



### The Raw Milk Conundrum: Microbiology and Ecology of Raw Milk

Michele Jay-Russell, DVM, MPVM, DACVPM Western Institute for Food Safety and Security University of California, Davis

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## **Objectives**

- Define raw milk
- Review the microbial composition of raw milk
- Discuss pathogen survival and growth in raw milk
- Evaluate "farm to fork" strategies to enhance raw milk safety and quality
- Define future research, extension, and education needs relating to the growing interest in raw milk consumption





## **Definition of Raw Milk**

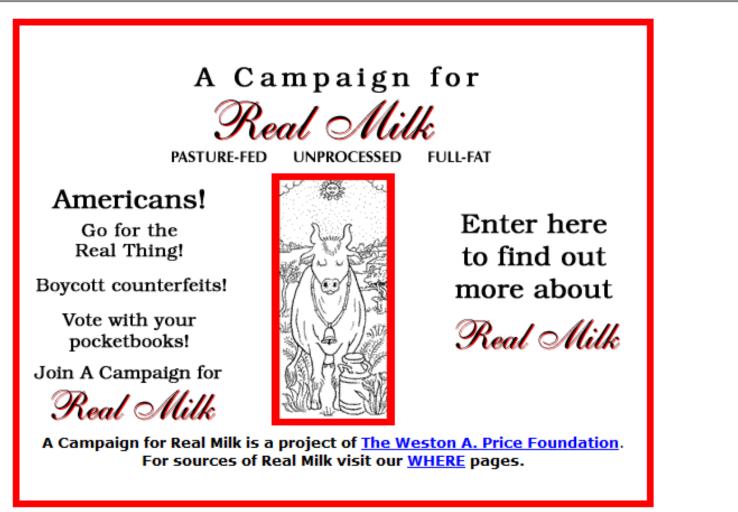
- Unpasteurized (no heat treatment)
  - Legal: licensed/inspected
  - Illegal: underground market; "bathtub cheese"
  - In between? Cow-leases







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L (RAW) MILK!	👌 🔻 🔊



## "Real Milk" Values

- Can save family farms
- Products contain no additives
- Contains butterfat, and lots of it
- Is not homogenized
- Is not pasteurized
- Comes from real cows that eat real feed

Source: Weston A. Price Foundation





### How do microorganisms contaminate milk?

- A healthy animal's milk is usually sterile when secreted from the mammary gland
- Contamination may occur from:
  - Mastitis
  - Systemic disease (e.g., bovine tuberculosis)
  - Skin microflora
  - Environment: feces, dirt, processing equipment
  - Vectors (flies)
  - Human carriers





# Factors that influence survival and growth of microorganisms in food

### Intrinsic

- Nutrient availability
- pH (acidity)
- Water availability
- Microbial community

### **Extrinsic**

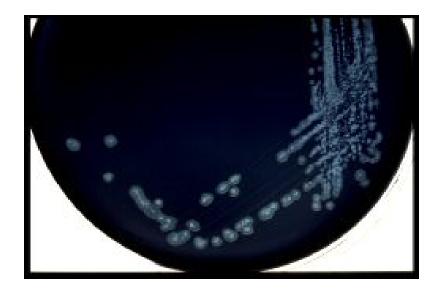
- Temperature
- Atmosphere



#### MILK (raw or pasteurized):

Rich in nutrients
Neutral pH
Excellent medium for microbial survival and growth





### Types of microorganisms that can contaminate dairy products

- Spoilage (gram negative and gram positive psychrotrophic bacteria, especially pseudomonads)
- Fecal indicators (coliform and non-coliform)
- Pathogens
- "Beneficial bacteria?"





