INCIDENCE OF LISTERIA MONOCYTOGENES IN DAIRY AND FOOD PRODUCTS OF ANIMAL ORIGIN IN CENTRAL REGION OF PENINSULAR MALAYSIA

MARINA A.R.*, ISMAIL M., NURILYANA M.T., MOHD FHAROK Y. AND AZZURA A.L.

Veterinary Public Health Laboratory, Jalan Nilai-Banting, Bandar Baru Salak Tinggi, 43900 Sepang, Selangor
* *Corresponding author*: marina@dvs.gov.my

ABSTRACT. *Listeria monocytogenes* (*L. monocytogenes*) is a food-borne pathogen which causes listeriosis, an illness characterised by meningitis, encephalitis and septicemia, especially in immuno-compromised individuals, pregnant women, newborns, and the elderly. It has been reported that several outbreaks of listeriosis were due to consumption of contaminated food products, such as dairy, meat, vegetable and seafood. *L. monocytogenes* is notable for its ability to grow at refrigeration temperatures, unlike most enteric pathogens. This has considerable significance for food safety as it means chilling to 4 °C could not be relied upon to prevent the growth of the organism to a dangerous level. The study aims to determine the incidence of *L. monocytogenes* in selected food products of animal origin obtained from selective food processing plants under Veterinary Health Mark (VHM) certification scheme in central Peninsular Malaysia. A total of 130 samples were collected from January to August 2018 from 19 processing plants in four states of Malaysia. The food samples comprising of 30 dairy milk products (ice-cream, butter and cheese) and 100 meat products (chicken frankfurter, smoked chicken frankfurter, chicken sandwiches and chicken Lyoner) were analysed for the presence of *L. monocytogenes* using selective enrichment and isolation protocol. *L. monocytogenes* was not detected in any of the samples. More comparative studies are needed to detect the existence of this pathogen from a variety of food and the environment. This is to ensure the appropriate prevention and control measures are implemented that will help to prevent *L. monocytogenes* food contamination in the food industry.

Keywords: Listeria monocytogenes, incidence, processing plants, dairy products, meat products

INTRODUCTION

Listeria genus is a facultative anaerobic, non-spore forming, motile, gram-positive rod-shaped bacterium with rounded ends. The size of the rods varies from 0.4 µm to 0.5 µm in diameter and from 0.5 µm to 2.0 µm in length. It is catalase positive, oxidase negative and expresses a beta-haemolysin which destroys red blood cells. This bacterium exhibits characteristic tumbling motility when viewed with a light microscopy (Farber and Peterkin, 1991). Although *L. monocytogenes* is actively motile by means of peritrichous flagella at room temperature (20 °C to 25 °C), the

organism does not synthesise flagella at body temperature (37 °C).

This bacterium is widespread in the environment and can survive under extreme conditions such as high concentration of salts, wide temperature range (-0.4 °C to 45 °C), pH between 4 and 9.6, water activities ≥0.92 and the ability to form biofilm (Kanarat *et al*., 2011). *L. monocytogenes* biofilms have been observed to resist cleaning, disinfectant, desiccation and UV light (Doijad *et al*., 2015). Furthermore, these bacteria has the ability to form biofilms with other microorganisms in the production environment such as *Psedomonas* or *Staphylococcus,* making it more resistant to cleaning and disinfection agents (Kurpas *et al*., 2018).

It is found in soil, water, sewage, dust, plants, animals and food (Schlech III, 1989). Soil is the most common and widespread medium for pathogen growth since it provides a cool and moist environment. In addition, the plant and faecal material in the soil supply important nutrients and facilities for the bacteria to grow (Doyle and Buchat, 2007).

L. monocytogenes is carried in the intestinal tract of 5 to 10 percent of healthy humans. In addition to humans, 17 avian species and more than 42 mammalian species (wild and domestic) harbour this bacterium. It has also been isolated from crustaceans, fish, oysters, tick and flies (Todar, 2008). Therefore, healthy animals act as reservoirs of pathogenic strains of *L. monocytogenes* involved in the contamination of food products.

