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DELLA LOMBARDIA E DELL'EMILIA ROMAGNA
"BRUNO LIBERTINI"
ENTE SANITARIO DI DIRITTO PUBBLICO

LA NOSTRA
ESPERIENZA,
LA VOSTRA
SICUREZZA.



Quantitative assessment of exposure to milk pathogens



ECVPH Annual Conference 2017,
Perugia/Italy, 17-19. Oct. 2018



Topics

- **The raw milk market in Italy: regulation and production data**
- **Raw milk and biological hazards**
- **Quantitative Risk Assessment related to consumption of Raw milk**



Raw Milk: definition

- **Regulation (EC) No 853/2004 lays down specific hygiene rules for food of animal origin for food business operators.**
- Annex I: Definitions

Raw milk" means milk produced by the secretion of the mammary gland of farmed animals that has not been heated to more than 40°C or undergone any treatment that has an equivalent effect".



Raw Milk: legislation

- **REGULATION (EC) N° 852/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29 April 2004 on the hygiene of foodstuffs**

recognizes that primary producers of milk are responsible for compliance with the hygiene requirements by requiring the implementation of appropriate measures that can identify, prevent and control the hazards associated with the same production and that these measures are codified, registered and made available to the competent authority control.



Raw Milk: legislation

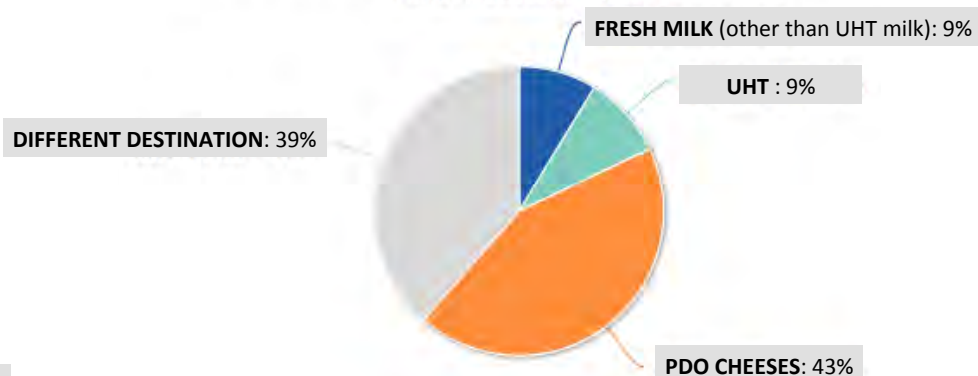
- **REGULATION (EC) No 853/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs**

oblige primary producers of raw milk to meet the requirements concerning the health status of dairy animals, the structural requirements of the farms (premises, equipment, milking, collection, transport of milk and staff hygiene) and the microbiological requirements raw milk.



Raw Milk: production and intended use in Italy

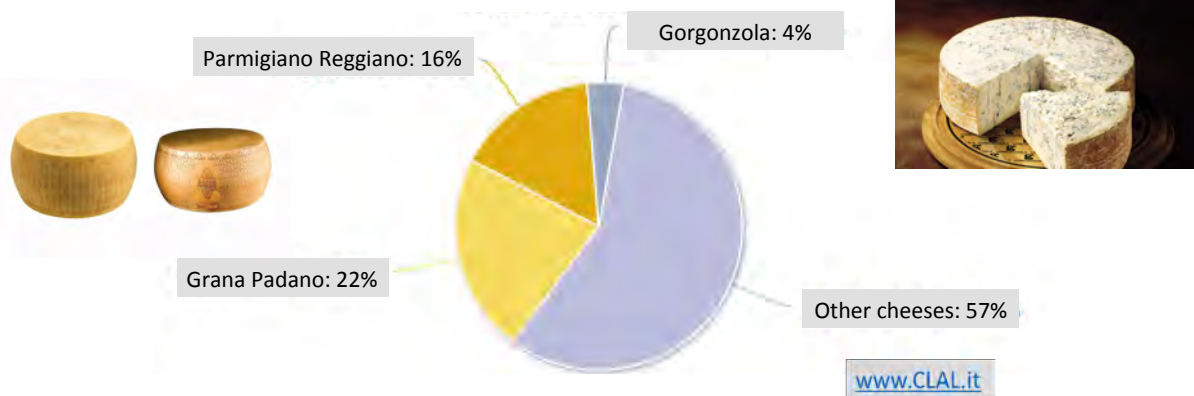
- **Italy, 2016: intended use of total milk available**





