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PATHOGENS IN MILK & MILK PRODUCTS I

This bulletin includes technical and latest development on products, systems, techniques etc. reported in journals, companies' leaflets and books and based on studies and experience. The technical information on different issues is on different areas of plant operation. It is hoped that the information contained herein will be useful to readers.

The theme of information in this issue **Pathogens in Milk & Milk Products I**. It may be understood that the information given here is by no means complete.

In this issue …

1. INTRODUCTION

Milk is an excellent food and protective medium for pathogens, whose growth depends mainly on temperature and competing microorganisms and their metabolites. Several of them produce toxins, and many are spore-formers. Their disease producing capacity depends upon the initial load of infection in the milk and on the subsequent dilution, processing, time lapse before the milk is consumed, and other factors.

Pathogenic microorganisms in milk are derived from the dairy animal itself, the human handler or the environment. One of the most important extraneous sources of infection is a contaminated water supply. Insects, rodents, dirt, manure and poor sanitation are other sources of pathogens in milk and its products.

With due care in milk production and handling, the modern processing facilities and good hygienic practices, pathogens can be controlled. There have, however, been reported cases of outbreak of disease attributed to pathogens in milk products. It is therefore necessary to take maximum care to ensure that the product is safe for consumption. This needs thorough understanding of products, processes, equipment, environment, and pathogens, amongst others. This issue of the Technews, and the next one, catalogue the important pathogens in milk with brief descriptions of their characteristics.

2. Bacillus anthracis

	Characteristics	Description
1	**General**	Unusually large rod shaped, non motile, spore forming, capsulated, gram positive bacterium
2	**Source**	Diseased animal Air, soil
3	**Pathogenicity** • **Humans** • **Animals**	Anthrax (a fatal disease) Cutaneous (skin infection), inhalational (affecting lungs) and gastrointestinal forms of infection Anthrax
4	**Growth parameters** • **Temperature**	 7 ^{0}C to 49 ^{0}C
5	• **Shedding in milk** • **Growth in milk** • **Associated dairy foods**	No. An animal with anthrax either ceases to lactate or gives milk that is bloody, yellowish or visibly abnormal. No May be raw milk
6	**Inactivation parameters**	Vigorous boiling for 2 to 3 minutes.[(1)]
7	**Control measures**	Elimination of infected animals from the food chain. Products from diseased and dying animals should be rejected for human consumption. Thorough cooking of animal products offers protection from vegetative cells of *B. anthracis.*

3. Bacillus cereus

	Characteristics	Description
1	**General**	Rod shaped, aerobic, spore forming, gram positive bacterium.
2	**Source**	Air, water, fodder or feed, soil, udder, milking equipment etc.
3	**Pathogenicity**	Foodborne illness (infection / intoxication)
	• **Humans**	Diarrheal illness (resembles *C. perferingens* food poisoning), Emetic illness (resembles staphylococcal food poisoning)
	• **Infectious dose**	Food poisoning due to enterotoxin production at high population levels (more than 10^6/ g) particularly in starchy food [(2)]
	• **Toxin type**	Heat labile enterotoxin (produced in small intestine) [(3)], Heat stable (120 ^{0}C for 90 minutes) [(4)] emetic toxin (produced in food) [(3)]
4	**Growth parameters[(3)]**	
	• **Temperature**	7 ^{0}C to 49 ^{0}C (Mesophilic organism capable of growing at 7 ^{0}C to 12 ^{0}C)
	• **Water activity**	0.93 minimum
	• **pH**	4.3 to 9.3

