utilized in the manufacture of Spanish goat cheese (Tornadijo *et al*. 1993), and its survival has been monitored in Crottin goat cheese (Tamagnini *et al*. 2005).

Unless present in extremely large numbers, *Y. enterocolitica* does not survive pasteurization (71.8C for 18 s) (Bottone 1999). The postpasteurization contamination and the ability of this bacterium to grow at refrigeration (4C) or environmental (25C) temperatures could facilitate its survival in goat cheese.

The detection of microorganisms in cheese can be performed by either culture-dependent or culture-independent methods (Jany and Barbier 2008). The culture-dependent methods allow culturing, isolating and identifying of microorganisms based on their phenotypic characteristics. The culture-independent methods such as the polymerase chain reaction (PCR) are based on the direct analysis of DNA. The plasmid-borne *yadA* gene has been used as a PCR target in the detection of pathogenic *Y. enterocolitica* since it encodes the polymeric protein YadA that functions as an adhesin and complement resistance factor (Biedzka-Sarek *et al*. 2008).

The present study was aimed to assess the detection limits of *Y. enterocolitica* in artificially contaminated goat cheeses using a culture method and a nested PCR targeting the *yadA* gene, and to evaluate the survival of potentially pathogenic *Y. enterocolitica* strains in goat cheese samples stored at 4° and 22C. In addition, the presence of indigenous *Y. enterocolitica* and total coliforms (TC) and fecal coliforms (FC) in the microflora of goat cheese manufactured in our region was investigated.

MATERIALS AND METHODS

Samples

A total of 36 goat's milk cheeses weighing approximately 300 g each were purchased from June to December 2008 in retail stores in San Luis, Argentina. These cheeses were elaborated by a local manufacturer following artisanal methods that utilize pasteurized goat milk, calcium chloride, rennet, lactic starters and salt for producing a medium-hard paste. According to manufacturer information, characteristics of these goat cheeses were: 38% maximum moisture, 1.7% sodium chloride, 33% fat and a pH ranging from 5.35 to 6.0. After purchase, cheeses were delivered to the laboratory and processed immediately.

Thirty goat cheeses were processed as follows: each cheese was divided in two portions, one portion was used for assessing *Y. enterocolitica* detection limits by culture and by nested PCR, and the other one was used to investigate the presence of indigenous *Y. enterocolitica* and TC and FC in the microflora of goat cheese. The remaining six cheeses were used in *Y. enterocolitica* survival studies.

Bacterial Strains

The following strains were used: *Y. enterocolitica*, W1024 O:9 pYV (+) kindly provided by Dr. G. Cornelis (Catholic University of Louvain, Belgium), and two local strains, *Y. enterocolitica* 2/O:9 pYV (+) and *Y. enterocolitica* 3/O:3 pYV (+), both isolated in our laboratory from chicken eggshell and feces of a symptomatic patient, respectively. Organisms were maintained at 4C on trypticase soy agar slants (TSA, Merck Laboratories, Darmstadt, Germany). Prior to each experiment, loop inocula were transferred to trypticase soy broth (TSB, Merck), incubated at 22C for 24 h and spread on Mac Conkey agar (MAC, Merck) before inocula preparation.

Investigation of Indigenous *Y. enterocolitica* and TC and FC in Goat Cheese

The presence of indigenous *Y. enterocolitica* and TC and FC coliforms was investigated in uninoculated goat cheese. *Y. enterocolitica* was searched by enrichment of 25-g samples in 225 mL of phosphate buffered saline pH 7.6 added with 1% sorbitol and 0.15% bile salts and incubated 21 days at 4C. After isolating on MAC for 48 h at 22C, presumptive *Yersinia* colonies were subjected to Gram staining and biochemical tests.

TC and FC were investigated in Mac Conkey broth (Merck) at 35C for 48 h by the three-tube most probable number procedure. Presumptive results of TC were confirmed in brilliant green lactose broth (Merck) at 35C for 24 h. FC were confirmed in *Escherichia coli* (EC) broth (Merck) at 44.5C for 24 h, and subsequent isolation on eosin methylene blue agar (Merck). Suspect EC colonies were studied by Gram-staining and biochemical tests.

