Prevalence of Listeria monocytogenes in Chicken Production Chain in Thailand

Sasitorn Kanarat*  Wongkwon Jitnupong  Jananya Sukhapesna

Abstract

This study was conducted from 2004 to 2009 to investigate the prevalence of Listeria monocytogenes in the chicken production chain in Thailand from primary production stages to processing plants. A total of 14,670 samples were taken from 43 breeder farms, 32 hatcheries, 1331 broiler farms, 22 slaughterhouses and 22 ready-to-eat (RTE) chicken products from processing plants. Various types of samples were collected: soiled litter, water, and chicken feed from breeder and broiler farms; swabs and meconium from hatcheries; cloacal swabs and fresh frozen chicken meat from slaughterhouses; and RTE chicken products from processing plants. This study showed that there was no L. monocytogenes contamination in the chicken production chain except in slaughterhouses and processing plants. This implies that the chickens did not carry the organism into slaughterhouses, and consequently primary production practices were not responsible for the contamination of end products. This suggests that the observed L. monocytogenes contamination in 59 (2.5%) and 2 (0.2%) samples of fresh frozen chicken meat and RTE chicken products, respectively, was due to breakdowns in the application of good hygienic and/or good manufacturing practices.

Keywords: chicken production chain in Thailand, Listeria monocytogenes, Prevalence

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Introduction

Listeria monocytogenes is a gram-positive psychrotrophic bacterium, which grows over a temperature range of \(-1.5°C\) to \(45°C\) at pH level between 4.4 and 9.4 and at water activities \(\geq 0.92\) (Fenlon et al., 1996; Samelis and Metaxopoulos, 1999; Moretro and Langsrud, 2004; WHO, 2004; Giannou et al., 2009; Sergelidis and Abrahim, 2009; Valderrama et al., 2009; Yvonne and Wiedmann, 2009). The organism is widespread in the environment and is resistant to various environmental conditions which allow it to survive longer under adverse conditions than most other non-spore forming food-borne bacteria. It is found in meats, poultry, vegetables, dairy products, fishery products, soil, water, sewage and decaying vegetation and can be isolated from humans, domestic animals, raw agricultural commodities, food processing environments and the home (Samelis and Metaxopoulos, 1999; Tompkin et al., 1999; Yucel and Gundogan, 2004; Tauer et al., 2005; Pappelbaum et al., 2008; Samelis and Silva et al., 2008; Garrido et al., 2009; Moretro and Langsrud, 2004; WHO, 2004; Guerrieri et al., 2009; Kushwaha and Moraru, 2009). Its ability to survive and proliferate at refrigeration temperatures in foods makes this pathogen a serious problem for many refrigerated RTE products with a long shelf life (Aaron and Moraru, 2009; Yvonne and Wiedmann, 2009). L. monocytogenes is an opportunistic pathogen that can adversely affects individuals with a severe underlying disease or condition, pregnant woman, unborn or newly delivered infants and the elderly. It causes listeriosis which is a relatively rare but serious disease with the highest hospitalization rate amongst known food-borne pathogens of 91% and a fatality rate with the highest hospitalization rate amongst known food-borne pathogens of 91% and a fatality rate among the food-borne diseases ranging from 20 to 30% (WHO, 2004; Sergelidis and Abrahim, 2009). In the EU and USA, L. monocytogenes is responsible for a high number of food product recalls. It has been claimed that the severity of listeriosis and the very frequent involvement of newly delivered infants and the elderly. It causes listeriosis which is a relatively rare but serious disease with the highest hospitalization rate amongst known food-borne pathogens of 91% and a fatality rate among the food-borne diseases ranging from 20 to 30% (WHO, 2004; Sergelidis and Abrahim, 2009). In the EU and USA, L. monocytogenes is responsible for a high number of food product recalls. It has been claimed that the severity of listeriosis and the very frequent involvement of L. monocytogenes can adhere to all of the materials commonly used in the food industry and form a biofilm (Moretro and Langsrud, 2004; Silva et al., 2008; Guerrieri et al., 2009; Kushwaha and Muriana, 2009). Its ability to survive and proliferate at refrigeration temperatures in foods makes this pathogen a serious problem for many refrigerated RTE products with a long shelf life (Aaron and Moraru, 2009; Yvonne and Wiedmann, 2009). L. monocytogenes is an opportunistic pathogen that can adversely affects individuals with a severe underlying disease or condition, pregnant woman, unborn or newly delivered infants and the elderly. It causes listeriosis which is a relatively rare but serious disease with the highest hospitalization rate amongst known food-borne pathogens of 91% and a fatality rate ranging from 20 to 30% (WHO, 2004; Sergelidis and Abrahim, 2009). In the EU and USA, L. monocytogenes is responsible for a high number of food product recalls. It has been claimed that the severity of listeriosis and the very frequent involvement of industrially processed foods cause the greatest social and economic impact among the food-borne diseases (Moretro and Langsrud, 2004; WHO, 2004). Contaminated food is the principle route of transmission of listeriosis and has been estimated to be the source in as many as 99% of the cases (Moretro and Langsrud, 2004; WHO, 2004; Sauders et al., 2009). Potential sources for introduction of L. monocytogenes to food-processing plants include raw materials, ingredients, water, people, animals and equipment.
Mecocium from 10 day-old chicks were collected and Litter Samples collected at breeder and broiler farms: to December 2009. Litter was moisturized with half-Fraser broth (Oxoid Co. Ltd., hatcheries were swabbed with sterile gauze incubator and equipment used in each of the 32: Incubator trays of each Samples collected at hatcheries farms. Incubator trays of each

