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TERAMO

Campylobacter
Laboratorio Nazionale di Riferimento

Problematiche di isolamento di *Campylobacter* da latte

Teramo 3 luglio 2019

Campylobacter nel latte

- Campylobacter può essere contenuto nel tratto intestinale degli animali
- Contaminazione fecale del latte
- La trasmissione del *Campylobacter* attraverso il latte può essere facilmente controllata tramite la pastorizzazione
- Ma… i consumatori chiedono «latte crudo»….
- Studi sulla contaminazione da Campylobacter nel latte crudo riportano valori dallo 0,4 al 12% dei campioni
- Tipo di campione: latte di massa o filtri linea di mungitura

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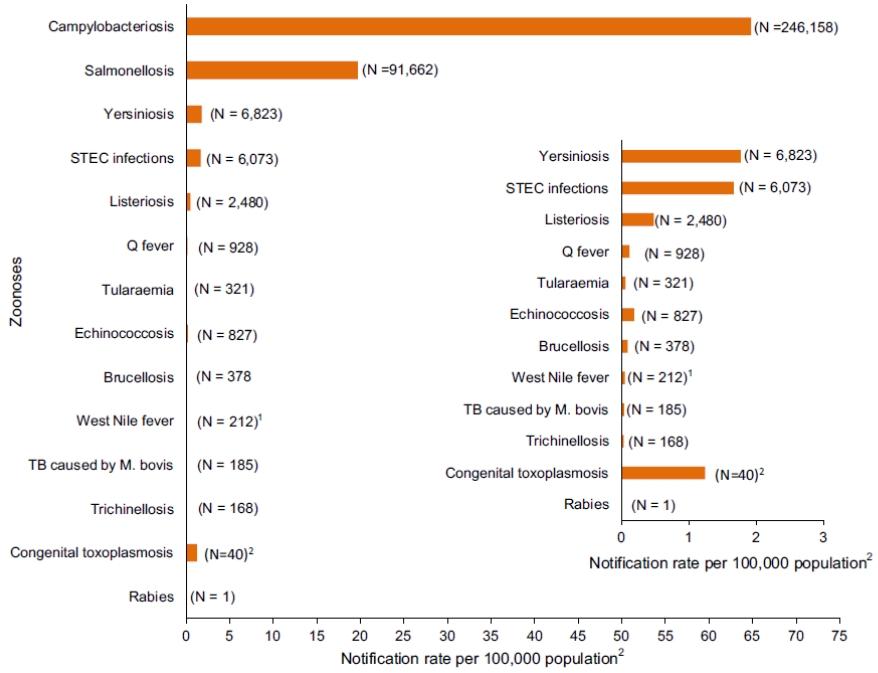
Report zoonosi ,2018

EFSA journal 2018;16(12):5500

Casi riportati EU 2017



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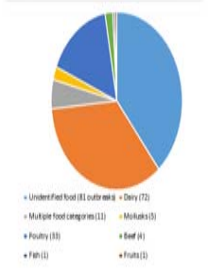
EFSA Journal 2018;16(12):5500

Outbreak 2017 EU da Campylobacter

Alimento	N° focolai	% totale
Latte	18	54,5
Prodotti derivati dal latte	2	6,1
Carne di pollo/p. derivati	8	24,2
carne rossa/prodotti	2	6,1
carne mista /pollo	2	6,1
carne e p. a base di carne	1	3,0
	33	100

Foodborne Campylobacter Outbreaks by Food Category, 2010-2015



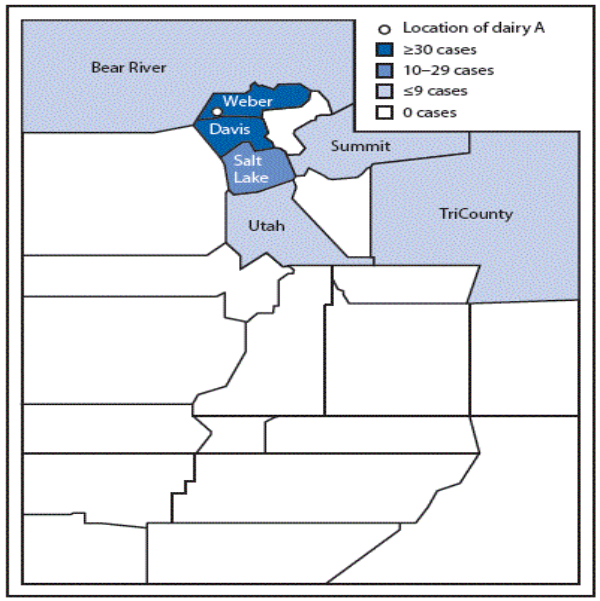
atte: 1554 campioni in 9 paesi:P1,9%

Centers for Disease Control and Prevention
MMWR
Morbidity and Mortality Weekly Report
Weekly / Vol. 65 / No. 12
April 1, 2016

