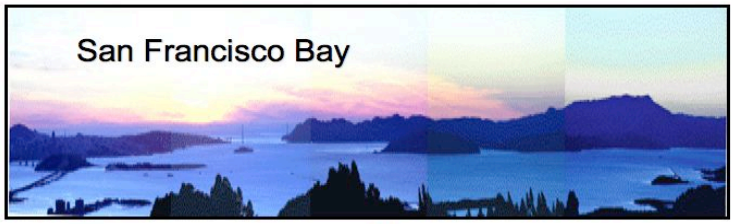
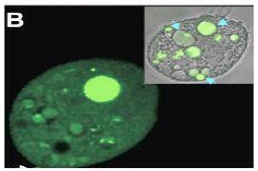
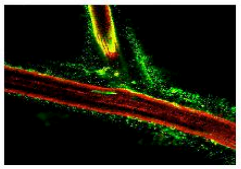






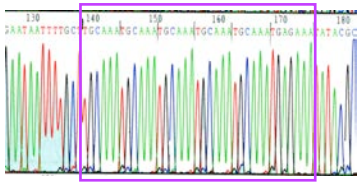
AVMA 146th Annual Convention, Seattle, WA

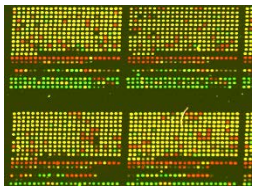
July 11-14, 2009

"Raw Milk Conundrum:The Interplay of Science, Policy and Free Choice"

"Advances in microbiological and molecular assays for assessing raw milk"

Robert Mandrell, Ph.D.
Research Leader, Produce Safety and Microbiology Research Unit
USDA, Agricultural Research Service, Western Regional Research Center
800 Buchanan Street, Albany, CA 94710





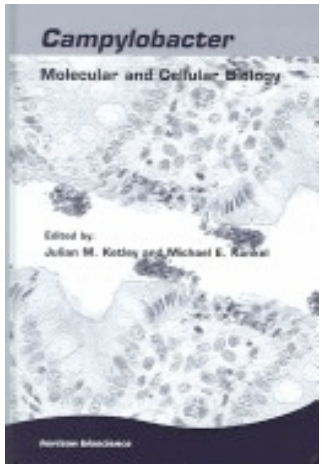
Raw Milk Conundrum

Summarized from “Marler Blog” (www.marlerblog.com)

- Pros
  - ”Protective effect” against allergies, tooth decay, pathogens (anti-bacterial)
  - Complex microflora (“Hygiene hypothesis”); induced immunity
  - Nutritional and fertility advantages
- Cons
  - **GI illness: sporadic and outbreak**
  - Costs to public health sector, productivity, dairy industry

Raw milk outbreaks

- Headrick, et al. The epidemiology of raw milk-associated foodborne disease outbreaks reported in the United States, 1973 through 1992. Am J Public Health 88:1219-21.
  - 46 outbreaks
  - 1733 illnesses
  - *Campylobacter* (57%), *Salmonella* (26%), *E. coli* O157 (2%), *Staphylococci* (2%)

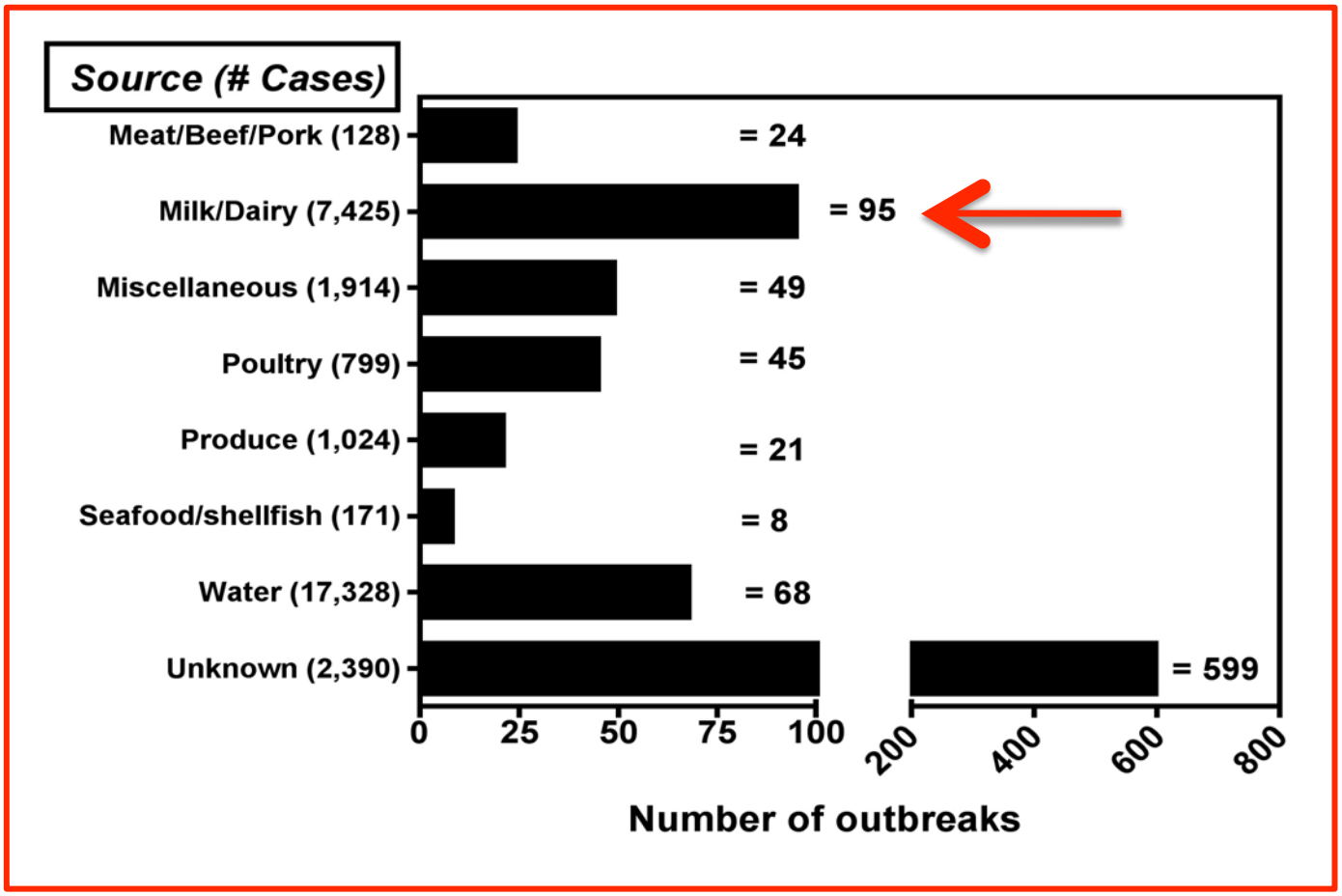


Chapter 6

Prevalence of *Campylobacter* in the Food and Water supply: Incidence, Outbreaks, Isolation and Detection

