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Risk factors involved in the contamination of Swiss cheeses by *Listeria monocytogenes* and coagulase-positive staphylococci

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Abstract

In order to fulfil the requirements of the European Union to facilitate the trade between Switzerland and European countries, an annual national monitoring programme of milk and dairy products (NMPD) was initiated in 2003. The aims of the NMPD are to assess the prevalence of microbial hazards in dairy products and to identify products and practices with a higher risk. Data collected from 2003 to 2010 are described and analysed in this paper. The focus is kept on *Listeria* and on staphylococci, but because of an unequal dataset, no significant results could be shown for *Listeria*. The results point out that the type of dairy (alpine, artisanal, farm-based or industrial) as well as the type of cheese (soft, semi-hard, hard or fresh) plays a role in the contamination of final products by coagulase-positive staphylococci. It was found that fresh and soft cheese samples were more frequently tested positive in alpine dairies opposed to all other dairy types. More positive samples of semi-hard cheese were found in farm-based dairies compared to the other processing types. No positive samples were discovered in cheeses produced in industrial dairies. Hard cheeses have rarely been tested positive (0.17 % for *Listeria monocytogenes*, 0.75 % for coagulase-positive staphylococci). The influence of the variable “heat treatment of milk” (values “pasteurized” or “unpasteurized”) used for cheese production could not be proved to be an additional risk factor even if a tendency can be pointed out, meaning that cheese made from raw milk did not significantly show a higher risk than cheese made from pasteurized milk. The results presented in this study emphasis the importance of good hygiene practices throughout the food chain since they might prevent contamination and/or bacterial growth of the manufactured products.

Keywords

Cheeses, Dairies, Listeria, Milk, Monitoring programme, Staphylococci

1 Introduction

Switzerland produces approximately 185,331 tons of cheese products per year, 34.4 % of which are exported, mainly to the EU (82.4 %) (Anonymous 2015). The equivalence of their respective food hygiene legislations facilitates the trade between Switzerland and the EU. In order to fulfil the requirements of the EU, an annual national monitoring programme of milk and dairy products (NMPD) was initiated in 2003. The aims of the NMPD are to assess the prevalence of microbial hazards in dairy products and to identify products and practices with a higher risk. Additionally, the surveillance of milk and milk products provides valuable information on the hygienic state of milk products manufactured in Switzerland and form the basis for practical recommendations regarding food safety in processing of milk. The results are reported yearly and distributed among the authorities and the dairy sector. Qualitative risk assessments (RA) (Brülisauer 2005; Menendez Gonzalez 2011) and results obtained from the previous year allowed refining of the sampling scheme over the years. Previous studies (Brülisauer 2005; Jakobsen 2011; Zweifel 2005) stated that the type of processing plant (where the milk is manufactured into dairy products) as well as the type of dairy product itself has an influence on the safety of the final product. In this study the risk was defined as being the likelihood of a product to be contaminated above a microbiological threshold value and passed on to consumers.

In order to ensure a high quality output from this programme, a working group (WG) was created, encompassing experts of the Federal Veterinary Office (FVO)[1], the Swiss Association of Cantonal Chemists, Agroscope Institute for Food Science IFS, the Federal Office of Public Health (FOPH)[1] and the Federal Food Chain Unit. The cantonal authorities were in charge of sampling different hazard/product combinations every year and analysed the milk products according to the instructions of the WG. In case of positives samples, the cantonal authorities were responsible for applying necessary follow-up measures. Finally the results were reported in the annual NMPD publication, printed outbound for theauthorities, the dairy sector and is since 2010 additionally available on the internet website of the FVO.

In relation to human health, the following food-borne pathogens were considered within the NMPD framework: *Salmonella* spp., *Campylobacter* spp., Shigatoxin-producing *Escherichia coli*, *Listeria monocytogenes* (LM) and the family of coagulase-positive staphylococci (CPS) encompassing *Staphylococcus aureus*. The investigated dairy products were predominantly cheeses, and also yoghurt, cream and butter.