Raw Milk: production and intended use in Italy

- Italy, 2016: raw milk destinations for PDO cheeses production



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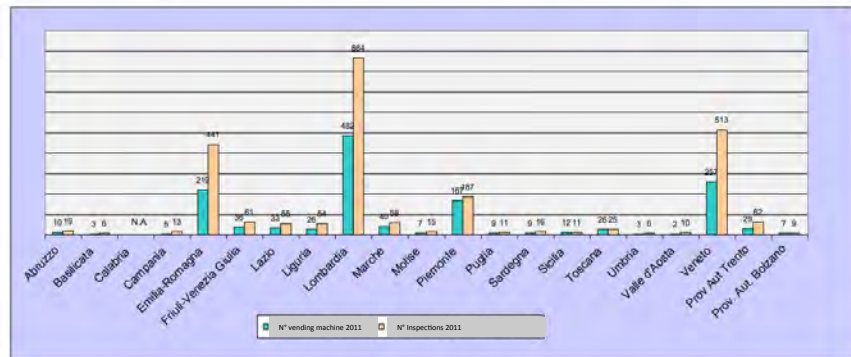
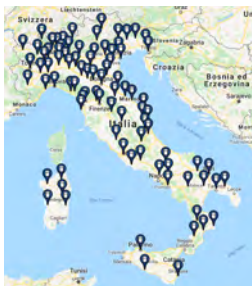
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Raw milk for direct human consumption

Total number of raw milk vending machines in Italy:

993 (2018) - 28% from 2011 (1373)



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8



Raw milk and biological hazards

Summary of microbiological hazards associated with raw cow milk

Organism	Shed directly in milk [#]	Severity of illness [§]	Implicated in foodborne illness
<i>Bacillus cereus</i>	×	Moderate	++
<i>Campylobacter jejuni/coli</i>	✓	Severe [^]	++
<i>Clostridium perfringens</i>	×	Severe [^]	+
<i>Coxiella burnetii</i>	✓	-	+
<i>Cryptosporidium parvum</i>	×	Severe [^]	+
Enterohaemorrhagic <i>E. coli</i>	✓	Severe	++
<i>Listeria monocytogenes</i>	✓	Severe [^]	++
<i>Salmonella</i> spp.	✓	Serious	++
<i>Staphylococcus aureus</i>	✓	Moderate	++
<i>Streptococcus</i> spp.	✓	-	+
<i>Toxoplasma gondii</i>	✓	-	++
<i>Yersinia enterocolitica</i>	✓	Serious	+

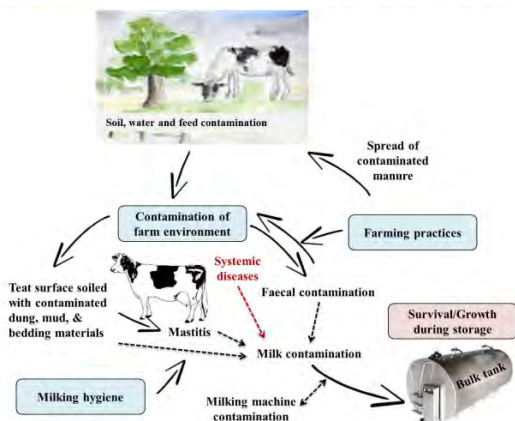
Key:
[#] Transmission through udder; mastitis etc - No data/unknown + Rare
[^] Susceptible sub-populations § Based on ICMSF (2002) ++ More common

Food Standards Microbiological Risk Assessment of Raw Cow Milk 2008



Raw milk and biological hazards

Main routes of contamination



Main food-borne outbreak linked with raw milk consumption

Year	Pathogen	Member State	Number of outbreaks	Number of cases
2007	<i>Campylobacter</i> spp.	The Netherlands	1	18
		Denmark	2	12
		Finland	1	4
		Germany	1	14
		Hungary	2	32
2008	<i>Campylobacter</i> spp.	Austria	1	2
		Germany	1	45
		The Netherlands	1	8
2009	Shiga toxin-producing <i>E. coli</i> (STEC)	Germany	1	23
		No outbreaks associated with raw milk were reported		
2010	<i>Campylobacter</i> spp.	Slovakia	1	6
		Germany	3	42
		Ireland	1	3
		The Netherlands	1	4
		Hungary	1	2
2011	<i>Campylobacter</i> spp.	Germany	3	32
		Sweden	1	13
2012	<i>Campylobacter</i> spp.	Denmark	1	11
		Germany	1	3
		Finland	2	22
		Finland	1	8
		Shiga toxin-producing <i>E. coli</i> (STEC)		

Source: Data on food-borne outbreaks included in this table were extracted from the EFSA 2000-2014 database on 10 November 2014.