5	• **Shedding in milk** • **Growth in milk** • **Associated dairy foods**	No Yes These organisms are common contaminants of raw milk, and invariably present in pasteurized milk, dried milk. Rapid growth of vegetative cells during periods of temperature abuse is presumably responsible for high incidence of this organism in milk during summer months. Presence of *B. cereus* in powdered milk probably poses the greatest public health concern because both pasteurization and spray-drying induce germination and outgrowth of spores in the reconstituted product *B. cereus* can give rise to "bitty" cream and sweet curdling in pasteurized milk, due to the spores surviving the pasteurization treatment and the vegetative cells resulting from these. Exceptionally heat resistant spores of *B.cereus* can spoil UHT milk. [(2)]
6	**Inactivation parameters**	Heat sterilization, done in an autoclave or by ultra high treatment, is enough to reduce *B. cereus* spore population to a level to ensure no public health risk and microbiological stability of

		the product. Increase in pasteurization temperatures above 78 ^{0}C reduces the shelf life of milk due to activation of spores of *B. cereus.*
7	**Control measures**	Widespread occurrence of *B. cereus* in the natural environment ensures its continued recovery from milk and other dairy products during all stages of production.. However dairy related outbreaks of *B. cereus* poisoning are readily prevented by sanitary handling, minimizing contamination of raw milk at the farm level and storing both fluid and reconstituted milk at temperatures less than 4 ^{0}C. Bactofugation of raw milk could be useful in removing spores of this organism.

4. Brucella abortus

	Characteristics	**Description**
1	**General**	Short rod shaped, pleomorphic, non motile, strictly aerobic, non spore forming, gram negative bacterium.
2	**Source**	Diseased animals (mammals) mainly localized in lymphoid system and reproductive organs.

		Vaginal discharge from infected cattle is an important source. Wool, hay, dust, soil, air etc.
3	**Pathogenicity** • **Humans** • **Animals**	Infection Brucellosis (malta fever, undulant fever) Brucellosis results in abortion in cattle and subsequent infection of mammary glands.
4	**Growth parameters** • **Temperature** • **pH**	 20 to 40 ^{0}C (optimum 37 ^{0}C) Above 4.0
5	• **Shedding in milk** • **Growth in milk** • **Associated dairy foods**	*Brucella* can persist in the udders of cows for many years following an abortion and can be intermittently shed at levels upto 15000 organisms / ml for as long as 5 months. (4) No Cheese and butter. Although it is destroyed by pasteurization, if post pasteurization contamination with infected milk occurs, it dies-off only slowly during manufacture and ripening of cheese and butter and could, therefore, be a hazard in soft cheese which has a short ripening period. (2)

6	**Inactivation parameters**	At low concentration in liquid media, *Brucella* are fairly heat sensitive. Low concentration of the organism in milk is readily inactivated by pasteurization (HTST or Flash method) or prolonged boiling for 10 minutes.(5) *Brucella* is reported to be readily killed in milk at 65 ^{0}C for 15 seconds with an initial count of up to 10^6 cells / ml. (1) *Brucella* is fairly sensitive to ionizing radiation and is readily killed by normal sterilizing doses of gamma rays. Also killed within hours when exposed to sun.
7	**Control measures**	Preventing dairy-related cases of brucellosis is based on: a) Specific programmes combining vaccination and / or test and isolation and slaughter of seropositive animals b) General non-specific good management practices and hygiene measures that reduce exposure potential c) Mandatory pasteurization and d) Ageing of cheeses, which are legally prepared from raw milk, for at least 60 days (4).

5. Campylobacter jejuni

	Characteristics	Description
1	**General**	Slender, spirally curved, obligate microaerophilic, motile , non spore forming, gram negative bacterium
2	**Source**	Gastrointestinal tract, reproductive organs and oral cavity of wild and domesticated animals. The bovine intestinal tract remains the primary reservoir for *C. jejuni* and consequent shedding in faeces. Faecal contamination of milk during and after milking is regarded as the primary route of contamination.
3	**Pathogenicity**	Infection
	• **Humans**	Enteritis, most common and frequent cause of bacterial diarrhea, and possibly ulcers.
	• **Infective dose**	Minimum of 500 bacteria [5] 400 to 500 bacteria [6] 500 to 800 bacteria [4]
	• **Animals**	Veterinary disease in poultry, cattle and sheep
	• **Toxin type**	Enterotoxin (produced in host) [3]
4	**Growth parameters**	
	• **Temperature**	31 ^{0}C to 45 ^{0}C (optimum 42 ^{0}C to 45 ^{0}C) (Mesophilic organism incapable of growing below 12 ^{0}C)

