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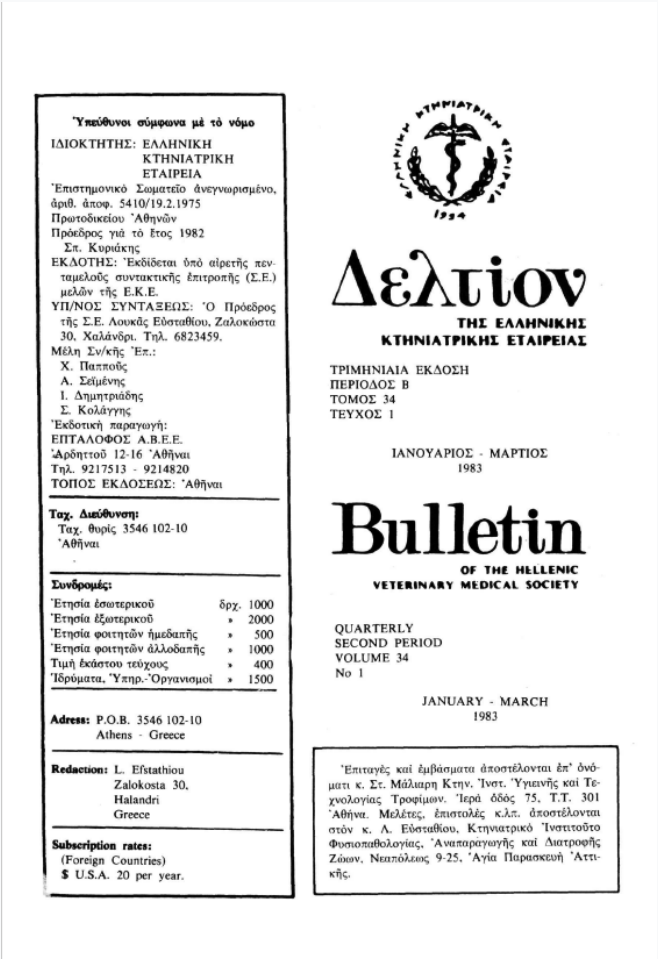

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Recent trends in foodborne diseases of bacterial origin

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RECENT TRENDS IN FOODBORNE DISEASES OF BACTERIAL ORIGIN*

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A. General Epidemiological Considerations

Foodborne and waterborne diseases are a major group of juman illnesses. Yet international statistics indicate that only a small fraction of the total population suffers some sort of food poisoning during any calendar year. Today reliable foodborne disease surveillance systems exist only in a few countries publishing data on a regular basis.[39,42,43] The mild nature of many of the food poisonings and the habit in many countries of treating these diseases empirically is another factor contributing to the lack of knowledge on their true incidence. A retrospective study evaluated outbreaks that occurred in Canada and the USA.[17] The median ratio of estimated cases to initially reported cases was found to be × 25:1. Based on this ratio and the systems of data transmission, the estimates of annual food and waterborne disease cases for 1974 to 1975 were from 150,000 to 300,000 for Canada and 1,400,000 to 3,400,000 in the USA.

Though statistical data are limited, they permit evaluations leading to a better understanding of the outbreaks, contributing factors, and trends.

Epidemiologic trends can be summarized as follows: 1) Microbiological agents remain the major cause of foodborne illness. 2) *Salmonella sp., S. aureus, C. perfringens* and *V. parahemolyticus* remain the most prevalent causes but their individual significance may differ with location. 3) A large number of outbreaks are of unknown etiology. 4) Foods of animal origin are incriminated more than other types of foods. 5) Common places where foods are mishandled are, in decreasing frequency, food service establishments, homes, and food-processing establishments. 6) the five most common factors contributing to foodborne disease outbreaks in the USA in order of frequency of occurence include α) inadequate cooling of foods, b) lapse of a day or more between preparing and serving, e) infected persons handling foods which are not

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subsequently heat-processed, c) inadequate time and/or temperature during heat processing of foods and e) insufficiently high temperature during storage of hot foods.[6]

