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Survival of *Listeria Monocytogenes* in Cheese Made of Unpasteurized Goat Milk

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Tham, W.: Survival of *Listeria monocytogenes* in cheese made of unpasteurized goat milk. *Acta vet. scand.* 1988, 29, 165–172. – Food-borne infections with *Listeria monocytogenes* have been reported during recent years, cheese being mentioned as one of the foods responsible. A classical opinion is that cheese represents a very inhospitable environment for pathogens due to antagonism by the starter culture of lactic-acid-producing organisms. In order to study the survival of *L. monocytogenes* in goat cheese, cheeses were made with the addition of *L. monocytogenes* cultures. The maximum survival time for *L. monocytogenes* was 18 weeks in two of the cheeses. It is concluded that *L. monocytogenes* has the ability to survive in semi-soft cheese made of unpasteurized goat milk during normal curing (2–3 months).

goat cheese; food-borne infections.

Introduction

Listeria monocytogenes is widely distributed in nature (Seeliger & Jones 1986). Humans and animals are frequently exposed to this organism and consequently *L. monocytogenes* may sometimes be harboured in the intestinal canal and thus excreted to the environment (Weis & Seeliger 1975). It causes disease in man and many species of animals (Seeliger & Jones 1986) but cases of illness are sporadic and more rarely epidemic. The infection may lie dormant awaiting the onset of predisposing factors. Especially neonates and immunodeficient persons have little resistance against this opportunistic pathogen (Barza 1985, Fleming et al. 1985). The pattern of disease is remarkably similar in man and animals (Seeliger 1972) and involves meningitis, encephalitis, septicemia, endocarditis and abortion (Seeliger & Jones 1986). *L. monocytogenes* has, furthermore,

been found as a causative agent of mastitis in cows (Wramby 1944, de Vries & Strikwerda 1957, Hyslop & Osborne 1959). The microorganism has also been found in milk from healthy cows (Amtsberg et al. 1969, Vizcaino & Garcia 1975) as well as from healthy goats (Løken et al. 1982).

Little is known about the source of human infection (Bowmer et al. 1979, Schlech et al. 1983, Fleming et al. 1985) although it would appear that food contamination is a likely candidate. The literature mentions coleslaw (Schlech et al. 1983), celery, tomato, lettuce (Ho et al. 1986), milk (Burn 1936, Potel 1953/54, Bowmer et al. 1979, Fleming et al. 1985) and cheese as sources of infection leading to illness or even death. During the first 6 months of 1985 an increased occurrence of listeriosis was noted in Los Angeles and Orange Counties, California. Consumption of Mexican-style fresh cheese from one

particular manufacturer was significantly associated with outbreaks of the disease (James *et al.* 1985). Since this event the Food and Drug Administration in the USA has increased the number of examinations of both imported and domestic cheeses for *L. monocytogenes*. Due to frequent isolations of *L. monocytogenes* the Food and Drug Administration has announced the recall of many cheese lots, consisting mainly of soft cheeses. However, no confirmed outbreaks of illness due to the consumption of these cheeses have been reported (Anonymous 1986). *Listeria* as a food-born pathogen has been highlighted in a review by Ciesielski *et al.* (1987).

It is considered that curing of cheese leads to a decrease or even the loss of pathogenic microorganisms due to antagonism by the starter culture of lactic-acid-producing organisms (Bryan 1983, Northolt 1983). The aim of this study was to investigate the survival of *L. monocytogenes* in semi-soft cheese, made of unpasteurized goat milk, throughout its normal curing time, which is generally 2–3 months.

Materials and methods

Cultures

Three different strains of *L. monocytogenes* were used (Table 1). Strain W had been isolated from a naturally contaminated eight

weeks old goat cheese. The strain was tested for cell shape, Gram reaction, hemolytic reaction on bovine and human blood agar, tumbling motility at 20°C, methyl-red and Voges-Proskauer reactions, fermentation of glucose and rhamnose, hydrolysis of esculine and production of catalase (Seeliger & Jones 1986). The two others were ATCC strains (no. 9493 and 15313). The strains were cultivated on blood agar (Blood agar base from Oxoid containing 5% v/v sterile defibrinated human blood) which was incubated at 37°C for 24 h. From each strain one colony was transferred to 10 ml Brain Heart Infusion (Difco) which was incubated at 37°C for 24 h. Of the culture obtained, 1 ml was used for bacterial counts by cultivation of serial dilutions on blood agar (37°C, 24 h). The remaining 9 ml were poured into 4000 ml goat milk (0.23% v/v) (Table 1).