	• **Water activity** • **pH**	0.987 minimum 6.0 to 9.5 (optimum 6.5 to 7.5)
5	• **Shedding in milk** • **Growth in milk** • **Associated dairy foods**	No. Evidence supporting shedding by naturally infected cows is limited. Experimentally induced mastitis in dairy cows has led to the excretion of up to 10^5 cells / ml in milk over a period of 7 days. (4) No Raw milk that has been exposed to faecal contamination and improperly pasteurized milk.
6	**Inactivation parameters**	Heating to an internal temperature of 60 ^{0}C for 10 minutes. (6) Proper LTLT (61.7 ^{0}C for 30 minutes) and HTST (71.7^{0}C for 15 seconds) pasteurization offer complete protection against the spread of milk borne campylobacteriosis. (4) Normal levels of oxygen in air will inhibit the growth of this organism. (6)
7	**Control measures**	Sanitary handling, processing, preparation and storage of foods. Infection can be reduced through hand washing with soap and hot running water for at least 18 seconds before food preparation and between handling of raw and prepared foods. (6)

		Because of broad distribution of *C. jejuni* in all kinds of domestic animals and the environment more intensive measures are necessary to keep the organism from livestock animals.

6. Clostridium botulinum

	Characteristics	Description
1	**General**	Rod shaped, strictly anaerobic, gas forming, spore forming, gram positive bacteria
2	**Source**	Soil and water. Spores widespread in nature with soil serving as the primary reservoir. Few domestic farm animals including cattle are faecal carriers of this organism. Vegetation, animal feed and farm produce are also frequently contaminated.
3	**Pathogenicity** • **Humans** • **Toxin type**	Food borne illness (intoxication) Botulism: Ingestion of toxin affects peripheral nervous system and results in respiratory failure and death Neurotoxin of 8 types - second most powerful biological poison known to humans (produced in food) [3]

4	**Growth parameters** [3]	
	• **Temperature**	3.3 ^{0}C to 45 ^{0}C (non proteolytic types) (Psychrotrophic organism capable of growing at temperatures of less than 5 ^{0}C) 10 ^{0}C to 48 ^{0}C (proteolytic types) (Mesophilic organism capable of growing at 10 ^{0}C to 12 ^{0}C)
	• **Water activity**	0.97 minimum (non proteolytic), 0.93 min. (proteolytic)
	• **pH**	5.0 to 9.0 (non proteolytic), 4.6 to 9.0 (proteolytic)
5	• **Shedding in milk**	No
	• **Growth in milk**	Yes
	• **Associated dairy foods**	Frequent contaminants of raw and pasteurized milk. Sterilized milk in cans, cheeses particularly anaerobically packaged cheeses and processed cheese spreads
6	**Inactivation parameters**	Toxins are reported to be destroyed by heating at 85 ^{0}C for 15 minutes [6] 80 ^{0}C for 30 minutes [1] or boiling for few minutes [1] Spores are destroyed completely at: [6] 100 ^{0}C for 360 minutes 105 ^{0}C for 120 minutes 110 ^{0}C for 36 minutes 115 ^{0}C for 12 minutes 120 ^{0}C for 4 minutes

7	**Control measures**	Since the probability of contamination of raw food with *C. botulinum* is high, prevention of botulism from processed food must rely upon any of the measures as under: (1) a) complete destruction of spores by thermal treatment or irradiation b) complete inhibition of the growth by physical or chemical methods c) inactivation of preformed toxins by cooking the food before consumption and d) active or passive immunization. Although contamination of products with spores of *C. botulinum* cannot be prevented, the threat of toxin production in anaerobically packed cheeses and processed cheese spreads can be eliminated by carefully controlling the pH, moisture content, water activity, phosphate level and nisin content of the finished product Proper canning of food, refrigeration and sanitation is also important.

