

## Emerging Pathogens in Dairy Industry

Mogha K.V.<sup>1\*</sup>, Chaudhari A. R.<sup>2</sup>, Subrota H.<sup>1</sup>

<sup>1</sup>Department of Dairy Science, S.M.C. College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India

<sup>2</sup>Dairy Chemistry, Mansinhbhai Institute of Dairy & Food Technology, Mehasana, Gujarat, India

### Abstract

*The epidemiology of food borne disease is changing due to change in food habits of the consumers. New pathogens have emerged, and some have spread worldwide which includes Salmonella, Escherichia coli O157:H7, Campylobacter, and Yersinia enterocolitica. These pathogens have reservoirs in healthy food animals, from which they spread to an increasing variety of milk and milk products. If raw milk is consumed as such or if there is problem in steps of milk processing than it results in severe case of outbreaks of food borne illness due to consumption of contaminated milk and milk products made with it. This review mainly focuses on the emerging pathogens in dairy industry and rapid methods to detect them and control measures necessary to prevent this pathogens entering the milk processing area and thereby in milk products.*

**Keywords:** Food borne disease, milk products, emerging pathogens, reservoirs, outbreaks

\***Author for Correspondence** E-mail: k\_v\_mogha@yahoo.co.in

### INTRODUCTION

The term food safety is often used nowadays. What's the concern? There are more than 200 known diseases which are transmitted through food by a variety of agents that include bacteria, fungi, viruses, and parasites. According to public health and food safety experts, each year millions of illnesses in our country and throughout the world can be traced because of pathogens emerging from milk and milk products. So should the dairy industry be concerned about food safety? And for these, there are several good reasons such as:

- i. Bulk tank milk contains several foodborne pathogens that cause human disease,
- ii. Outbreaks of disease in humans have been traced to the consumption of raw unpasteurized milk and have also been traced back to pasteurized milk,
- iii. Raw unpasteurized milk is consumed directly by dairy producers and their families, farm employees and their families, neighbours, etc.,
- iv. Raw unpasteurized milk is consumed directly by a much larger segment of the population via consumption of several types of cheeses including ethnic cheeses

manufactured from unpasteurized raw milk,

- v. Entry of foodborne pathogens via contaminated raw milk into dairy food processing plants can lead to persistence of these pathogens in biofilms and subsequent contamination of processed food products,
- vi. Pasteurization may not destroy all foodborne pathogens in milk, and
- vii. Faulty pasteurization will not destroy pathogens.

This review mainly focus on the prevalence of foodborne pathogens (primarily *Campylobacter jejuni*, Shiga-toxin producing *E. coli* (STEC), *L. monocytogenes*, and *Salmonella*) in milk and in the dairy environment, the role of foodborne pathogens as mastitis-causing bacteria and their impact on milk quality, and to discuss public health and food safety issues associated with foodborne pathogens found in the dairy environment.

Milk and products derived from milk of dairy cows can harbour a variety of microorganisms and can be important sources of foodborne pathogens.

The presence of foodborne pathogens in milk is due to direct contact with contaminated sources in the dairy farm environment and to excretion from the udder of an infected animal. Food safety is a pressing issue for governments, food processors, retailers and consumers worldwide and this sense of urgency is felt not only in the meat or seafood industries, but also in the dairy industry. The quality and safety of milk and dairy foods are of primary concern to the dairy industry and the obligation of dairy industry personnel. Food safety begins on the farm and continues through processing and transportation processes until the milk or milk product is consumed. There is an emphasis on farm control of foodborne pathogens because the farm environment is a reservoir for many microorganisms.

In order to reduce the incidence of foodborne illness associated with milk and dairy products, a pathogen reduction program must begin on the farm and be applied throughout the food chain. There are two categories of food safety concerns in the dairy industry. These include microbiological hazards and chemical hazards, primarily foodborne pathogens with regard to the former and chemical related concerns such as; antibiotics, pesticides and herbicides.