Cheese-making

In order to study cheese with domestic bacterial flora of various characters, fresh unpasteurized goat milk with no provable *Listeria* bacteria, was obtained from 3 different goat stocks. The milk from each stock was divided into 2 parts and 4 l of each of the 6 parts so formed was poured into a pan which was kept in a waterbath. When the milk reached + 20°C, the culture of lactic-acid-producing organisms (*Leuconostoc cre-*

Table 1. The cultures of *Listeria monocytogenes* added to the different samples of cheese milk.

Goat stock	Milk part/cheese	Organism	Added number per ml milk
A	I	<i>Listeria monocytogenes</i> ATCC 15313	450 000
A	II	„ „ ATCC 9493	600 000
B	III	„ „ ATCC 15313	1 000 000
B	IV	„ „ W	1 200 000
C	V	„ „ ATCC 15313	2 000 000
C	VI	„ „ ATCC 9493	2 060 000

moris, *Streptococcus lactis*, *S. cremoris* and *S. diacetylactis*) and the *L. monocytogenes* broth cultures (Table 1) were added. From now onwards the cheese-making required a good 3 h including curdling, cutting of the curd, stirring and heating, draining off the whey and pressing the curd. During this process the temperature of the curd reached a maximum of + 37°C. The 6 cheeses made were each packed in a plastic bag and cured at + 12°C. The cheese-making procedure is outlined in a time schedule (Table 2).

Determination of *L. monocytogenes*

Every second week during the period of investigation, a 10 g cube, consisting of material from both the surface and the interior,

was cut from each cheese. Using a stomacher, the cube was macerated and blended with 90 ml Brain Heart Infusion (Difco) containing 8 % NaCl. Ten-fold serial dilutions (10^{-2} – 10^{-5}) of 1 ml macerate were made in peptone water. The macerate and each dilution were then pour-plated in 1 ml portions into blood agar (Blood Agar Base from Oxoid containing 5 % v/v sterile defibrinated human and ovine blood respectively). Incubation was performed at 37°C for 48 h and presumptive *L. monocytogenes* colonies were counted. From each of the various platings, 5 presumptive *L. monocytogenes* colonies were isolated and submitted to the same confirmatory tests as strain W.

The remaining macerate was incubated at

Table 2. The cheese-making procedure.

Time from start (min)	Procedure
0	4000 ml milk was poured into a stainless pan placed in a waterbath. Heating.
60	When the milk reached + 20°C the culture of lactic-acid-producing organisms*) and the <i>L. monocytogenes</i> broth culture (Table 1) were added. Heating.
120	When + 30°C was reached, 20 ml rennet (Ostlöpe, Apoteksbolaget) diluted with 20 ml sterile water was thoroughly mixed with the milk to start curdling.
165	The firm jelly-like curd was broken into pieces of about 1 cm ³ with a kitchen beater in order to release the whey from the curd. Heating. The curd was kept in motion by stirring to prevent the pieces of curd from sticking together and forming lumps.
225	+ 37°C was reached and maintained until the curd had attained the desired degree of firmness.
265	The whey was gradually ladled off. The curd and the remainder of the whey were placed into a colander where more whey was drained off. Then the curd was lined with muslin cloth and placed in a 0.5 l plastic container with holes in the bottom. A plastic piece of the same size as the internal measurements of the container was placed on top of the curd and weight was applied for 1 h. The cheese obtained was then kept in a salt brine (16–18 %w/v) for 10 h. Finally, the cheese was packed in a plastic bag and cured at + 12°C.

*) 40 ml fresh whole goat milk which has been boiled for 1 h, cooled and then mixed with 10 ml fermented whole cow milk (filmjök, a dairy product marketed in Sweden). The mixture was incubated at room temperature for 24 h.

+ 4°C according to *Gray et al.* (1948) – a cold enrichment method recommended by *Hayes et al.* (1986) and *Doyle & Schoeni* (1987). This cold enrichment sample was examined for *L. monocytogenes* after 3, 4, 5 and 6 weeks as follows. Portions of 0.2, 0.5 and 1.0 ml were pour-plated into, and a portion of 0.2 ml was surface-plated onto, blood agar (human and ovine erythrocytes respectively). A 0.2 ml portion of the cold enrichment sample was also added to 5 ml *Listeria* Enrichment Broth (Merck). All samples were incubated at 22°C for 48 h. One loopful from the *Listeria* Enrichment Broth was then spread onto *Listeria* Selective Agar (Merck) and onto blood agar (human and ovine erythrocytes respectively). The plates were incubated at 37°C for 48 h. From each of the various platings, 5 presumptive *L. monocytogenes* colonies were isolated and submitted to the same confirmatory tests as strain W.