There are seven species: *L. monocytogenes, L. ivanovii, L. seeligeri, L. welshimeri, L. innocua, L. murrayi* and *L. grayi*. *L. monocytogenes* and *L. ivanovii* are pathogenic, the former causing disease in humans and animals, and the latter in animals. The other species are non-pathogenic (Rocourt, 2004). *L. monocytogenes* is one of the important food-borne pathogens of concern for human infection associated with listeriosis (Ryser and Marth, 2004). It can cause severe infections such as meningitis, meningoencephalitis, septicemia, miscarriage and death (McLauchlin, 1996). Even though human listeriosis is a rare disease with incidences ranging from 1.6 to 6 cases per million population, the overall mortality rate amongst cases of listeriosis is about 20% and can be as high as 40% in susceptible persons (Rocourt *et al.,* 2000).

L. monocytogenes is divided by serotyping into 13 different serotypes. However, majority of the human listeriosis are caused by serotypes 1/2a, 1/2b and 4b (Curtis and Lawley, 2003). According to Doyle and Beuchat (2007), *L. monocytogenes* serotype 4b strains are responsible for 33 to 50 percent of sporadic human cases worldwide and all major food-borne outbreaks since the 1980s in Europe and North America.

Changes in food production and demands of a growing society have increased the number of incidences of food-borne illnesses (Doyle, 2000). Changes in eating habits and how foods are produced have seen a growth in the sale of foods which are highly processed, having an extended shelf life and which may be consumed without further cooking. It is just such foods which are more likely to be

vehicles for the transmission of this infection (McLauchin, 1996).

L. monocytogenes has also developed as a food-borne pathogen due to several other factors. One of the factors is the cold storage condition which provides adequate temperature and condition for the growth and survival of *L. monocytogenes*. The population increase of the elderly, and of immunocompromised, acquired immune deficiency syndrome (AIDS), transplant and cancer patients, has contributed in the development of *L. monocytogenes* as an emerging food-borne pathogen (Vitas et *al.*, 2004). However, one of the main factors for the increase of the emergence of *L. monocytogenes* is the production of minimally processed foods, mainly ready-to-eat and heat-and-eat convenient food products, which may not be heated to temperatures to properly destroy the pathogen (Liu, 2008).

Foods can become contaminated with *Listeria* at any stage in the food chain, from the farm through processing and distribution, to the consumer's kitchen, especially in moist environments. *L. monocytogenes* can be found in a wide variety of food such as unprocessed foods of animal origin like raw milk, meat, poultry and fish (Farber and Peterkin, 1991). It can also be found in processed and ready-to-eat foods like pasteurised milk, cheese, ice-cream, fermented raw meat sausages, raw and cooked poultry, raw meats and raw and smoked fish. These bacteria are also reported found on fresh fruits, vegetables and seafood (Jinneman *et al.*, 2004).

Cooked, chilled, ready-to-eat meat, poultry, and sausage products are now a particular concern, and *L. monocytogenes* has been isolated from samples of these foods in many countries (Farber and Peterkin, 2004). Ready-to-eat meats may be contaminated with *L. monocytogenes* at low levels during the manufacturing process. However, it has huge opportunities to multiply to unsafe levels during refrigerated storage of the products, during distribution and at the consumer's kitchen. Because these products are usually fully cooked during manufacturing and are usually consumed without further heating, they present high risks to the consumer, if they are contaminated with *L.monocytogenes* at the processing facility (Swaminathan and Gerner-Smidt, 2007).

Even though *Listeria* species other than *L. monocytogenes* are not pathogenic to humans, their presence in food may be considered as a useful indicator of poor hygienic practice in the food establishment, which could lead to an increased risk of contamination with pathogenic *Listeria* species (Greenwood *et al.*, 2005).

