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Review

Listeria monocytogenes in foods: Incidences and possible control measures

F. Adzitey and N. Huda*

School of Industrial Technology, Universiti Sains Malaysia, Minden, 11800, Pulau Pinang, Malaysia.

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The bacterium *Listeria monocytogenes* is a Gram-positive, intracellular, ubiquitous, and facultative food-borne pathogen of humans and animals. They may occur naturally in environmental sources such as soils, faeces and intestinal tracts of animals and humans. The pathogen causes listeriosis characterized by gastroenteritis, meningitis, abortion, and sometimes death in systemic cases. Neonates, infants, immunocompromised individuals, pregnant women, and the elderly in particular are most susceptible to listeria infections. In recent times, contamination of foods by *L. monocytogenes* has become a major concern to all stake holders in the food industry and the health sector. Their infection has been associated with a number of food-borne outbreaks resulting from the consumption of various foods especially, cooked and chilled ready-to-eat foods. A review on *L. monocytogenes* and its association with foods is important to create more awareness on the need to reduce their colonisation, transmission, cross contaminations and infections.

Key words: *Listeria monocytogenes*, listeriosis, food.

INTRODUCTION

Listeria monocytogenes is one of the most important food-borne pathogens of humans. It is a Gram-positive, rod-shaped, non-spore-forming, and facultative anaerobe bacterium (Vazquez-Boland et al., 2001; Sukhadeo et al., 2009). *L. monocytogenes* has been described as opportunistic pathogen affecting mainly children, pregnant women, the aged and immune-challenged individuals (Schlech, 2000; Liu, 2006). In addition a wide variety of animals including sheep, cattle, goats, pigs, rabbits, mice, birds, and fish are also infected (Ireton et al., 2006). The pathogen is also responsible for listeria infections that can lead to abortion, bacteraemia, sepsis, and meningoencephalitis (Khelef et al., 2006; Sukhadeo et al., 2009). The incidence of listeriosis is relatively rare and represents less than 0.1% of all food-borne illnesses but causes infections with very high mortalities (20 to 30% deaths) (Mead et al., 1999; Ireton 2006).

In 1992 the number of cases of listeriosis in the United States was 1,550, which costs the government $142,581 per patient and $221,000,000 globally (Khelef et al., 2006). In England, 2 cases were reported by Gillespie et al. (2006) from the consumption of hospital sandwiches. In 2006, EFSA (2007) report revealed 6 cases of listeriosis from the consumption of hard cheese in Germany. Major outbreaks of listeriosis in the USA and Europe have also been reported from the consumption of turkey deli meat, pork deli meat, hot dogs, corn salad, chocolate milk, rice salad, deli meat, shrimps, Mexican cheese, soft cheese, pasteurized milk and cole slaw, with known and unknown perinatal cases and mortality rates (Khelef et al., 2006; Arun, 2008). However in Japan, Okutani et al. (2003) reported that the incidence of listeriosis has been very low for the past 40 years compared to that of Western Europe and North America.

Nevertheless, various foods and environmental samples have been implicated in the spread of *L. monocytogenes*. Thus the pathogen is repeatedly found in meat and meat products, raw milk, soft cheese and pasteurised dairy products, vegetables, and fish and fish products. For example, *L. monocytogenes* has been isolated from sheep, goat and cow milk (Rahimi et al., 2010), chopping boards, cleaning cloths, mincing machine, poultry meat and meat products (Mahmood et

*Corresponding author. E-mail: nrlhd@usm.my. Tel: +604-6532112. Fax: +604-6573678.

al., 2003), cooked meats, cured meats, smoked salmon, soft cheese and vegetables (Vitas et al., 2004), ready-to-eat foods (Aurora et al., 2008) and raw and pasteurized egg samples (Rivoal et al., 2010). This has led to the setting up of microbiological criteria or recommendations for the occurrence of *L. monocytogenes* in foods in some countries. In the USA, a zero tolerance of *L. monocytogenes* in 25 grams of food have been recommended (Shank et al., 1996) while in Canada, a tolerance level of below 100/g for some foods and a zero tolerance for others (Farber et al., 1996) is being adhered to. In Europe, it is stated and recommended that, *L. monocytogenes* must not be present in levels above 100 cfu/g during the shelf life of a product, and products in which the growth of the bacterium is possible, it must not contain *L. monocytogenes* in 25 g at the time when they leave the production plant unless the producer can demonstrate, to the satisfaction of the competent authority, that the product will not exceed 100 cfu/g limit throughout shelf life (ESFA, 2010).