The aims of our study are to analyse and assess the data collected within the framework of the NMPD from 2003 to 2010. The results will point out which kind of processing premises and which kind of dairy products represents the highest risk for the consumer. Moreover, it is assessed if the variable “heat treatment of milk” (pasteurized or unpasteurized) used for cheese production plays a role in the contamination of the final product. Furthermore, it is of interest to evaluate the congruency between the prediction made in the RA (Menendez Gonzalez 2011) and the results obtained in this study, i.e. to verify if the “at risk” predicted hazard/sample combinations are positive more often compared to samples deemed a “negligible risk”. This would underline the utility of risk assessments used for surveillances of dairy products.

2 Material & methods

2.1 Data selection

Data collected from 2003 to 2010 for the NMPD have not been sampled in order to perform multi-year statistical analyses. Sampling scheme was adapted from year to year based on RA and on the results of the previous year by the WG, resulting in inadequate number of samples for predefined pathogens. We therefore focus our analyses on LM and CPS where sufficient data are available over the years.
In this evaluation, the likelihood of contamination of 4 types of cheeses is assessed: hard, semi-hard, soft and fresh cheese (Table 1). Data collection of 2008 to 2010 contain information on the heat treatment of milk intended for cheese production (pasteurized and unpasteurized) which is also taken

[1] FVO and the Food Safety Division of the FOPH merged 2014 to the new Federal Food Safety and Veterinary Office (FSVO).

into account. The processing term “thermised milk” (more than 40 °C and less than 72 °C for at least 15 sec. and a positive phosphatase test), first recognized as such in the NMPD 2010, is combined with unpasteurized milk for statistical analyses (Verordnung über Lebensmittel tierischer Herkunft, 2005a).

Table 1: classification of the 4 types of cheeses and the 4 types of processing premises

Hard cheese	< 500-540 g/kg MFFB*
Semi-hard cheese	540-650 g/kg MFFB*
Soft cheese	
Fresh cheese	> 650 g/kg MFFB*

Alpine dairies	are summer dairies producing and selling less than 10,000 kg of processed milk
Farm-based dairies	are dairies producing and selling less than 200,000 kg of processed milk
Artisanal dairies	are dairies producing and selling less than 2 million kg of processed milk
Industrial dairies	are dairies producing and selling more than 2 million kg of processed milk

*Moisture content of fat free basis

Processing premises are grouped in 4 different categories, mainly depending on their production profile (Table 1). The classification was defined in the federal regulation on the quality assurance in artisanal dairy processing (Anonymous 1999) and applied until 2006, when it was replaced by a new classification. But for the NMPD, the former classification was maintained, because the different types of dairies can be considered as risk groups.
In this study a sample is considered positive if it contains more than 100 CFU per gram for LM (at the end of the shelf-life) and more than 10,000 CFU per gram for CPS (for raw milk cheese) (Anonymous 2005b). Since 2008, additionally, CPS positive samples of the NMPD should be tested to reveal the presence or absence of enterotoxins.
A total of 11,433 microbiological analyses are integrated in the various models, 7,621 for CPS and 3,812 for LM. Out of those test results, 161 revealed positive for CPS and 16 for LM. In the years 2008 to 2010, 29 of the 46 positive detected CPS samples were analysed for the presence of enterotoxins. All samples are negative.
Samples positive for CPS were found each year, in contrast to LM, which was not detected in 2007, 2009 and 2010 (Table 2).

Positive samples were detected in all types of cheeses, except in fresh cheese samples, which have never been tested positive for LM (Table 2). The highest percentage of CPS positive samples was found in soft and semi-hard cheese (2,3 to 4,7 %, and 1,6 to 4,4 % resp.).

No positive samples were discovered in cheeses produced in industrial dairies. Cheeses from farm-based dairies have all been tested negative for LM, but 22 were positive for CPS. Indeed, alpine and farm-based dairies reached the highest percentage of CPS positive samples (4,6 resp. 3,1 %). Finally, positive samples for LM as well as for CPS were detected in both artisanal and alpine dairies (Table 2).