Campylobacter jejuni Infections Associated with Raw Milk Consumption — Utah, 2014

Kenneth R. Davis, MPH[1]; Angela C. Dunn, MD[2]; Cindy Burnett, MPH[1]; Laine McCullough[1]; Melissa Dimond, MPH[1]; Jenni Wagner, MS[3]; Lori Smith[3]; Amy Carter[4]; Sarah Willardson, MPH[5];

"Outbreak in northern Utah latte crudo A: 99 cases di campylobacteriosi (59 confermati e 40 probabili) 10 ospedalizzati, 1 morto



Campylobacter prompts health alert for raw milk in Ohio

by NEWS DESK

December 4, 2016 | US News | 2 Comments

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Outbreak of Fluoroquinolone-Resistant *Campylobacter jejuni* Infections Associated with Raw Milk Consumption from a Herdshare Dairy — Colorado, 2016

Weekly / February 9, 2018 / 67(5);146–148

Alexis Burakoff, MD[1,2]; Kerri Brown, MSPH[3]; Joyce Knutsen[2]; Christina Hopewell[3]; Shannon Rowe, MPH[4]; Christy Bennett[5]; Alicia Cronquist, MPH[2] (View author affiliations)

More than 50 Campylobacter cases linked to raw milk

By Joseph James Whitworth
02-Jan-2017 - Last updated on 02-Jan-2017 at 09:24 GMT

RELATED TAGS: Raw milk, Milk

More than 50 cases of Campylobacter have been linked to unpasteurised (raw) milk from a farm in Kendal, UK.

Campylobacter illnesses in Wales linked to raw milk

By Joseph James Whitworth
14-Aug-2017 - Last updated on 15-Aug-2017 at 06:05 GMT

RELATED TAGS: Wales, Epidemiology

Public Health Wales is investigating four cases of Campylobacter linked with milk from Penlan y Môr farm

Foodborne Pathog Dis. 2017 Jun;14(6):326-332. doi: 10.1089/fpd.2016.2257. Epub 2017 Mar 28.

Outbreak of Campylobacteriosis Following a Dairy Farm Visit: Confirmation by Genotyping.

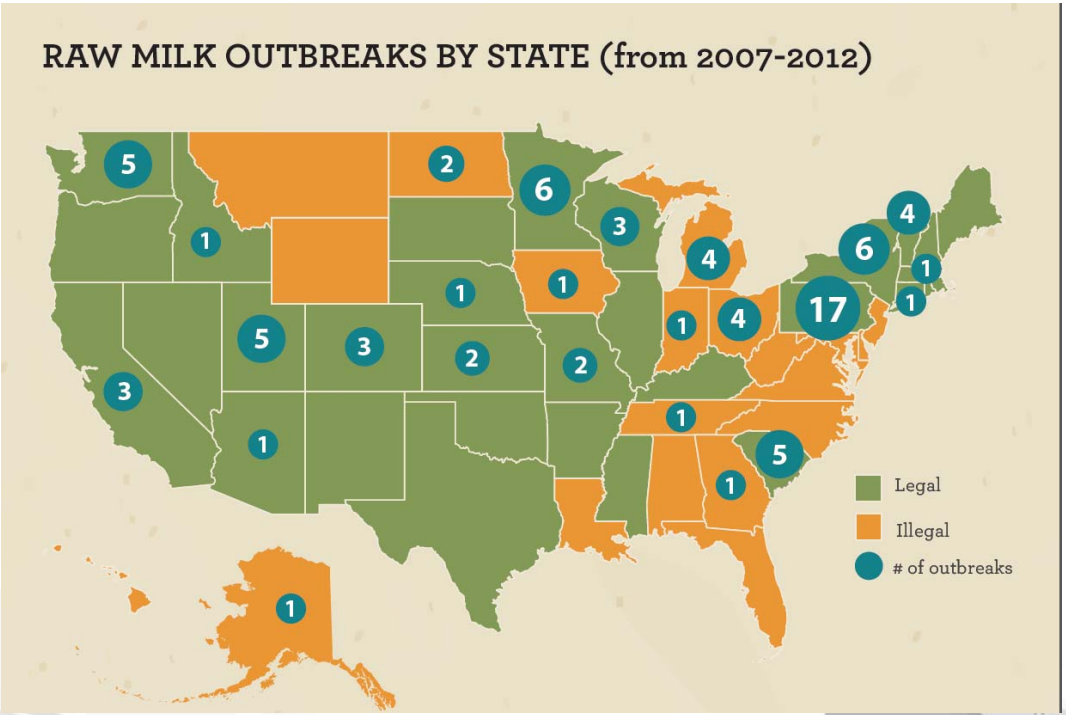
Lahti E[1], Rehn M[2,3], Ockborn G[4], Hansson I[1], Ågren J[1], Engvall EO[1], Jernberg C[2].