On a global basis the incidence of food poisoning is expected to increase because of 1) Changes in eating habits from home-prepared meals to commercial meals in restaurants, schools, day care centers, and other fast mass feeding establisments; 2) Mass production of processed foods and their nation-wide or international distribution exposing large segments of the population to potential dangers; 3) Increased consumption of slightly heated products of animal origin because of ignorance of undue faith in the protective effect of food inspection; 4) Trends toward «natural» food consumption resulting in availability of foods which have not undergone any terminal heat treatment (consumption of raw milk); 5) Rapid expansion of tourism to places where the local food service establishments are unprepared to acomodate huge numbers of visitors. The tourist is unaware of local food handling practices and unable to take precautionary measures. 6) Increasing migrant worker movement which is characterized by numerous changes in socioeconomic, ecological, hypien Vic and feeding conditions; 7) Improvement in the national Public Health services and surveillance systems allowing better investigation, analysis and reporting of disease; 8) Improvement in consumer awareness resulting in better recognition of foodborne cases presently escaping reporting; 9) Introduction of new technology in food preservation and preparation with safety at times getting lower priority than marketability. This is quite common in developing nations where small food industries cannot afford the cost of food safety evaluation programs, government agencies are not equipped to do it either, and consumers are unaware of appropriate handling.

B. Foodborne Diseases Due to Bacteria

Numerous bacterial species are responsible for foodborne diseases. For some the main road of transmission to humans is through foods. Other organisms are transmitted to man by other means but are sometimes foodborne. The proof of transmission by foods in inconclusive for a number of bacteria and still unknown for others. In this paper the discussion will be limited to recent trends and developments with respect to major and some recently recognized bacterial agents.

1. Salmonellosis

Salmonellosis remains one of the three most common foodborne diseases. The true incidence of the disease is by far greater than reported. The annual estimate of cases for Canada is 150,000 and for the USA 740,000[17], with medical costs in the USA exceeding 1.2 billion dollars. [43] The overall incidence of

no-host specific human salmonellosis in the USA has increased over the last 10 years though typhoid fever has been declining steadily.
The major source of salmonella problem in man is still derived from food of animal origin. Most of the outbreaks are due to the no-host specific salmonellae. Of the 1700 serotypes less than 100 represent over 80% of the isolations. Yet any exotic serotype may be introduced to a country and eventually become dominant, first among food animals and then among humans.
In 1969-1970 S. *agona* emerged as a public health problem in UK, Israel and the Netherlands[7,32] and by 1976 it had become the third most frequently isolated serotype from human sources and fourth from non-human sources in the USA.

The gastrointestinal (C.I.) system of food animals remains the major reservoir. Most of the isolated samonellae in feces are no-host specific serotypes while most animals are symptomless carriers. Normally fed and watered animals are able to eliminate rapidly even large levels of salmonellae entering the G.I. tract with feed while starved animals fed low levels of samonellae shed the agent for a prolonged time. Long travel from ranches to slaughterhouses and feeding in slaughterhouse environments increases the number of salmonella shedding animals. Overall, stress as water and feed deprivation, fatigue, transportation, conditions disturbing the normal flora of the gut, or disease allows easier colonization and multiplication of the pathogen in the G.I. tract. Feeding subtherapeutic levels of antibiotics to animals reduces to various degrees the numbers of salmonella being shed into the environment. Antibiotics may affect the normal flora of the gut and permit, once the feeding is interrupted, attachment and colonization by Salmonella. Use of G.I. tract flora of animals in the feed early in life plays an important role in minimizing salmonella colonization of the gut.[26] Contaminated animal feeds remain the major source of salmonella infection and spread.[48] Factors responsible for feed contamination appear to be: contaminated ingredients, inadequate sanitation, airborne dispersion, insufficient cooking and most often recontamination of the finished product. Pelletization reduces the problem but increased energy costs caused cancellation of this process in some places. Increased environmental pollution, expanded urbanization in areas where animals are raised, ineffectiveness of sewage treatment to destroy existing salmonella and recent trends in using treated waste water and sewage sludge for irrigation and soil fertilization are becoming newer contributing factors to salmonella spread among animals.[27,48].
Development of salmonella free herds and flocks is a key to success as efforts in some countries have shown. High prevalence of salmonella in animal feces increases cross contamination in the slaughterhouse environment and the finished product. Current practices still allow product contamination.[7,9,32] Scalding tanks, defeathering and dehairing machines and cooling tanks are major places of cross contamination. Recent trends point toward a simultaneous scalding-defeathering system using steam, and cooling approaches other than i-

ce. Further cross contamination takes place during evisceration, packaging, mechanical deboning, further processing, and cutting and slicing in markets. Prevalence of salmonellae in fresh poultry and red meat at the retail level remains high.[7] Salmonella incidence in non-fat dry milk has remained the same (less than 1%) over the last 10 years in the USA[32] but extensive reduction in other foods has been achieved.

