

Listeria monocytogenes in Chicken Meat

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Abstract *Listeria monocytogenes* is the causative agent of listeriosis, an infection that gives rise to bacteremia and meningitis that can be propagated to humans via food contamination. The chicken-meat and derivatives processing industries are common sites of this pathogen, and the great challenge is in controlling this hazard to avoid economic and public health losses. A literature review on *L. monocytogenes* and implications to the chicken supply chain, poultry slaughterhouses, and public health was conducted. The review was compiled with the main papers published around the world in the last 15 years containing the key words *Listeria monocytogenes*, *poultry*, *meat*, *chicken*, *broilers*, and *listeriosis*, using the main publishers of online journals. The collected information was discussed and it was concluded that poultry can be asymptomatic carriers of *L. monocytogenes* and introduce contamination in slaughterhouses, which can become a persistent problem in poultry slaughterhouses due to its capacity to form biofilms on many different materials, causing cross-contamination in chicken meat and its derivatives. Carcasses, cuts, or giblets of chilled or frozen chicken *in natura* are sources of contamination by *L. monocytogenes* and can transmit listeriosis to humans.

Keywords: *poultry*, *aviculture*, *listeriosis*, *slaughterhouses*, *biofilms*

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1. Introduction

Listeria monocytogenes is the causative agent of listeriosis, a foodborne disease that can lead to bacteremia and meningitis. Manifestation of symptoms can occur after ingestion of foods contaminated by 100 to 1000 cells [1], affecting mainly elderly people, newborns, pregnant women, and immunocompromised individuals [2].

According to the Centers for Disease Control and Prevention [3], approximately 48 million people fall ill every year after consuming contaminated food, generating an index of 128,000 hospitalizations and 3,000 deaths per year. Of this total, 1,600 are listeriosis cases, with a rate of 260 deaths.

Contaminated foods are the major source of transmission of this microorganism, whose presence has been reported in different foods such as raw and/or pasteurized milk, cheeses, chicken meat, fish, and processed meat products [4].

In the food industries, this microorganism can multiply within food grade lubricants, machinery, treadmills, countertops, utensils, floors, drains, sewers, rubbers, welds, wall cracks, lining, air filters, metal structures, employees' clothes, hoses, tools, sponges, brushes, or other sites where disinfection is not effective; additionally, it can grow at chilling temperature and form biofilms on the most diverse materials [5,6,7], which makes its elimination a difficult task in this industry, ultimately causing cross-contamination.

The chicken-meat and derivatives processing industries are common sites for this pathogen. The great challenge of poultry-processing companies is to control this hazard to avoid economic and public-health losses stemming from product recalls [8] or condemnation by the veterinary inspection service.

In the present study, a literature review was conducted on *L. monocytogenes* and the implications to the commercial poultry supply chain, poultry slaughterhouses, and public health. The review includes the main papers published worldwide in the last 15 years containing the key-words *Listeria monocytogenes*, *poultry*, *meat*, *chicken*, *broilers*, and *listeriosis* using the main publishers of online journals. The data were presented, interpreted and discussed, and conclusions were drawn on the subject.

2. *Listeria monocytogenes* and Pathogenicity

The genus *Listeria* comprises fifteen species and six subspecies; noteworthy examples are *Listeria welshimeri*, *L. seeligeri*, *L. monocytogenes*, *L. innocua*, *L. grayi*, and *L. ivanovii* [9,10,11,12].

L. monocytogenes was first described in 1924 by Murray, Webb, and Swam, who isolated a microorganism responsible for causing a clinical case of mononuclear leukocytosis in rabbits and named it *Bacterium monocytogenes* [13]. In 1927, Pirie isolated a similar organism from rodent livers, later naming it *Listerella hepatolytica*. The term *L. monocytogenes* was only coined in 1940 [14].

L. monocytogenes is a gram-positive, halotolerant, facultative anaerobic, non-spore forming, non-H₂S producing bacterium of bacillus morphology with a great ability to adapt to and survive in adverse environmental conditions, developing at temperatures ranging from 3 to 50°C, withstanding pH variations (between 4.3 and 9.4) and a water activity of 0.92 [15,16]. The biochemical classification of the *Listeria* species is catalase-positive, oxidase-negative, glucose-fermentative, which produces lactic acid and does not reduce nitrate to nitrite [17]. For differentiation between species, carbohydrate-fermentation tests are applied, e.g. dextrose, rhamnose, mannitol, and xylose. Beta-hemolysis production in blood agar is also a widely adopted differentiation proof protocol [18] (Table 1).

