

## Prevalence of *Listeria monocytogenes* in Chicken Production Chain in Thailand

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### *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.

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

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## บทคัดย่อ

### ความชุกโรคของเชื้อ *ลิสเตอร์เรีย โมโนซัยโตจีเนส* ตลอดห่วงโซ่การผลิตเนื้อไก่ และผลิตภัณฑ์ของประเทศไทย

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การศึกษาความชุกโรคของ *ลิสเตอร์เรีย โมโนซัยโตจีเนส* ตลอดห่วงโซ่การผลิตเนื้อไก่ และผลิตภัณฑ์ของประเทศไทย ตั้งแต่ปี พ.ศ. 2547-2552 โดยเก็บตัวอย่างจำนวน 14,670 ตัวอย่างจากฟาร์มพ่อแม่พันธุ์ 43 ฟาร์ม โรงฟักไข่ 32 โรงฟัก ฟาร์มไก่กระທ 1331 ฟาร์ม โรงฆ่าสัตว์ปีก 22 โรงงาน และโรงงานแปรรูปเนื้อไก่ 22 โรงงาน ตัวอย่างที่ใช้ในการศึกษา ได้แก่ วัสดุรองพื้น อาหารไก่ น้ำกิน และน้ำใช้ในฟาร์มพ่อแม่พันธุ์ และฟาร์มไก่กระທ ตัวอย่างสาส์ป้ายเชื้อจากถาดไข่ฟัก และมูลของลูกไก่ที่ฟักออกใหม่ในโรงฟักไข่ ตัวอย่างสาส์ป้ายเชื้อจากกันไก่ก่อนฆ่า และเนื้อไก่แช่แข็งจากโรงฆ่าสัตว์ปีก และตัวอย่างผลิตภัณฑ์เนื้อไก่สำเร็จรูปพร้อมบริโภคจากโรงงานแปรรูปเนื้อไก่ ผลการศึกษาค้นนี้ ไม่พบการปนเปื้อนเชื้อ *ลิสเตอร์เรีย โมโนซัยโตจีเนส* ในตัวอย่างที่เก็บจากฟาร์ม และโรงฟักไข่ แต่สามารถแยกเชื้อดังกล่าวจากตัวอย่างเนื้อไก่ และผลิตภัณฑ์เนื้อไก่ จำนวน 59 (ร้อยละ 2.5) และ 2 (ร้อยละ 0.2) ตัวอย่าง ตามลำดับ บ่งชี้ว่าไก่ไม่ได้นำเข้าสู่โรงฆ่าสัตว์ปีก ซึ่งเป็นการชี้ว่าการปฏิบัติในขั้นตอนการผลิตไก่ชั้นต้น ไม่ใช้สาเหตุการปนเปื้อนของเชื้อในเนื้อไก่และผลิตภัณฑ์เนื้อไก่พร้อมบริโภค แต่การปนเปื้อนเชื้อ *ลิสเตอร์เรีย โมโนซัยโตจีเนส* อาจเกิดจากความบกพร่องในการจัดการสุขอนามัย และสุขาภิบาลที่ดีภายในโรงงาน และ/ หรือ ขาดขั้นตอนการผลิตที่ดี

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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 Abraham, 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; Joseph et al., 2009). It is capable of growing during refrigerated storage (Junttila et al., 1988; Farber et al., 1989; Hudson et al., 1994; Panagou, 2008; Aurora et al., 2009; Giannou et al., 2009; Karakolev, 2009). *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 Abraham, 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