Author information

Abstract

In April-May 2014, an outbreak of campylobacteriosis occurred after a preschool visit to a dairy farm in the South Western part of Sweden. During the visit, a meal, including unpasteurized milk, was served. A retrospective cohort study using a web-based questionnaire was performed among the participants (n = 30) of the farm visit. A total of 24 of the 30 (80%) cohort members completed the questionnaire. Eleven cases were identified, and Campylobacter jejuni was isolated from eight of them. Seven of the cases were 2- to 7-year-old children. We found the highest attack rates among those who usually drink milk (45%) and those who consumed unpasteurized milk during the farm visit (42%). No cases were unexposed (risk ratio incalculable). As result of the farm investigation, Campylobacter was isolated from cattle on the farm. Genotyping with pulsed-field gel electrophoresis and whole genome sequencing confirmed that human and cattle isolates of C. jejuni belonged to one cluster. Thus, cattle on the farm are considered the source of infection, and the most likely vehicle of transmission was contaminated unpasteurized milk. We recommend consumption of heat-treated milk only and increased awareness of the risk of consuming unpasteurized milk.

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™



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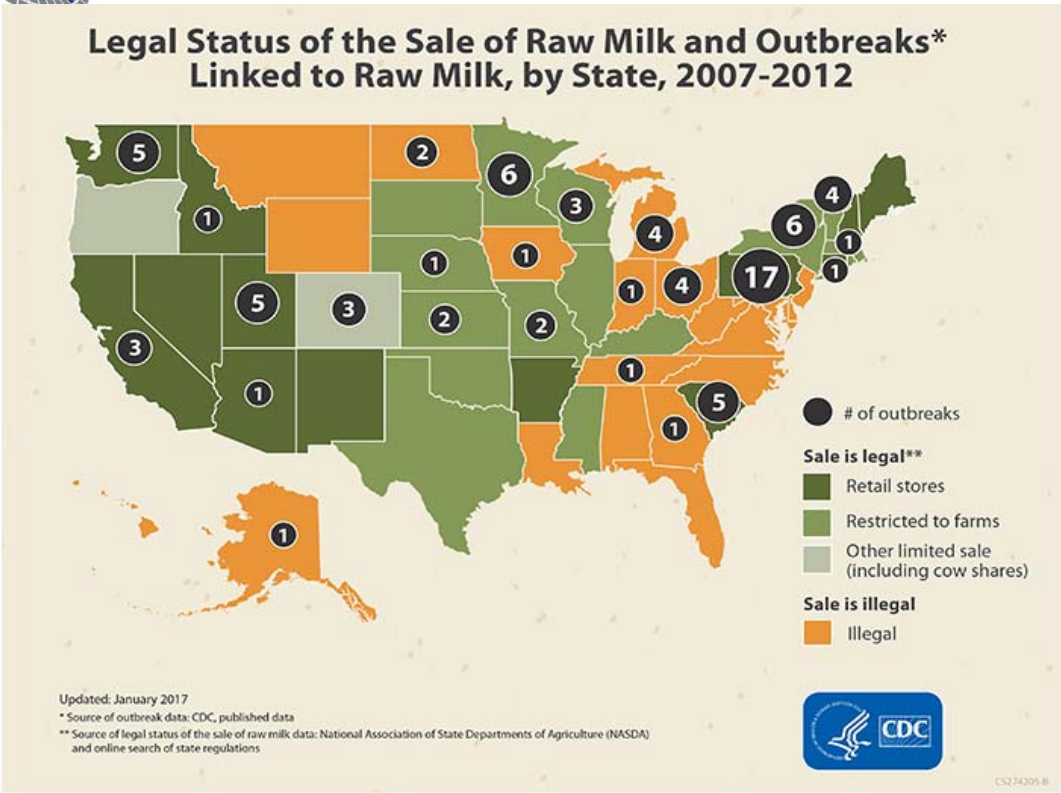