For easy, rapid and efficient methods of isolating and identifying *L. monocytogenes*, both standard culture and several PCR based techniques have been employed (Johansson, 1998; Choi and Hong, 2003; Becker et al., 2005; Loncarevic et al., 2008; Aurora et al., 2009). Such protocols and techniques are essential for the purpose of epidemiological studies, clinical and treatment purposes. Food safety continues to be an increasing concern to consumers and listeria infection is an important public health problem.This review briefly discusses *L. monocytogenes*, incidences, isolation techniques, and possible measures to reduce transmissions and infections. In this text *L. monocytogenes* is used for both the singular and the plural form.

LISTERIA MONOCYTOGENES AND THEIR SOURCES

L. monocytogenes is a Gram-positive, rod-shaped, facultative anaerobe, and intracellular bacteria with a low G+ C (36 to 42%) content and without capsule (Vazquez-Boland et al., 2001; Sukhadeo et al., 2009). It is catalase positive, L-Rhamnose positive, oxidase negative and motile at 10 to 25°C (Arun, 2008; Sukhadeo et al., 2009). The pathogen is 1 to 2 µm long and may exist as single or double cells but display long chains depending on the growth (Arun, 2008). It has the ability to grow in a wide range of temperature (1 to 45°C), although optimal growth occurs at 30 to 37°C (Swaminathan et al., 1995). *L. monocytogenes* is also tolerant and survives in extreme conditions like a wide pH range (4.1 to 9.6), high salt (10%) concentrations and in the presence of antimicrobial agents (Liu et al., 2005; Arun, 2008). *L. monocytogenes* belongs to the genus Listeria, which is closely related to *Bacillus*, *Clostridium*, *Enterococcus*, *Streptococcus*, and *Staphylococcus* (Vazquez-Boland et al., 2001; Sukhadeo et al., 2009). Furthermore, there are six species under the genus Listeria notably; *L. monocytogenes, L. ivanovii* subsp. *ivanovii, L. ivanovii* subsp. *londoniensis, L. seeligeri, L. innocua, L. welshimeri* and *L. grayi.* Out of these only *L. monocytogenes* have been authentically reported by many researchers as human and animal pathogen. *L. ivanovii* has also been identified as pathogenic for animals but, mainly in sheep and cattle; and on rare occasions, *L. ivanovii* and *L. seeligeri* have been associated with human infections (Rocourt and Cossart, 1997).

There are 13 distinct O-antigenic patterns, in *L. monocytogenes* which comprises the serovars; 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 4e, 4ab, and 7 (Arun, 2008). Serovars 1/2a, 1/2b, and 4b (most virulent) are responsible for 98% of the outbreaks (Arun, 2008). There are also three lineages; Lineage I (Highly pathogenic, with epidemic clones and responsible for most outbreaks e.g. 1/2b, 3b, 4b, 4d, 4e), Lineage II (medium pathogenic, sporadic cases e.g. 1/2a, 1/2c, 3c, 3a) and Lineage III (low and rarely cause human diseases e.g. 4a, 4c) (Arun, 2008). The opportunistic and ubiquitous food-borne pathogen occupies several environmental niches (Vazquez-Boland et al., 2001). Sukhadeo et al. (2009) reported that the natural habitat of *Listeria monocytogenes* is thought to be decomposing plant matter, in which they live as saprophytes. Nevertheless, *L. monocytogenes* can be found in the intestinal tract of animals such as cattle, goats, sheep, poultry, fish, rabbits, mice, pets and wild animals. They may also occur in soils, water, effluents, plants, vegetables and faeces of animals and humans. It has been reported that one out of five percent healthy humans serves as a carrier of this pathogen (Arun, 2008). *L. monocytogenes* can also colonize various inert surfaces and can form biofilms on food-processing surfaces (Roberts and Wiedman, 2003).

TRANSMISSION AND INFECTION

The primary vehicle for listeria infection is food. Normally, meat and meat products, vegetables, fish, dairy products, minimal processed food and ready-to-eat foods are potential source of transmission (Arun, 2008). The infective dose for one to get listeriosis has been estimated to be the consumption of food containing about 100 to 10^6 cells, depending on the immunological status of the host (Arun, 2008).The rate of infection is also affected by bacterial virulence, size of ingested inoculum, and underlying host defences (Sukhadeo et al., 2009).