# Examples of microorganisms and toxins isolated from raw milk that may cause disease

Staphylococcal enterotoxins Brucella spp. Campylobacter spp. Streptococcus Coxiella burnetti (Q fever) Tick-borne encephalitis virus Cryptosporidium Toxoplasma E. coli O157:H7/EHEC Yersinia enterocolitica Listeria monocytogenes Mycobacterium bovis (bovine tuberculosis) Mycobacterium avium subspecies paratuberculosis **Rabies virus** Salmonella spp. Shigella





# Surveys of pathogens in bulk tank (pooled) raw milk in the US

- Campylobacter jejuni
- *E. coli* O157:H7/STEC
- Listeria monocytogenes
- Salmonella spp.
- Yersinia enterocolitica

- <1% 12.3%
- <1 3.8%
- 2.7 6.5%
- <1% 8.9%
- 1.2 6.1%

LeJeune et al, Clin Infect Dis 2009





# Survey of raw bovine colostrum in Pennsylvania

- Salmonella detected in 8 of 55 (15%) samples
- Streptococcus agalactiae (1000 colony-forming units [CFU]/mL) detected in 1 of 55 (2%) samples



- The mean standard plate count (977,539 CFU/mL), coliforms (323,372 CFU/mL), and non-coliforms (111,544 CFU/mL) counts in colostrum were considerably higher than raw bulk tank milk counts reported previously from Pennsylvania
- Farm size did not influence the bacteriological quality of colostrum.
- Conclusions: Collection, handling, and storage of colostrum need to be addressed to improve bacteriological quality of colostrum intended not only for feeding calves but also for human consumption.

Houser et al, Foodborne Pathog Dis 2008





### Escherichia coli O157:H7 Infections in Children Associated with Raw Milk, California, 2006: Raw Milk and Colostrum Product Testing

#### Raw colostrum/chocolate colostrum: fecal coliforms ranged 210-46,000 MPN/g

	Standard plate counts			Coliform counts		
Product sample	>15,000 CFU/mL (n)	>250,000 CFU/mL (n)	Range (CFU/mL)	>10 coliforms/mL (n)	>1,500 coliforms/mL (n)	Range (coliforms/mL)
Raw skim milk (n = 13)	11	11	2,900 to >10,000,000	12	9	75 to >10,000
Raw whole milk (n = 18)	13	11	1,800 to >9,000,000	15	4	0 to >10,000
Raw colostrum (n = 4)	4	4	2,000,000 to >8,000,000	4	2	110 to >10,000
Raw chocolate colostrum (n = 3)	3	3	263,000 to 1,200,000	3	2	98 to >20,000
Raw cream (n = 11)	9	7	1,800 to 12,000,000	10	6	39 to 6,200
Raw kefir (n = 3)	3	3	320,000 to 9,000,000	3	0	12 to 270
Raw butter (n = 4)	3	2	110,000 to >4,000,000	4	3	110 to >3,300

TABLE. Microbial testing results for dairy A raw milk product samples with code dates of September 17 through October 9, 2006 - California

#### MMWR June 13, 2008





### Survival and Growth of Foodborne Pathogens in Raw Milk and "Competitive Exclusion"

 <u>Ecology</u>: The competitive exclusion principle, sometimes referred to as Gause's Law of competitive exclusion or just Gause's Law, states that two species that compete for the exact same resources cannot stably coexist.





# Survival and Growth of Foodborne Pathogens in Raw Milk and "Competitive Exclusion" cont.

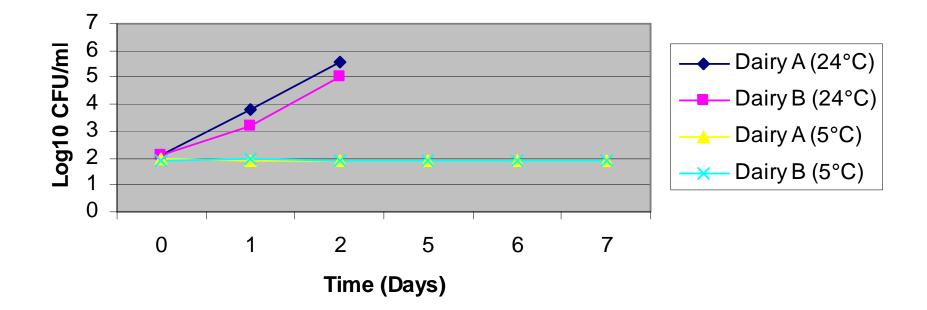
- <u>Microbiology</u>: In 1973, Nurmi and Rantala introduced a technique to increase the resistance of baby chicks to *Salmonella* infection by inoculating them orally with adult fowl intestinal contents. This technique has since been termed "competitive exclusion" or CE.
- More generally, the term competitive exclusion is used to describe the process by which beneficial bacteria exclude bad bacteria or pathogens. CE implies the prevention of entry and establishment of a bacterial population into the gut. To succeed, the "good" bacteria must be better suited to establish or maintain itself in that gut environment.