7. Clostridium perfringens

	Characteristics	Description
1	**General**	Rod shaped, anaerobic, gas forming, spore forming, gram positive bacterium
2	**Source**	Intestinal tract of man and animals as the primary source. Faecal matter, water, soil and dust
3	**Pathogenicity**	Foodborne illness (infection)
	• **Humans**	Gastroenteritis, hemorrhagic colitis
	• **Infectious dose**	Large number of bacteria must be ingested for illness to occur.
	• **Animals**	Possibly bovine mastitis [(2)]
	• **Toxin type**	Number of soluble substances capable of causing toxic effect.
4	**Growth parameters**	
	• **Temperature**	10 ^{0}C to 52 ^{0}C (Mesophilic organism capable of growing at 10 ^{0}C to 12 ^{0}C)
	• **Water activity**	0.93 minimum
	• **pH**	5.0 to 8.5
5	• **Shedding in milk**	No
	• **Growth in milk**	Yes
	• **Associated dairy foods**	Milk, cheese etc.
6	**Inactivation parameters**	Vegetative cells are destroyed above 60 ^{0}C [(6)] Spores are destroyed by heating at 100 ^{0}C for few minutes to 4 hours.[(6)]

7	**Control measures**	As it is impossible to reduce the incidence of *C. perferingens* in nature, it must be accepted that it will be present in many foods. The prevention must be concerned not only with destruction, but also with the control of germination of spores and subsequent multiplication of vegetative cells in processed foods. Effectively controlled by rapid cooling of cooked and heat processed foods, prompt refrigeration of leftover food, proper refrigeration and sanitation. Reheating of leftover foods to 60 ^{0}C destroys the living organism. [6]

8. Coxiella burnetii

	Characteristics	Description
1	**General**	Pleomorphic, short rod, non motile, non spore forming, gram negative bacterium
2	**Source**	Many cold and warm-blooded animals (primary host) and anthropods (vectors) like ticks, lice, flies and bed bugs. Amniotic fluid and infected fetuses are the most intensive source of infection Most

		transmission takes place by the faeces, which desiccate and may infect wild and domestic animals in particular via dust, food stuff, litter etc. [5] Because of high degree of contamination of the environment, *C. burnetii* becomes airborne and infects animals.
3	**Pathogenicity** • **Humans** • **Animals**	Infection Q -fever (an intracellular parasite) Q -fever
4	**Growth parameters** • **Temperature**	 Optimum 37 ^{0}C
5	• **Shedding in milk** • **Growth in milk** • **Associated dairy foods**	Yes No Milk, butter and soft cheese
6	**Inactivation parameters**	*Coxiella bunetii* has a high degree of resistance to chemical and physical agents, including dessication. It is slightly more heat resistant than Mycobacterium tuberculosis, usually considered to be killed by pasteurization treatments, complete inactivation may not be accomplished. [2] It is reported to survive temperatures of 63 ^{0}C for 30 to 40 minutes but killed completely at temperature of 71.7 ^{0}C for 15 seconds. [1]

7	**Control measures**	Milk borne Q fever can be prevented by animal hygiene and immunization and effective pasteurization of milk.

This issue provided brief account of seven important pathogens. The next issue would include details of six more pathogens.

References:

(1) Riemann, H. and Bryan, F.L. (1979), Food-borne Infections and Intoxications, 2nd Edition, Academic Press, London.

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(3) Jackson, S.L. et al (1999) Advances in Experimental Medicine and Biology (vol. 459) Impact of Processing on Food Safety, Kluwer Academic – Plenum Publishers, New York, pp 9 to 20.

(4) Marth, E.H. and Steele, J.L (1998) Applied Dairy Microbiology, Marcel Dekker Inc. New York, pp 263 to 369.

(5) IDF (1994), Significance of Pathogenic Microorganisms in Raw Milk, International Dairy Federation, Brussels.

(6) Marriot, N.G. (1999), Principles of Food Sanitation, 4th Edition, Aspen Publishers Inc., Maryland, pp 11 to 50.

Next Issue: Pathogens in Milk & Milk Products II

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