William G. Miller and Robert E. Mandrell

2006. In, *Campylobacter*: New Perspectives in Molecular and Cellular Biology. Horizon Scientific Press, Norfolk, UK.



Outbreaks- *Campylobacter* and milk, 1978-present

Milk and Dairy	No. cases	Location	Date	References
Milk, raw	100	UK	Mar-78	(Robinson, DA and Jones, DM, 1981)
Milk, raw	63	UK	Oct-78	(Robinson, DA et al., 1979, Robinson, DA and Jones, DM,

Milk, unpasteurized, 3500 cases, UK-1979

Milk, raw	148	Scotland	Jan-79	(Porter, IA and Reid, TM, 1980, Robinson, DA and Jones, DM, 1981)
Milk, raw	13	UK	Mar-79	(Robinson, DA and Jones, DM, 1981)
Milk, unpasteurized	3500	UK	Mar-79	(Robinson, DA and Jones, DM, 1981, Jones, PH et al., 1981)
Milk, unpasteurized	>75	UK	Apr-79	(Robinson, DA and Jones, DM, 1981)
Milk, raw	4	UK	Jun-79	(Robinson, DA and Jones, DM, 1981)
Milk, fecal contam	14	UK	Aug-79	(Robinson, DA and Jones, DM, 1981)
Milk, raw	75	UK[i]	Jan-80	(Robinson, DA and Jones, DM, 1981)
Milk, raw	30	UK	Mar-80	(Robinson, DA and Jones, DM, 1981)
Milk, raw	40	UK	Mar-80	(Robinson, DA and Jones, DM, 1981)
Milk, raw	2	UK	Apr-80	(Robinson, DA and Jones, DM, 1981)
Milk, raw	106	USA (OR)	Jan-81	(Finch, MJ and Blake, PA, 1985)
Milk, raw	103	USA (KS)	Mar-81	(Finch, MJ and Blake, PA, 1985, Kornblatt, AN et al., 1985)
Milk, raw	190	USA (AZ)	Apr-May-81	(Taylor, DN et al., 1982, Finch, MJ

Milk, raw, 500 cases, Switzerland-1981

				Potter, ME et al., 1983)
Milk, raw	3	USA (ME)	Oct-81	(Finch, MJ and Blake, PA, 1985)
Milk, raw	14	USA (AZ)	Oct-81	(Finch, MJ and Blake, PA, 1985)
Milk, raw	14	USA (ME)	Oct-81	(Finch, MJ and Blake, PA, 1985)
Milk, raw	>500	Switzerland	Nov-81	(Stalder, H et al., 1983)
Milk, raw	50	UK	Nov-81	(Wright, EP et al., 1983)
Milk, raw	22	UK	Jan-82	(Wright, EP et al., 1983)
Milk, raw	46	USA (MD)	Apr-82	(Finch, MJ and Blake, PA, 1985)
Milk, raw	32	USA (MI)	May-82	(Finch, MJ and Blake, PA, 1985)
Milk, raw	32	USA (ME)	Jun-82	(Finch, MJ and Blake, PA, 1985)
Milk, raw	16	USA (WI)[ii]	Jun-82	(Finch, MJ and Blake, PA, 1985, Klein, BS et al., 1986)
Milk, raw	15	USA (VT)	Sep-82	(Finch, MJ and Blake, PA, 1985)
Milk, raw	4	USA (VT)	Oct-82	(Finch, MJ and Blake, PA, 1985)
Milk, raw	57	USA (PA)	May-83	(CDC, 1983)
Milk, raw (goat)	6	USA (WA)	Jul-83	(Harris, NV et al., 1987)

Milk, unpasteurized, 332 cases, UK-1987

Milk, unpasteurized	35	USA (VT)	Apr-86	(Birkhead, G et al., 1988)
Milk, raw	~110	USA[iii]	78-86	(Tauxe, RV et al., 1988, Riley, LW and Finch, MJ, 1985)
Milk, unpasteurized	332	UK (Multi)[iv]	87	(Sockett, PN, 1991)
Milk, heat-treated	526	UK (Multi)[v]	87	(Sockett, PN, 1991)
Milk, heat-treated	14	UK (Multi)	89	(Sockett, PN, 1991)

Milk, fecal contam	104	USA (OK)	88	(Sails, AD et al., 2003)
Milk,raw	87	USA (KS)	88	(Sails, AD et al., 2003)
Milk, raw	13	USA (WA)	Mar-90	(CDC, 2003a)
Milk, raw	2	USA (WA)	Apr-90	(CDC, 2003a)
, raw	42	USA (TX)	May-90	(CDC, 2003a)
, raw (goat)	3	USA (WA)	Jul-91	(CDC, 2003a)
, raw (susp.)	11	USA (ME)	Feb-92	(CDC, 2003a)
	23	USA (NY)	Apr-92	(CDC, 2003a)
Milk, milk bottles	23	UK	May-92	(Pebody, RG et al., 1997)
Milk, raw	50	USA (MN)	May-92	(CDC, 2003a)
Milk, ra				
Milk, pa				
Milk, ra				
Dairy, c				
Milk, ra				
Milk, ra				
Milk, pe bottles				
Milk, ra				
Milk				
Milk				
Milk, ra				
Milk				
Milk				
Milk, raw	38	Austria	Sep-98	(Lehner, A et al., 2000)
Milk, raw	3	Sweden	98	(SMI, 1999)
Milk, raw	5	Sweden	98	(SMI, 1999)
Milk	11	Germany	Apr-99	(Reintjes, R et al., 1999)
Milk, raw	2	USA (WA)	Jun-99	(CDC, 2003a)
Milk, raw	2	USA (TX)	Feb-00	(CDC, 2003a)
Milk, raw	4	USA (ID)	Feb-00	(CDC, 2003a)
Milk, raw	21	USA (OK)	Feb-00	(CDC, 2003a)
Milk, raw	19	USA (WI)	May-00	(CDC, 2003a)
Milk, unpasteurized	8	USA (MN)	Jun-00	(MDH, 2000)
Milk, raw	8	USA (MN)	Jun-00	(CDC, 2003a)
Milk, raw	42	USA (ID)	Jun-00	(CDC, 2003a)
Milk, raw	11	USA (OK)	Jun-00	(CDC, 2003a)
Milk, raw	3	USA (PA)	Aug-00	(CDC, 2003a)
Milk, raw	39	USA (NY)	Sep-00	(CDC, 2003a)
Milk, raw	4	USA (MN)	Mar-01	(MDH, 2000, CDC, 2003b)
Milk, raw	75	USA (WI)	Nov-01	(CDC, 2002, CDC, 2003b)
Milk, raw	13	USA (UT)	Jan-02	(Peterson, MC, 2003)
Milk, raw	28	Netherlands	Apr-02	{van den Brandhof, 2004 #455}
Milk, raw	3	UK	Oct-Dec-02	(CDR, 2003)
Milk, custard[ii]	81	Spain	May-03	(Jiménez, M et al., 2005)
Dairy, cheese	11	USA (CA)[ii]	Oct-03	(DCDC, 2004)
Milk, pasteurized	>1600	USA (CA)	May-06	(CDHS-DCDC, 2006)
Milk, raw, cheese	19	USA (KA)	Oct-07	(MMWR, 2009)
Total	**7,425**			