The final objective is to elucidate if the variable “Heat treatment of milk” (with the values “pasteurized” and “unpasteurized”) is a potential risk factor. To perform this analysis, the available data set is limited to the results obtained in the years 2008 to 2010 of the NMPD, since the distinction between different milk heat treatments was not reported until 2008. For this purpose the focus is kept on CPS as the number of results obtained for LM is too small. A total of 2,033 samples were collected, 1,410 originated from cheese made from unpasteurized/thermised milk and 623 from cheese made from pasteurized milk.

Table 2: Description of the dataset (years 2003 to 2010) for the variables “Year”, “Cheese type” and “Dairy type”

Variable		Samples analysed for CPS		Samples analysed for LM	
		Positive samples / total samples	95 % CI prevalence [%]	Positive samples / total samples	95 % CI prevalence [%]
“Year”	2003	29/2,060	[0.9 - 2.0]	5/1,272	[0.1 - 1.0]
	2004	26/1,004	[1.7 – 3.8]	5/479	[0.4 – 2.6]
	2005	34/1,011	[2.3 – 4.7]	*	*
	2006	5/626	[0.3 – 2.0]	4/575	[0.2 – 1.9]
	2007	21/881	[1.5 – 3.7]	0/222	0
	2008	16/592	[1.6 – 4.4]	2/524	[0.1 - 1.5]
	2009	13/694	[1.0 – 3.3]	0/139	0
	2010	17/753	[1.4 – 3.7]	0/601	0
“Cheese type”	Hard	2/1,143	[0.0 – 0.7]	2/268	[0.0 – 3.0]
	Semi-hard	101/3,795	[2.2 – 3.2]	8/2,177	[0.2 – 0.8]
	Soft	37/1,078	[2.5 – 4.7]	6/870	[0.3 – 1.6]
	Fresh	21/1,605	[0.8 – 2.0]	0/497	0
“Dairy type”	Alpine	85/1,863	[3.7 – 5.6]	5/983	[0.2 – 1.3]
	Artisanal	54/3,940	[1.0 – 1.8]	11/1,981	[0.3 – 1.0]
	Farm-based	22/703	[2.0 – 4.8]	0/323	0
	Industrial	0/1,115	0	0/526	0

*No sample was analysed for LM in 2005
CI: Confidence Interval

Due to the risk based approach no hard cheese had been sampled from 2008 to 2010. The majority of microbiological analyses concerned semi-hard cheese (65 %) and alpine and artisanal dairies (73 %). In our study more CPS positive samples were detected in cheeses made from unpasteurized/thermised milk (3 % in average) than from pasteurized milk (0.5 % in average) (Table 3).

Table 3: Description of the dataset (years 2008 to 2010) for the variable “Heat treatment of the milk” (values “pasteurized” and “unpasteurized”) per type of cheese and type of dairy regarding contamination by CPS

Variable		CPS positive samples / total samples analysed		95 % CI prevalence	
		unpasteurized	pasteurized	unpasteurized	pasteurized
“Cheese type”	Semi-hard	34/1219	0/103	[2.0 – 3.9]	0
	Soft	7/167	2/184	[1.8 – 8.8]	[0.2 – 4.3]
	Fresh	1/24	1/336	[0.2 – 23.1]	[0.0 – 1.9]
“Dairy type”	Alpine	26/571	2/66	[3.1 – 6.7]	[0.5 – 11.5]
	Artisanal	8/628	0/212	[0.6 – 2.6]	0
	Industrial	0/21	0/244	0	0
	Farm-based	8/190	1/101	[2.0 – 8.4]	[0.0 – 6.2]

CI: Confidence Interval

2.2 Statistical analyses

In order to identify risk factors associated with contaminations by CPS or LM of different types of cheese, logistic regression models were developed using a forward pre-selection of variables using univariate analyses. The following variables are considered: “Hazard” (LM or CPS), “Year” (2003 to 2010), “Cheese type” (hard, semi-hard, soft and fresh cheese), “Dairy type” (alpine, artisanal, farm-based and industrial dairy) and “Heat treatment of the milk” (pasteurized and unpasteurized/thermised). Odds ratios (OR) are calculated on significant results in order to assess the strength of association between variables with regard to contamination probability.