FOODFACTS

From the U.S. Food and Drug Administration

The Dangers of Raw Milk:

Unpasteurized Milk Can Pose a Serious Health Risk

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Milk and milk products provide a wealth of nutrition benefits. But raw milk can harbor dangerous microorganisms that can pose serious health risks to you and your family. According to an analysis by the Centers for Disease Control and Prevention (CDC), between 1993 and 2006 more than 1500 people in the United States became sick from drinking raw milk or eating cheese made from raw milk. In addition, CDC reported that unpasteurized milk is 150 times more likely to cause foodborne illness and results in 13 times more hospitalizations than illnesses involving pasteurized dairy products.

Raw milk is milk from cows, sheep, or goats that has not been pasteurized to kill harmful bacteria. This raw, unpasteurized milk can carry dangerous bacteria such as *Salmonella*, *E. coli*, and *Listeria*, which are responsible for causing numerous foodborne illnesses.

These harmful bacteria can seriously affect the health of anyone who drinks raw milk or eats foods made from raw milk. However, the bacteria in raw milk can be especially dangerous to **people with weakened immune systems, older adults, pregnant women, and children**. In fact, the CDC analysis found that foodborne illness from raw milk especially affected children and teenagers.

"Pasteurized Milk" Explained

Pasteurization is a process that kills harmful bacteria by heating milk to a specific temperature for a set period of time. First developed by Louis Pasteur in 1864, pasteurization kills harmful organisms responsible for such diseases as listeriosis, typhoid fever, tuberculosis, diphtheria, and brucellosis.

Research shows no meaningful difference in the nutritional values of pasteurized and unpasteurized milk. Pasteurized milk contains low levels of the type of nonpathogenic bacteria that can cause food spoilage, so storing your pasteurized milk in the refrigerator is still important.

Raw Milk & Pasteurization: Debunking Milk Myths

While pasteurization has helped provide safe, nutrient-rich milk and cheese for over 120 years, some people continue to believe that pasteurization harms milk and that raw milk is a safe, healthier alternative.

Here are some common myths and proven facts about milk and pasteurization:

- Pasteurizing milk **DOES NOT** cause lactose intolerance and allergic reactions. Both raw milk and pasteurized milk can cause allergic reactions in people sensitive to milk proteins.
- Raw milk **DOES NOT** kill dangerous pathogens by itself.
- Pasteurization **DOES NOT** reduce milk's nutritional value.
- Pasteurization **DOES NOT** mean that it is safe to leave milk out of the refrigerator for extended time, particularly after it has been opened.
- Pasteurization **DOES** kill harmful bacteria.
- Pasteurization **DOES** save lives.

Raw Milk and Serious Illness

Symptoms and Advice

Symptoms of foodborne illness include:

- Vomiting, diarrhea, and abdominal pain
- Flulike symptoms such as fever, headache, and body ache

While most healthy people will recover from an illness caused by harmful bacteria in raw milk — or in foods made with raw milk — within a short period of time, some can develop symptoms that are chronic, severe, or even life-threatening.

If you or someone you know becomes ill after consuming raw milk or products made from raw milk — or, if you are pregnant and think you could have consumed contaminated raw milk or cheese — see a doctor or healthcare provider immediately.

The Dangers of Listeria and Pregnancy

Pregnant women run a serious risk of becoming ill from the bacteria *Listeria*, which can cause miscarriage, fetal death, or illness or death of a newborn.

If you are pregnant, consuming raw milk — or foods made from raw milk, such as Mexican-style cheese like Queso Blanco or Queso Fresco — can harm your baby even if *you* don't feel sick.