### **EMERGING FOODBORNE PATHOGENS IN MILK**

Several surveys are carried out to detect pathogens present in milk and also the line studies are performed to check whether the milk processing lines are cleaned properly. The prevalence of foodborne pathogens in milk is influenced by numerous factors such as; farm size, number of animals on the farm, hygiene, farm management practices, variation in sampling and types of samples evaluated, differences in detection methodologies used, geographical location, and season.

However, in spite of the variation, all of these surveys demonstrated quite clearly that milk can be a major source of foodborne pathogens of human health significance [1]. Also many surveys have detected foodborne pathogens in bulk tank milk. Results of those studies have shown clearly that prevalence rates of foodborne pathogens including *C. jejuni*,

STEC, *L. monocytogenes*, and *Salmonella* spp. in milk vary considerably. Jayarao *et al.* [2] collected bulk tank milk from 131 dairy herds in eastern South Dakota and western Minnesota and these were examined for the presence of foodborne pathogens. *Campylobacter jejuni*, shiga-toxin producing *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., and *Yersinia enterocolitica* were detected in 9.2, 3.8, 4.6, 6.1, and 6.1% of bulk tank milk samples, respectively. Similarly a research was carried out by Rohrbach *et al.* [3]. They collected milk samples from 292 farm bulk tanks and these were analyzed for selected bacteria. It was seen that frequency of bacterial isolation present was *Listeria monocytogenes* 12 (4.1%), *Campylobacter jejuni* 36 (12.3%), *Yersinia enterocolitica* 44 (15.1%), and *Salmonella* 26 (8.9%). Some of the emerging pathogens in milk and milk products are given below.

#### ***E. coli* O157:H7**

The human pathogen which was recognized first in 1982 was *E. coli* O157:H7 with the two outbreaks in the United States by consumption of undercooked hamburgers from a fast-food restaurant chain. The pathogen was since then emerged as a major cause of bloody and nonbloody diarrhea. In addition to bloody diarrhea, *E. coli* O157:H7 infection is the most common cause of the hemolytic uremic syndrome, the leading cause of acute kidney failure in children in the United States. Consumption of raw milk and untreated water has been implicated in outbreaks, and person-to-person transmission is also well documented.

#### ***Listeria monocytogenes***

The unique among Gram-positive bacteria in which it possesses lipopolysaccharide, which served as an endotoxin is *L. monocytogenes*. Infection by *L. monocytogenes* causes the disease listeriosis. The manifestations of listeriosis include septicemia, meningitis (or meningoencephalitis), encephalitis, corneal ulcer, pneumonia, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion (2nd/3rd trimester) or still birth. Influenza-like symptoms, including persistent fever, usually precede the onset of the mentioned disorders. Gastrointestinal symptoms such as; nausea,

vomiting, and diarrhea may precede more serious forms of listeriosis or may be the only symptoms expressed. Gastrointestinal symptoms were epidemiologically associated with use of antacids or cimetidine.

The onset time to serious forms of listeriosis is unknown but may range from a few days to three weeks. The onset time to gastrointestinal symptoms is unknown but probably exceeds 12 h. This organism is found naturally in the environment. Consuming raw milk could lead to contracting this organism. It could cause symptoms like flu-like illnesses to meningitis. It may also cause abortion in pregnant women. It has a mortality rate of 30% of those who are infected. (Pasteurisation destroys this organism).

### ***Helicobacter pylori***

*H. pylori* is a cork-screw shaped Gram-negative microaerophilic bacterium which is found to be present in the stomach and duodenum and is the most common bacterial infection of man. Many of those carrying the bacterium have little or no symptoms and are apparently well, but all without exception have inflammation of the stomach lining, a condition which is called 'gastritis'. Gastritis is the underlying condition which eventually causes ulcers and other digestive complaints. If a person has had an *H. pylori* infection constantly for 20–30 years, it can lead to cancer of the stomach. This is the reason that the World Health Organisation's (WHO) International Agency for Research into Cancer (IARC) has classified *H. pylori* as a 'Class- I-Carcinogen' i.e., in the same category as cigarette smoking is to cancer of the lung and respiratory tract. This organism may be transmitted through contaminated milk. Infection with the organism is a powerful predisposing factor to the development of stomach cancer (Pasteurisation destroys this organism).