Outbreaks of salmonellosis due to raw milk are still reported. Political strength of food fadists presents a problem in the universal application of milk pasteurization. Surveillance in the USA has remained a powerful tool for protecting the consumer by discovering problem plants and on-going epidemics but it has done little to control the endemic problem from which epidemics emerge. Inadequate cooling, process failure, and cross contamination of working surfaces in the kitchen environment remained the most important factors contributing to outbreaks of salmonellosis.[6]

The pathogenesis of salmonella-mediated diarrheal disease is still unclear. Recent findings tend to implicate a heat labile toxin. Though we are still far from controlling salmonellosis in animals and man, progress includes food handler and consumer education, development of salmonella free birds, better understanding of the thermal destruction in food and feeds, and improvements in surveillance systems, animal management, and slaughterhouse practices.[7,8,9,12,42]

2. Escherichia Coli Diarrhea

Escherichia coli organisms are a part of the normal human and animal intestinal flora. They appear soon after birth and are usually confined to the distal portion of the large bowel where they are the predominant aerobic organisms. Some strains of *E. coli* are able to cause diarrheal diseases in man and animals after ingestion, by at least three mechanisms which are partially understood.[45] These strains have the ability to attach and multiply in the small intestine producing illness by invading epithelial tissue, producing one or more enterotoxins or by adherance to and destruction of the microvilli without inviasion. All three mechanisms of pathogenicity result in diarrhea. Three groups of *E. coli* have been recognized as important diarrheal pathogens: *Enterotoxigenic E. coli* (ETEC), *Enteropathogenic E. coli* (EPEC), and *Enteroinvasive E. coli* (EIEC). *Enterotoxigenic E. coli* is a major cause of illness in children in the developing world, is the most common cause of traveller's diarrhea and is responsible for severe cholera-type disease in children and adults in a few cholera-endemic areas. ETEC's produce a heat labile (LT) enterotoxin immunologically related to cholera toxin and/or a heat stable (ST) enterotoxin. The mode of action of both toxins is well understood.[11,45] A relatively large inoculum is required to cause illness and decreased gastric acidity may increase susceptibility.

Transmission is through water, food, and person-to-person. Large outbreaks

from water and food (aboard ships) have been reported. Production of both toxins is controlled by plasmids which are easily transferrable to recepient strains at least in the laboratory. Certain serofermentative types of ETEC are adapted as carriers of these plasmids. Adherence or attachment of the bacteria to the intestinal surface is facilitated by surface protein antigens originating in the pili and plasmid controlled. These colonization antigens in ETEC of animal origin have been studied extensively while those of human origin have only recently been discovered. Parenteral or oral administration of pili offers protection to oral challenge by the corresponding ETEC strain, at least in animals. These findings are of great importance for the development of immunity and protection against ETEC diarrheas in humans. The possible relationship and transfer of plasmids between animal and human strains of ETEC have not been explored extensively. It is presumed that humans are the major reservoir of the organisms responsible for human disease.

Enteropathogenic E. coli's have been responsible for epidemic infantile enteritis with mortality as high as 50% in some cases. Decline in the incidence of the disease is attributed mainly to improvements in personal and environmental hygiene in homes and institutions. With aging, children show increased prevelence antibodies to EPEC suggesting acquisition of immunity and explaining the rarity of EPEC disease in older children and the high frequency of EPEC carriers among adults. A few water and foodborne outbreaks have occurred recently in adults.[45]The pathogenesis is still unclear. EPEC's produce no LT or ST. They colonize the small intestine and caused severe diarrhea in adult volunteers. Animal model work suggests involvement of a «toxin». *Enteroinvasive E. coli* serotypes have been reported causing dysentery-like disease in man. School, hospital, water and cheeseborne outbreaks have been reported from a number of countries. EIEC's produce no LT or ST, invade the intestinal epithelium of laboratory animals, and show extensive biochemical and antigenic resemblance to Shigella. The pathogenesis and epidemiology of EIEC diarrhea is still unclear.