Samples collected from 1331 broiler and 1506, 2200 and 1346 samples of soiled litter, water and chicken feed, respectively, were collect ed from 43 breeder farms 869 samples of soiled litter, water and chicken feed, respectively, were collect ed from 43 breeder farms. Altogether 1888, 2327 and 1273 samples of cloacal swabs, frozen chicken meat and RTE chicken products correspondingly were collected from 22 slaughterhouses and 22 RTE processing plants.

Transportation of the Samples: Samples were transported to the laboratory immediately after collection in a portable cooler at 3±2°C and microbiological analysis was carried out upon arrival or within 24 hours.

Testing, isolation and confirmation procedures for L. monocytogenes: For food and soiled litter samples, 25-g portions were mixed in stomacher bags with 225 ml of half-Fraser broth (Oxoid Co. Ltd., Basingstoke, UK) and stomached in a Seward model 400 stomacher (Seward, Bohemia, NY) for 2 min at 220 rpm. For swab samples, half-Fraser broth was added to the swabs at the ratio of 9:1 (weight by weight) and manually mixed by carefully squeezing of swabs for several seconds by hand after addition of half-Fraser broth. For water samples, 100 ml of water was added to 150 ml concentrated half-Fraser broth leading to the final concentration equal to single strength. All samples were incubated at 30°C for 24±3 hours. After the enrichment, the rest of the analytical steps were performed according to BAX® System PCR screening kit for L. monocytogenes (BAX® System) and ISO 11290-1 (ISO, 2004). The selective plating agar used were Listeria Ottaviani Agosti Agar (ALOA) (AES Laboratoire, Combourg, France) and PALCAM (Oxoid Co. Ltd., Basingstoke, UK) and incubated at 37±1°C for 24 to 48 hours. For each selective agar up to 5 colonies with phenotypic characteristics typical for L. monocytogenes were subcultured onto horse blood bi-layer Columbia agar (Oxoid Co. Ltd., Basingstoke, UK) and then incubated at 37±1°C for 24±3 hours. Weakly beta-hemolytic colonies were then subcultured onto trypticase soy agar (Oxoid Co. Ltd., Basingstoke, UK) with 5% sheep’s blood and then incubated for at 37±1°C for 24±3 hours. The identification to species level based on Rocourt et al. (cited by Junntilla et al., 1988) including CAMP test, Gram staining, catalase reaction, motility test, and carbohydrate utilization was subsequently performed for phenotypic characterization and species confirmation.

Results
From this study 3059, 1071, 5052, 4215 and 1273 samples taken from 43 breeder farms, 32 hatcheries, 1331 broiler farms, 22 chicken slaughterhouses and 22 chicken processing plants, respectively were analyzed for the presence of L.
monocytogenes. It was surprising that there was no L. monocytogenes isolated from the samples taken from farms and hatcheries and from 1888 samples of cloacal swabs collected from chickens entering slaughterhouses, whereas 59 (2.5%) samples of frozen chicken meat and 2 (0.2%) samples of RTE chicken products were found to be contaminated with L. monocytogenes. The study showed that there was no correlation of the L. monocytogenes contamination in the samples of frozen chicken meat and RTE chicken products. L. monocytogenes was isolate from 2.0%, 5.2%, 1.4%, 1.2%, 0% and 2.1% of frozen chicken meat samples in year 2004, 2005, 2006, 2007, 2008 and 2009 respectively but only the samples of RTE chicken products collected in year 2004 and 2006 were found contaminated with the organism at the rate of 0.6% and 0.2%. The prevalence of L. monocytogenes in the samples analyzed is shown in Table 1 and Figure 1.

Figure 1: Results of the study on the prevalence of Listeria monocytogenes at slaughter house and processing plants.

Table 1: Results of the study on the Prevalence of Listeria monocytogenes at each stage of chicken production chain.

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Discussion

It can be concluded from this study that *L. monocytogenes* contaminated in frozen chicken meat and RTE chicken products did not come from the primary production stages. These findings are in agreement with the study of Fenlon et al. (1996), who found *L. monocytogenes* in both frozen and chilled birds tested immediately after processing, but did not find in poultry faeces.