Neonates, infants, pregnant women, immunocompromised individuals (AIDS, cancer, organ transplant patients etc) and the elderly are most susceptible to listeriosis (Schlech, 2000; Liu, 2006). Although listeriosis is rare (less than 0.1% of all food-borne illnesses) with a very high mortalities (20 to 30%) (Mead et al., 1999;

Ireton, 2006); mortality can be as high as 75%, in high risk persons (Khelef et al., 2006). The common symptoms of listeriosis include fever, watery diarrhea, nausea, headache, and pains in joints and muscles (Arun, 2008). Clinical manifestations of invasive listeriosis are usually severe and include abortion, sepsis, meningoencephalitis, neuro-encephalitis, chorioamnionitis, gastroenteritis and bacteraemia (Khelef et al., 2006; Sukhadeo et al., 2009).

ISOLATION AND DETECTION OF *LISTERIA MONOCYTOGENES*

Proficient and dependable techniques for the isolation and identification of *L. monocytogenes* in food samples are very important to facilitate clinical and epidemiological studies. Most people have relied on the standard culture method. The standard culture method basically involves enrichment in one or more listeria enrichment broth(s), followed by plating onto one or more listeria selective agar(s) and appropriate biochemical confirmation. Enrichment broths used for isolating *L. monocytogenes* include Listeria enrichment broth and modified Fraser broth. Plating is done using PALCAM, ALOA, *L. monocytogenes* blood agar, Chromogenic agar, Oxford agar, and Lithium chloride-phenylethanol-moxalactam agar. Aragon-Alegro et al. (2008) and Loncarevic et al. (2008) have validated various listeria medium for detecting and enumerating *L. monocytogenes* in foods and feeds.

Presumptive *L. monocytogenes* are purified on Trypticase soy agar, with 0.6% yeast extract prior to confirmation tests. Confirmation tests have been achieved using Gram staining, carbohydrate utilization, motility, and haemolysis tests; and perhaps the use of *L. monocytogenes* antisera. Incubation of both enrichment and plating is done at 30 to 35°C for 24 to 48 h under aerobic conditions. Standard culture methods detected viable *L. monocytogenes* and yields isolates that can further be studied and characterized. More details of the methods for isolating and detecting *L. monocytogenes* have been described by Pagotto et al. (2001) and Hitchins (2001).

Rapid methods based on the antibodies and DNA *of L. monocytogenes* has also been developed to characterize this pathogen to the strain level. Such methods can be categorised broadly into immunological (for example latex agglutination test, ELISA), nucleic acid (polymerase chain reaction (PCR) based methods) and growth-based methods. For instance, Choi and Hong (2003) used a rapid competitive polymerase chain reaction (cPCR) for direct enumeration of *L. monocytogenes* in sterile milk artificial contaminated with the foodborne pathogen. Furthermore, Aurora et al. (2002) used a multiplex-PCR (to serotyped *L. monocytogenes*) and RAPD (for RAPD profiles to determine discrimination among isolates) to characterize *L. monocytogenes* and concluded that both methods allow rapid discrimination of *L. monocytogens* strains and could be relied on for typing *L. monocytogenes* strains. A review of such methods can also be found in Jeyaletchumi et al. (2010).

INCIDENCES OF *LISTERIA MONOCYTOGENES* IN FOODS AND REPORTED CASES

The main source of listeria infection in humans is thought to be from the consumption of infested food. Various kinds of foods have been implicated in outbreak of listeriosis. A summary of the incidences of *L. monocytogenes* in foods is shown in Table 1. From Table 1, the occurrence of *L. monocytogenes* varies from one food, one place, and one author, to the other. The table also reveals that various kinds of foods, processing equipments, and environmental samples can be vehicles for transmission of *L. monocytogenes*. The rate of transmission will depend on the initial load and the handling precautions. *L. monocytogenes* were absent in some food samples analysed, suggesting that such foods were processed and handled under hygienic conditions.

The highest (100%) occuccurence of *L. monocytogenes* was observed by Johansson (1998) from spiked soft cheeses. The isolation of *L. monocytogens* from frozen and pasteurized samples confirm the ability of the pathogen to survive under refrigeration conditions and possible re-contamination of foods under poor processing and handling conditions, making it more complex to produce listeria-free foods. In addition, Table 1 shows that most work on *L. monocytogenes* have concentrated on milk and milk products, meat and meat products, and ready-to-eat foods. Few studies considered environmental and processing equipments although they are also important vehicles for transmission of *L. monocytogenes* and should not be overlooked. Shade (1992) showed that *L. monocytogenes* persists in the food processing (for example meat and dairy) environments, especially in cool damp places, conveyers, floors and drains despite vigorous sanitary regimes. Not only have *L. monocytogenes* been isolated from foods, but also they have cause a number of infections from the consumption of contaminated foods.