FDA *Safety* ■ *Health* ■ *Science* ■ *Nutrition*

August 2012

According to the CDC, from 1993 through 2012, 127 outbreaks linked to raw milk or raw milk products like ice cream, soft cheese, or yogurt. They resulted in 1,909 illnesses and 144 hospitalizations.

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Prevalenza di Campylobacter nel latte

Prevalenza%		Matrice	dove
2,91%	Shivani et al,2015		
0,4-12,3% (studio 1982-2001)	Oliver et alt.2005		
1,5	Bianchi DM	Latte crudo (macchine)	Italy
12%	Bianchini et al,2014	Latte crudo (macchine)	Italy
6,45%	Giacometti et al,2012	Latte crudo+ filtri	Italy
2%	Jayarao et al, 2016	Latte crudo	Usa
12-13%	Ollson,Sva	Filtri (InCary Blair)	Sweden 2016,2017

Journal of Food Protection

International Association for Food Protection.

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Journal of Food Protection
October 2016, Vol. 79, No. 10, pp. 1775-1783

Campylobacter spp. Prevalence and Levels in Raw Milk: A Systematic Review and Meta-Analysis
T. CHRISTIDIS, K. D. M. PINTAR, A. J. BUTLER, A. NESBITT, M. K. THOMAS, B. MARSHALL, and F. POLLARI
https://doi.org/10.4315/0362-028X.JFP-15-480

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Sopravvivenza di Campylobacter nel latte

AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology®

Appl Environ Microbiol. 1982 Nov; 44(5): 1154–1158.

PMCID: PMC242162
PMID: 6897503

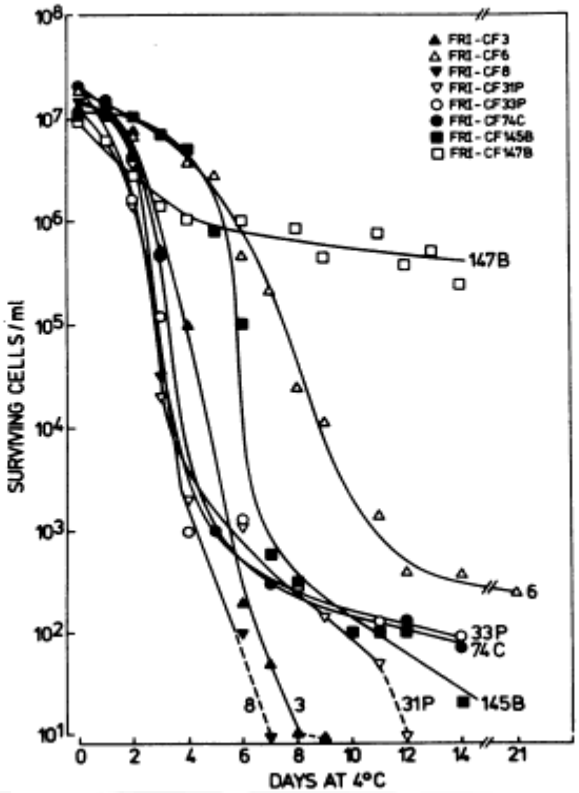
Prevalence and survival of Campylobacter jejuni in unpasteurized milk.

M P Doyle and D J Roman

Abstract

Campylobacter jejuni was isolated from 1 to 108 (0.9%) milk samples obtained from the bulk tanks of nine grade A dairy farms and from 50 of 78 (64%) cows producing grade A milk. Survival of eight Campylobacter strains in unpasteurized milk (4 degrees C) varied greatly: the most tolerant strain showed a less than 2-log10 decrease in viable cells after 14 days, and the most sensitive strain showed a greater than 6-log10 decrease after 7 days. One strain was still recoverable 21 days after the inoculation of milk. Inactivation of the different strains corresponded with an increase in milk aerobic plate count and a decrease in milk pH; however, no absolute correlation could be made between the rates of change of these parameters and the rates of campylobacter inactivation. When held at 4 degrees C, C. jejuni was most stable in brucella broth, died most rapidly in unpasteurized milk, and was inactivated at an intermediate rate in sterile milk. Our results indicate the presence and possible persistence of C. jejuni in raw grade A milk and reaffirm the need for pasteurization of milk.