### ***Campylobacter jejuni***

The genus *Campylobacter*, (meaning 'twisted bacteria') first discovered in 1963 describes Gram-negative, spiral, microaerophilic bacteria. Motile, with either uni- or bi-polar flagella, the organisms have a characteristic spiral/corkscrew appearance and are oxidase-

positive. This organism is found naturally in soil, water, and farm waste and in the digestive tract of animals. *Campylobacter jejuni* is now recognized as one of the main causes of bacterial foodborne disease in many developed countries. At least a dozen species of *Campylobacter* have been implicated in human disease, with *C. jejuni* and *C. coli* the most common. *C. fetus* is a cause of spontaneous abortions in cattle and sheep, as well as an opportunistic s in humans. *Campylobacteriosis* is an infection by *campylobacter*. It produces an inflammatory, sometimes bloody, diarrhea, periodontitis or dysentery syndrome, mostly including cramps, fever and pain. The infection is usually self-limiting and in most cases, symptomatic treatment by reposition of liquid and electrolyte replacement is enough in human infections. The use of antibiotics, on the other hand, is controversial. In recent years there have been reports of severe outbreaks of enteritis in the UK attributed to *Campylobacter jejuni*. In all cases consumption of unpasteurised milk was implicated. Symptoms are profuse diarrhoea (sometimes bloody), stomach cramps, nausea, dizziness and fever (Pasteurisation destroys this organism).

### ***Mycobacterium avium* spp. *paratuberculosis* (MAP)**

*Mycobacterium avium* ssp. *paratuberculosis* (MAP) is a Gram-positive, acid-fast and facultative anaerobic, intracellular bacterium. It is a fastidious microorganism that requires the growth factor mycobactin J for *in vitro* growth. *Mycobacterium avium* ssp. *paratuberculosis* (MAP) is the causative agent of paratuberculosis, or Johne's disease, a chronic granulomatous enteritis that affects all ruminants worldwide. Since the isolation of MAP from intestinal tissue of human patients bearing Crohn's disease, there has been a debate on the possibility of this agent playing a role in the etiology of Crohn's disease. Milk could be the potential vehicle for transmission to humans. *Mycobacterium avium* ssp. *paratuberculosis* has already been detected in milk samples worldwide. MAP is likely to be present in most bulk milk. Public health concerns about the presence of MAP in milk and dairy products, as cheese. It is most interesting that the organism has been shown

to some extent to survive the pasteurisation of milk.

### **Tick-borne Encephalitis**

It is a tick-borne viral infection of the central nervous system affecting humans as well as most other mammals. It is caused by the tick-borne encephalitis virus. The virus can infect the brain (encephalitis), the membrane that surrounds the brain and spinal cord (meningitis) or both (meningoencephalitis). It is transmitted by the bite of infected deer- or sheep ticks or (rarely) through the nonpasteurized milk of infected cows. Sexual transmission has been documented in mice with vertical transmission to progeny. Sexual transmission with humans has never been documented. The virus can, besides being tick-borne, also be transmitted by consumption of contaminated raw goat, sheep and cow milk, and is thus a food-borne zoonotic disease.

### ***Enterobacter sakazakii***

*Enterobacter sakazakii* is a Gram-negative, rod-shaped pathogenic bacterium. It is a rare cause of invasive infection with historically high case fatality rates (40–80%) in infants. It can cause bacteraemia, meningitis and necrotising enterocolitis. Environmental low level contamination of the milk powder during processing is likely to be the source of the organism. *E. sakazakii* infection has been associated with the use of powdered infant formula, with some strains able to survive in a desiccated state for more than 2 years.

Sepsis (bacteria in the blood), meningitis (inflammation of the lining of the brain), or necrotizing enterocolitis (severe intestinal infection) are common symptoms of *Enterobacter sakazakii* infection exhibited by infants. These can be accompanied by seizures, brain abscess, hydrocephalus, developmental delay, and death.