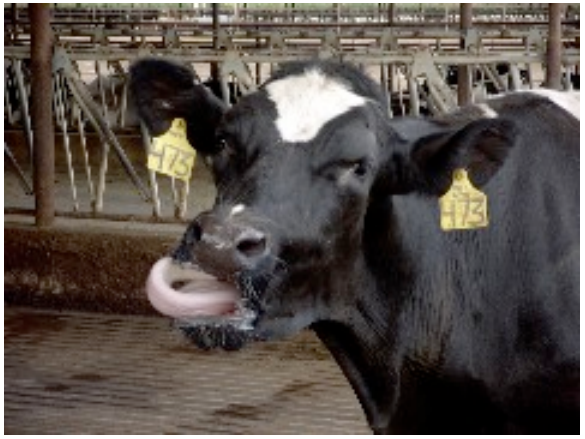
[i] Represents 2 outbreaks.

May-2006, 2nd largest *Campylobacter* outbreak in US history? (CA prison dairy)

Minimal Infectious Dose (MID)

- Minimal Infectious Dose depends upon:
  - Virulence of the strain
  - Immune-status of the host and host specificity
  - Complexity of the contaminated sample (food) ingested with pathogen
  - Exposure does not always result in an illness
- *C. jejuni*
  - 500 cells in a single “volunteer” (Robinson, 1981)
  - ~500 cells in volunteers (Black *et al.,* 1988)
- *E. coli* O157:H7
  - 31 to 35 cells, children and adults (Teunis et al. 2004)
- Theoretically, milk provides an even distribution of MIDs

Surveillance and outbreaks



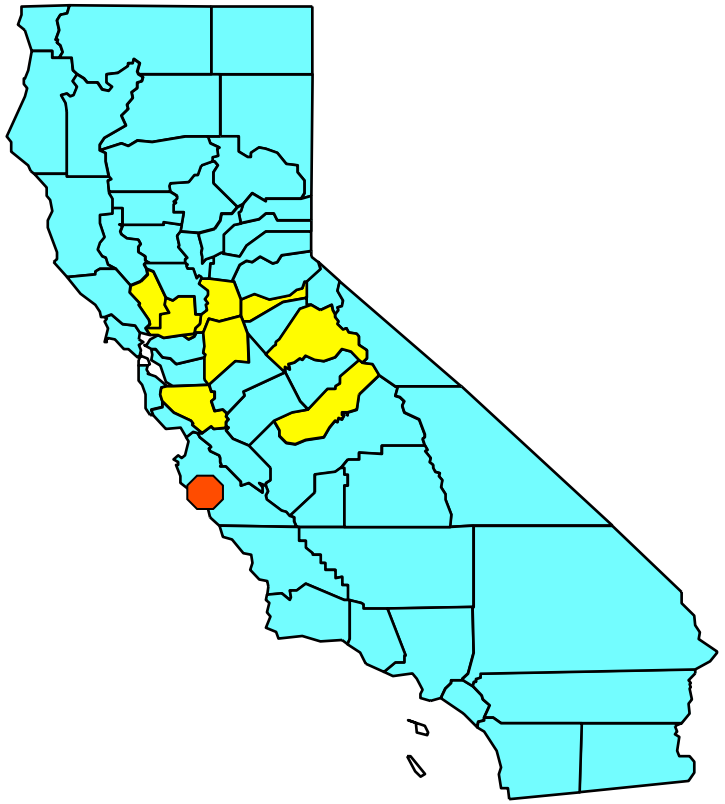
- Epidemiology is critical
- Microbiology
  - Isolation of pathogen from complex samples (milk)
    - Enrichment culture
    - ImmunoMagnetic separation (IMS)
    - Selective and/or chromogenic media
    - Subculture of suspect colonies
    - Test many (10-50 cfu) to increase chance of finding outbreak strain (“needle in the haystack”)
  - Genotyping to identify strains
  - Molecular identification without culture (PCR)

Molecular characterization of *Campylobacter jejuni* strains linked to recent milk-related outbreaks and surveillance of California Central Valley dairy environments

Michele Jay, William Miller, Emma Yee,
Anna Bates, Paul Rossitto and Robert Mandrell

C. jejuni Outbreak 1

- Correctional facility with on-site dairy ('Dairy A')
- Onset dates of May 13–26, 2006
- 1,644 ill inmates/11 facilities
- Pasteurized milk from Dairy A only common food/beverage
- No *Campylobacter* isolated from milk
- Largest US milk-related *Campylobacter* outbreak; 2nd largest *Campylobacter* outbreak ever in US.



Campylobacter sample collection

Farm Investigation

- Environmental samples were collected between Dec 2006 and Jan 2007
  - Cattle feces
  - Flush alley water
  - Bulk tank raw milk
  - Dairy lagoons

C. jejuni CFU on selective medium: the start of isolation

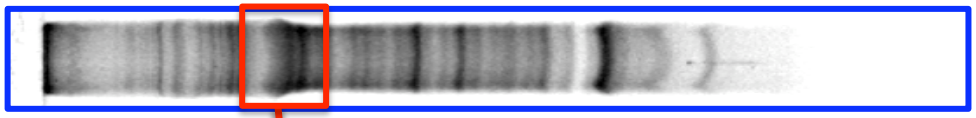
How many CFU should you pick to find the "needle in the haystack"?

