MICROBIOLOGY of RAW MILK
Introduction

- Milk and other dairy products are of superior quality and safety
Microbial in Raw Milk

GENERAL ASPECTS

Milk is a good source of nutrients and edible energy, not only for mammals but for numerous microorganisms, which thus can grow in milk. These microorganisms are primarily bacteria, but some molds and yeasts can also grow in milk.
Milk as Growth Media

- Carbon and Nitrogen Sources
- Minerals and Micronutrients
- Growth Inhibitors
  - Immunoglobulins
  - Lysozyme and lactoferrin,
  - The lactoperoxidase–thiocyanate–H2O2 system,
- Lysozyme is an enzyme (EC 3.2.1.17) that hydrolyzes the structural polymer peptidoglycan of the bacterial cell wall, in particular, splitting the linkage between the N-acetyl muramic acid and N-acetyl glucosamine units; this may cause lysis of the bacteria.
- Lactoferrin binds Fe, thus reducing the activity of Fe$^{2+}$ ions, which are needed by several bacteria. Possibly some fragments of lactoferrin produced by proteolysis exhibit other antimicrobial activities.
Some Genera of Bacteria Possibly Associated with Milk

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micrococcaceae</td>
<td>Micrococcus</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus</td>
</tr>
<tr>
<td>Lactobacillaceae</td>
<td>Lactococcus</td>
</tr>
<tr>
<td></td>
<td>Streptococcus</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus</td>
</tr>
<tr>
<td>Bacillaceae</td>
<td>Bacillus</td>
</tr>
<tr>
<td></td>
<td>Clostridium</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>Escherichia</td>
</tr>
<tr>
<td></td>
<td>Salmonella</td>
</tr>
<tr>
<td>Pseudomonadaceae</td>
<td>Pseudomonas</td>
</tr>
</tbody>
</table>
Microbial Growth – The Growth Curve

(a) Lag phase
(b) Logarithmic phase
(c) Stationary phase
(d) Death phase

Total cells in population:
- Few cells
- Live cells
- Dead cells

Logarithm (10^x) of viable cells

Time (hours)
Physical Requirements

- Temperature
  - psychrophiles (cold loving microbes)
    - range: 0 C - 20 C
  - mesophiles (moderate temp. loving microbes)
    - range: 20 C - 40 C
  - thermophiles (heat loving microbes)
    - range: 40 C - 100 C
## Generation Time (h) of Some Groups of Bacteria in Milk

<table>
<thead>
<tr>
<th></th>
<th>Temperature (°C)</th>
<th>5</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid bacteria</td>
<td>&gt;20</td>
<td>2.1</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Pseudomonads</td>
<td>4</td>
<td>1.9</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Coliforms</td>
<td>8</td>
<td>1.7</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Heat-resistant streptococci</td>
<td>&gt;20</td>
<td>3.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Aerobic sporeformers</td>
<td>18</td>
<td>1.9</td>
<td>0.45</td>
<td></td>
</tr>
</tbody>
</table>
Approximate Example of the Effect of the Keeping Temperature of Raw Milk on Its Count after 24 h, and on Its Keeping Quality (Initial Count $2.3 \times 10^3$ ml$^1$)

<table>
<thead>
<tr>
<th>Milk Held at (C)</th>
<th>Count after 24 h (ml$^1$)</th>
<th>Keeping Qualitya(h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>$2.5 \times 10^3$</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td>10</td>
<td>$1.2 \times 10^4$</td>
<td>89</td>
</tr>
<tr>
<td>15</td>
<td>$1.3 \times 10^5$</td>
<td>35</td>
</tr>
<tr>
<td>20</td>
<td>$4.5 \times 10^6$</td>
<td>19</td>
</tr>
<tr>
<td>30</td>
<td>$1.4 \times 10^9$</td>
<td>11</td>
</tr>
</tbody>
</table>

Keeping quality is defined here as the storage time during which milk remains suitable for processing (count not exceeding $1 \times 10^6$ ml$^1$).
SOURCES OF CONTAMINATION

- Milk is sterile when secreted into the alveoli of the udder. Microbial contamination occurs mainly during and after milking.
- Microorganisms in bulk tank milk originate from the interior of teats, the farm environment and surfaces of the milking equipment.
2. Environment

- the most common microbial sources in the farm environment are feeds, faeces, bedding material and soil.
- The strains and concentrations of microorganisms depends on the composition of the attached dirt and microbial concentration in the dirt.
Microorganisms

Contagious mastitis organisms
- *Staphylococcus aureus*
- *Streptococcus agalactiae*
- *Corynebacterium bovis*

Environmental mastitis organisms
- *Streptococcus uberis*
- *Streptococcus dysgalactiae*
- *Escherichia coli*
- *Klebsiella spp.*
soil: spores of *B. cereus*

The impact of feed as a hazard of microbial contaminants of raw milk:
- a source or transmission vehicle of pathogens causing infection in cattle,
- an important source of bacterial spores in raw milk.

Animal pathogens associated with feed:
- *L. monocytogenes*, *E. coli* O157:H7 and
- *Salmonella enterica*.

Spore-forming bacteria isolated from feeds belong to the genera *Clostridium* and *Bacillus*.