Food safety assurance systems play an important role in the implementation of a nation's food safety programmes and policy. Both food industry and the government shared commitment to assure the safety and quality of food along the food production chain from the farm to table. The food industry is responsible in providing and distributing safe food for consumers by establishing and implementing food safety assurance programmes whereas the government is committed in providing leadership, consultancy, expertise and training in food safety systems established and implemented by the food industry.

Veterinary Health Mark (VHM) is one of the certification schemes issued by the Department of Veterinary Services (DVS) under the Ministry of Agriculture and Agro-Based Industry Malaysia. The aim of this scheme is to encourage the food industry to adopt the implementation of good manufacturing practice (GMP), hazard analysis and critical control point (HACCP) and environmental control in ensuring the production of safe food products. This scheme also aims to enhance the verification system and veterinary health certification for animal-based products for the export market. It provides necessary food hygiene and sanitation requirements for abattoirs and processing plants of livestock such as the production of meat, dairy and its related products.

VHM is a mark of safety and quality awarded to abattoirs and animal based processing plants. Plants certified with VHM are allowed to print the VHM logo on the packaging of their products. This logo indicates that the food processing plants have achieved complete compliance to the minimum standard of hygiene and sanitation, quality assurance and food safety systems. The logo is promoted as a marketing tool that creates customer confidence in product safety and wholesomeness. Currently, 177 premises throughout Malaysia are participants of this programme.

In order to verify the implementation of GMP and HACCP, regular monitoring through National Surveillance Food Safety Programme has been conducted annually by DVS. Samples of products and environment will be taken during this programme and send to the respective laboratory for testing. Review audit will be conducted every year in order to renew the VHM certification. This study aims to determine the incidence of *L. monocytogenes* in selected food products of animal origin obtained from animal based processing plants under Veterinary Health Mark (VHM) certification scheme in central Peninsular Malaysia.

MATERIALS AND METHODS

Sample Collection

A total of 130 samples were collected from January to August 2018 from 19 processing plants under the VHM certification scheme in four states of Malaysia (Selangor, Negeri Sembilan, Melaka and Wilayah Persekutuan Kuala Lumpur). The food samples comprising 30 dairy milk products (ice-cream, butter and cheese) and 100 meat products (chicken frankfurter, smoked chicken frankfurter, chicken sandwiches and chicken Lyoner). These samples were collected by DVS meat inspectors of the National Food Safety Monitoring Programme and sent to a veterinary public health Laboratory for testing.

Isolation and Identification of *Listeria monocytogenes*

Isolation and detection of Listeria *monocytogenes* from dairy and food products of animal origin were by a modified method of the Food Safety and Inspection Services, United States Department of Agriculture (FSIS USDA) Microbiology Laboratory Guidebook (USDA, 2019). A 25

gram of sample was pre-enriched in 225 ml of University of Vermont (UVM) broth (1:10 dilution) and stomached for approximately 2 minutes. The test portion was then incubated at 30 ± 2 °C for 20 h to 26 h. A 0.1 ml of the UVM broth was transferred to 10 ml Fraser broth which was further incubated at 35±2 °C for 18 h-24 h. The inoculum was cultured on chromogenic selective agar, Agar Listeria according to Ottaviani and Agosti (ALOA) and incubated at 35±2 °C for up to 48 h. After incubation, ALOA agar plates were examined for typical *L. monocytogenes* colonies (blue-green colonies with halo).

RESULTS AND DISCUSSION

A total of 130 samples were analysed for the presence of *L. monocytogenes*, which accounted for 30 dairy milk products (ice-cream, butter and cheese) and 100 meat products (chicken frankfurter, smoked chicken frankfurter, chicken sandwiches and chicken Lyoner). The study showed that there was no *L. monocytogenes* contamination in the dairy meat products received from all of the processing plants under the VHM certification scheme. The food processing plants were found to have complied with the standard of hygiene and sanitation, quality assurance and food safety systems.

