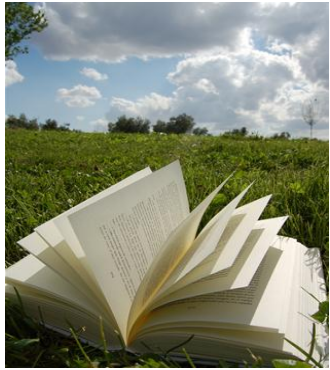


**Microbiological hazards associated
with consumption of raw milk**

Antonella Maugliani



Raw milk consumption in Italy: Regulations



- Not regulated at the EU level
- Direct selling by producers allowed in Italy
- General hygiene rules according to Regulation (EC) No 853/2004
- Specific rules at national level
 - Microbiological criteria for *Salmonella*, *Listeria*, *Campylobacter*, VTEC O157, *St. aureus*
 - Indication on the vending machine that milk should be consumed after boiling.

Raw milk vending machines

Data of the consumption of raw milk in Italy:

1424 vending machines (as of 2 November 2012)

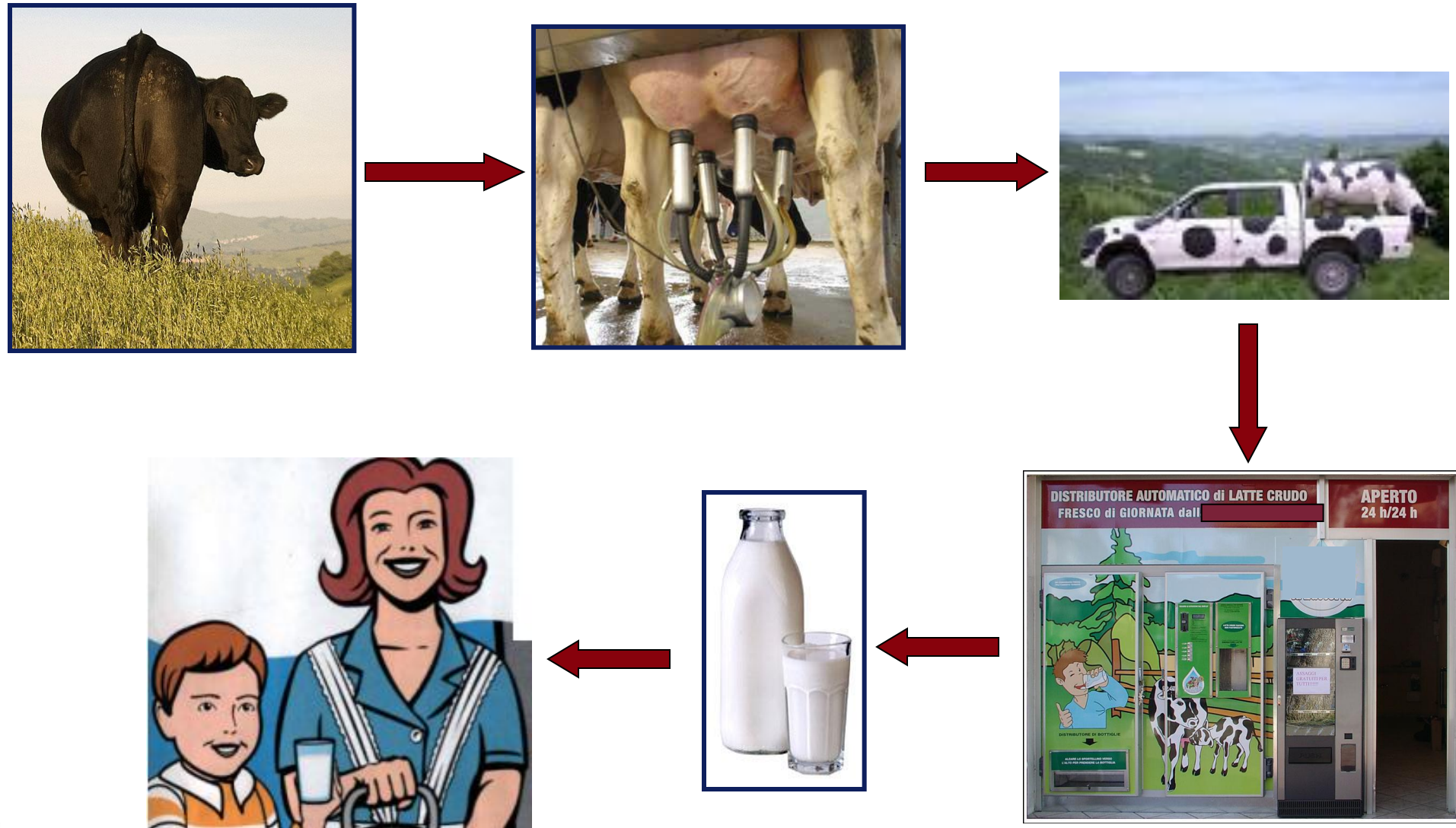
Why raw milk consumption became popular: natural lifestyle and ecological flavor, richer in nutrients, less expensive

Why milk should NOT be consumed raw: health hazards

www.milkmaps.com



Raw milk production line



Research project

RF-IZV-2008-1142936 - Risk-benefit analysis as a tool to support and evaluate nutritional strategies