Schleifer JH, World's Poultry Science J 1985 Canadian Poultry Consultants





#### Salmonella Survival and Growth in Fresh Raw Milk at Room Temperature and Refrigeration

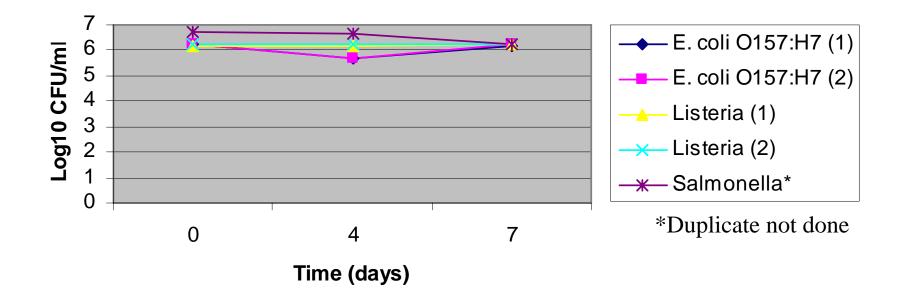


Source: Linda Harris and Candice Lin, UC Davis (unpublished data)





#### Survival and Growth of Foodborne Pathogens in Fresh Raw Milk during Refrigeration

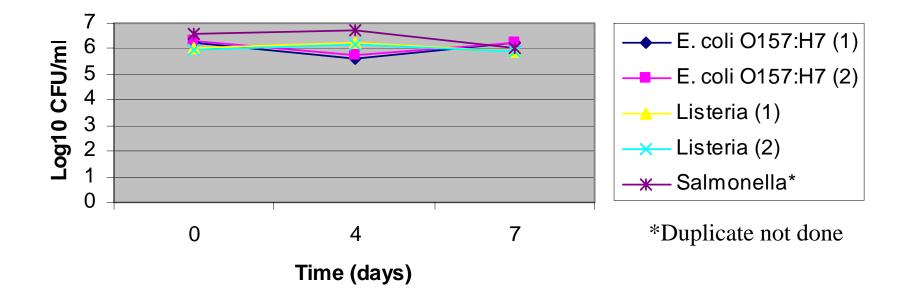


Source: BSK Laboratories (unpublished data)





#### Survival and Growth of Foodborne Pathogens in Fresh Raw Colostrum During Refrigeration



Source: BSK Laboratories (unpublished data)





## Conclusions

- No significant growth or reduction in numbers of Salmonella (both studies), *E. coli* O157:H7, and *Listeria* during refrigeration
- 3+ log increase in *Salmonella* at room temperature (UCD study)
- No evidence of "competitive exclusion" in either preliminary study





## **Internet Interpretation**

- "When 7 logs (10 million counts) of pathogens were added to one-milliliter samples of organic raw milk they would not grow. In fact they died off.
- The listeria drop was less dramatic and was similiar to the E. Coli O157:H7 samples that were studied, but they also did not grow and declined substantially over time.
- The lab concluded: "... organic raw milk and colostrum do not appear to support the growth of pathogens..."