3. Yersinia Enterocolitica

Yersinia enterocolitica has been implicated in sporadic cases of human disease since 1939. The agent is an important food and waterborne pathogen with world-wide distribution. Gastroenteritis in humans is the major expression of the disease. Limited studies from certain developed nations implicated *Y. enterocolitica* as responsible for 1-3% of all human enteritis cases. *Y. enterocolitica* is a psychrotolerant bacterium. Five biotypes have been recognized with human pathogenic strains belonging mainly to types 2,3 and 4. Biotype 5 has most commonly been observed in animal epizootics while Biotype 1 includes mostly «environmental» non-human pathogenic strains. Strains causing human disease have belonged almost exclusively to serotypes 03, 08,09.[39,45]

The pathogenesis of the disease remains unclear. Plasmid-controlled ability to invade animal and human cells has been shown. A heat stable enterotoxin possibly plasmid-mediated is produced by certain strains while others may be both invasive and enterotoxigenic. Low temperature incubation (30^0C-4^0C) favors enterotoxigenesis and invasiveness.[39] The activity of the toxin is similar to the ST of *E. coli* with stability of 30 min. at 121^0C.

Millions of cells are needed to cause disease. Production of ST is subiquitous in *Y., enterocolitica* with the highest prevalence among strains associated with human infections. The epidemiology of the disease is unclear. It is considered a zoonosis with the major route of transmission through food or water contaminated by feces, urine, or insects. Contact with sick animals and people may be another route. Of many carrier animals, swine seem to be the major reservoir for human infections.[4,39,46] Swine are the only recognized food animal which recurrently harbor pathogenic *Y. enterocolitica*. Studies in Belgium and Denmark have shown that 3-5% of pigs are intestinal carriers of serotype 3, while pig throat and tongue cultures were positive for up to 53%. Foods of animal origin and water have been repeatedly contaminated by *Y. enterocolitica*.[5,39,46] Prevalence up to 18% has been observed in raw milk. Most strains isolated from foods other than pork meat have been classified into the environmental strain category which rarely shows invasiveness. Since the pathogenic factors causing human disease are not well understood the public health significance of these strains cannot be critically evaluated. During the 1970's at least six large community outbreaks of *Y.enterocolitica* infections have been reported[39]. In only one outbreak was the foodborne transmission proven.

Studies in food have shown that small initial inoculum can reach millions after storage at 0-2^0C for 2 weeks. This psychrophilic organism presents the food industry with a unique problem in maintaining food safety. Recent trends to extend the shelf life of fresh meat by cold storage under vacuum or hypobaric and controlled atmosphere[41] may have implications on its safety with respect to *Y. enterocolitica*.

4. Campylobacter Fetus Enteritis

The major significance of *Campylobacter fetus* to human health has been exposed only in the last 4 years.

C. fetus ssp. *intestinalis* is an orally transmitted agent causing abortion in both cattle and sheep.[13,36] Free living wild birds are involved in the transmission of the disease. The agent can also infect humans, generally causing a bacteremic illness in compromised patients.

Campylobacter fetus ssp. *Jejuni* is a normal gut inhabitant of many animals and has been associated with animal disease. The agent has been established as a cause of zoonotic infectious enteritis in humans[34] occurring with a frequency rivaling that of Salmonella. International studies have implicated the organism

as a cause of up to 40% of human diarrheas, especially in children.[10,13] Epidemiologic evidence links the disease with direct human contact with infected chickens, dogs and possible other animals.[10,18,20,35] Person-to person transmission is suspected when sanitation and personal hygiene are not well practiced. Untreated patients shed the agent in feces for about 2-5 weeks after an attack of *Campylobacter enteritis.* [10] It seems that food and waterborne *Campylobacteriosis* is the usual way of human infections. Recently unpasteurized and raw certified milks and water were implicated in several huge outbreaks in UK and USA.[20] Due to a limited culturing of stools the true incidence remains unknown.