In England and Wales, Gillespie et al. (2006) reported a total of 48 cases of listeriosis resulting from the consumption of butter and hospital sandwiches. In France, de Valk et al. (2001) reported listeriosis in 42 patients due to the consumption of pork rillettes and jellied pork tongue. In the USA 13 case from the consumption of Mexican style soft cheese (MacDonald et al., 2005), 93 cases from the consumption of cooked turkey (Olsen et al., 2005; Gottlieb et al., 2006), 16 cases from the consumption of Sliced cooked turkey (Frye et al., 2002) and 108 cases from the consumption of frankfurters (Mead et al., 2006) have been reported. In

Table 1. Occurences of *Listeria monocytogenes* in foods and other important sources.

Samples	No. analysed	No. of positives	% Positive	Reference
Raw egg samples	144	25	17	Rivoal and others (2010)
Pasteurized egg samples	144	4	3	
Fresh broiler cuts	142	73	51	
Sliced or unsliced sausages and ham	24	19	79	
Frankfurters and pates	44	5	11	
Vacuum-packed smoked and cold-salted fish	110	22	20	
Spiked soft cheeses	6	6	100	
Environmental samples from dairies	69	4	6	Johansson (1998)
Fish-processing plants	24	5	21	
Yoghurts	5	0	0	
Animal feeds	5	0	0	
Hard and semi-hard cheeses	11	0	0	
Fresh cheeses	36	0	0	
Fresh poultry meat	40	2	5	
Fresh poultry boneless	40	1	3	
Frozen poultry meat	40	3	8	
Frozen chicken nugget	40	5	13	Mahmood and others (2003)
Frozen chicken burgers	40	3	8	
Chopping board	40	6	15	
Mincing machine	40	4	10	
Cleaning cloth	40	7	18	
Preserved fish products (Not heat treated)	335	35	10	
Preserved meat products (heat treated)	328	77	23	
Heat-treated meat products	772	45	6	Nørrung and others (1999)
Raw fish	232	33	14	
Raw meat	343	106	31	
Poultry products (raw)	58	34	59	Lawrence and Gilmour (1994)
Poultry products (cooked)	94	0	0	
Raw meats	295	103	35	
Raw chicken	158	57	36	
Raw cow milk	340	23	7	
Raw sheep milk	202	6	3	
Frozen vegetable	1750	31	2	Vitas and others (2004)
Cooked meats	396	35	9	
Cured meats	345	23	7	
Smoked salmon	100	28	28	
Soft cheese	99	1	1	
Raw ground beef	100	52	52	
Raw chicken legs	100	34	34	
Raw pork chops	98	24	24	
Fermented sausage	100	4	4	Bohaychuk and others (2006)
Roast beef	101	0	0	
Turkey breast	100	3	3	
Beef wieners	100	5	5	
Chicken wieners	101	3	3	

Table 1. Continued.

Beef whole pieces	4231	217	5	
Beef minced	49	11	22	
Pork whole pieces	4421	355	8	
Pork minced	104	20	19	
Chicken whole parts	331	49	15	Okutani and others (2004)
Chicken minced	53	22	42	
Raw milk	139	7	5	
Cheese	19	0	0	
Retail cheese	5	0	0	
Raw whole milk (Conventional method)	81	13	16	Vanegas and others (2009)
Raw whole milk (Real time-PCR)	81	21	26	
Ham/salami/bacon/luncheon meat	17	3	18	
Fish fingers/fish cake	16	3	19	
Tuna fish	5	0	0	
Coleslaw/vegetable salad	50	2	4	
Ice-cream	61	0	0	
Yoghurt	40	0	0	
Cheese/cheese spread	103	0	0	Ng and Seah (1995)
Milk/cream	17	0	0	
Sandwiches/bun	11	0	0	
Duck rice/chicken rice/char siew rice	71	0	0	
Fried chicken/chicken parts	26	0	0	
Meat filling	3	0	0	
Smoked mussels	2	1	50	
Milk samples	2060	105	5	Kalorey and others (2008)
Raw cow milk	90	1	1	
Raw sheep milk	62	4	6	
Raw goat milk	60	1	2	
Raw camel milk	48	0	0	
Commercial cheese	30	0	0	
Traditional cheese	60	9	15	Rahimi and others (2010)
Commercial ice cream	28	0	0	
Traditional ice cream	40	2	5	
Commercial doogh	10	0	0	
Traditional doogh	20	0	0	
Commercial butter	15	0	0	
Traditional butter	25	1	4	
Vacuum-packed smoked salmon	102	11	11	
Vacuum-packed smoked trout	40	10	25	
Vacuum-packed deli meat products	220	6	3	Garrido and others (2001)
Opened deli meat products	200	17	9	
Vacuum-packed pâté	120	1	1	
Opened pâté	41	0	0	