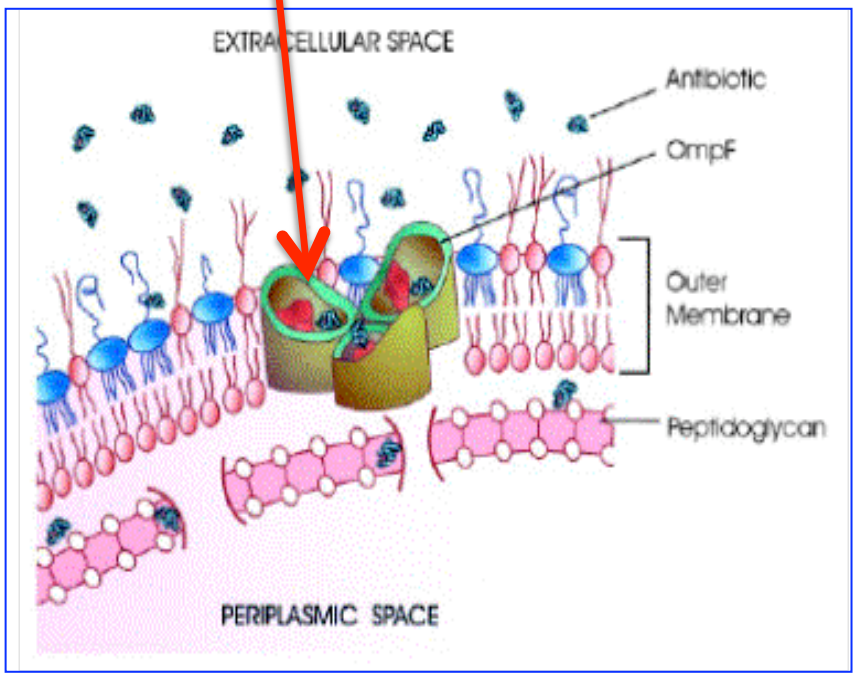
Campylobacter sample collection

Farm Investigation

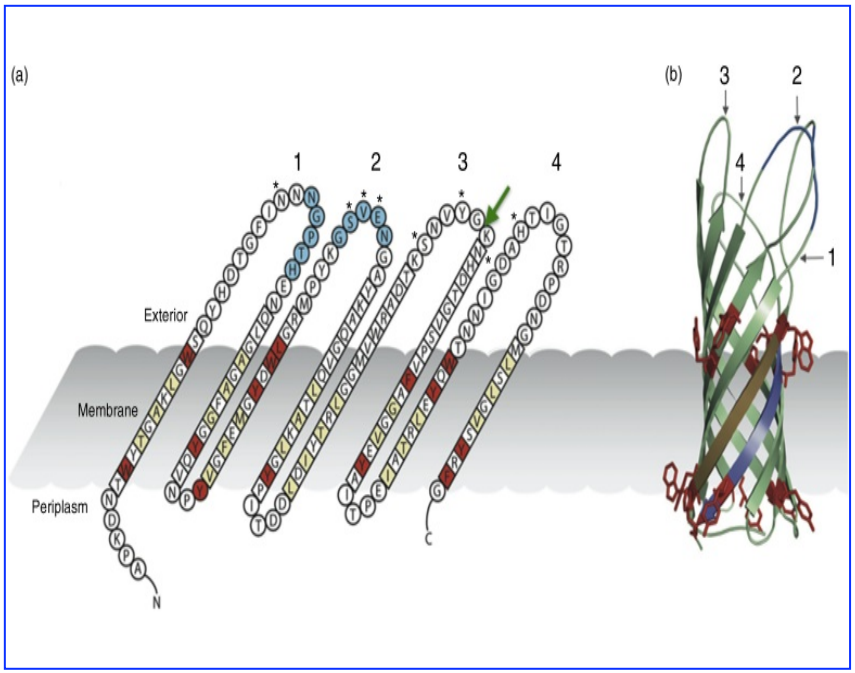
- Multiple colony picks
  - 6-12 suspect *Campylobacter* from each positive sample
  - 52 isolates confirmed *C. jejuni*
  - Major outer membrane protein (Cmp/MOMP) typing: identified multiple isolates as potential outbreak strain
  - Multilocus sequence typing (MLST) ST-21

MOMP typing (Cmp)





Outer Membrane



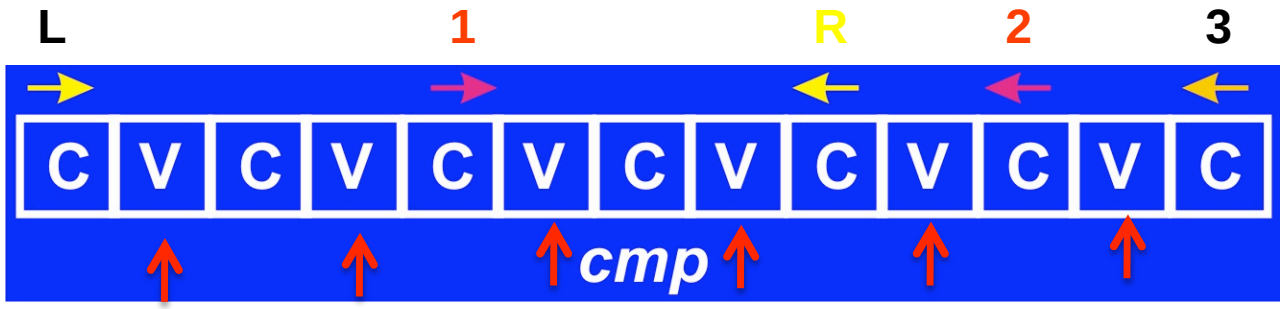
Hypervariable loops in OMP

Related strains will have identical DNA sequence for this gene

Methods

MOMP (*cmp*) typing

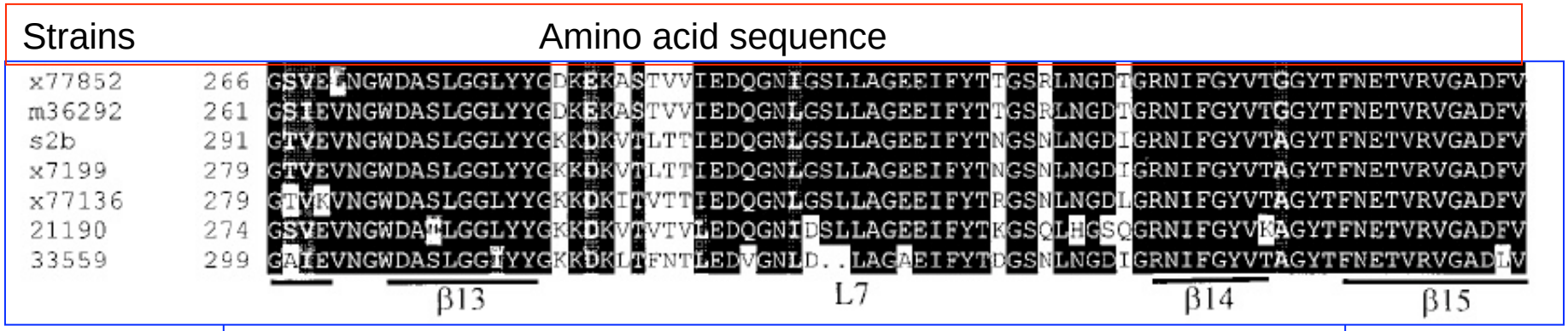
- *cmp* gene encodes the *Campylobacter* MOMP
- Sequence polymorphisms make *cmp* typing an epidemiological tool