(Moretro and Langsrud, 2004; Pappelbaum et al., 2008). *L. monocytogenes* is commonly isolated from various types of processed meats. It can survive in dry sausage and grow well in high pH cooked meat and poultry products (cited by Samelis and Metaxopoulos, 1999). Post processing contamination of RTE food products with *L. monocytogenes* from environmental sources has been identified as the main source of *L. monocytogenes* contamination of RTE products in processing plants (Tompkin et al., 1999; Yvonne and Wiedmann, 2009). The results of numerous studies suggest that *L. monocytogenes* is unlikely to be completely eradicated from the food processing environment but it can be controlled effectively through good hygiene and good manufacturing practices (Tompkin et al., 1999; WHO, 2004; Lekroengsin et al., 2007; Keeratipibul and Ledroengsin, 2008; Keeratipibul and Ledroengsin, 2009; Kushwaha and Muriana, 2009). Thailand is one of the leading exporters of poultry meat products. The export in 2009 was worth 52,734 million baht. *L. monocytogenes* has been one of the causes responsible for product non-compliances (Anon, 2010). In Thailand, most of the studies on *L. monocytogenes* contamination have been conducted only in food processing plants but not in primary production stages. Therefore, the objectives of the present study were to investigate the prevalence of *L. monocytogenes* at each stage of the poultry production chain in Thailand and to identify the source of *L. monocytogenes* contamination in primary production stages.

### Materials and Methods

**Collection of Samples:** A total of 14,670 samples were randomly collected on a monthly basis from 43 chicken breeder farms, 32 hatcheries, 1331 broiler farms, 22 chicken slaughterhouses and 22 RTE chicken products processing plants from January 2004 to December 2009.

**Samples collected at breeder and broiler farms:** Litter soiled with fecal drops (soiled litter) were collected from the floor of chicken houses of each farm by taking from 60 positions per house and pooled in to one sample. Collection of soiled litter samples at broiler farm was undertaken when the chickens were 30 days of age. Simultaneously, a sample of 100 ml of the animals' drinking water for chicken, a sample of 100 ml of water for cleaning equipment used in the farm and a sample of 100 g of chicken feed were collected from each farm. Altogether 998, 1192 and 869 samples of soiled litter, water and chicken feed, respectively, were collected from 43 breeder farms and 1506, 2200 and 1346 samples of soiled litter, water and chicken feed, respectively, were from 1331 broiler farms.

**Samples collected at hatcheries:** Incubator trays of each incubator and equipment used in each of the 32 hatcheries were swabbed with sterile gauze moisturized with half-Fraser broth (Oxoid Co. Ltd., Basingstoke, UK) and pooled in to one sample. Meconium from 10 day-old chicks were collected and

pooled in to one sample. A total of 548 and 523 samples of swabs and meconium respectively were collected from 32 hatcheries.

**Samples collected at chicken slaughterhouses and ready-to-eat (RTE) chicken processing plants:** At each slaughterhouse a pooled sample of cloacal swabs taken from 60 chickens from each farm (at entry to the slaughterhouses) and 5 samples of frozen chicken meat were collected. Five samples of frozen RTE products were collected from each RTE chicken processing plant. 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  $3\pm 2^{\circ}\text{C}$  and microbiological analysis was carried out upon arrival or within 24 hours.