Source: http://www.realmilk.com/safety-raw-milk.html





## **Infectious Dose**

1 milliliter ~ 20 drops

• Salmonella

~1,000-10,000 cells (some strains <100)

Listeria monocytogenes ~1,000 cells

Campylobacter

~500 cells

<50

• E. coli O157:H7





#### Survival of Campylobacter jejuni in milk

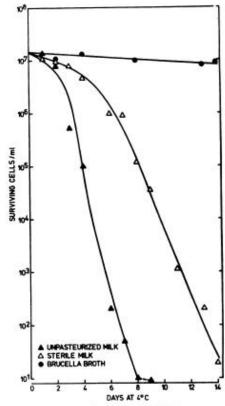
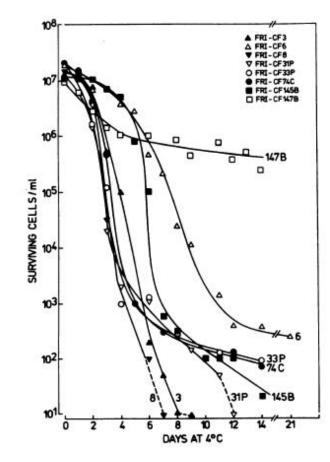


FIG. 2. Survival of C. jejuni FRI-CF3 in unpasteurized milk, sterile milk, and brucella broth at 4°C.



**Doyle and Roman, Applied Environ Microbiol 1982** 







- C. jejuni died off in parallel in both raw (~8 days) and sterile (~14 days) milk at refrigeration; the authors speculated that the more rapid die-off in raw milk may be due to antibacterial properties such as lactoperoxidase in raw milk
- Despite the more rapid inactivation of *C. jejuni* in raw milk, enough cells survived for about a week to potentially cause illness (infectious dose = ~500 cells)

Doyle and Roman, Applied Environ Microbiol 1982





## **Conclusions...cont.**

- The survival of *C. jejuni* in raw milk was highly variable depending on the strain:
  - range from a 6 log reduction of viable cells in 7 days for the most sensitive strain to a 2 log reduction in 14 days for the more tolerant strains
  - One C. jejuni strain survived 21 days in raw milk
- In sum, the study shows that most *C. jejuni* strains die off in both raw and sterile milk by the end of the shelf life
  - In general, Campylobacters are more fastidious (fragile) than other pathogens such as Salmonella and E. coli O157:H7
  - Die-off may partially explain the difficulty in recovering *C. jejuni* from milk during outbreak investigations

#### Doyle and Roman, Applied Environ Microbiol 1982





Type of milk	Storage time - (h)	Survivor numbers (log <sub>10</sub> cfu/ml) at various storage temperatures (°C)				
		5	10	15	20	
Raw	24	3.00	2.88	2-40	ND	
	48	2.40	2.40	ND	ND	
Pasteurized	24	3.51	3-30	2.88	3.00	
	48	3.68	3-48	3.10	ND	
UHT	24	3.30	3-30	3.24	2.40	
	48	3.30	3.00	2.40	2.40	

 Table 1. Survival of Campylobacter jejuni in goat's milk stored at four different temperatures\*

\* Milk samples were inoculated to give an initial viable count of  $\log_{10}$  5.23 cfu/ml.

ND, No viable units detected; UHT, ultra-heat-treated.

#### Simms et al, Letters Applied Microbiol 1989





### Is raw milk a probiotic?

- "The term *probiotic* should be used only for products that meet the scientific criteria for this term – namely, products that contain an adaquate dose of live microbes that have been documented in target-host studies to confer a health benefit.
- Probiotics must be identified to the level of strain, must be characterized for the specific health target, and must be forulated into products using strains and doses shown to be efficacious."

Sanders et al, Clin Infect Dis 2008





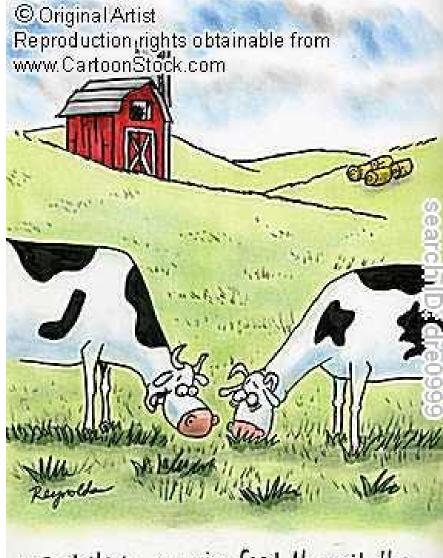
# Does an animal's diet impact fecal shedding of pathogens?