*Clostridium* species: *C. tyrobutyricum*, *C. butyricum*, *C. beijerinckii* and *C. sporogenes*. 
SOURCES OF CONTAMINATION

3. Milking equipment

- Contamination of milk via the milking equipment occurs when
  (a) microorganisms adhere to surfaces of the milking equipment and
  (b) milk residues that remain in the equipment after the cleaning cycle
Mikroorganisms on milking equipment:

- In general, microorganisms originating from the farm environment (e.g. soil, faeces, bedding and feeds)
- *S. aureus* has been recovered from surface of milking equipment
- Cleaning the milking equipment at low temperatures or cleaning without sanitisers gives rise to fast growing gram-negative rods like coliforms and *Pseudomonas*
Spoilage Organisms

- **Psychrotrophs**
  - 3-7°C storage
  - Proteolytic and lipolytic

- **Sources**

- **Spoilage “Rates”**
  - Pasteurization Kills all Pathogens, not necessarily all spoilage
  - Recontamination can occur from equipment
  - Spoilage occurs at $10^6$ to $10^7$
Spoilage-Psychrotrophs

- Off-Flavors and Odors
- Texture and Appearance Changes
- Protein
  - Bitter and Putrid Flavors
  - Coagulation
- Fat
  - Rancid and Fruity Flavors
Fermentative Spoilage

- Lactic Acid Bacteria
  - *Lactobacillus*
  - *Lactococcus*
  - *Leuconostoc*
  - *Enterococcus*
  - *Pediococcus*
  - *Streptococcus*

- Coliforms
  - LAB Usually out compete
MIKROBA PATOGEN

- Bacillus cereus
- Listeria monocytogenes
- Yersinia enterocolitica
- Salmonella spp.
- Escherichia coli O157:H7
- Campylobacter jejuni
- Mycobacterium bovis,
- Mycobacterium tuberculosis
Control

- Prevent Contamination
- Rapid Cooling
- Refrigeration
- Cleaning and Sanitizing
- Pasteurization
- Prevent Recontamination of Pasteurized Products
Milkborne diseases

Pasteurization of milk

Milk Quality

00  49  99

00  29  49  69  89  99
Historical perspective

- **1900-1940s**: Tuberculosis, Bang’s disease, Diptheria, Typhoid fever highly prevalent
- **1930s**: Pasteurization of milk
- **1940-1960s**: Eradication of animal diseases (Brucellosis, Tuberculosis)
- **1970s**: Few human cases reported
- **1990s**: Few human cases still reported

Why?
Improved farm hygiene and milking practices

Improved milk handling and processing technology

Educated consumers and higher consumer awareness on food safety

Still......................
Why do milkborne illnesses occur?

- Faulty pasteurization of fluid milk
  - Defective pasteurizer -- less likely

- Post-pasteurization contamination of milk and milk products
  - -- likely

- Raw milk consumption
  - Rural communities with access to raw milk
  - Holistic / new age / back to nature - city folks?
  - Visitors on farms
  - raw milk products
Health risks associated with raw milk?

- Several reports on foodborne outbreaks due to consumption of raw milk

- *Escherichia coli* O157:H7
- *Salmonella typhimurium* DT104
- *Campylobacter jejuni*
- *Listeria monocytogenes*
- *Yersinia entercolitica*
- *Salmonella spp.*
How do pathogens get into raw milk?
How do Pathogens gain access to a farm?

Most important sources of infection:

- Replacement trucks
- Heifer/cows
- Calves
- Birds/pests
- Feed
- Water
- Visitors
Other routes of entry
Putting it all together!
Salmonella

- Occur in humans, warm and cold blooded animals, food and environment
- Pathogenic to humans and many animals
- Causes typhoid, enteric fevers, gastroenteritis and septicaemia
- More than 2200 types of Salmonella
- Prevalence in bulk tank milk ?????
Salmonella typhimurium DT 104

- Emerging foodborne pathogen
- Detected in several countries
- Why is DT 104 of concern?
  - Multiple antibiotic resistance
    - Ampicillin
    - Chloramphenicol
    - Streptomycin
    - Sulfonamides
    - Tetracycline

- DT 104 has been isolated from:
  - poultry, swine, cattle and wild animals, and from bulk tank milk
Campylobacter jejuni

- Inhabitant in the reproductive tract, intestine, and oral cavity to humans and animals
- Excreted in large numbers in feces
- 1983- Campylobacteriosis associated with raw milk consumption in PA
- Prevalence in bulk tank milk ?????
Escherichia coli

- Occur as normal flora in the lower part of intestine of warm blooded animals
- Toxin producing strains of *E. coli* are important agents of food-borne illnesses
- Emerging pathogen *E. coli* O157:H7; dairy cattle considered as reservoirs.
- Prevalence of enterotoxigenic *E. coli* in bulk tank milk ???????
Yersinia enterocolitica

- Occur in a broad spectrum of habitats including man, animals, rodents, birds, soil, water, dairy products and other foods.
- *Yersinia enterocolitica* causes chronic diarrhea and severe septicaemia
- Prevalence of *Y. entercolitica* in BTM ????

Natural hosts
Listeria monocytogenes

- Widely distributed in the environment
- *Listeria monocytogenes* pathogenic to humans and animals
- Several reported outbreaks following consumption of raw milk
- Prevalence of *L. monocytogenes* in bulk tank milk ?????