Other determinations

Every fourth week the macerate and dilutions were also investigated for the following: Total aerobic count on Tryptone Glucose Extract Agar (Difco) incubated at 30°C for 72 h; enterococci on Slanetz and Bartley Medium (Oxoid) incubated at 44°C for 48 h; coagulase positive staphylococci on Baird-Parker Medium (BBL) incubated at 37°C for 48 h (production of coagulase was used as a confirmatory test); coliform bacteria on Violet Red Bile Agar (Difco) incubated at 37°C for 24 h. The pH of the macerate was measured with a pH meter (Orion Research, model 701 A).

Results

Determination of L. monocytogenes

L. monocytogenes could be isolated from cheeses V and VI by direct plating which made quantitative determination possible

(Fig. 1). From cheeses II, III and IV *L. monocytogenes* could be isolated only through the cold enrichment method (Fig. 2). From cheese I *L. monocytogenes* could not be isolated either by direct plating or after enrichment (Fig. 2).

Other determinations

Cheeses I, II, III and IV contained maximum numbers of 25 million enterococci, 60 000 coagulase positive staphylococci and 3 million coliform bacteria per gram. These bacteria were more or less present throughout the investigation.

Cheeses V and VI contained maximum numbers of 10 000 enterococci, 1 000 coagulase positive staphylococci and fewer than 10 coliform bacteria per gram. After 10 weeks the enterococci and the coagulase positive staphylococci were also fewer than 10 per gram.

The total aerobic counts for all the cheeses ranged from 10^6 to 10^9 per gram depending on sampling occasion. The total aerobic counts for cheeses V and VI are depicted in Fig. 1. The pH varied within the interval of 5.5 to 6.5 in all the cheeses, the lower levels generally being in the beginning of the curing and the higher levels at later stages.

Discussion

In cheeses V and VI, *L. monocytogenes* could be quantitatively determined for as long as 18 weeks. *Ikonomov & Todorov* (1964) report that *L. monocytogenes* survived for 10 weeks in cheese made of unpasteurized ewe's milk when the cheese was stored at 18–20°C and *Sipka et al.* (1974) that the number of *L. monocytogenes* in a cheese made of naturally contaminated, unpasteurized cow milk, increased dramatically during the curing – from 52 *L. monocytogenes* per g to 1 million in 14 days, before declining gradually to 100 000 after 4 weeks.

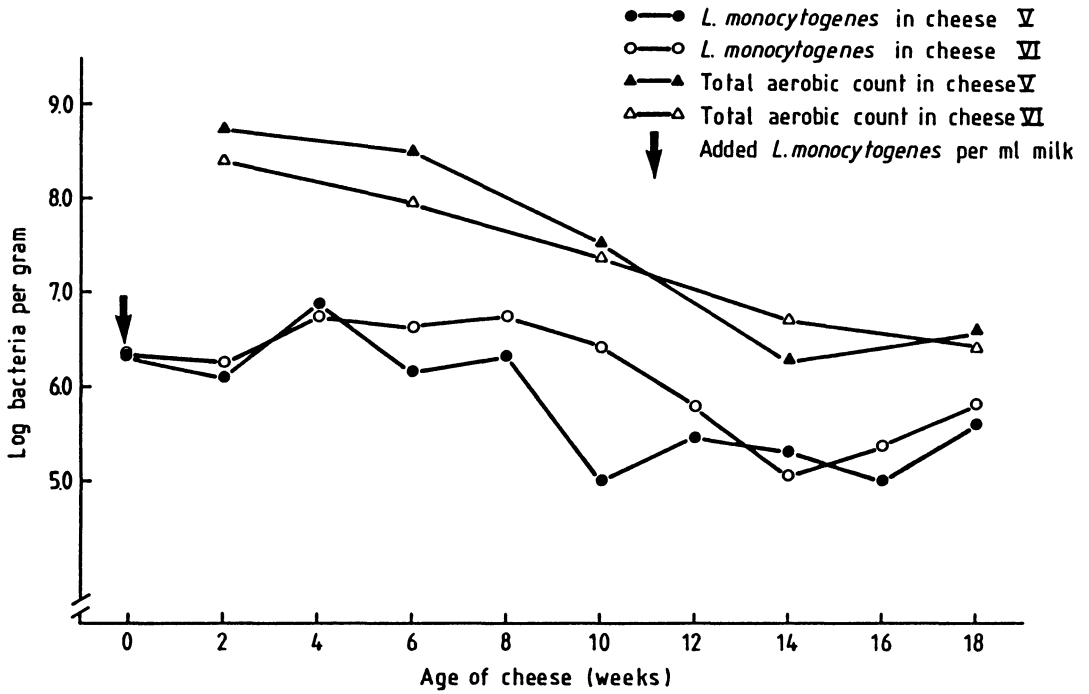


Figure 1. Variations in numbers of *L. monocytogenes* and in total aerobic counts in cheeses V and VI.