Japan, Makino et al. (2005) reported a total of 38 cases from the consumption of cheese.

MEASURES TO REDUCE *LISTERIA MONOCYTOGENES* IN FOODS

Strategies to reduce *L. monocytogenes* in foods and consequently listeriosis will depend much on hygienic and sanitary production and processing practices. This is to reduce the colonisation, transmission and cross-contamination of *L. monocytogenes* among foods and the environment. An effective control measure for this pathogen has to target the farm, processing plants and the environments. At these all these stages, strict adherence to standard operating measures must be practised. In farming, livestocks should be reared in clean dry environments. Soils in particular should not be moist or damp as that will provide a conducive environment for the growth of this pathogen. Livestock houses should be thoroughly cleaned, and disinfected on regularly basis. Prevent entering of wild animals (which may serve as reservoirs) into the farm especially in areas where feeds are stored.

In processing plants, there is the need for each company to set up processing and environmental monitoring plans for *L. monocytogenes*. Such plans must be specified in the HACCP plan of the company. Monitoring plans should lay emphasize on sanitation practices, processing and packaging operations, personnel hygiene, and routine testing programs for *L. monocytogenes*. If the pathogen is found during monitoring, investigations must be carried out immediately to determine the source to prevent further transmissions. The management needs to set clear policies and train employees so that they understand the importance of proper sanitary practices. Practices such as moving people and equipment from raw material areas to finished product areas, not wearing clean gloves, handling unsanitary utensils or equipment and then touching finished products should be avoided. Cooling units should have dehumidifying properties in order to limit moisture in this area. Packing materials should also be palletized and covered until used. In retail display, temperatures of refrigerators should be monitored on regular basis, avoid mixing products from different sources, and products should be well packaged for display. Expired products should be disposed off immediately. Further education of consumers on food safety issues is recommended. Also foods should be well cooked or heated (in case of ready-to-eat foods) before being eaten.

The use of bioprotective meat starter cultures such as *L. rhamnosus* E-97800, *L. rhamnosus* LC-705 and *L. plantarum* ALC01 in some sausages could help reduce the number of *L. monocytogenes* if they are present (Työppönen et al., 2003). Such cultures express antilisterial activity against *L. monocytogenes.* In addition, combined effect of ozone and organic acid treatment was found to reduce the initial population levels of these pathogens on food samples such as mushroom (Yuk et al., 2007). Nitrite injures listeria (Nyachuba et al., 2007), suggesting that nitrite could reduce the number of Listeria cells. Min et al. (2005) found that whey protein isolate (WPI) films/coatings incorporated with lactoperoxidase system (LPOS) prevented the growth of *L. monocytogenes* in smoked salmon. *L. monocytogenes* viable counts were not detectable when ultra high pressure homogenisation (UHPH) was used to treat grape juice (Velázquez-Estrada et al., 2010). The use of a steam treatment system is very effective in controlling *L. monocytogenes* (Bremer et al., 2002).

CONCLUSION

L. monocytogenes is ubiquitous, opportunistic and a very important food-borne pathogen that continues to pose worries to the food industry and health authorities. Their infection is severe in high risk individuals. The main source of infection is through the consumption of contaminated food. Ready-to-eat foods, meat and meat products, and milk and milk product are the major source of outbreaks and most research has concentrated in this area. Their ability to survive in refrigeration and wide environmental conditions increases the plight of achieving zero or minimal tolerant of *L. monocytogens* in foods. Reliable and accurate isolation and detection techniques are important in the surveillance of *L. monocytogenes* and listeriosis. Standard and hygienic operating methods in the farming, processing and marketing of foods are the way forward to reduce the incidence of listeriosis.

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