All statistical analyses are performed using the language and environment for statistical computing R (R Development Core Team, 2012).

3 Results

3.1 Analyses of the single factors “Year”, “Cheese type” and “Dairy type”

The factor “Year” does not show any significant results. Thus, years are grouped for all tests.

Data for the years 2003 to 2010 are plotted as mean CPS contamination frequencies of the four cheese types for each type of dairy (Figure 1). One can read in this figure that the 2 positive hard cheese samples

originated from alpine dairies. Moreover, it was found that fresh and soft cheese samples were more frequently tested positive in alpine dairies opposed to all other dairy types. More positive samples of semi-hard cheese were found in farm-based dairies compared to the other processing types.

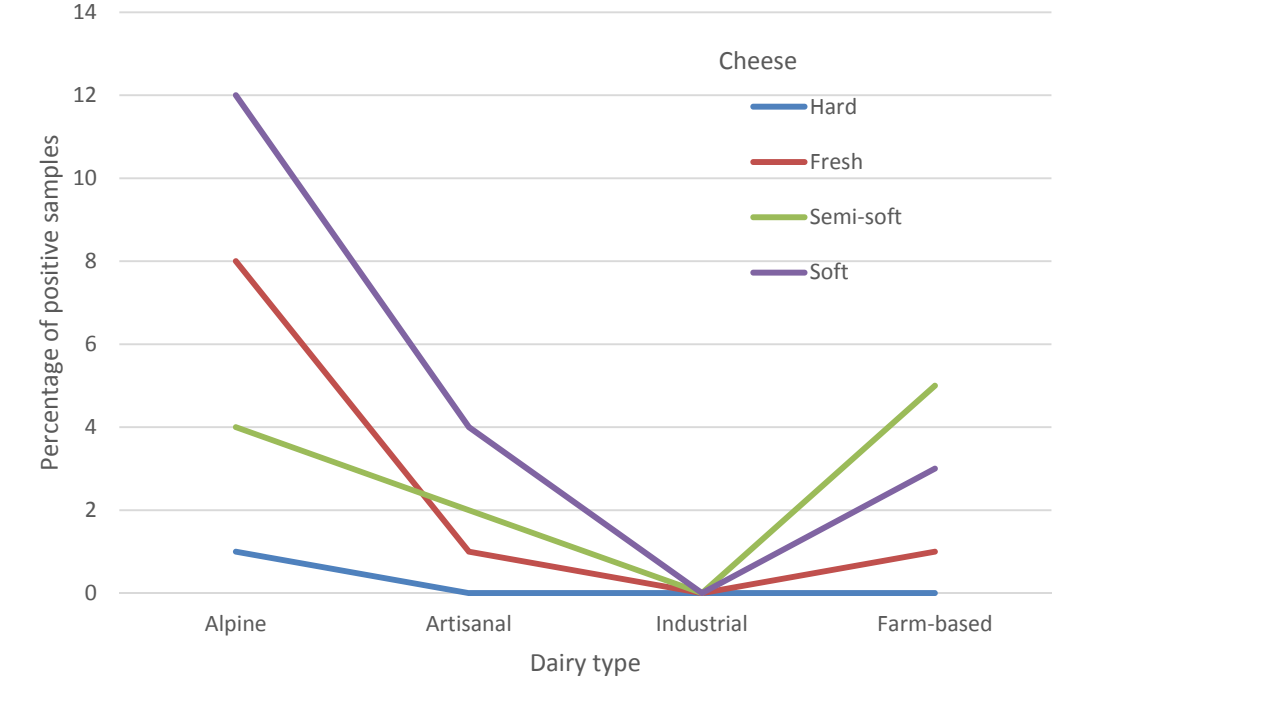

Figure 1: Percentage of CPS contamination of the 4 different types of cheeses according to their origin of production

3.2 Logistic regression with 2 factors as covariates: "Cheese type" & "Dairy type"

A logistic regression model with the covariates "Dairy type" and "Cheese type" is applied as a basis in order to calculate the Odd's Ratio (OR) presented in Table 4. Only the results with significant p-values are reported. Since all results for LM are not significant, OR calculation could only be applied on CPS. The types of dairies have all been compared with each other but the results for the industrial dairies were not included, as no contamination was detected.