A prevalence of up to 100% in chicken and turkey ceca, in chicken meat[21] at the supermarket level and 43% in chicken carcasses after 3 weeks storage in the freezer was noticed.[33] Over 33% of carcasses after chilling in chlorinated water (50-340 ppm chlorine) overnight remained positive.[21] The agent declines rapidly in various environments and food. The extent of multiplication in foods is unknown. In 10% reconstituted nonfat dry milk we have found that after 180 min. heating at 63°C there were still 10^2 to 10^4 viable cells/ml out of an initial inoculum of 10^8/ml. Surviving cells did not differ from the original in heat resistance.

In man, the principal site of infection seems to be the small intestine but infection may not be limited to this site. Antibody titers quickly reach a maximum and gradually decline with time.[10] Infection, as judged from stool isolations are more prevalent in the warmer months of the year. The significance of this is obscure but it broadly parallels the trend in Salmonella infections at least in UK and USA.

The pathogenesis and epidemiology of the disease in man is still unclear. Serology and biotyping have failed to identify a pathogen as belonging to a particular type. The significance of *C. jejuni* isolation from foods of animal origin is still unclear.

5. Vibrio Parahaemolyticus.

This halophilic marine organism first recognized in Japan has been found now to be an important cause of diarrhea in many parts of the world, and it has occasionally been associated with extraintestinal infections.[4,31,46] Two clinical syndromes have been described, one characterized mainly by waterly diarrhea and the other by dysentery. Incubation time may also differ. Both syndromes are usually self limited. The mechanism of the disease remains unclear. A heat stable hemolysin, a heat-labile toxic factor, and ability to adhere and invade the intestinal tissues of humans are suspected virulence factors[4]. Isolates from wounds were non-hemolytic. No particular serotype has been associated with human illness. The seasonal incidence of gastroenteritis correlates well with the ability of the organism to grow rapidly in warm temperatures. In winter, sea-

water is frequently free of *V. parahaemolyticus*. Low numbers are found in the sediment where the organism begins to proliferate and it is released to the water and zooplankton as the ambient temperature rises. The causative foodstuff is associated directly or indirectly with seafish and seawater.
The main vehicle is raw fish and shellfish. Cooked food can be contaminated by hands, raw materials, equipment and seawater. Cross contamination has caused big outbreaks aboard cruising ships. With a generation time as short as 8 minutes the organism can proliferate rapidly in unrefrigerated foods reaching infective levels of $> 3X10^5$. Fresh water and fresh water fish have been implicated in India. *V. parahaemolyticus* has been found in the stools of patients but not in the stools of healthy individuals. The role of symptomless excretors is unknown and person-to-person transmission has not been described. Control of the infection in man is based mainly on limiting multiplication of the agent in seafoods and on preventing secondary contamination of already cooked seafood from raw materials.

6. Staphylococcal Food Intoxication

The disease remains a world wide problem. Five serologically distinct enterotoxins (A,B,C,D and E) are responsible for the same syndrome. The purified toxins are proteins resistant to proteolytic enzymes and heating. ($D_{121} = 10$)[3,24]. Production of these enterotoxins is limited to *S. aureus strains*, mutants or variants. Most strains produce one or more of these toxins in yields varying from $< 1\mu$ g/ml for A, D and E to $> 50\ \mu$ g/ml for B and C. Production of B and C seems to be controlled by chromosomal genes while A,D and E by plasmids. No physiological characteristic of the bacterium correlated 100% with enterotoxigenesis in foods. Enterotoxins A and D are the most frequent causes of food poisoning. B enterotoxin is often produced by multiple antibiotic resistant strains and by strains associated with post-operative wound infections. Field data indicate that amounts of 1 μg may cause disease. Implicated foods contain at least 10^6 cells/g. The pathogenesis of the disease remains unclear. Circulating antibodies usually are not protective. The nasopharynx and skin of man and animals remain the major reservoir of *S. aureus* with human origin strains implicated in outbreaks more frequently. Prevalence of over 50% has been shown repeatedly in food handlers. Cured meats are the most common foods implicated in outbreaks, followed by bakery and dairy products.[3,8] Major factors contributing to the problem are inadequate cooling, preparing foods a day or more before serving and infected food handlers. Post process contamination is more common than process failure. Staphylococci cannot compete effectively with the normal food flora and their growth is more luxurious in cooked rather than raw foods. Heavy initial staphylococcal contamination and absence of efficient competition by other bacteria, or starters in some fermented foods like cheese and salami has resulted in a number of food poisoning outbreaks. Computer assisted least cost formulations by the meat industry may

lead to use of low quality meat with high staphylococcal counts. Lactic acid bacteria starters may not be effective in all sausage formulations.[14,15] Also use of unpasteurized milk and starter failure due to phages may cause problems in the cheese industry. Ham is the single most commonly implicated food ite because of the recontamination of a cooked product with salt acting as a solective agent for *S. aureus* growth. The problem will be minimized by food handler education, environmental and personal hygiene, minimal contact with food, milk pasteurization, use of starters and inhibition of growth by refrigeration.