Detection limits of *Y. enterocolitica* in goat cheese by culture and nested PCR

Inocula Preparation. One colony of each strain was taken from MAC and used to inoculate a flask containing 100 mL of TSB and then incubated 24 h at 22C. The inoculum concentration was standardized at optical density measured at λ 600 nm 0.2 (Metrolab VD 40 Spectrophotometer, Lab. Rodriguez Corswant, Bernal, Argentina) and estimated in 8.59 ± 0.93 log cfu/mL by plating on TSA.

Sample Inoculation. Twenty-five grams of goat cheese samples individually placed in sterile plastic bags were surface inoculated with 1 mL aliquots of decimal dilutions corresponding to each *Y. enterocolitica* strain prepared as described above, submerged in 225 mL of each of three enrichment broths, immediately homogenized in a stomacher (IUL Instruments, Königswinter, Germany) for 90 s, and incubated at 22C for 18 h. The final

bacterial concentrations ranged from 1×10^4 to 1×10^1 cfu/g of goat cheese. Enrichment broths were: TSB (pH 8.2, Merck), modified Rappaport broth (MRB, pH 5.2, Merck) and a formulated in our laboratory broth (FLB, pH 8.2, composition g/L: proteose peptone 5, yeast extract 5, sorbitol 5, sodium pyruvate 1.5 and distilled water). A total of three broths per three *Y. enterocolitica* strains per four dilutions were prepared.

Culture. After 0, 3 and 18 h enrichment, TSB, MRB and FLB aliquots were withdrawn from each flask and subcultured on MAC to perform *Y. enterocolitica* counts. One to four small and smooth lactose-negative colonies were confirmed for *Y. enterocolitica* species by Gram-staining and biochemical screening.

Nested PCR. After 0, 3 and 18 h enrichment, TSB, MRB and FLB aliquots were withdrawn from each flask to perform DNA extraction. One milliliter of enriched sample was centrifuged at 10 000 × g for 5 min. The pellet was suspended in 150 μL of Triton ×100 (1% in TE buffer 1 ×, 1 mM Tris–HCl, 0.1 mM ethylenediaminetetraacetic acid, pH 8). The suspension was boiled for 15 min and then centrifuged at 12,000 × g for 5 min to remove the bacterial debris. A 50 μL aliquot of the supernatant containing DNA was transferred to other Eppendorf tube and stored at 4C.

The nested PCR method of Kapperud *et al*. (1993) modified by Lucero Estrada *et al*. (2007) was used. Five microliters of the template were used for the first PCR step, and 2 μL of the first PCR product was used as template in the second PCR step. Two sets of oligonucleotide primers (Invitrogen, Buenos Aires, Argentina) based on the nucleotide sequence of the *yadA* gene were used. The first primer pair integrated by the following oligonucleotides: YadA1 5′-TAA GAT CAG TGT CTC TGC GGC A-3′ and YadA2 5′ -TAG TTA TTT GCG ATC CCT AGC AC-3′ was used under the following conditions: initial denaturation at 95C for 3 min followed by 40 cycles of denaturation at 95C for 30 s, annealing at 58C for 60 s and extension at 72C for 90 s, and then one cycle at 72C for 10 min. The second primer pair including YadA3 5′-GCG TTG TTC TCA TCT CCA TAT GC-3′ and YadA4 5′ -GGC TTT CAT GAC CAA TGG ATA CAC-3′ was used under the following cycle conditions: initial denaturation at 95C for 3 min followed by 20 cycles of denaturation at 95C for 30 s, annealing at 62C for 60 s, and extension at 72C for 90 s. A final extension at 72C for 10 min was done. PCR was performed in a programmable Gene Amp System 2400 Perkin Elmer thermocycler. The reaction mixture (50 μL) contained 1 U of Taq DNA-polymerase (Invitrogen), 1× PCR buffer (Invitrogen), 200 μmol/mL of each dNTP (Invitrogen) and 0.1 μmol/mL of each primer (Invitrogen). The size of the amplified second PCR product (about 529 bp) was determined in 1% agarose gel by comparison with the 100-bp molecular weight marker (Biody-

namics SRL, Buenos Aires, Argentina). Electrophoresis was carried out at 80 V for 40 min. The bands were visualized by ethidium bromide staining (0.5 μg/mL) in a UV transilluminator (UVP, Upland, CA).