OBJECTIVE

Evaluation of the microbiological and chemical risks related with the consumption of raw milk



**Regions
Involved in The
project**

Cases of HUS in Italy (February 2012)

Year	HUS Cases	Consumption of raw milk	%
2006	30	0	0
2007	37	3	8.2
2008	24	5	20.8
2009	46	5	10.9
2010	43	2	3.3
2011	40	1	2.2
2012*	8	2	25.0

DRINKING RAW MILK AS A RISK FACTOR

MATERIALS AND METHODS

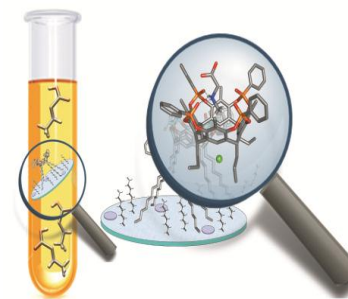
SAMPLING : at vending machines or directly from milk tanks

SCREENING : MOLECULAR APPROACH

VTEC presence of virulence genes and serogroups genes, based on Real Time PCR, as defined in the **ISO TS 13136**

MRSA presence of SA genes, associated with *S. aureus* species, and *mecA*, coding for resistance to methicillin, through duplex end point PCR

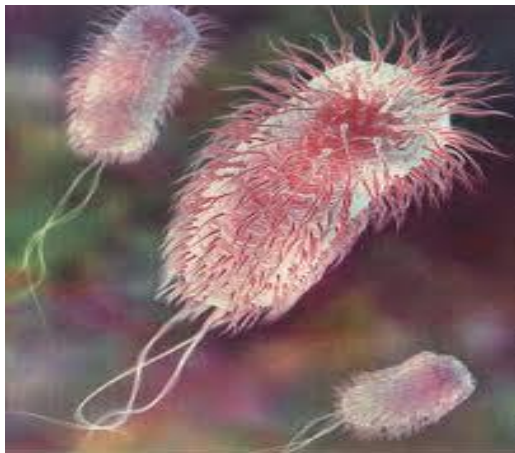
ISOLATION: FROM PCR POSITIVE SAMPLES



RESULTS OF MONITORING OF VTEC AND MRSA

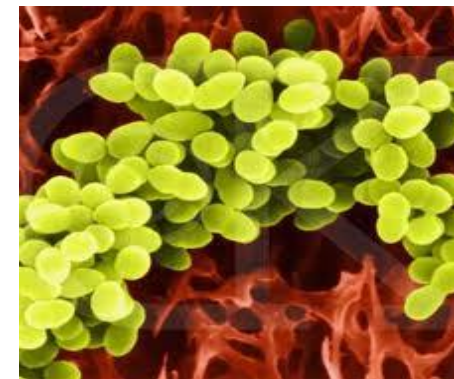
VTEC

- ✓ Samples = 657
- ✓ PCR positive = 35 (5,3 %)
- ✓ Confirmed (isolation) = 5(0,78 %)



MRSA

- ✓ Samples = 554
- ✓ PCR positive = 27 (4,87 %)
- ✓ Confirmed (isolation) = 6(1,08%)



VTEC RESULTS

8/35 samples positive in PCR for the following O-groups:

- ✓ **O145(1)**
- ✓ **O26(3)**
- ✓ **O157(1)**
- ✓ **O145-O103(1)**
- ✓ **O145-O103-O26-O157(1)**
- ✓ **O145-O103-O157(1)**

5 strains were isolated

- ✓ **O145= 1 strain**
- ✓ **O26= 2 strains**
- ✓ **O103= 1 strain**
- ✓ **O157= 1 strain**

Evaluation of the efficiency of nucleic acid extraction

- UHT milk samples diluted and spiked with 3.2×10^5 cfu/ml of VTEC O157
- Three different commercial systems of nucleic acid extraction
- 8 replicates of 1 ml for each extraction system
- Each extraction was analyzed in duplicate with real time PCR *rfaA* (O157)

Method A (Median)	Method B (Median)	Method C (Median)
Columns	Not immobilized resin	Not immobilized resin
Expected result 3.2×10^2 GU/rxn Obtained 0.4 GU/rxn	Expected result 3.2×10^2 GU/rxn Obtained 9.7 GU/rxn	Expected result 3.2×10^2 GU/rxn Obtained 20.3 GU/rxn
EFFICIENCY OF EXTRACTION METHOD		
0,12%	3%	6,5%

Extraction step: CONCLUSION



➔ The data on the extraction efficiency indicate a **Limit of Detection** between 6 and 350 UFC of VTEC per ml of milk with the systems used

➔ POSSIBLE UNDERSTIMATION OF THE PRECENCE OF PATHOGENS IN MILK

=

POSSIBLE UNDERSTIMATION OF RISK FOR THE CONSUMER



CONCLUSIONS -2



PATHOGENS ARE PRESENT IN RAW MILK AT VENDING MACHINES



DNA EXTRACTION REPRESENTS A CRITICAL STEP IN MOLECULAR ANALYSES OF FOOD



CONSUMPTION OF RAW MILK REPRESENTS A RISK FOR THE CONSUMERS

Thank you for your attention!