Significant morbidity in the form of neurological deficits can result from infection, especially among those with bacterial meningitis and cerebritis. *E. sakazakii* has a higher thermal resistance in comparison to other members of the Enterobacteriaceae family which might explain their high prevalence in powdered and prepared formula milk; they survive the spray drying of the milk

with hot air. Also high osmotolerance of the organism may be of importance for its survival. The technology of powdered milk production is one of the most critical parts in the prevention of the risk and breaking of the line of transmission.

### ***Bacillus cereus***

*Bacillus cereus* is an endemic, soil-dwelling, Gram-positive, rod-shaped, beta hemolytic bacterium. Some strains are harmful to humans and cause foodborne illness, while other strains can be beneficial as probiotics for animals. *B. cereus* bacteria are facultative anaerobes, and like other members of the genus *Bacillus* can produce protective endospores. *B. cereus* is responsible for a minority of foodborne illnesses (2–5%), causing severe nausea, vomiting and diarrhea. Generally, *Bacillus* foodborne illnesses occur due to survival of the bacterial endospores when food is improperly cooked. This problem is compounded when food is then improperly refrigerated, allowing the endospores to germinate. Bacterial growth results in production of enterotoxins, one of which is highly resistant to heat and to pH between 2 and 11; ingestion leads to two types of illness, diarrheal and emetic (vomiting) syndrome. The diarrheal type is associated with a wide-range of foods has an 8 to 16.5 h incubation time and is associated with diarrhea and gastrointestinal pain. The emetic form is commonly caused by rice that is not cooked for a time and temperature sufficient to kill any spores present, then improperly refrigerated.

## **SOURCES OF PATHOGENS IN MILK**

There are two main sources of food borne pathogens in dairy foods, contaminated raw milk and dairy products that have been contaminated during post-pasteurization processing or handling, by dairies, by those in the retail food chain or by the consumer.

### ***Raw Milk***

Milk obtained from the udder (aleveoli) of a healthy milch animal is almost sterile but beyond these stage of milk production, microbial contamination can generally occur from three main sources [4] from within the udder, from the exterior of the udder, and from the surface of milk handling and storage

equipment. The health and hygiene of the cow, the environment in which the cow is housed and milked, and the procedures used in cleaning and sanitizing the milking and storage equipment are all keys in influencing the level of microbial contamination of raw milk. Equally important are the temperature and length of time of storage, which allow microbial contaminants to multiply and increase in numbers. All these factors will influence the total bacteria count or Standard Plate Count (SPC) and the types of bacteria present in bulk raw milk. Thus it can be said that largest number of dairy related foodborne illness outbreaks can be attributed to contaminated raw milk. Of the known pathogenic bacteria in raw milk, *Campylobacter*, *Listeria* and *Salmonella* lead the pack, with verotoxigenic *E. coli* (VTEC), *Staphylococcus aureus*, and *Bacillus cereus* also implicated. Diseases such as; tuberculosis, diphtheria, scarlet fever and brucellosis have declined dramatically to being non-existent as a result of mandatory regulations requiring pasteurization and other strategies such as the implementation of a brucellosis eradication program.

*Mycobacterium paratuberculosis* is an emerging concern in the dairy industry, as well, particularly in light of the fact that while the other pathogens mentioned are effectively killed during pasteurization, *M. paratuberculosis* has been reported to survive this process. (It should be noted that there is currently a fierce debate as to whether this pathogen causes disease in humans, specifically Crohn's Disease). Despite the fact that consumption of contaminated raw milk poses a potential hazard to the consumer and that many foodborne illness outbreaks have been traced to this source, it remains legal to sell raw milk in some U.S. states. However, these states are under pressure to ban the sale of raw milk. In the province of Ontario, Canada, it is illegal to sell raw milk.