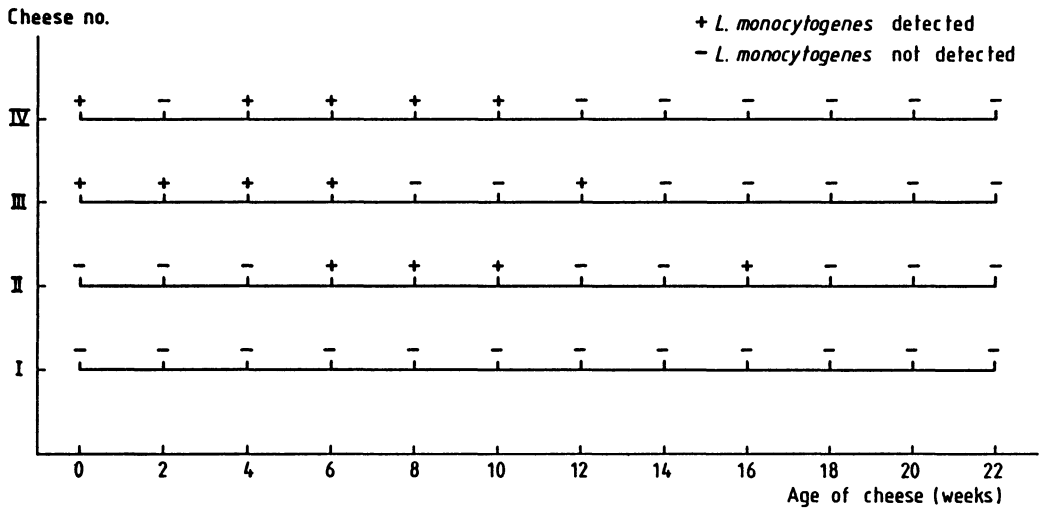


Figure 2. Isolations of *L. monocytogenes* from cheeses I-IV by the enrichment method.

After 6 weeks only 116 remained per g. The faster decline in the cheeses investigated by *Sipka et al.* (1974) than in cheeses V and VI in the present investigation may be due to the differences in pH (4.50 to 5.11 in their study, 5.55 to 6.20 in ours). Growth of *L. monocytogenes* is reported not to occur below pH 6 (*Seeliger & Jones* 1986). In one study (*Ryser & Marth* 1987), dealing with survival of *L. monocytogenes* in Camembert cheese, this organism was found to require a pH greater than 5.5 to multiply. These cheeses, however, were made of pasteurized cow milk.

The long survival time of *L. monocytogenes* in cheeses V and VI may, besides a suitable pH, be due to the initially low rate of some of the competitive flora and also to the gradual decrease of it (Fig. 1).

Despite the fact that all the six milk samples used in this study contained fairly large numbers (450 000 – 2 060 000/g) of *L. monocytogenes* (Table 1) compared to the naturally infected milks reported by *Dedié & Schulze* (1957) (50 000/ml) and *Donker-Voet* (1963) (2 000 – 20 000/ml), the organisms could not be recovered in one of the cheeses (Cheese I) and only after cold enrichment could they be detected in three others (Cheeses II, III and IV). However, the difficulty in cultivating *L. monocytogenes* in specimens containing a mixed bacterial population is well documented (*Donker-Voet* 1963, *Bojsen-Møller* 1972). Special prominence is given to the enterococci as "confusing" (*Seeliger* 1972) organisms since their nutrient and growth requirements (*Seeliger* 1972) as well as colony appearance are similar to those of *L. monocytogenes*. The normal goat cheese represents an environment of mixed microbial growth and the enterococci are often present in large numbers (*Tham et al.*, to be published).

In the literature, only one study has been found (*Comi et al.* 1987) where *L. monocytogenes* were found in goat cheese. This goat cheese was made under uncertain hygienic conditions.

Conclusion

L. monocytogenes is found capable of surviving throughout the common curing period of semi-soft cheese made of unpasteurized goat milk.

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Sammanfattning

Överlevnaden av Listeria monocytogenes i ost tillverkad av opastöriserad getmjölk.

Under senare år har ett antal livsmedelsburna infektioner orsakande av *Listeria monocytogenes*

rapporterats. Vid ett par av dessa har ost misstänkts vara det orsakade livsmedlet. Ost har av hävd ansetts utgöra en ogästvänlig miljö för patogena mikroorganismer p g a antagonistisk verkan från syrakulturerna.

Avsikten med föreliggande arbete var att studera överlevnaden av *L. monocytogenes* i ost tillverkad av opastöriserad getmjölk. *L. monocytogenes* ympades i mjölken och osten tillverkades sedan på sedvanligt sätt. Som längst kunde *L. monocytogenes* påvisas i 18 veckor i två av ostarna.

L. monocytogenes kan således finnas kvar efter den normala mognadstiden (2-3 månader).

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