- Identification of on-farm management practices that would reduce or eliminate foodborne pathogens in cattle and other livestock (including diet changes) is an active area of research, but many study results are inconclusive.
- Diez-Gonzalez (Science, 1998): study suggested that cattle could be fed hay for a brief period before slaughter to significantly reduce the risk of foodborne *E. coli* infection. Conclusion based on a hypothesis that grain feeding increases acid resistance of *E. coli* in cattle. Although they showed increased acid resistance in *E. coli* from grain-fed cattle, the sample size was small, and only "generic" *E. coli* strains were used, not *E. coli* O157:H7.









" Just think... our pies feed the soil, the soil feeds the grass, the grass feed us..."





# Does an animal's diet impact fecal shedding of pathogens...cont.

- Studies by other researchers worldwide have since found little difference in acid resistant *E. coli* O157:H7 among grain- verses grass-fed cattle, and some even found more *E. coli* O157:H7 shed by grass-fed animals.
- Outbreaks have traced back to grass-fed and pastured animals, as well as animals in feedlots.





# Examples of recent foodborne disease outbreaks and recalls linked to "grass only, pastured" cattle

Year	State	Pathogen	Vehicle	Illnesses	Comment
					Outbreak strain found in
2006	Multi	<i>E. coli</i> O157:H7	Baby spinach	205	cattle feces
			Raw		<i>E. coli</i> O157:H7 found in
2006	CA	<i>E. coli</i> O157:H7	milk/colostrum	6	heifers
					Outbreak strain found in
2007	WA	C. jenuni	Raw milk	5	milk
			Raw		Outbreak strain found in
2007	CA	C. jejuni	milk/colostrum	8	cattle feces
2007	CA	Listeria	Raw cream	0	Recall, no illnesses
2008	CA	C. jejuni	Raw cream	0	Recall, no illnesses
2009	СО	C. jejuni	Raw milk	8	Final report pending





# Fresh Raw Milk

(From Healthy Grass-fed Cows)





"Products from grass-fed animals are safer than food from conventionally-raised animals." Eatwild website

"When cows are not stressed (grass-fed and kept healthy) they simply do not slough off pathogens in their manure." Letter on the "Safety of Raw Milk," Real Milk website (5/19/04)

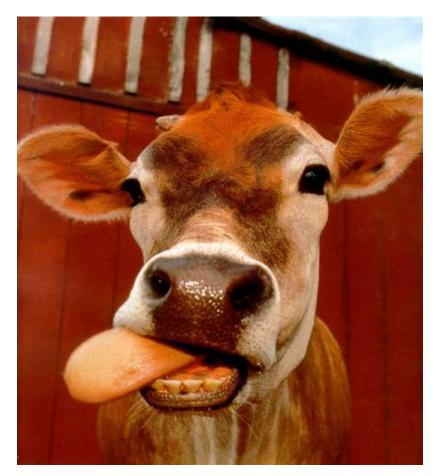
"The thought behind my post was that every lawmaker that votes on the new raw dairy bill should SEE the DIFFERENCE between CLEAN GRASS FED farms and the FILTHY confinement farms. Then ask them which dairy products will you feed your children and elderly parents? Don W. The Complete Patient (6/2/08)"





### **Future Directions**

- Research needs: on-farm factors affecting pathogen prevalence; survival of pathogens in raw dairy products for human consumption; improved diagnostic assays
- Risk assessment for 21<sup>st</sup> century raw milk "models" (herd shares, on-farm vs. retail sales)
- Is there a place for HACCP-like approaches to raw milk safety?
- Outreach/extension/education for raw dairy farmers and consumers







## **Questions?**