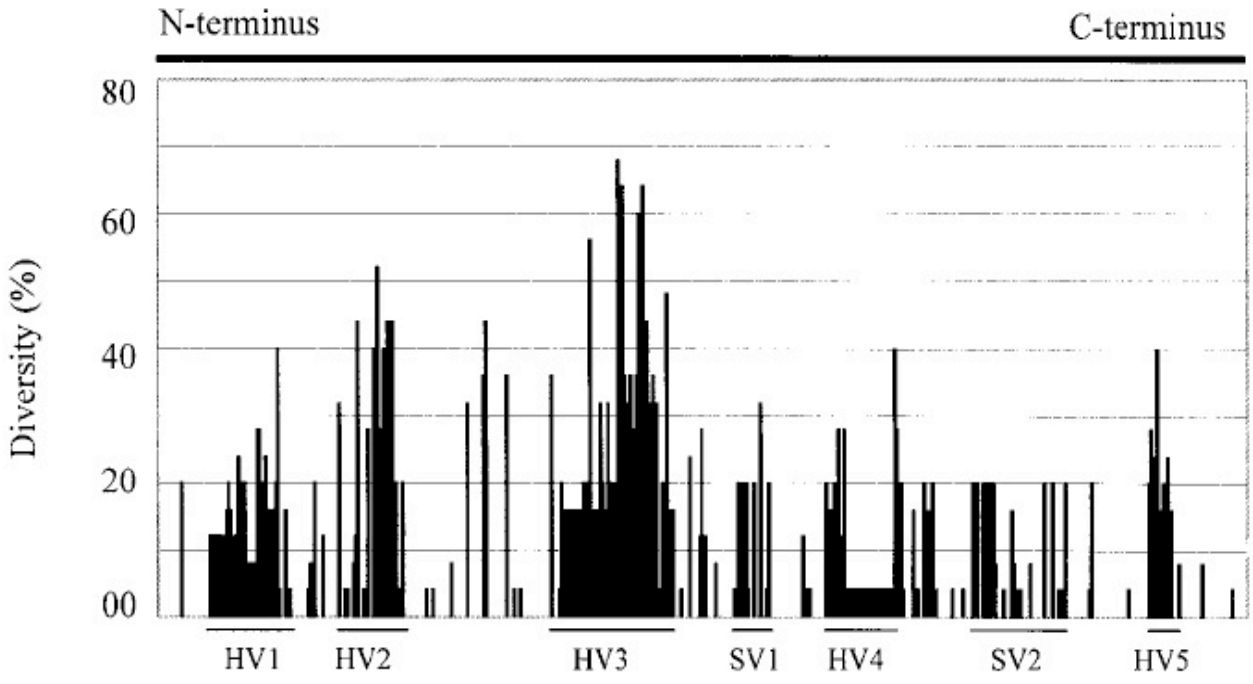


- Sequence *cmp* gene of large number of environmental strains: identify potential “outbreak strains”
- Further characterize “outbreak strains”
  - PFGE (*Sma*I, *Kpn*I) and MLST

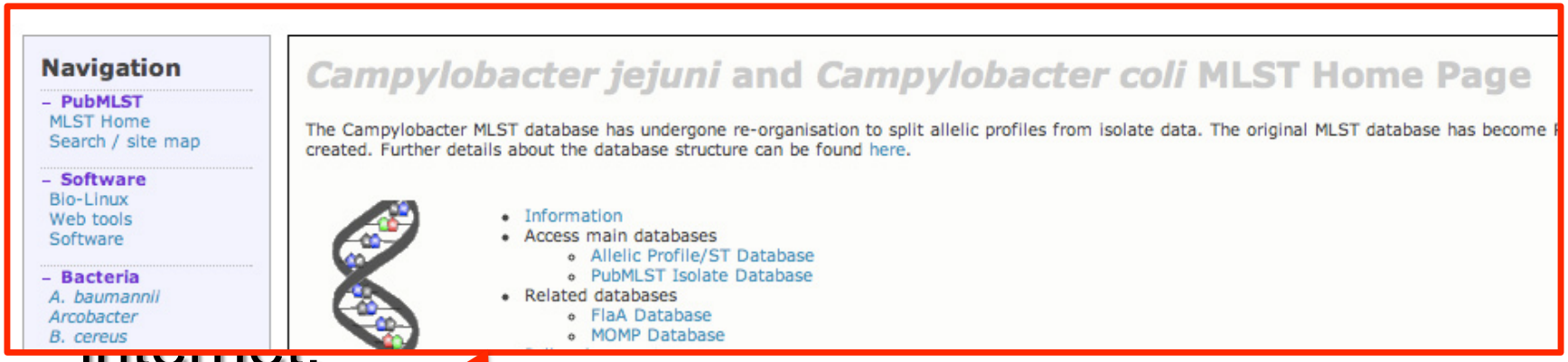
Sequence variation in hypervariable loops

Zhang et al, 2000, *Infect and Immun.*





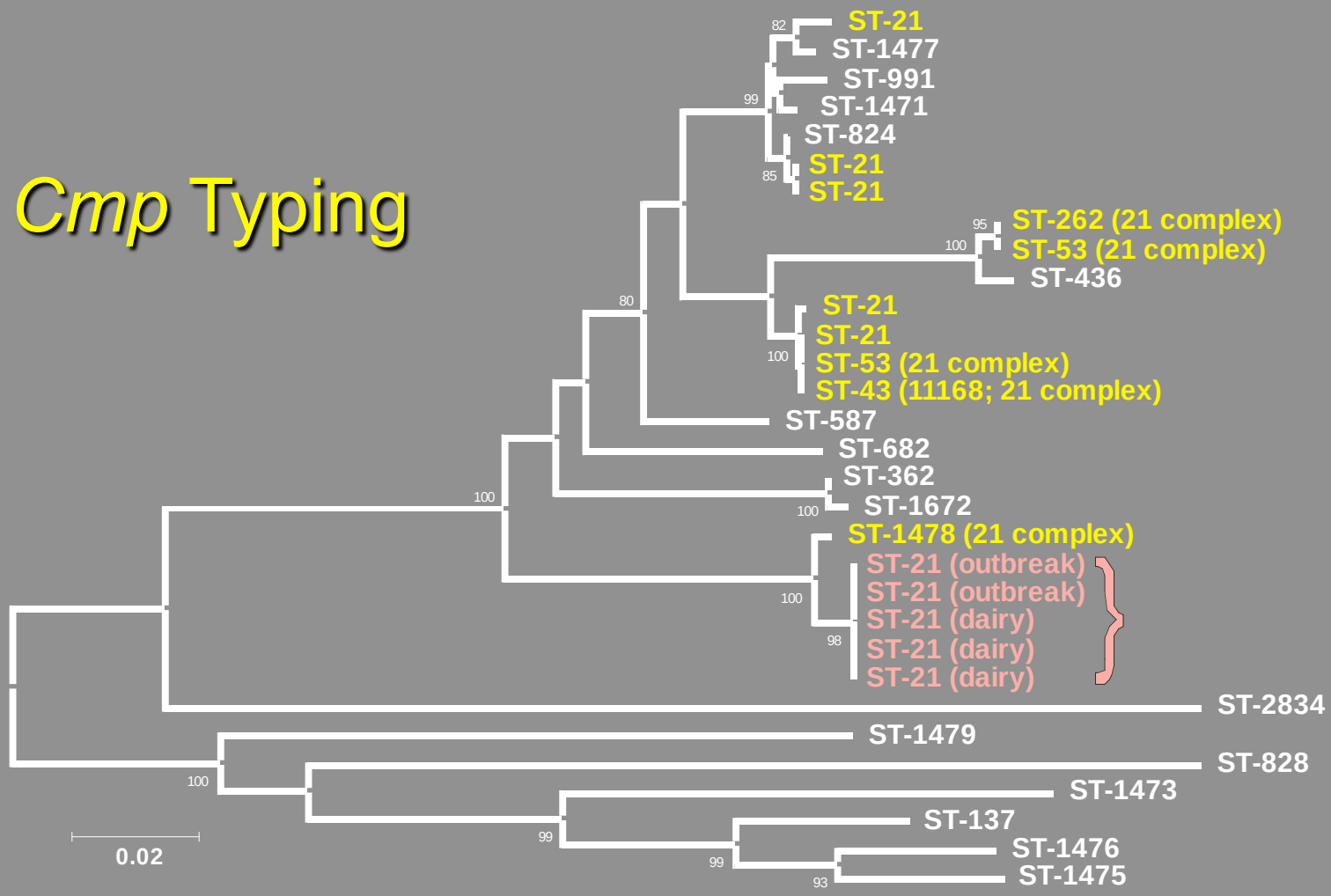
Multilocus Sequence Typing (MLST)