Sopravvivenza di Campylobacter nel latte crudo 4°C



- 9 ceppi diversi (uomo, aviari,bovini, maiale)
- 1 ml di brodocoltura 10E9 +99ml di latte crudo
- T 4°C,ogni 1-2 giorni (x14gg) conta Campylobacter, carica totale e pH

- Solo il 145B sopravvive in maniera eccezionale nel latte crudo a 4 °C ; (<2log dopo 14g).
- Ceppo 8 a >6log diminuzione di cellule vitali e scomparsa a 7 giorni
- Solo il ceppo 6 dopo 21 g ancora vitale (250UFC/ml)

Doyle et Roman 1982

Comparazione inattivazione (latte crudo, brucella broth,latte sterile)

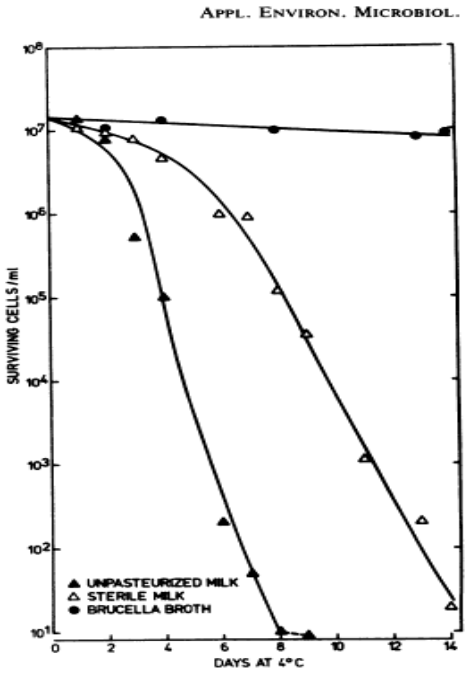


FIG. 2. Survival of *C. jejuni* FRI-CF3 in unpasteurized milk, sterile milk, and brucella broth at 4°C.

- Sopravvivenza maggiore in Brucella broth
- Nel latte sterile il tasso di morte è più basso che nel latte crudo ma stesso andamento
- C.jejuni è inattivato più rapidamente nel latte sterile (pH6,6-6,7) che in brodo Brucella (pH6,9)

Doyle et Roman 1982

Campylobacter e latte crudo

- Norme UE in fatto di igiene: gli Stati membri possono vietare o limitare l'immissione sul mercato di latte crudo destinato al consumo umano (cellule somatiche e coliformi??)
- Esperti EFSA gruppo scientifico sui pericoli biologici (BIOHAZ) : il latte crudo può essere una fonte di batteri nocivi, principalmente Campylobacter, Salmonella, ed Escherichia coli produttore della tossina Shiga (STEC)
- **27/33** focolai in EU tra il 2007 e il 2013 sono da ricondurre al consumo di latte crudo (4 latte di capra, 23 latte bovino) . Di questi, 21 sono stati attribuiti al Campylobacter spp. (in particolare il C. jejuni), 1 a Salmonella Typhimurium, 2 a E. coli STEC e 3 al TBEV (virus encefalite delle zecche).

Cos'è il latte crudo?

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Secondo la legislazione comunitaria, **per "latte crudo"** si intende il **latte prodotto dalla secrezione della ghiandola mammaria di animali di allevamento che non è stato riscaldato a più di 40°C** o sottoposto ad alcun trattamento avente un effetto equivalente (Allegato I, punto 4.1 del Reg. (CE) n 853/2004).

In Italia, può essere commercializzato solo con l'etichetta "da consumarsi previa bollitura", in base al Decreto legge n. 158 del 13/09/2012 (art.8).

Problematica di ricerca Campylobacter nel latte crudo

- Il pH del latte crudo si attesta attorno a 6,5-6,7
- Il pH è influenzato dalla crescita dei batteri acidi lattici

Un basso pH favorisce:

- La proliferazione dei batteri lattici acidi
- l'attività della perossidasi normalmente presente nel latte e che ha un effetto battericida sui batteri Gram negativi
- massima attività a pH 6

Modalità operativa

ISO 20172:2017 (2006)

8 Preparation of test samples

……in accordo con la ISO 6887(ogni parte)

ISO 6887 (all parts),Mycrobiology of the food chain – Preparation of test samples , initial sospensione and decimal dilution for microbiological examination

ISO 6887. Part 5:Specific rules for the preparation of milk and milk products

ISO 6887-5:2010 “Specific rules for preparatio of milk and milk products”