Even though *L. monocytogenes* was not detected from the food products, there is a possibility that contamination can arise from workers, equipment or the processing plant environments. Although the processing plants had HACCP in place, it could not guarantee the prevention of cross-contamination of raw material and final products with *L. monocytogenes*.

A study done by Kanarat *et al.* (2011) showed that there was no *L. monocytogenes* contamination in the chicken production chain except in slaughterhouses and processing plants. Only 0.2% of RTE chicken products were found to be contaminated with *L. monocytogenes*. Raw meat, other raw materials, contaminated equipment and utensils, workers and plant environments might be the contamination sources of *L. monocytogenes*. These findings were in agreement with the study by Leong *et al.* (2014), who found that *L. monocytogenes* prevalence of 4.6% was seen in all samples analysed with similar rates seen in food and environmental samples.

Although there was no significant finding in the occurrence of *L. monocytogenes* in ready-to-eat food in this study, a study is recommended in the processing plants, which could be a possible source of *L. monocytogenes* contamination. Kanarat *et al.* (2011) reported that the floor drains of the slaughterhouses and the processing plants, from which *L. monocytogenes* was isolated in samples, were found contaminated with *L. monocytogenes*. This clearly showed that *L. monocytogenes* from the environment was identified as the main source of *L. monocytogenes* contamination in ready-to-eat products in the processing plants (Kozak *et al.*, 1996).

Biofilms formed by these organisms on various kinds of food processing surfaces including stainless steel, glass and rubber are difficult to eliminate by cleaning or disinfection (Kurpas *et al.*, 2018). The common area involved in biofilm development are floors, freezers, warehouses waste water pipes and on surfaces in

processing plants which have contact with food or raw products. It is important to distinguish the potential transmission route of *L. monocytogenes* in order to protect the food and production environment from contamination. Environmental sampling of food-contact surfaces should be conducted in order to monitor the effectiveness of microbiological control programmes.

Several studies have indicated that the control of *L. monocytogenes* must be directed towards preventing its establishment and growth in the environment (Khan I. *et al.,* 2016). Processing equipment is one of the major sources of cross-contamination and amplification of the level of *L. monocytogenes* contamination, which emphasised the importance of good personal hygiene and sanitation and good manufacturing practices. Moreover, a disinfection programme should address more thorough and accurate cleaning practices and continuous education of processing plant workers in the specific control of *L. monocytogenes* in plant environments.

CONCLUSION

The results obtained in this study showed that there was no incidence of *L. monocytogenes in* all the samples tested. It is also important to do comparative studies from a variety of food and the environment of the processing plants to detect the existence of this pathogen through implementation and regular monitoring of GMP and HACCP. This is to ensure that the appropriate prevention and control measures are implemented to help prevent *L. monocytogenes* food contamination in the food industry.