Quantitative Risk Assessment: definition

Quantitative Risk Assessment (QRA) "is use of measurable, objective data to determine asset value, probability of loss, and associated risk(s)".

In this study QRA considered the presence of pathogens (*Campylobacter spp.*, *Salmonella spp.*, *L. monocytogenes*, *E. coli* O 157 H:7) in dairy farms, the field handling conditions of raw milk during distribution and delivery to the consumer, consumer habits, and the behavior of pathogens throughout the food chain.



Quantitative Microbiological Risk Assessment

Journal of Food Protection®, Number 8, August 2011, pp. 1224-1294



Quantitative Risk Assessment of Listeriosis Due to Consumption of Raw Milk

USA

Authors: Latorre, Alejandra A.; Pradhan, Abani K.; Van Kessel, Jo Ann S.; Karns, Jeffrey S.; Boor, Kathryn J.; Rice, Daniel H.; Mangione, Kurt J.; Grohn, Yrjo T.; Schukken, Ynte H.



AUS

Microbiological Risk Assessment of Raw Cow Milk

Ministry for Primary Industries
Mauritius Airline

NZ

Assessment of the microbiological risks associated with the consumption of raw milk

MPI Technical Paper No. 2014/12

ISSN No. 078-6470-4/2014-4 (online)
ISBN No. 978-0-478-3921-4 (paper)

June 2013

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Research Note

Quantitative Risk Assessment of Verocytotoxin-Producing *Escherichia coli* O157 and *Campylobacter jejuni* Related to Consumption of Raw Milk in a Province in Northern Italy

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Foodborne Pathogens in Raw Milk Sold by Vending Machines in Italy

Prevalence of *Salmonella* and *L. monocytogenes* isolated from raw milk samples in seven Italian regions, 2008 through 2011, and beta distributions from the univariate analysis

Pathogen, region	No. of negative samples	No of positive samples	Prevalence (%)	Beta distribution (%)	5th percentile (%)	95th percentile (%)
<i>Salmonella</i>						
Emilia Romagna	1,398	1	0.07	0.14	0.03	0.34
Lazio and Tuscany	626	6	0.96	1.11	0.53	1.88
Lombardy	10,788	7	0.06	0.07	0.04	0.12
Marche	196	0	0.00	0.51	0.03	1.51
Piedmont	826	2	0.24	0.36	0.10	0.76
Sicily	288	1	0.35	0.69	0.12	1.63
Veneto	1,280	1	0.08	0.16	0.03	0.37
Pearson's χ^2 (6) = 43.1139, P = 0.000						
<i>L. monocytogenes</i>						
Emilia Romagna	1,398	3	0.21	0.29	0.10	0.55
Lazio and Tuscany	627	4	0.64	0.79	0.31	1.45
Lombardy	10,561	54	0.51	0.52	0.41	0.64
Marche	194	1	0.52	1.02	0.18	2.41
Piedmont	817	11	1.35	1.47	0.85	2.22
Sicily	307	5	1.63	2.26	1.07	3.80
Veneto	1,277	5	0.39	0.47	0.21	0.82
Pearson's χ^2 (6) = 19.6106, P = 0.003						

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Journal of Food Protection, Vol. 76, No. 11, 2013, Pages 1902-1907

13



Foodborne Pathogens in Raw Milk Sold by Vending Machines in Italy

Prevalence of *E. coli* O157:H7, and *C. jejuni* isolated from raw milk samples in seven Italian regions, 2008 through 2011, and beta distributions from the univariate analysis

Pathogen, region	No. of negative samples	No of positive samples	Prevalence (%)	Beta distribution (%)	5th percentile (%)	95th percentile (%)
<i>E. coli</i> O157:H7						
Emilia Romagna	1,400	1	0.07	0.14	0.03	0.34
Lazio and Tuscany	602	9	1.50	1.66	0.90	2.59
Lombardy	10,849	9	0.08	0.09	0.05	0.14
Marche	177	0	0.00	0.56	0.03	1.67
Piedmont	827	1	0.12	0.24	0.04	0.57
Sicily	182	0	0.00	0.54	0.03	1.62
Veneto	1,058	4	0.38	0.47	0.19	0.86
Pearson's χ^2 (6) = 75.0332, P = 0.000						
<i>C. jejuni</i>						
Emilia Romagna	1,374	8	0.58	0.65	0.34	1.05
Lazio and Tuscany	589	5	0.85	1.02	0.44	1.77
Lombardy	10,698	22	0.21	0.21	0.15	0.29
Marche	191	0	0.00	0.52	0.03	1.55
Piedmont	810	18	2.22	2.34	1.54	3.27
Sicily	293	0	0.00	0.34	0.02	1.01
Veneto	1,274	0	0.00	0.08	0.00	0.23
Pearson's χ^2 (6) = 98.6775, P = 0.000						