Survival of *Y. enterocolitica* in Goat Cheese

For studying the survival of *Y. enterocolitica* during the storage of goat cheese at 4 and 22C, a three-strain *Y. enterocolitica* cocktail was prepared by combining equal volumes of suspensions of each strain standardized as described above. Six whole cheeses weighing approximately 300 g each were fractionated in 12 portions of 150 g each and homogenized in stomacher (IUL Instruments). Two portions, one stored at 4C and another stored at 22C, were used as negative controls. Homogenates of each one of the 10 remaining 150-g cheese portions were inoculated at a final concentration of 1×10^6 *Y. enterocolitica* cfu/g. A group of five 150-g cheese portions was stored at 4C and the other one was stored at 22C. At 0, 1, 2, 3, 5, 7, 15, 30, 45, 100 and 120 storage days, samples of 10 g inoculated cheese were taken from each storage group and individually placed into 90 mL of TSB at 22C for up to 30 min. Then, 100 μL aliquots were plated on MAC for *Y. enterocolitica* counts and 1 mL volumes were subjected to DNA extraction for nested PCR. When *Y. enterocolitica* was not detected by direct culture on plating media, 24 h TSB enrichment at 22C was performed.

Statistical Analysis

Three replicates of each experiment were performed on different days. Results of plate counts were transformed to $\log_{10}$ cfu/g, and mean values were compared by analysis of variance (Statistix version 3.5 software, Statistix Analytical Software, Tallahassee, FL). Differences were based on confidence level equal or higher than 95% ($P \leq 0.05$ was considered statistically significant). For survival experiments, bacterial counts obtained after each storage day at 4 and 22C were compared with counts obtained on day 0 and the variations of *Y. enterocolitica* populations were estimated as $[\log \text{cfu/g}_{(\text{day } 0)} - \log \text{cfu/g}_{(\text{day } x)}]_{\text{temperature}}$.

Differences between *Y. enterocolitica* counts at different temperatures for each storage day were calculated as $[\log \text{cfu/g}_{(\text{temperature } X)} - \log \text{cfu/g}_{(\text{temperature } Y)}]_{\text{day}}$.

RESULTS

Investigation of Indigenous *Y. enterocolitica*, TC and FC

The investigation of indigenous *Y. enterocolitica* in microflora of goat cheese produced negative results. Estimations of TC ranged from

0.68 ± 0.18 log CT/g to 3.04 ± 0.26 log CT/g (four positive samples). Levels lower than 1 log FC/g (*E. coli*) were detected in two samples.

Detection Limits of *Y. enterocolitica* in Artificially Contaminated Goat's Cheese

A previous enrichment step in TSB, MRB or FLB was included before assessing *Y. enterocolitica* detection limits in goat cheese in order to promote the growth of the target organism. Two protocols (culture and nested PCR) were evaluated for their ability to detect *Y. enterocolitica* strains in the enriched samples.

By culture, no growth was observed on plating medium at 0 h enrichment (nonenriched samples). At 3 h enrichment, the lowest limit of detection observed was 1×10^3 cfu/g for *Y. enterocolitica* B2 O: 9 contaminated samples enriched in TSB and FLB. At 18 h enrichment, the lowest limit of detection corresponded to 1×10^2 cfu/g by culture on MAC for all strains (Table 1). MRB produced *Y. enterocolitica* counts approximately 2 log cfu/g lower than those obtained from TSB and FLB (data not shown).