7. Botulism

The global problem of botulism though limited to a few outbreaks and cases per year greatly concerns the consumer, the industry and the regulatory agencies. This is mainly due to the high mortality, the potential of a wider distribution of canned products often manufactured by the millions in a single plant and the extensive publicity through communication media resulting in the financial death of the manufacturer. Three forms of botulism are recognized: food intoxication, wound infection, and infant botulism.

Food intoxication is caused by consumption of preformed type A,B,E and F toxins of *C. botulinum.*[30,38] Early disease detection, use of antitoxins and supportive therapy has minimized mortality in recent years. While past exposure does not protect from future attack, resistance among individuals varies. Circulating antibody has been found in normal people, but its role is unclear.[30] Infant botulism was reported first in California in 1976.[1]
Since then increasing numbers of cases have been reported in this and other countries in both breast fed and formula fed infants of < 8.5 months of age. Only two deaths have been reported. The cost for one case was $ 300,000. This disease is due to consumption of *C. botulinum* type A,B or F (one case) organisms or spores which manage to colonize the G.I. tract of the infants and produce toxin for reasons that are still unclear. Toxin and organisms are excreted in the feces for a long period of time even in the absence of symptoms. Toxin was detected in the serum only in one case. On epidemiological grounds and isolations, honey was incriminated as the source of the spores in some cases. It is recommended that honey should not be fed to infants under 1 year of age. It is suspected that infant botulism has a microbial ecological basis. There is possibly a microbial antibotulinal barrier which develops with age or it is easily displaced. Wound botulism remains a rare disease.
Eight *C. botulinum* neurotoxins have been recognized as A,B,C_1C_2,D, E,F and G. All but G have been purified and studied extensively.[30,38]
A strain may produce more than one type of toxin. High prevalence of spores has been found in soil, seamud, raw foods of plant origin and very low (<1%) in fresh meat. *C. botulinum* can multiply in certain types of soil, especially tho-

se rich in organic matter, but it has not been shown how important this is in the ecology of the organism at least for those types to which man is susceptible. Spores found in animal feces, contribute relatively little to pollution of the soil. Decaying carcasses of animals and birds seem to play a role in soil pollution with spores and the epidemiology of botulism of mammals and birds. No epidemiologic relationships between animal and human botulism have been demostrated.

Botuligenic foods are usually a)raw or food subjected to some form of preservation process, b) have a pH > 4,6 and have been stored under conditions conducive to toxigenesis and c) ingested without adequate heating to destroy the relatively heat-labile toxins. The thermal destruction of the toxins has been reexamined recently.[49] Inadequate home processing of foods by canning, salting, smoking, pickling or fermenting and drying remains the key contributor to present day botulism. World wide inflation contributes also to more home food preservation and indirectly to botulism.

The early caning industry adopted heat processes that ensure killing of botulinum spores with a margin of safety of less than one surviving spore out of 10^{12} in low acid foods.[15] Outbreaks of botulism from commercially canned foods are rather infrequent. Nevertheless experience shows that an outbreak of botulism may require only something slightly unusual, such as a change in food formulation, under-processing, defects in can-seaming operations or a change in packaging technology.

In recent years there has been a rapid increase in the use of controlled atmospheres in cold storage of plant and animal origin foods to extend shelf life and facilitate intercontinental shipping. Use of vacuum packaging, hypobaric atmosphere (reduced pressure) or atmospheres modified by CO_2, O_2, and N_2 affect the lag phase, rate of growth and selection of spoilage bacteria.[41] The effect of these new preservation technologies upon the growth of the psychrotolerant non-proteolytic type B,E and F *C. botulinum* in cold storage temperature has not been evaluated. Temperature abuse has shown the potential of type A and B growth.