Samples from alpine dairies are more frequently contaminated with CPS than samples from other dairies. The likelihood of contamination is estimated to be 3.5 times higher in alpine dairies than in artisanal dairies. Cheeses from artisanal dairies revealed a 2 times lower probability of contamination than cheeses from farm-based dairies. The different types of cheeses are also unequally likely to be contaminated with CPS. The cheese posing the highest relative risk for consumers is soft cheese, followed by semi-hard and fresh cheese. The latter do not significantly differ from each other. The results of this study indicate that hard cheese is the safest type of cheese regarding the investigated hazards CPS & LM (Table 4).

Table 4 Odds Ratio of significant comparisons of CPS positive results between products within a category

Variable	Comparison	Odds Ratio (+/- 95 % CI)	p-value
"Dairy type"	Alpine vs artisanal	3.5 (2.5 - 5.1)	<0.001
	Farm-based vs artisanal	2 (1.2 - 3.3)	<0.01
"Cheese type"	Soft vs hard	28.5 (8.6 – 176.6)	<0.001
	Semi-hard vs hard	12 (3.6 – 70.5)	<0.001
	Fresh vs hard	10.7 (3.1 – 67.5)	<0.01

CI: Confidence Interval

3.3 Multivariate generalized linear model including the factor "Heat treatment of milk"

A multivariate analysis with three covariates ("Dairy type", "Cheese type" and "Heat treatment of milk") is performed for CPS on the years 2008 to 2010. Tested as a single factor, the variable "Heat treatment of milk" (pasteurized or unpasteurized) is highly significant for CPS (p-value < 0.01) but not when tested as a covariate. With an insignificant p-value of 0.12, a tendency can be pointed out for cheese made with unpasteurized/thermised milk to be 3.1 times more contaminated as those produced with pasteurized milk (Table 5).

Table 5 Odd's Ratio of significant comparisons between products within a category 2008 to 2010

Variable	Comparison	Odds Ratio (+/- 95 % CI)	p-value
"Dairy type"	Alpine vs Artisanal	4.7 (2.1 - 10.6)	<0.001 ***
	Farm-based vs Artisanal	3.6 (1.4 - 9.6)	<0.01 **
"Cheese type"	Soft vs Semi-hard	2.2 (1.0 - 4.8)	<0.05 *
"Heat treatment of milk"	Past. vs unpast.	3.1 (2.2 - 4.1)	0.12

CI: Confidence Interval

* = Significant
** = Very significant
*** = Extremely significant

4 Discussion

The analyses show that types of dairy and types of cheeses are risk factors for CPS contamination of dairy products. Only the industrial dairies are a priori, not a risk factor for contamination. In contrast, alpine dairies are the processing premises showing the highest probability to produce final products contaminated with CPS as shown in Figure 1. Regarding cheese types, soft cheeses are 28 times more likely to be contaminated than hard cheeses, followed by semi-hard and fresh cheeses. As expected, hard cheeses belong to the safest type of cheese regarding CPS contamination. Even if not all CPS positive samples were tested for enterotoxins, no positive result was found, meaning that those samples would not have affected the consumer health (Becker 2007). This assertion should be handled carefully because the staphylococcal enterotoxins detection targets the most common toxins (Boss 2011). Thus, toxins produced by rarer CPS genotypes would not be detected yet.

Although the data are not significant for LM, the low rate of LM contamination of cheeses (<1 % for all categories, 0.4 % on average) is noteworthy. No sample was tested positive in industrial or in farm-based dairies. Even better, no sample was tested LM positive in 2007, 2009 and 2010 which demonstrates the usefulness of the prevention measures implemented by the authorities and the dairy industry. The treatment applied on the rind of hard cheese as well as microbiological analyses are also contributing to the rarely occurrence of this pathogen (Schaffner 2003).
A variation of contamination over the years could not be thoroughly showed. Consequently, it has been admitted that the variable "year" had no effect or that the dataset was not big enough to highlight it.