#### **Post Pasteurization Contamination**

It is clear that contamination of dairy product can occur post pasteurization. This may be as a result of cross contamination of finished product with raw product, inadequate sanitation procedures in the plant environment,

or inadequately sanitized equipment. Since the introduction of pathogens can occur at any stage along the post pasteurization processing, distribution, storage and handling chain, a system of good laboratory methods for detecting and tracing sources of microbial contamination, as well as control measures for preventing such contamination are essential elements of the dairy industry food safety program. Generally, Gram negative bacteria are absent when the milk is pasteurized in dairy industry but it when this milk is stored at refrigeration temperatures the spores present in the raw milk get germinated also the major contamination sources which are difficult to detect as the production system is complicated and contains dead ends, pockets and traps with the potential to act as a permanent reservoir for spoilage bacteria, e.g., valves, shafts and gaskets that may be difficult both to clean and to sample [5, 6]. Most of the dairies have effective cleaning-in-place (CIP) systems but they might occasionally fail to disinfect hidden reservoirs. Similarly, the filling procedure is critical for the recontamination of the pasteurized milk by Gram-negative psychrotrophic bacteria [7].

Eneroth *et al.* in [8] studied the contamination of pasteurized milk by *Bacillus cereus* during the filling process in two dairy plants. Samples of pasteurized milk were taken at four different sites along the production line. The samples were stored at 7°C for 7 d, or at 10°C for 5 d, before plate counting and random selection of *B. cereus* isolates. It was observed that more *B. cereus* of different RAPD types was growing in the consumer packages than in samples taken just before the filling machine. Several RAPD types found in the consumer packages were not detected in the samples taken just before the filling machine.

#### **RECENT OUTBREAKS**

Several food borne illnesses occur due to consumption of milk and milk products from dairy industry contaminated with pathogens. Recently an *E. coli* outbreak took place in 2014 when four children drank milk from the dairies supplying raw milk in Kentucky and were hospitalized for the same. It is generally seen that pasteurization eliminates pathogens and consumption of nonpasteurized dairy

products is mostly uncommon, dairy-associated disease outbreaks continue to occur. To determine the association of outbreaks caused by nonpasteurized dairy products with state laws regarding sale of these products. It was observed that in dairy-associated outbreaks during 1993–2006, 121 outbreaks

was for which the product's pasteurization status was known; among these, 73 (60%) involved nonpasteurized products and resulted in 1,571 cases, 202 hospitalizations, and 2 deaths. A total of 55 (75%) outbreaks occurred in 21 states that permitted sale of nonpasteurized products (Table 1).

**Table 1: Recent Outbreaks due to Milk and Milk Products Consumption.**

Month/Year	Location	Details	Reference
April 2012	Missouri	14 sick from raw milk ( <i>E. coli</i> )	[9]
April 2012	Oregon	21 sick from <i>E. coli</i> associated with raw milk consumption	[10]
October 2011	Indiana	0 illnesses. Recall of pasteurized cheeses because of potential <i>Listeria</i> contamination	[11]
September 2011	Georgia	0 illnesses. Improper pasteurization of milk	[12]
August 2011	Virginia	0 illnesses. recall of pasteurized cheddar cheese spread with possible <i>Salmonella</i> contamination	[13]
March-August 2011	Pennsylvania	16 illness from <i>Yersinia enterocolitica</i> from glass-bottled pasteurized milk and ice cream	[14]
June 2011	New York	No illnesses from pasteurized queso fresco cheese contaminated with <i>Staphylococcus aureus</i>	[15]

## RAPID METHODS ADOPTED BY DAIRIES

Microbial analysis of foods is an integrated part of management of microbial safety in the food chain. Both control authorities and individual food business operators use microbial analysis for the purpose of monitoring of the actual situation and trend analysis in order to detect emerging risks. Also for compliance testing to defined microbiological criteria or assessment of the performance of management strategies based upon Hazard Analysis Critical Control Points (HACCP), microbial analysis is a valuable tool. Standardized methods (e.g., ISO methods) are acknowledged as the reference analytical methods for official control. Rapid and automated methods for the screening and detection of pathogens are useful application in a dairy industry HACCP program. Recently, there has been a move in the dairy industry toward the use of rapid methods to quickly screen raw milk for bacteria levels as a general indicator of adequate sanitation and milk quality.