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Journal of Food Protection, Vol. 76, No. 11, 2013, Pages 1902-1907

14



Field handling conditions of raw milk sold in vending machines

Experimental evaluation of the behavior of *L. monocytogenes*, *E. coli* O157:H7, *S. Typhimurium* and *Campylobacter jejuni*

Italian Journal of Animal Science 2012; volume 11:e24

Pathogenic microbial population count during storage at 4°C and at variable temperatures (mean±SD Log CFU mL⁻¹ of 9 data: 3 replicates x 3 batches).

Phase	Sampling, total time	<i>Listeria monocytogenes</i>		<i>Escherichia. coli</i> O 157:H7		<i>Campylobacter jejuni</i>		<i>Salmonella Typhimurium</i>	
		4°C±0.5	Variable conditions	4°C±0.5	Variable conditions	4°C±0.5	Variable conditions	4°C±0.5	Variable conditions
For <i>L. monocytogenes</i> , <i>E. coli</i> and <i>Salmonella</i> the risk analysis must evaluate the prevalence, and the ability to grow during the transport and shelf life	T0, 0 h	2.23±0.03	2.18±0.03	2.40±0.09	2.14±0.02	1.92±0.06	1.92±0.06	1.98±0.19	1.88±0.09
	T1, 5 h	2.29±0.41	2.30±0.11	2.42±0.05	2.28±0.14	1.82±0.25	1.98±0.01	1.77±0.12	1.94±0.10
	T2, 27.5 h	2.35±0.20	2.45±0.24	2.17±0.16	3.15±0.30	1.74±0.13	1.97±0.07	1.78±0.11	2.00±0.01
	T3, 28 h	2.41±0.06	2.50±0.13	2.28±0.09	2.90±0.02	1.84±0.06	1.96±0.07	1.51±0.07	2.72±0.24
	T4, 96 h	2.61±0.02	3.25±0.31	2.10±0.13	3.97±0.28	1.72±0.07	1.28±0.18	1.85±0.25	3.20±0.06
After boiling	T5, 96 h	nd	nd	nd	nd	nd	nd	nd	nd

Time and temperature of incubation in each phase during storage at variable temperatures: *5 h at 7°C±0.5; *22.5 h at 11°C±0.5; *30 min at 30°C±0.5 air temperature; *68 h at 12°C±0.5; nd, not detected.

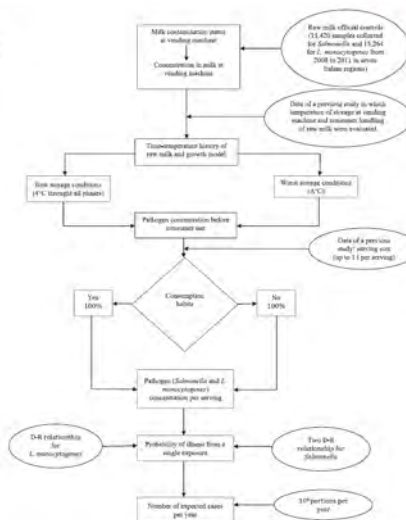
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15



Quantitative Risk Assessment related to consumption of Raw milk: the model

Flow chart of the model used to estimate the probability of illness from a single exposure to contaminated raw milk and the number of illness cases expected each year.



Journal of Food Protection, Vol. 78, No. 1, 2015, Pages 13-21
Journal of Food Protection, Vol. 75, No. 11, 2012, Pages 2031-2038

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16



Quantitative Risk Assessment related to consumption of Raw milk: *Listeria monocytogenes* and *Salmonella* spp.