- MLST for *Campylobacter* species developed by Dingle et al; JCM, 2001
- 7 housekeeping genes (*aspA, glnA, gltA, glyA, pgm, tkt, and uncA)*, ~420 bp each = ~3000 bp total sequence for comparison
- Database: >7800 isolates, ~4000 profiles, time and source

Comparison of Cmp types among *C. jejuni* ST-21 strains and other representative isolates

Cmp Typing



Test new genotyping methods in other suspected *C. jejuni* raw milk outbreaks

- *C. jejuni* Outbreak 2
  - 5 cases, Washington state
  - Dec, 2007
  - Linked epidemiologically to consumption of raw milk from "Dairy C"
  - MLST ST-806

- *C. jejuni* Outbreak 3
  - 8 cases, California
  - Onset dates of Nov 23 - Dec 5, 2007
  - All 8 patients reported drinking raw milk/raw chocolate colostrum from "Dairy B"
  - MLST ST-1244
  - PFGE pattern from Dairy B cattle fecal isolates indistinguishable from case-patient isolate

Conclusions

- Isolation of the outbreak strains from dairy environment provides evidence that the source of contamination for each outbreak was at the dairy
- Cmp typing provides rapid triaging
- DNA fingerprinting methods (MLST) provide higher resolution for screening environmental isolates
- Cmp + MLST typing = 8 loci; provides added discrimination
- Persistent and/or predominant strains may exist in the dairy environment

Outbreak 4:

Raw milk suspected,
but no isolates available

Only molecular methods

- *C. jejuni* Outbreak 4

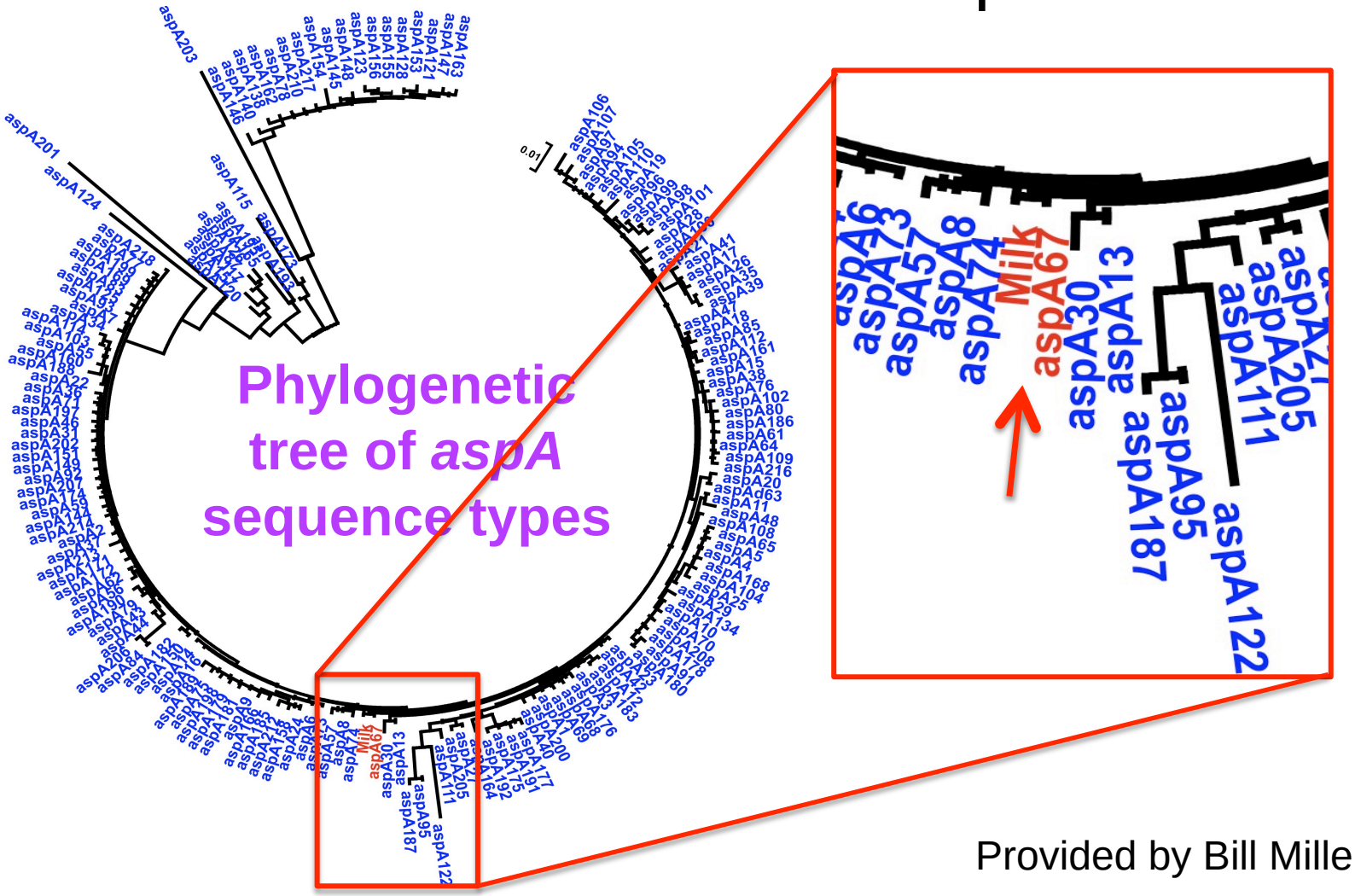
  (Karon et al, presented at 2009 ICEID, Atlanta, GA)