The analysis of foods for the presence of both pathogenic and spoilage bacteria is a standard practice for ensuring food safety and quality. Alternative microbiological methods may help the industry to find new ways of obtaining reliable results more efficiently to ensure food

safety. The alternative and rapid method which can be used to detect pathogens are modified and automated conventional methods; Bioluminescence; Cell counting methods; Impedimetry; Immunological methods; Nucleic acid-based assays. Bai *et al.* [16] developed a method based on amino-modified silica-coated magnetic nanoparticles (ASMNPs) and polymerase chain reaction (PCR) for rapidly and sensitively detecting foodborne pathogens in raw milk. After optimizing parameters such as pH, temperature, and time, a trace amount of genomic DNA of pathogens could be extracted directly from complex matrices such as raw milk using ASMNPs. These magnetically separated complexes of genomic DNA and ASMNPs were directly subjected to single PCR (S-PCR) or multiplex PCR (M-PCR) to detect single or multiple pathogens from raw milk samples. *Salmonella Enteritidis* (Gram-negative) and *Listeria monocytogenes* (Gram-positive) were used as model organisms to artificially contaminate raw milk samples.

## CONTROL MEASURES

Control measures for preventing contamination begin at the farm level and are implemented during animal production with adequate environmental sanitation, separating infected or sick animals from young calves and other healthy animals, as well as

the implementation of Good Manufacturing Practices (GMPs) during milking and storage. Milking must be performed using procedures that will prevent contamination of milk by microorganisms from the udder and teats. Cold storage at 4°C and strategies for ensuring that all equipment that comes into contact with milk is properly sanitized are other important control measures implemented at the dairy processing and after processing stages of production. For example, separation of raw milk from finished product and adequate environmental sanitation in both dairies and retail stores will reduce the incidence of post-pasteurization contamination. While the key control measures—maintenance of high sanitation standards, GMPs and the prevention of cross contamination of finished dairy product by raw milk help immensely in the control of microbiological hazards, the implementation of a Hazard Analysis and Critical Control Points (HACCP) plan provides better control of food safety hazards.

While HACCP is not mandatory for the milk and dairy product industry, it has been voluntarily implemented in many dairy processing plants across North America. This science based approach in which potential sources of food safety hazards—chemical, biological and physical—in the operation are identified, critical control points (CCPs) established, monitoring procedures instituted and verification that control measures are working effectively can be implemented at both the farm and processing levels. One of the key benefits of HACCP is an increased ability to identify problems before they occur, which results in the opportunity for the producer or processor to establish control measures critical to food safety aims.

On the farm, for example, biological CCPs, primarily pathogens, can be identified at critical junctures during animal production, milking and storage. Control measures for identified hazards might include the development and implementation of an on farm sanitation program that ensures the cleanliness of equipment and tools used for milking, and the cleanliness of the water used on the farm so that it meets the standards for potable water. Different standard hygiene

programmes like GMP (Good Manufacturing Practices), GLP (Good Laboratory Practice), and GHP (Good Hygienic Practices) can be implemented in dairy processing plants to prevent emerging pathogens to enter dairy products.

## CONCLUSION

The challenges to providing a safe and nutritious food supply are complex because all aspects of food production – from farm to fork – need to be considered. Given the considerable national/international demand for food safety and the formidable challenges of producing and maintaining a safe food supply, food safety research and educational programs has taken on a new urgency.

In today's dairy industry, potentially harmful pathogenic contamination can occur at any stage of milk production: on the farm, in the processing plant, in the retail store, and even in the consumer's home. Therefore, steps must be taken to control microbial contamination at various points along the food chain. As indicated, HACCP implementation is an essential component of the control measure arsenal available to ensure food safety in the dairy industry.

Emerging rapid and automated methods are being developed and improved upon to help the dairy industry to effectively address emerging microbial challenges and concerns. The implementation of HACCP from farm to retail distribution of milk and dairy products is a must for food safety assurance in the dairy industry.

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