Products from alpine dairies are found to pose the highest risk for microbial contamination. One reason for this might be that employees in small processing premises can be involved in both farm and dairy tasks, which facilitates the transfer of pathogens from one environment to another (Rosengren 2010). Maintaining the cooling chain is another critical aspect relating to food hygiene that is more difficult to accomplish in an isolated environment such as an alp, as are other hygiene and safety rules. Therefore cheese makers should stop producing certain types of cheese such as soft cheeses as advised by Agroscope IFS (Berger and Jakob 2012a). Moreover, milk processing is performed less often due to a decrease in milk production at the end of the alp season, which is why the manufacture of cheese made from raw milk must be strictly regulated (Berger and Jakob 2012b).

Contamination of dairy products can happen primarily via contaminated raw milk or via cross-contamination. According to Kells and Glimour (2004), *Listeria* can be found on many objects used to manufacture cheese as well as on the floor. Loncarevic

(2005) also demonstrated that there are multiple sources for contamination with CPS. This conclusion is based on the detection of a wide variety of CPS strains discovered within the same sample with animal, environmental and human origins. Little (2008) showed that the microbiological safety of cheese is also influenced by equipment and environmental hygiene during manufacture, packaging and handling. At any rate it is related to the type of dairy, probably depending on the size of processing plant, its configuration, training of staff, quality management, etc. (Menendez Gonzalez 2011).

Soft cheeses are more at risk than other cheese types regarding contamination with CPS. This is why the FOPH recommends pregnant women not to eat soft cheese or semi-hard cheese regardless of previous heat treatment of the manufactured milk. Because soft cheeses contain more water than other types of cheese, they provide a very favorable environment for the development of germs (Berger and Jakob 2012b). In addition, the rind of a soft cheese, potentially infected secondarily, is fully eaten, which is not the case when consuming semi-hard and hard cheeses.

The risk assessment (RA) conducted by Menendez Gonzalez et al. (2011) categorized each type of final product in 5 levels: high, medium, low, very low and negligible. Most of the hazard/product combinations originating from industrial and artisanal dairies were considered to represent a negligible or very low relative risk. Opposed to that, hazard/product combinations originating from farm-based and alpine dairies were categorized together as representing a high and medium relative risk. The results of this multi-year assessment support the conclusions of the RA. The only difference between the predictions of the RA and the results of the present study is that alpine dairies manufacture more frequently contaminated final products than farm-based dairies do, which was not considered in the original RA. The other assumptions made in the RA fit perfectly to the results of this multi-year assessment, which proves that the risk-based sampling was efficient and that one can rely on risk assessments for future monitoring programmes, adapting them every year according to recent findings.

In Switzerland, 65 to 70 % of cheeses are made from raw milk (Flammer 2010), thus it seems important to consider the subject. Obtained results in the framework of the NMPD do not significantly highlight the importance of pasteurization since it was established that the type of dairy and the type of cheese (and not the heat treatment of milk) are the most significant risk factors on contamination of dairy products with CPS. This assertion may depend on the size of the subset from the years 2008 to 2010. Even if the microbiocidal effect of pasteurization is clearly established, post-contaminations occur.

5 Conclusion

Only very few LM positive samples were detected in the last 2 years (2009-2010). The last known Swiss outbreak of LM occurred in 2006 (Bille 2006) and could be kept under control quickly. Prevention measures implemented by the national authorities appear to have had a positive effect on the occurrence of this pathogen. Nevertheless, it is important to educate cheese makers in order to maintain a high disease awareness. It is also important to note that pasteurization of raw milk does not eliminate the risk of consuming a contaminated dairy product. Publication of annual reports provides maintenance of knowledge concerning the contamination of cheese by various pathogens as LM and CPS by keeping the awareness high and by showing an effective and active surveillance from the cantonal and federal authorities. Despite this, since 2011, there has been an interruption of the NMPD. This monitoring programme may be included within the National Control Plan which includes several projects. Swiss authorities are considering several options.

The results presented in this study emphasize the importance of good hygiene practices throughout the food chain since they might prevent contamination and/or bacterial growth of the manufactured products.

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