REFERENCES

1. Curtis L. and Lawley R. (2003). *Micro-Facts. The working companion for food microbiologists*. Fifth edition. Leatherhead Food International. Cambridge, UK.
2. Doijad S.P., Barbuddhe S.B., Garg S., Poharkar K.V., Kalorey D.R., Kurkure N.V., Rawool D.B. and Chakraborty T. (2015) Biofilm-forming abilities of *Listeria monocytogenes* serotypes isolated from different sources. *PLoS ONE* **10(9):** e0137046. doi: 10.1371/journal.pone.0137046
3. Doyle P.M. (2000). Food safety issues arising at food production in a global market. *Journal of Agribusiness.* **18(1):** 129-133
4. Doyle P.M. and Beuchat L.R. (2007). *Food Microbiology: Fundamentals and Frontiers*. ASM Press, Washington DC, pp 457-491
5. Farber J.M. and Peterkin P.I. (2004). Incidence and behaviour of *Listeria monocytogenes* in meat products. In: *Listeria, Listeriosis and Food Safety.* Ryser E.T. and Marth E.H. (eds). Third edition. Marcel Dekker Inc., New York, USA. 505-564
6. Greenwood M., Willis C., Doswell P., Allen G. and Pathak K. (2005). Evaluation of chromogenic media for the detection of *Listeria* species in food. *Journal of Applied Microbiology* **99:** 1340-1345
7. Kanarat S., Jitnupong W. and Sukhapesna J. (2011). Prevalence of *Listeria monocytogenes* in chicken production chain in Thailand. *Thai J Vet Med.* **41(2):** 155-161
8. Khan I., Jangrez K., Miskeen S., Tango C., Park Y.S. and Oh D.H. (2016). Prevalence and control of *Listeria monocytogenes* in the food industry – a review. *Czech Journal of Food Sciences*. **34:** 469-487. doi: 10.17221/21/2016-CJFS
9. Jinnerman K.C., Wekell M.M. and Eklund M.W. (2004). Incidence and behaviour of *Listeria moncytogenes* in fish and seafood products. In: *Listeria, Listeriosis and Food Safety.* Ryser E.T. and Marth E.H. (eds). Third edition. Marcel Dekker Inc., New York, USA. pp. 601-630
10. Kozak J., Balmer T., Bryne R. and Fisher K. (1996). Prevalance of *Listeria monocytogenes* in foods: Incidence in dairy products. *Food Control*, **7(4-5):** 215-221.
11. Kurpas M., WIeczorek K. and Osek J. (2018). Ready-to-eat meat products as a source of *Listeria monocytogenes. J Vet Res* **62(1):** 49-55
12. Leong D., Alvarez-Ordóñez A. and Jordan K. (2014). Monitoring occurrence and persistence of Listeria monocytogenes in foods and food processing environments in the Republic of Ireland. *Front. Microbiol.* **5:**436. doi: 10.3389/fmicb.2014.00436

13. Liu D. (2008). Preparation of *Listeria monocytogenes* specimens for molecular detection and identification. *International Journal of Food Microbiology*. **122:** 229-242
14. McLauchlin J. (1996). The relationship between *Listeria* and listeriosis. *Food Control*, **7(4-5):** 187-193
15. Rocourt J. (2004). The Genus *Listeria* and *Listeria monocytogenes*: phylogenetic position, taxonomy and identification. In: *Listeria, listeriosis and food safety*. Third edition. Ryser E.T. and Marth E.H. (eds). Marcel Dekker Inc., New York, USA. pp. 1-20.
16. Rocourt J., Jacquet C. and Reilly A. (2000). Epidemiology of human listeriosis and seafoods. *International Journal of Food Microbiology* **62:** 197-209
17. Ryser E.T. and Marth E.H. (2004). *Listeria, listeriosis and food safety*. Third edition. Marcel Dekker Inc., New York, USA.
18. Schlech III W.F. (1989). Methods to determine the virulence of *Listeria* strains. *International Journal of Food Microbiology*. **8:** 273-276
19. Swaminathan B. and Gerner-Smidt P. (2007). The epidemiology of human listeriosis. *Microbes and Infections*. **9(10):** 1236-1243
20. Todar K. (2008). *Listeria monocytogenes*. In: *Todar's Online Textbook of Bacteriology*. Accessed on 12 February 2018 http://textbookofbacteriology.net/Listeria.html
21. USDA (2019). MLG8.11: Isolation and identification of *Listeria monocytogenes* from red meat, poultry, ready-to-eat siluriformes (fish) and egg products, and environmental samples. In: *Microbiology Laboratory Guidebook*. United States Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, Laboratory QA Staff.
22. Vitas A.I., Aguado V. and Garcia-Jalon I. (2004). Occurence of *Listeria monocytogenes* in fresh and processed food in Navarra (Spain). *International Journal of Food Microbiology* **90:** 349-356