By nested PCR, the lowest limit of detection after 0 h enrichment was 1×10^3 cfu/g for all the *Y. enterocolitica* strains from all enrichment broths. At 3 h enrichment, the lowest concentration detected was 1×10^2 cfu/g for *Y. enterocolitica* W1024 from TSB and MRB, and for *Y. enterocolitica* B3 O:3 from all the broths. At this time, the detection limit of *Y. enterocolitica* B2 O: 9 corresponded to 1×10^3 cfu/g from MRB and TSB. At 18 h enrichment, all samples contaminated at level of 1×10^2 cfu/g were *yadA* positive (Table 1).

Survival of *Y. enterocolitica*

No significant variations of the number of *Y. enterocolitica* cells were observed at 4C ($P > 0.05$) during the first 15 days of storage (Fig. 1). However, a decrease of 1.4 log cfu/g in *Y. enterocolitica* levels as compared with the initial count (5.76 ± 0.18 log cfu/g) was observed on day 30. This was the last sampling day when *Y. enterocolitica* was detected by culture and nested PCR. In the following days, *Y. enterocolitica* was undetectable in goat cheese by both methods. Negative results were also obtained after 24 h TSB enrichments. At 22C storage, *Y. enterocolitica* counts remained without significant changes from day 0 (5.45 ± 0.49 log cfu/g) to day 5 (5.51 ± 0.55 log cfu/g). *Y. enterocolitica* detection was possible up to day 7 by using culture and PCR methods (Fig. 1).

Significant differences in *Y. enterocolitica* counts were also observed between both temperatures of storage. On day 7, bacterial counts at 22C were

TABLE 1.
DETECTION OF THREE *YERSINIA ENTEROCOLITICA* STRAINS FROM CONTAMINATED GOAT CHEESE BY USING A CULTURE TECHNIQUE AND NESTED PCR

Enrichment broth	Inoculum cfu/g	Culture on plating agar*			Nested PCR		
		W1024	B3 O:3	B2 O:9	W1024	B3 O:3	B2 O:9
0 h–enrichment†							
TSB	1×10^3	–	–	–	+	+	+
	1×10^2	–	–	–	–	–	–
	1×10^1	–	–	–	–	–	–
MRB	1×10^3	–	–	–	+	+	+
	1×10^2	–	–	–	–	–	–
	1×10^1	–	–	–	–	–	–
FLB	1×10^3	–	–	–	+	+	+
	1×10^2	–	–	–	–	–	–
	1×10^1	–	–	–	–	–	–
3 h–enrichment							
TSB	1×10^3	–	–	+	+	+	+
	1×10^2	–	–	–	+	+	–
	1×10^1	–	–	–	–	–	–
MRB	1×10^3	–	–	–	+	+	+
	1×10^2	–	–	–	+	+	–
	1×10^1	–	–	–	–	–	–
FLB	1×10^3	–	–	+	+	+	–
	1×10^2	–	–	–	–	+	–
	1×10^1	–	–	–	–	–	–
18 h–enrichment							
TSB	1×10^3	+	+	+	+	+	+
	1×10^2	+	+	+	+	+	+
	1×10^1	–	–	–	–	–	–
MRB	1×10^3	+	+	+	+	+	+
	1×10^2	+	+	+	+	+	+
	1×10^1	–	–	–	–	–	–
FLB	1×10^3	+	+	+	+	+	+
	1×10^2	+	+	+	+	+	+
	1×10^1	–	–	–	–	–	–

* Mac Conkey agar.
† Nonenriched samples.
+, detection; –, no detection; TSB, trypticase soy broth; MRB, modified Rappaport broth; FLB, broth formulated in our laboratory; PCR, polymerase chain reaction.

1.11 log cfu/g lower than those corresponding to samples stored at 4C. On day 15, differences between temperatures increased since *Y. enterocolitica* counts were nearly 5 log cfu/g lower at 22C than at 4C. On day 30, high levels of *Y. enterocolitica* were still detectable from 4C stored samples, whereas the pathogen was undetectable from 22C stored samples (Fig. 1).