Estimated concentrations of *Salmonella* and *Listeria monocytogenes* in milk after the best and worst storage conditions and after boiling: minimum, mean, and maximum estimated concentrations

Journal of Food Protection, Vol. 78, No. 1, 2015, Pages 13-21

	Estimated concn (CFU/ml) of <i>Salmonella</i>				Estimated concn (CFU/ml) of <i>Listeria monocytogenes</i>			
	BS	WS	BBS	BWS	BS	WS	BBS	BWS
Minimum	4.42×10^{-7}	2.90×10^{-6}	1.33×10^{-16}	8.19×10^{-16}	4.58×10^{-8}	1.09×10^{-7}	1.26×10^{-15}	2.35×10^{-15}
Mean	0.25	2.05	8.53×10^{-11}	7.33×10^{-10}	3.18×10^{-3}	7.53×10^{-3}	4.82×10^{-10}	1.15×10^{-9}
Maximum	560	5,323	1.31×10^{-7}	6.44×10^{-6}	7	15	5.04×10^{-7}	1.53×10^{-6}

This data emphasize, yet again, that boiling of milk before drinking is a simple but effective tool to protect consumers against the risks of illness inherent in the consumption of raw milk

^a BS, best storage conditions; WS, worst storage conditions; BBS, best storage conditions and boiling; BWS, worst storage conditions and boiling.



Quantitative Risk Assessment related to consumption of Raw milk: *Listeria monocytogenes* and *Salmonella* spp.

This study clearly show how consumer behavior could affect the probability and number of salmonellosis cases and, in general, the risk of illness.

The RA models predicted no human listeriosis cases per year either in the best or worst storage conditions and with or without boiling raw milk.

The annual estimated cases of salmonellosis depend on the dose-response relationships used in the model, the milk storage conditions, and consumer behavior in relation to boiling raw milk or not. The estimated salmonellosis cases can't be assessed due to wider variability in selling and consuming data



Output QRA models

Salmonella

- FSANZ (2009) in children
 - Scenario 1 (at farm direct consumption): 17 cases on 100,000 daily portions;
 - Scenario 2 (domestic consumption): 55 cases on 100,000 daily portions;
 - Scenario 3 (domestic consumption after botling and on shop selling) : 153 cases on 100,000 daily portions (130 adulti).
- Soboleva (2013) 8 cases on 100,000 daily portions (in all different scenarios)



Quantitative Risk Assessment related to consumption of Raw milk: *E. coli* O157 and *Campylobacter jejuni* in North Italy

Estimated *E. coli* and O157:H7 *C. jejuni* levels in raw milk under the best and worst storage conditions and after boiling

Measure ^b	Estimated level (CFU/ml) ^c							
	<i>E. coli</i> O157:H7				<i>C. jejuni</i>			
	BS	WS	BBS	BWS	BS	WS	BBS	BWS
Minimum	1.02×10^{-8}	1.06×10^{-8}	1.35×10^{-13}	1.95×10^{-13}	1.52×10^{-8}	3.44×10^{-9}	1.34×10^{-13}	4.08×10^{-14}
Mean	6.69×10^{-4}	4.90×10^{-3}	2.97×10^{-7}	1.99×10^{-6}	6.66×10^{-5}	3.49×10^{-5}	6.66×10^{-9}	3.49×10^{-9}
Maximum	7.84×10^{-1}	12.14×10^0	3.05×10^{-4}	3.33×10^{-3}	5.06×10^{-2}	7.19×10^{-1}	3.87×10^{-4}	1.64×10^{-4}

The estimated *E. coli* O157:H7 level increased in the worst storage scenario, whereas estimated *C. jejuni* levels decreased because of the more pronounced decrease in *C. jejuni* in raw milk stored at higher temperatures.

Boiling greatly reduces the estimated levels of both *C. jejuni* and *E. coli*

^a BS, best storage conditions; WS, worst storage conditions; BBS, best storage conditions and boiling; BWS, worst storage conditions and boiling.



Quantitative Risk Assessment related to consumption of Raw milk: *E. coli* O157 and *Campylobacter jejuni* in North Italy

For every 10,000 to 20,000 consumers each year, the models predicted for the best and worst storage conditions, respectively,

- campylobacteriosis 2.12 and 1.14 cases;
- HUS 0.02 and 0.09 cases in the 0- to 5-year age group and 0.1 and 0.5 HUS cases in the .5-year age group.

Journal of Food Protection, Vol. 75, No. 11, 2012, Pages 2031–2038



Conclusion

- Considering QMRA available in Europe on bovine milk there's a clear evidence of risks associated with raw milk consumption for *Campylobacter spp.*, *Salmonella Typhimurium*, *STEC*
- *L.monocytogenes* doesn't represent a quantitative relevant risk in bovine raw milk
- Pathogen-free raw milk is difficult to obtain, but the risk of illness could be significantly reduced by using simple control measures:
 1. high gmp level at farm
 2. a strict monitoring of temperatures by farmers during raw milk distribution;
 3. enhanced educational efforts to the consumers for the importance maintain cold chain, reduce domestic shelf life and first of all boiling raw milk

Thank you for your attention

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