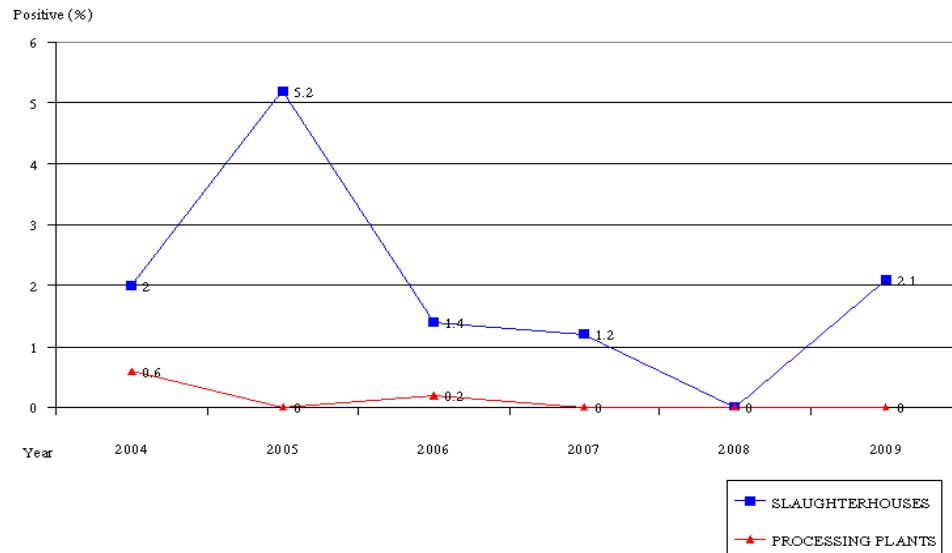
**Testing, isolation and confirmation procedures for *L. monocytogenes*:** For food and soiled litter samples, 25-g portions were mixed in stomacher bags with 225 ml of half-Fraser broth (Oxoid Co. Ltd., Basingstoke, UK) and stomached in a Seward model 400 stomacher (Seward, Bohemia, NY) for 2 min at 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  $30^{\circ}\text{C}$  for  $24\pm 3$  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 agars used were *Listeria* Ottaviani Agosti Agar (ALOA) (AES Laboratoire, Combourg, France) and PALCAM (Oxoid Co. Ltd., Basingstoke, UK) and incubated at  $37\pm 1^{\circ}\text{C}$  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  $37\pm 1^{\circ}\text{C}$  for  $24\pm 3$  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 at  $37\pm 1^{\circ}\text{C}$  for  $24\pm 3$  hours. The identification to species level based on Rocourt et al. (cited by Junttila 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**

Location	2004		2005		2006		2007		2008		2009		2004 - 2009	
	Total no.	Positive (%)	Total no.	Positive (%)	Total no.	Positive (%)	Total no.	Positive (%)	Total no.	Positive (%)	Total no.	Positive (%)	Total no.	Positive (%)
<b>BREEDER FARMS</b>														
No. of farms	32	0	43	0	42	0	21	0	7	0	20	0	43	0
Soiled litter	112	0	252	0	416	0	112	0	7	0	99	0	998	0
Water for drinking	105	0	240	0	350	0	104	0	7	0	89	0	895	0
Water for cleaning	41	0	74	0	131	0	28	0	1	0	22	0	297	0
Feed	108	0	236	0	329	0	99	0	7	0	90	0	869	0
<b>HATCHERIES</b>														
No. of hatcheries	21	0	32	0	29	0	12	0	7	0	12	0	32	0
Swabs	73	0	150	0	198	0	62	0	7	0	58	0	548	0
Meconium	71	0	143	0	186	0	60	0	7	0	56	0	523	0
<b>BROILER FARMS</b>														
No. of farms	183	0	363	0	500	0	154	0	10	0	121	0	1331	0
Soiled litter	207	0	404	0	594	0	158	0	10	0	133	0	1506	0
Water for drinking	205	0	405	0	592	0	158	0	10	0	133	0	1503	0
Water for cleaning	107	0	193	0	340	0	0	0	1	0	56	0	697	0
Feed	207	0	406	0	590	0	0	0	10	0	133	0	1346	0
<b>SLAUGHTERHOUSES</b>														
No. of establishments	22	0	22	0	22	0	22	0	7	0	22	0	22	0
Cloacal swabs	227	0	495	0	731	0	215	0	24	0	196	0	1888	0
Frozen chicken meat	304	6 (2.0%)	632	33 (5.2%)	870	12 (1.4%)	258	3 (1.2%)	26	0	237	5 (2.1%)	2327	59 (2.5%)
<b>PROCESSING PLANTS</b>														
No. of plants	22	0	22	0	22	0	10	0	7	0	22	0	22	0
RTE chicken product	158	1 (0.6%)	355	0	515	1 (0.2%)	47	0	20	0	178	0	1273	2 (0.2%)

### 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., 1980). The manufacturing temperature that kills *Salmonellae* 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 (Tompkin 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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