	Preparazione	pH	Campione test (ISO 6887-5 + 10272:2017)
2010	Addizionare un diluente di uso generale (9x)	Aggiustare a neutro	1ml di latte + 9 ml di diluente , dovrebbero essere miscelati con 90 ml di brodo di arricchimento
ISO/ DIS	Fare diluizione o porre direttamente nel brodo di arricchimento	Aggiustare a neutro	Come sopra o 10 ml inoculati direttamente in 90 ml del brodo di arricchimento

ISO 6887-5:2010 versus Bacteriologycal analytical manual BAM

	Preparazione	pH	Campione test (ISO 6887-5 + 10272:2017)
2010	Addizionare un diluente di uso generale (9x)	Aggiustare a neutro	1 ml di latte + 9 ml di diluente , miscelati con 90 ml di brodo di arricchimento
ISO/ DIS	Fare diluizione o porre direttamente nel brodo di arricch.	Aggiustare a neutro	Come sopra o 10 ml inoculati direttamente in 90 ml del brodo di arricchimento
BAM	Centrifugare 50 ml e risospendere il pellet (senza grasso) in 10 ml di brodo	Aggiustare a neutro nel sito di campiona mento e poi in laboratorio	Pellet disciolto in 10 ml di brodo e poi in 90 ml di Brodo di arricchimento (Bolton)

ISO 6887/2010; ISO 10272-1:2017

Soluzione di uso generale (ISO 6887:2010)

- peptone salt solution pH 7.0 ±0,2 a 25°C
- quarter –strenght Ringer solution pH 6,9±0,2 a 25°C
- peptone solution pH 7.0 ±0,2 a 25°C
- Buffered peptone water pH 7.0 ±0,2 a 25°C

Brodo di arricchimento (ISO 10272:2017) parte B

- Brodo Preston pH 7.4 ±0,2 a 25°C

Bacteriological analytical Manual cap.8
Campylobacter

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Preparazione campioni (Milk, frozen dairy products)

Raw milk.

- Controllo del pH al sito di prelievo usando cartine per pH (6-8 range).
- Se il pH è <7,6 addizionare NaOH sterile (1-2 N) e aggiustare il pH a7.5 ± 0.2.
- Ripetere il controllo al laboratorio e aggiustare a pH 7.5 ± 0.2 con NaOH sterile 1-2 N (se occorre) .
- Procedere con il metodo

Bacteriologca analiticala manual cap.8
Campylobacter

Milk Products

(Sec. C. 2.h)

↓

Adjust pH to 7.6

↓

Centrifuge 50 g
(12,000 x g, 40 min)

↓

Discard supernate

↓

Mix pellet with 10 ml Bolton broth with antibiotics

↓

Pellet mix in 90 ml Bolton broth

↓

PREENRICHMENT (Sec. D.1)
35°C, 4 h (microaerobic)

↓

ENRICHMENT (Sec. D.2)
42°C, 20-44 h (microaerobic)

↓

Streak 24 and 48 h enrichments to Isolation agar

Problematica riscontrata

???Visto la bassa carica nel latte, i volumi normalmente esaminati sono corretti?

- campioni di latte di massa 1000 ml o 100 ml (suddiviso in aliquote da 10 ml ciascuno)
- Ogni aliquota è stata esaminata con ISO 10272-1:2017 e in PCR Real time dal brodo di arrichimento
- Primo problema: un campione (controllo positivo,con ceppo ATCC) risultato negativo
- Verifica del problema……

Problematica riscontrata

- Misurato il pH del terreno…….non conforme (pH attorno a 6.0)
- Nuova prova con pH adeguato, saggiando lo stesso ceppo come controllo positivo è stato risolto.
- Quindi il controllo del pH in realtà andrebbe sempre fatto altrimenti si potrebbero avere falsi campioni negativi

Prove effettuate

Per i campioni da 1000 ml divisi in 100 aliquote da 10 ml solo 1-2 aliquote sono risultate positive sia all'isolamento che alla PCR Realtime

	Latte di massa	filtri	UC da 10 ml esaminati	Positivi latte	Positivi Filtri
A	100 ml	Si(1)	10	0	0
B	1000 ml	Si (5)	100	1	0
C	100	Si (5)	10	0	1
D	1000 ml	Si(5)	100	0	1
E	1000 ml	-	100	2	-
F	1000 ml	-	100	0	-