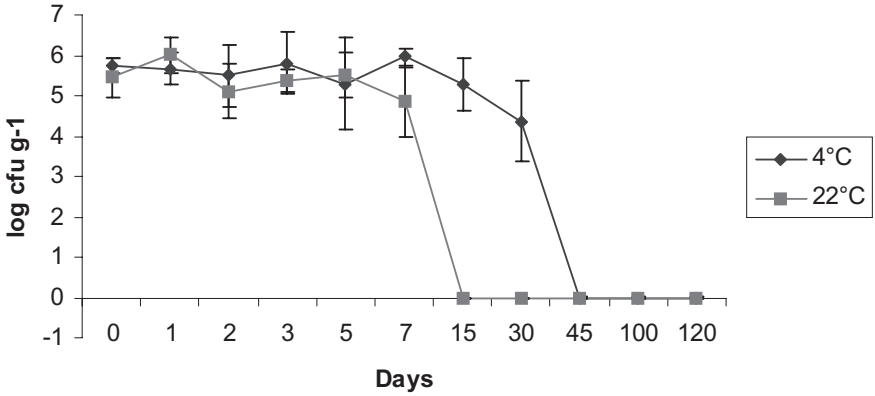

FIG. 1. SURVIVAL OF *YERSINIA ENTEROCOLITICA* IN GOAT CHEESE STORED AT 4 AND 22C

DISCUSSION

This is the first survey of *Y. enterocolitica* in goat cheese manufactured in our region. The AFC establishes microbiological regulations for cheeses of any origin whose moisture contents are between 36 and 46%. Thus, values of up to 500 coliforms per gram at 45C are acceptable in these products. FC are considered indicators for hygienic quality and may correlate with the presence of pathogenic bacteria such as *Y. enterocolitica* (Yucel and Ulusoy 2006). Specific requirements for *Y. enterocolitica* have not been yet established by AFC. Even though coliforms were detected in samples analyzed in the present study, the investigation of indigenous *Y. enterocolitica* produced negative results. Tamagnini *et al*. (2005) evaluated the microbiological quality of five samples of Crottin, a soft goat cheese made in Argentina, and reported absence of TC, *E. coli* and *Y. enterocolitica*. Araya *et al*. (2008) performed a bacteriological evaluation of goat milk and cheese in Costa Rica. They related the absence of TC and FC in cheese to good manufacturing practices. Bonetta *et al*. (2008) performed microbiological analysis on samples of Robiola di Roccaverano cheese, a typical Italian goat milk cheese. They reported coliform levels varying between 4.23 and 6.20 log cfu/g and *E. coli* counts lower than 1 log cfu/g. None of the 27 samples studied by these authors contained pathogenic bacteria. The presence of *E. coli* strains in goat cheese could be explained by factors such as inadequate control of temperature during milk pasteurization, the later addition of raw milk or the bacterial contamination during ripening and storage (Klinger and Rosenthal 1997).

Several studies have reported the use of PCR in addition to culture for the detection of *Y. enterocolitica* from culture media or foods when a low number of pathogen is present (Lantz *et al*. 1998; Knutsson *et al*. 2002; Lambertz *et al*. 2007). Lambertz *et al*. (2007) evaluated a method which consisted of an overnight enrichment followed by detection of pathogenic *Y. enterocolitica* by PCR and culture on a selective agar medium. They obtained a detection limit of at least 25 cfu/g in food by a single PCR. Fredriksson-Ahomaa *et al*. (1999) reported more positive results by nested PCR than by culture when they studied the prevalence of *yadA*-positive *Y. enterocolitica* in contaminated pig tongues and minced meat in Finland. In the present study, nested PCR was more effective than culture in detecting *Y. enterocolitica* from nonenriched goat cheese samples (0 h enrichment). Detection of nonviable cells might be irrelevant for food safety considerations (Lambertz *et al*. 2007); however, a positive result reveals possible contamination events during manufacturing or storing. In this work, three different enrichment broths were used. The selective characteristics of MRB are known. Additionally, two nonselective broths, TSB and FLB, were turned into selective media by preparing them at a final high pH. Since *Y. enterocolitica* can tolerate a wide range of pH, an advantageous growth of this species in the presence of background flora was expected. Using 3 h enrichment, *Y. enterocolitica* detection by culture showed no significant enhancement as compared with 0 h enrichment. Only *Y. enterocolitica* 2/O:9 at level of 1×10^3 cfu/g was detected from TSB and FLB enrichments. At this time, levels of 1×10^2 cfu/g for *Y. enterocolitica* W1024 and 3/O:3 strains were detected by nested PCR. Lantz *et al*. (1998) employed an enrichment step in different media to detect low inocula of *Y. enterocolitica*. The negative PCR results obtained by these authors at the beginning of the enrichment period were just due to low number of target bacteria. Nevertheless, they reported positive results by multiplex PCR after 6 to 8 h enrichment depending on the *Y. enterocolitica* concentration against different levels of background flora.

