CAMPYLOBACTER IN MILK
(OR: CHERCHEZ LES CAMPYLOBACTERS IN MILK...)

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12th EURL Campylobacter workshop
Nantes, France, 14-15 September, 2017
WHY SAMPLE MILK?

• Outbreak situations, search for source of infection

• Quality tests, eg certification programmes

• Research, prevalence studies, risk assessment

- Type and number of samples could vary, depending on sampling situation
CAMPYLOBACTER AND MILK

- Campylobacteriosis - mainly sporadic cases, but outbreaks occur and are often associated with consumption of contaminated (raw) milk or water
- *Campylobacter* spp - in the intestine of healthy cattle
- Risk for fecal contamination of milk
- Management, strict milking routines, hygiene – impact on milk quality
- Pasteurization - effective way to improve milk safety
- But – consumers ask for ’raw milk’ and products made from unpasteurized milk – ’healthier’, better taste…….
In 2015, 26 MS reported a total of 4,362 food-borne outbreaks, including waterborne outbreaks.

Most of the outbreaks reported were caused by bacterial agents (33.7% of all outbreaks), in particular Salmonella (21.8%) and Campylobacter (8.9% of all outbreaks).

In 2015, 17 MS reported a total of 385 food-borne Campylobacter outbreaks.

The most frequently reported food vehicle was raw milk.

(Cf USA)
MILK, CHEESE AND DAIRY PRODUCTS

Data from 25 outbreaks are included: Austria (1), Finland (1), France (3), Germany (12), the Netherlands (3) and the United Kingdom (5). Number after the label refers to the number of outbreaks.

**Figure 80:** Distribution of food vehicles in strong-evidence outbreaks caused by *Campylobacter* (excluding waterborne outbreaks) in the EU, 2015
MILK ASSOCIATED OUTBREAKS

Typical situation (in Sweden):
Farm visit by group of children who are offered raw milk to drink.
A number of persons become sick with diarrhea

- Samples from patients, milk, farm animals and environment
- Laboratory analysis including genotyping of isolated Campylobacter
- Epidemiological and laboratory results confirm/support that the outbreak was caused by the raw milk
EU LEGISLATION (EG) 853/2004

Allows small amounts of raw milk to be sold….

A Member State may, of its own initiative and subject to the general provisions of the Treaty, maintain or establish national rules:

(a) **prohibiting or restricting** the placing on the market within its territory of raw milk or raw cream intended for direct human consumption; or

• (b) **permitting the use,**

• National rules may include some certification programme/routine tests of raw milk for sale

(However, these routine tests are often only somatic cell and coliform counts, which do not ensure the safety of the milk!)
PREVALENCE OF CAMPYLOBACTER IN MILK

Oliver et al 2005, a summary of studies 1982-2001: the prevalence of *C. jejuni* in BTM ranged from 0.4% - 12.3%

In later studies, *isolation rates fall within this range*,
Examples: Bianchini et al 2014, Italy – 12%
Giacometti et al 2012, Italy – 6.45%*
Jayarao et al 2006, USA – 2%
Two Swedish studies – 12%* /13%*

Type of sample, BTM or *in-line milk filters*
BULK TANK MILK, BTM
STANDARD METHODS

• ISO 10272:2006 or 2017, Part 1 (detection)
• Volume of sample could vary, but enriched in Bolton broth (dilution 1:10), and continue according to the ISO standard
• NMKL 119, 3rd ed 2007 – in principle the same procedure as in ISO 10272
Under C. 2.h, preparation of samples
Milk, frozen dairy products

**Raw milk.** Instruct the investigator to test raw milk at the collection site by using a sterile pipette to place test portion onto pH test paper (pH 6-8 range). If the pH is below 7.6, add sterile 1-2 N NaOH and gently to adjust it to 7.5 ± 0.2. Immediately upon receipt in the laboratory, test the pH of the dairy sample with pH test paper and adjust to pH 7.5 ± 0.2 with sterile 1-2 N NaOH if necessary. Centrifuge a 50 g portion at 20,000 × g for 40 minutes. Discard supernatant and dissolve pellet (not fat layer) in 10 ml enrichment broth. Transfer pellet to 90 ml enrichment broth
Prevalence and Survival of *Campylobacter jejuni* in Unpasteurized Milk

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Received 7 May 1982/Accepted 8 July 1982

*Campylobacter jejuni* was isolated from 1 of 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°C) varied greatly: the most tolerant strain showed a <2-log_{10} decrease in viable cells after 14 days, and the most sensitive strain showed a >6-log_{10} 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°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.
EX PREVALENCE STUDIES BTM

• Jayarao et al 2006. 248 dairy herds USA
  • BTM samples, volume ~120 ml, shipped on ice, analysed within 36h, BAM procedure (pH adjustment?)
  • C. jejuni isolated from 5/248 BTM samples (2.0%).

• Bianchini et al 2014. 282 dairy herds, Italy
  • BTM samples, volume? As part of routine monitoring programs (local authority), transported chilled, analyses within 24h, in principle as ISO 10272
  • C. jejuni isolated from 34/282 BTM samples (12%)

Transport: Cool and Quick!
IN-LINE MILK FILTERS, ADVANTAGES

• Three - to 10-fold-higher pathogen* isolation rates have been reported from milk filter samples than from BTM samples (refs in Giacometti et al 2012).

• Concentration of low numbers of bacteria in the milk filter

• Procedures could still vary:

• Giacometti et al 2012, Italy

• Milk filters covered in milk and processed within 6h, BAM procedure, culture and PCR (on enriched samples) for detection. *Campylobacter* in 24/378 milk filters (6.45%)

• Two Swedish studies (2016, 2017)

• Milk filters in Cary-Blair transport medium, chilled and analysed within 24-48h, ISO 10272. *C. jejuni* in 12-13%
LABORATORY ANALYSES COULD VARY (AS WELL)

- Detection/identification – "standard" cultural and biochemical methods
- PCR – note: on enriched samples
- ELISA
- MALDI-TOF

- For strain characterisation: PFGE, MLST, WGS, etc.
THANK YOU FOR YOUR ATTENTION!

AND GOOD LUCK IN YOUR SEARCH FOR CAMPYS

Cherchez les campylobacters…