Low acid canned foods produced commercially in the USA have had a remarkably good record during the last 50 years. Approximately 775 billion cans of food were produced through 1971. The unexpected outbreaks of 1970-74, due mostly to commercially canned mushrooms, forced the government and the industry to reexamine the state of the art. Some significant deficiencies were found in equipment and operating procedures used to can mushrooms. In 1973 FDA published a final order «Good Manufacturing Pratice Regulations for Thermally Processed Low-Acid Canned Foods in Hermetically Sealed Containers». [22] The regulations were supplemented by inspectional procedures to identify critical control points in the processing and included a hazard analysis of these points.

Of the 722 reported outbreaks of *C. botulinum* between 1899 and 1975 in

USA, 35 were due to high acid (pH< 4.6) foods (mostly home canned tomato products). What caused growth of *C. botulinum* in such foods is of concern since the organism cannot grow below pH 4.7. High acid foods are heated for the destruction of spoilage sporeformers and not *C. botulinum* spores which are 10 to 200 times more heat resistant. Though decreased pH increases the heat sensitivity of *C. botulinum* spores, surviving spores remain viable for a long time[25] and can germinate and grow if the pH changes to > 4.7 and temperature and food composition are also favorable. Certain microorganisms surviving the canning process or entering the can post processing, can utilize acids and increase the pH to values conducive for *C. botulinum* growth. New tomato varieties suitable for mechanical harvesting, some with pH's> 4.6, have been introduced recently. Home canning of such tomatoes may need higher F_0's to assure safety. A pH of 3.4-5.0 was found in home canned tomato products.

Fish farming is expected to increase due to worldwide dermand for more protein. Such intensive aquaculture may have serious implication on the ecology of *C. botulinum*. High prevalence of *C. botulinium,* mainly type E, reaching 100% in pond bottom material, has been shown. Feeds heavily contaminated, ponds with earthen bottoms, excess feeding, high fish populations during warm weather, and fish feces can contribute to *C. botulinum* growth and heavy product contamination.[19] Such products will need special processing methods to assure destruction of spores or inhibition of *C. botulinum* growth.

Cured meats are very popular roday. Their public health record, at least of those commercially processed, with respect to botulism has been great. This record has been attributed to the use of curing salts, low prevalence and numbers of *C. botulinum* in meats, interaction effects and heat processing or fermentation (use of starters and fermentable carbohydrate). As a result shelf stable canned cured meats, though being low acid foods, required F_0=0.1 to 0.6 instead of full botulinum cook of F_0=>2.5.15 Incured meats the probability oφ *C. botulinum* growth decreases with increasing levels of nitrite. The biochemical basis for nitrite-inhibition of *C. botulinum* has been reviewed.[2] Because of the potential of nitrosamine formation, use of nitrite and nitrate has been attacked. Numerous recent studies looked for alternatives. No food additive has been found to replace nitrite. Approaches to reduce the risk of nitrosamine formation and maintain safety against *C. botulinum* have been proposed and some have been implemented. Briefly these include: a) elimination of nitrate except in dry fermented sausages; b) reduction of the added nitrite along with addition of certain level of ascorbate, tocopherol and potassium sorbate; c) use of starter cultures, sugars and decreased levels of nitrate and nitrite in fermented meats; d) incorporation of lactic acid bacteria inside the packages of non-shelf stable products which may undergo consumer abuse; e) adjustment of water activity to > 0.93 or brine concentration to < 10% for dry cured non-heated products containing no nitrite; and f) preserving no-nitrite contained «cured meats» by freezing.[28,37,40]

8. Clostridium Perfringens Gastroenteritis.

Clostridium perfringens gastroenteritis remains a major foodborne disease problem.[9,43]

The disease is due mainly to the consumption of a cold or warmed poultry or other meat cooked the previous day, or even a few hours before consumption and allowed to cool slowly. Cooking kills the vegatative cells of *C. perfringens* type A but activates the surviving spores, which can eventually germinate and grow in the low redox of the cooked food. Ingested cells ($>10^6$/g) sporulate readily in the intestines and produce a heat labile, trypsin resistant enterotoxin (spore coat protein) which is responsible for the disease after its release from the lysed sporangia[18]. A direct relationship between degree of sporulation and yield of enterotoxin has been demonstrated with most type A strains