In our work, *Y. enterocolitica* was detected by nested PCR after 18 h enrichment in all broths. After 18 h enrichment at 25C, Lucero Estrada *et al*. (2007) established a lower detection limit (1 cfu *Y. enterocolitica*/mL) in artificially contaminated pork sausage and minced meat samples by nested PCR than that obtained for *Y. enterocolitica* contaminated goat cheese in this study. The gummy matrix of the cheese samples constitutes a challenge during the homogenization and handling steps before analysis. For instance, partial homogenization of samples may hamper cell lysis lowering DNA availability during the nucleic acid extraction (Jany and Barbier 2008).

The time point of cheese contamination is a very critical question regarding the possibility of survival and persistence of pathogens in foods. In this study, *Y. enterocolitica* was added on the already produced goat cheese

simulating a cross-contamination subsequent to the manufacture process. We found that *Y. enterocolitica* survived for at least 30 days in goat cheese stored at 4C.

Tamagnini *et al*. (2005) suggested that due to its pH 4.9, a 60.5% water content and a low amount of NaCl (1.30%), Crottin goat's cheese might be a suitable substrate for the growth of *Y. enterocolitica.* However, survival but no growth of *Y. enterocolitica* along 42 days in Crottin cheese was observed by these authors. De Lamo-Castellvi *et al*. (2005) studied the survival of the *Y. enterocolitica* pathogenic serotypes O:1, O:3 and O:8 in pasteurized cow milk cheese stored at 8C, and reported that bacterial counts decreased below the detection limit at day 45 probably due to the low pH produced by lactic bacteria during cheese ripening. The control of background microflora by cold storage (4C) as well as the active production of bacteriocins at 22–25C by different lactobacilli species (Miteva *et al*. 1998; Hernandez *et al*. 2005; Lavermicocca *et al*. 2008) could explain differences in the decrease of the *Y. enterocolitica* levels in cheese. Since *Y. enterocolitica* is a psychrotroph, the storage temperatures of cheeses play an important role on its survival. The survival of *Y. enterocolitica* in goat cheese at levels nearly 5 log cfu/g up to day 7 at 22C and day 30 at 4C after cheese contamination arises concern about a potential hazard for the consumer health. *Y. enterocolitica* infective dose of 10^9 cfu has been reported (Doyle 1990).

In conclusion, the microbiological analysis of goat cheese produced in our region revealed absence of *Y. enterocolitica*. *Y. enterocolitica* detection limits in artificially contaminated goat cheese by a culture method and by nested PCR depended on the inoculum size as well as the enrichment time. The low number of viable microorganisms might explain the different results observed at shorter times in the detection of *Y. enterocolitica* strains by culture and nested PCR. At 3 h enrichment, nested PCR was more sensitive than the culture technique in detecting *Y. enterocolitica* from all enrichment broths at levels varying between 1×10^2 and 1×10^3 cfu/g. At 18 h enrichment, *Y. enterocolitica* was detected at concentrations of 1×10^2 cfu/g by using both culture and nested PCR. Further studies should be performed for assessing the *Y. enterocolitica* survival in goat cheese after inoculating the organism at different production stages.

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