  - May-June, 2008
  - Raw milk, cow leasing program
  - 15 cases, California
  - No isolates were saved!
  - 1 patient with Guillain-Barré Syndrome (GBS)
  - 1 sample of 45 day old raw milk was available
  - Opportunity to test detection, limited genotyping and characterization methods

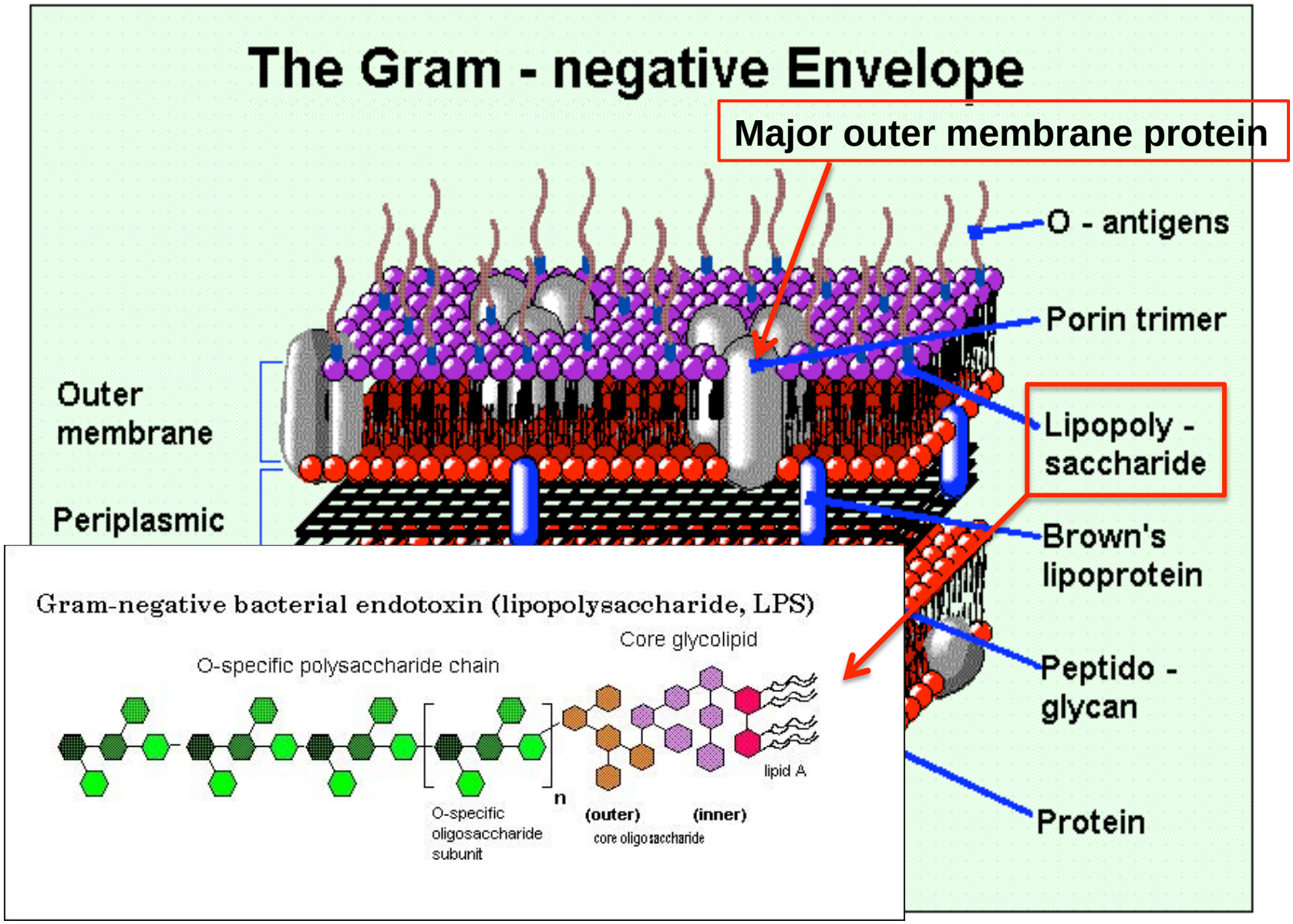
Analysis of milk samples

- Attempts to isolate *C. jejuni* failed
  - Tried enrichment, multiple media, atmospheres
- Milk: DNA purification using multiple kits
- PCR for MLST alleles
- PCR for genes in lipooligosaccharide (LOS) loci

A full *aspA* (aspartase) allele ("aspA67") was sequenced from PCR products from DNA isolated from raw milk sample



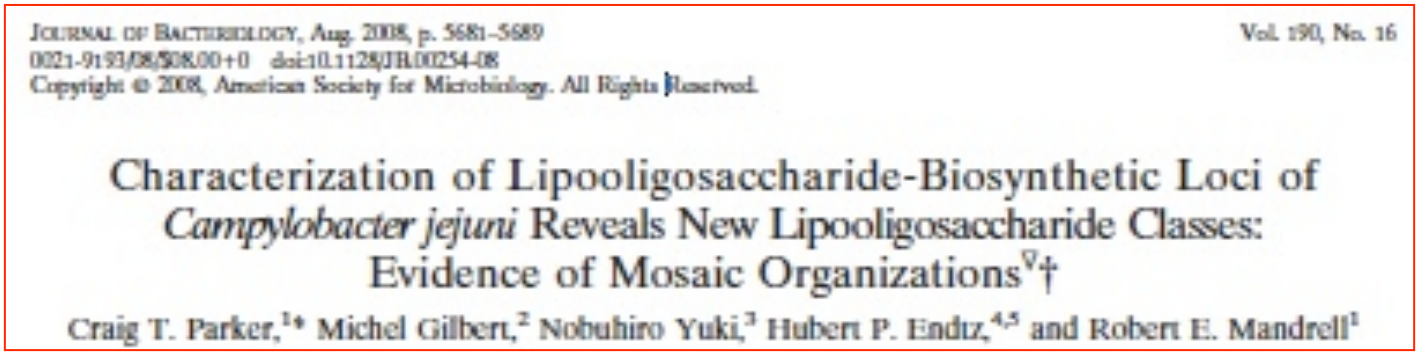
Provided by Bill Miller



From: www.bio.davidson.edu/total_membrane.gif

C. jejuni and Guillain-Barre Syndrome (GBS)

- Lipooligosaccharides (LOS) mimic mammalian gangliosides
- Mono-, Di-, Tri,-sialylated glycolipids: GM1a, GM1b, GM2, GD1a, GD1b, GD1c, GD3, etc.