It is not surprising that chicken feeds were not found to be contaminated with *L. monocytogenes* because the feed used for rearing chickens had been heat treated and pelleted. According to the Feed Quality Control Act of Thailand, the feed must be free from *Salmonella* contamination (Department of Livestock Development, 1999). The manufacturing temperature that kills *Salmonella* can also kill *L. monocytogenes*. To comply with Thailand standard farm regulation, drinking water for rearing chickens should have the same quality as potable water (Department of Livestock Development, 1999). Therefore, it should not contain any pathogens. In some farms, water used for cleaning equipment and for rearing chickens are from different sources so water from both sources were collected separately. However, there was no *L. monocytogenes* detected in either types of water. It is interesting to find that *L. monocytogenes* was not isolated from soiled litter collected from both breeder and broiler farms. This may be the reason why *L. monocytogenes* was not isolated from chicken cloacal swabs taken at the slaughterhouses.

At slaughterhouses, *L. monocytogenes* was not detected from the cloacal swabs taken from the chickens entering slaughterhouses, but was detected from 59 (2.5%) out of 2,327 samples of frozen chicken meat. This demonstrates that *L. monocytogenes* isolates did not originate from the chickens entering slaughterhouses but must have been due to contamination arising from workers, equipment and/or the processing plants environments. Two out of 1273 (0.2%) samples of frozen RTE chicken products were found positive for *L. monocytogenes*. The contamination source might come from raw chicken meat, other raw materials, contaminated equipment and utensils, workers and/or plants environments. Kerr et al. (1993) found that 7% of food workers carried *L. monocytogenes* in their study and demonstrated the prolonged survival of *L. monocytogenes* on the hands of volunteers. Though both the slaughterhouses and processing plants had HACCP in place, HACCP CCPs could not prevent cross contamination of chicken meat and RTE products with *L. monocytogenes*. Several studies have indicated that the control of *L. monocytogenes* must be directed toward preventing its establishment and growth in the environment and that the control through HACCP CCPs is impractical. A prerequisite program together with GMPs, sanitation and training toward specific control of *L. monocytogenes* are necessary tools for the control of *L. monocytogenes* (Tompkin et al., 1999; WHO, 2004).

Investigations conducted in the slaughterhouses and the processing plants, from which *L. monocytogenes* was isolated from the samples, revealed that the floor drains of those establishments were contaminated with *L. monocytogenes*, which is consistent with observations that *L. monocytogenes* from environmental sources has been identified as the main source of *L. monocytogenes* contamination of RTE products in the processing plants (Tomkin et al., 1999; Sauders et al., 2009). The elimination of the entry of contamination into the processing environment, is therefore, an important preventive measure as after the organism enters the plants, widespread distribution can easily occur during a production run (Kerr et al., 1993).

It is necessary for slaughterhouses and processing plants to establish a specific program to monitor *L. monocytogenes* or *Listeria* spp. to identify potential sources of contamination, so that specific cleaning and disinfecting procedures can be applied in the event that *L. monocytogenes* or *Listeria* spp. are detected in order to prevent spreading of the organisms to the products and to prevent the buildup of contamination on mechanical equipment as well as recontamination after heat process. Equipment where meat (food) debris can accumulate is a potential source of cross-contamination between carcasses and the food strains established in the processing environment is a significant possible cause of contamination and a more probable cause of widespread contamination compared to sporadic contamination of primary production origin (Fenlon et al., 1996). Fenlon et al. (1996) emphasized that processing is a major hazard of cross-contamination and amplification of the level of *L. monocytogenes* contamination, underlining the importance of good hygiene, good sanitation and GMPs in the food producing industry. Hand washing can eliminate *L. monocytogenes*; however, hand washing is not successful if large numbers of bacteria are present. Therefore, a suitable hand washing technique for food workers is essential to prevent *L. monocytogenes* cross-contamination (Kerr et al., 1993).

In summary, the study showed that there was no *L. monocytogenes* contamination in the chicken primary production stages while the organism was isolated from the samples taken from slaughterhouses and RTE processing plants. This implies that *L. monocytogenes* contamination in slaughterhouses and processing plants did not originate from primary production stages. It could be assumed that the observed *L. monocytogenes* contamination in 59 (2.5%) and 2 (0.2%) samples of fresh frozen chicken meat and RTE chicken products, respectively, might be due to breakdowns in the application of good hygienic and/or good manufacturing practices. If the assumption was correct, then there was a need to improve hygiene and manufacturing practices. Moreover, disinfection programs should be addressed more accurate cleaning practices and continuous education of slaughterhouses and RTE processing plant workers toward specific control of *L. monocytogenes* is also necessary.
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