Repeated heat shock of spores may lead to cultures with increased ability to sporulate and yield more toxin.[18,35] Upon storage the strains revert to low level sporulators in a few weeks by an unknown mechanism. Production of a serologically similar enterotoxin by certain type C and D strains has been shown.[18,35] Pure enterotoxin and antisera became available for the development of serodiagnostic techniques and detection of the toxin in stools of victims. Sporulation in foods is not as easy as in the intestinal tract. Certain foods can support sporulation and enterotoxin formation within a relatively short time of incubation. Such preformed toxin may contribute to earlier onset of symptoms. The *in vivo* action of the toxin remains unclear. Fluid accumulation in the small intestine and diarrhea are due to increased transient capillary permeability, increased vasodilation, tissue damage in the gut, and increased intestinal mobility. High prevalence of antienterotoxin in normal human sera has been described[18,35] but the mechanism of its formation and its role in protecting humans after reexposure to toxin is unclear.[23] The role of enterotoxin in the pathogenesis of enteritis necroticans due to *C. perfringens* type C is unknown. This disease is attributed to the beta toxin which is very sensitive to proteolysis. Low protein diet and presence of heat-stable trypsin inhibitors in sweet potatoes, a dietary staple, may reduce destruction of the toxin in the gut of people thus contributing to high prevalence of the disease in places like New Guinea.

All reported outbreaks of C. perfringens are due to type A which is widely distributed in nature with human and animal intestines the major reservoir. Prevalence of 60-100% has been shown in normal human and animal feces with counts as high as $5X10^6$/g.[18]. Shedding rates in animals are affected by disease, stress and husbandry methods Endogenous invasion of carcasses because of special animals handling before slaughtering was shown.

Enterotoxin production varies with strain, source and locale.

Enterotoxigenic strains are extemely prevalent in normal human populations regardless of sex, age, gastrointestinal féatures, or nutritional habits.

Most animal strains are enterotoxigenic, too. No serological or biochemical characteristics have been correlated with enterotoxin production. The organism is not dangerous when ingested in small numbers. In *C. perfringens* outbreak some or more of the following events were the major contributing factor: a) improper cooling; b) improper hot holding; c) food prepared a day or more before serving; and d) inadequate reheating.[8]

9. Bacillus Cereus Food Poisoning

Recent investigations have established *B.cereus* as a cause of two food poisoning syndromes in addition to other exointestinal infections in man and animals. One syndrome resembles *C.perfringens* food poisoning and it is characterized by diarrhea and long incubation (8-16 hours). The other resembles staphylococcal food poisoning and is characterized by vomiting and short incubation (1-5 hours).

A wide range of foods, but mostly meat and meat products, have been implicated in the diarrhea type outbreaks, although not very common in recent years. Inadequate cooking, survival, gerination and growth of spores during unsatisfactory post-cooking storage was the cause. Numerous outbreaks of the vomiting type mostly due to cooked rice have been reported recently from at least seven countries. The outbreaks were linked with the practice of preparing a large portion of rice in advance of serving and keeping it at room temperature. *B. cereus* spores survive the boiling and germinate and grow luxuriosly during storage at warm temperatures.

B. cereus is common in soil and vegetation and has been isolated from a wide variety of routine samples of food. A 90% prevalence was found in uncooked rice and 14%, and low numbers, was found in the feces of general human populations. One spore in at least 10^5 exhibits extreme heat resistance, at times 4 hours at 135°C.

Serotyping cannot distinguish cultures responsile for a particular disease syndrome. Important recent advances have been made on the pathogenesis of the disease. We now recognize at least two toxins as virulence factors.[16,44].

One is a heat labile, unstable trypsin sensitive, 50000 MW, protein enterotoxin synthesized during the exponential phase of growth of *B. cereus*. It causes fluid accumulation in rabbit ileal loop, altered vascular permeability in rabit skin, diarrhea in rhesus monkeys and kills mice IV.

The toxin is produced by most *B. cereus* strains at various levels, and is considered responsible for the diarrhea type outbreaks. The second toxin or emetic factor thought to be causing the vomiting symdrome is a small MW heat resistant toxin (90 min. at 129°C), probably not a protein which is elaborated particularly during growth in rice for reasons that have yet to he defined.

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