JOURNAL OF BACTERIOLOGY, Aug. 2008, p. 5681–5689 Vol. 190, No. 16
0021-9193/08/$08.00+0 doi:10.1128/JB.00254-08

Characterization of Lipooligosaccharide-Biosynthetic Loci of *Campylobacter jejuni* Reveals New Lipooligosaccharide Classes: Evidence of Mosaic Organizations▽†

Craig T. Parker,[1]* Michel Gilbert,[2] Nobuhiro Yuki,[3] Hubert P. Endtz,[4,5] and Robert E. Mandrell[1]

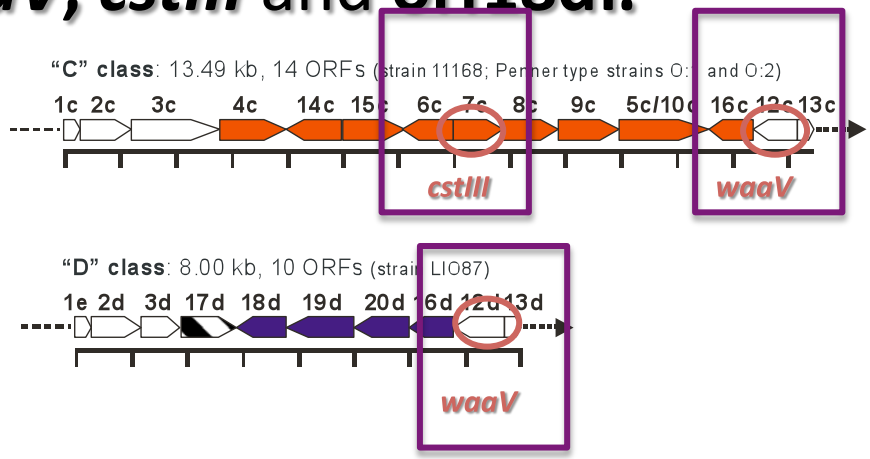
- PCR mapping of LOS genes in DNA from Milk sample

PCR amplification and sequencing of *C. jejuni* LOS genes from total DNA recovered from milk samples

Amplification

PCR products for LOS genes ***waaV, cstIII*** and **orf18df.**

- Sequencing of *waaV*
  - Sample 1 – 500 bp 100% identical to a **Class C LOS** gene
  - Sample 2 – 500 bp 100% identical to a **Class D LOS** gene



Sequencing of *cstIII*- a sialyltransferase gene

- Sample 1 – 127 bases 99% identical to a **Class C LOS** gene

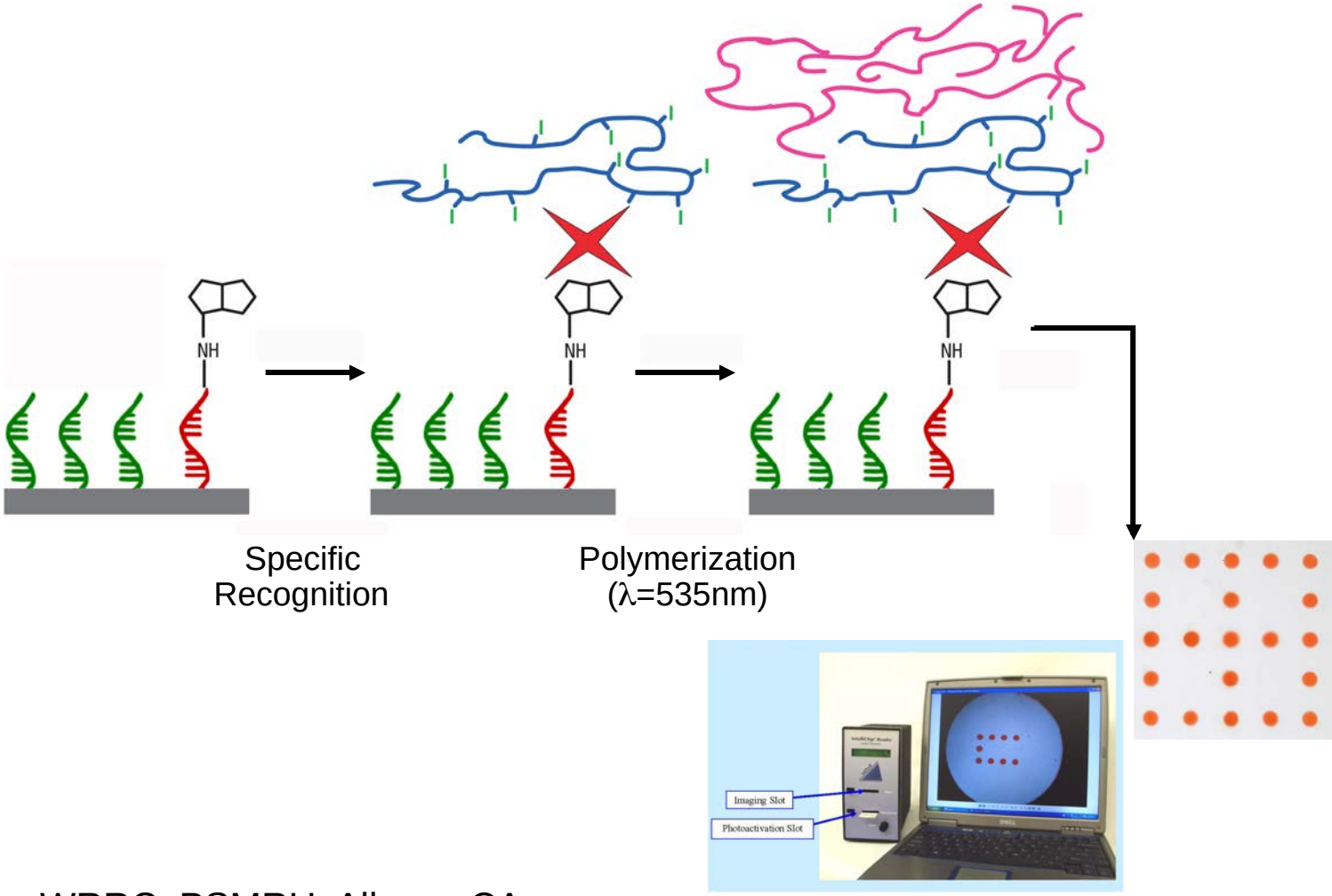
Craig Parker

Conclusions

- Genetic evidence of *C. jejuni* in raw milk
  - Complete *aspA* sequence (>477 bp)
  - Two *waaV* sequences (500 bp each)
  - 127 bp for *cstIII* (sialyltransferase)
- Evidence of mixed strain sample: two different *waaV* genes (class C and D LOS)
- Patient serum antibodies bind best to LOS of a GBS *C. jejuni* strain

Rapid and Cost-effective Methods for the Detection of Foodborne Pathogens by DNA Microarrays

Photopolymerization: A non-enzymatic signal amplification system



Beatriz Quiñones, WRRC, PSMRU, Albany, CA
Cooperative Agreement with InDevR, Inc., Boulder, CO