- Quindi la quantità esaminata è cruciale

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Laboratorio Nazionale di Riferimento

Problematiche di identificazione in PCR di C.jejuni

PCR C.jejuni (target gene hip0)

Wang et al,2002

primer multiplex	Sequenza (5′-3′)
CJF (25 pm) CJR (25 pm)	ACTTCTTTATTGCTTGCTGC GCCACAACAAGTAAAGAAGC
CCF (50 pm) CCR (50 pm)	GTAAAACCAAAGCTTATCGTG TCCAGCAATGTGTGCAATG
CLF (25 pm) CLR (25 pm)	TAGAGAGATAGCAAAAGAGA TACACATAATAATCCCACCC
CUF (100 pm) CUR (100 pm)	AATTGAAACTCTTGCTATCC TCATACATTTTACCCGAGCT
CFF (50 pm) CFR (50 pm)	GCAAATATAAATGTAAGCGGAGAG TGCAGCGGCCCCACCTAT
16Sf_entecra (50 pm) 16Sr_entecra (50 pm)	*GCTTAACACATGCAAGTCGA* *GTATTACCGCGCTGCTGG*

primer simplex	*Sequenza (5′-3′)*
P3F (1500-1521)	GGAAAAACAGGCGTTGTGGGGG (50 pm)
P3R (1887-1866)	CCGAAGAAGCCATCATCGCACC (50 pm)

Front Microbiol. 2016 Jun 13;7:887. doi: 10.3389/fmicb.2016.00887. eCollection 2016.

Tracing Back Clinical Campylobacter jejuni in the Northwest of Italy and Assessing Their Potential Source.

Di Giannatale E[1], Garofolo G[1], Alessiani A[1], Di Donato G[1], Candeloro L[2], Vencia W[3], Decastelli L[3], Marotta F[1].

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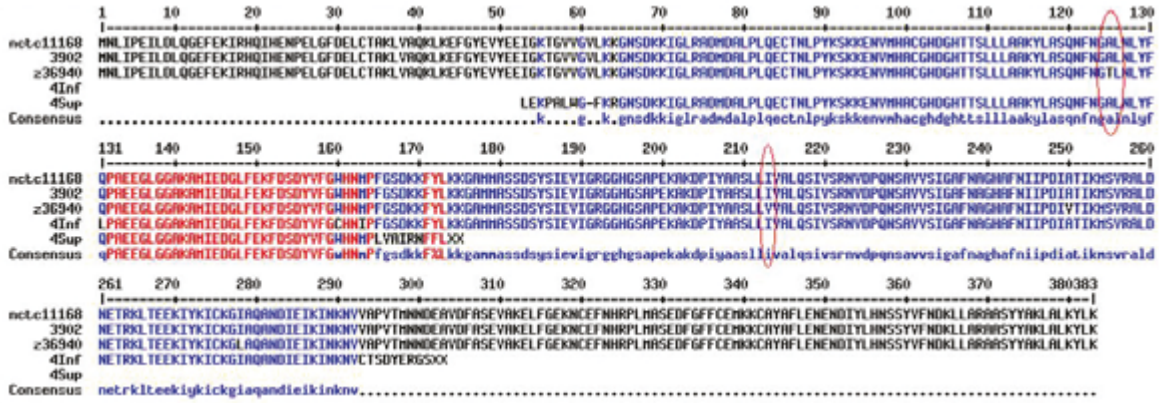
Analysis of the DNA sequences with the

con MultAlin software (http://multalin.toulouse.inra.fr/multalin/).



Il sequenziamento dei frammenti hipO dei ceppi di Campylobacter ha mostrato una differenza nei siti 125 e 213, in cui una treonina (T) e una valina (V) sono stati sostituiti rispettivamente con una adenina (A) e una isoleucina (I), a differenza del ceppo di riferimento usato da Wang (z36940).

Raccomandazioni

- Controllare sempre il pH del terreno dopo l'inoculazione del campione ed eventualmente correggerlo
- Aumentare la quantità di latte da esaminare e effettuare prove in parallelo con centrifugazione e successiva coltura
- Controllare la sequenza hip 0 per l'identificazione di C.jejuni

Cosa ci riproponiamo: studio in parallelo con 3 differenti metodi

- ISO 10272-1:2017
- Metodo BAM
- Filtrazione e successiva coltura

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Grazie per l'attenzione