After enduring the physiological barriers of digestion, in the intestine, the bacterium reaches the bloodstream of the host and has a typical facultative intracellular action, capable of proliferating in macrophages and in a wide variety of normally non-phagocytic cells, such as epithelial and endothelial cells, as well as in hepatocytes. In all these cell types, *L. monocytogenes* develops a characteristic life cycle, with the following stages: (I) early escape from the phagocytic vacuole (II), multiplication in the cytoplasm of the host cell, (III) motility by polymerization of the actin tail, (IV) pseudopod protrusion for bacterial movement, and (V) phagocytosis of the pseudopod and start of a new cycle [21].

Table 1. Biochemical characteristics of species of the genus *Listeria*

		<i>L. monocytogenes</i>	<i>L. innocua</i>	<i>L. ivanovii</i>	<i>L. seeligeri</i>	<i>L. welshimeri</i>	<i>L. grayi</i>
β- hemolysis		+	-	+	+	-	-
Catalase		+	+	+	+	+	+
Oxidase		-	-	-	-	-	-
Carbohydrate fermentation	Rhamnose	+	+/-	-	-	+/-	+/-
	Mannitol	-	-	-	-	-	+
	Xylose	-	-	+	+	+	-
	Dextrose	+	+	+	+	+	+

Source: Adapted from [19,20].

The invasion occurs by the binding of two proteins located on the surface of the microorganism to receptors located on the surface of the host cell. These proteins are called Internalin A and Internalin B. The former has E-cadherin, a glycoprotein, as its host cell receptor, whereas Internalin B has Met, a protein of the tyrosine kinase family, as the cell receptor [22,23]. The bacterium is then phagocytosed by the macrophages and shielded from damage within this cell due to the production of catalase and superoxide dismutase enzymes, which will protect it from the oxidative effects of the phagocyte [24]. To escape the phagocyte, *L. monocytogenes* performs lysis of the membrane through the action of listeriolysin O (LLO), a β-hemolysin that forms pores on the phagocytic membrane, allowing the phospholipases to degrade the phospholipids of the membrane [25].

After escaping the phagosome, the bacteria are surrounded by actin monomers, which rearrange themselves and reach one of the poles of the microorganism. This action is coordinated by a surface protein named ActA, that enables the association of the actin filaments in the cytoplasm of the host cell, thereby polymerizing the tail and allowing for intra- and intercellular bacterial movement [26,27]. When this tail reaches the cell membrane, it is then pushed to the outside and penetrates the adjacent cell, forming a double-layered vacuole. After about 5 min, the bacterium escapes into the intracellular space, beginning a new invasion cycle [28,29].

3. *Listeria monocytogenes* and Poultry Health

Listeriosis in birds was first reported in 1935, three years after its isolation from sick chickens [30]. The infection affects wild and domesticated birds such as ducks, geese, pheasants, canaries, and cockatiels, which

can be asymptomatic carriers of the agent and participate in the propagation of the pathogen in the environment [31,32]. Dissemination occurs through infected birds, by nasal and fecal secretions; however, total elimination of this pathogen does not occur in the eggs [31].

Although many poultry species are affected, the clinical disease is rare, and occasionally described [33]. Studies in Nairobi, Kenya, using samples from *swabs* of the cloaca and oropharynx, demonstrated the presence of 22.2% of positive poultry for *L. monocytogenes* [34], similar results were also found in Brazil, where 2.9 % of cloacal *swabs* samples were positive for this organism [32]. It is important to note that in both studies, surveyed poultry presented no clinical symptoms of listeriosis, these results further support the hypothesis that poultry's can be asymptomatic carriers of that pathogen and thus contribute to contamination of the production environment and processing [34].

Young birds are usually more susceptible and likely to develop listeriosis, which may assume the forms of either septicemic listeriosis or encephalitis [31]. However, listeriosis in chickens in the septicemic form occurs sporadically, presenting acute or chronic evolution. Because *L. monocytogenes* is an opportunist pathogen, the disease may appear secondarily to parasitosis [35].

Chronic infection may induce injuries in the heart, liver, and, rarely, in the brain, if it affects the central nervous system. Symptoms may include torticollis, blindness, tremors, stupor, and paresis [36]. In *post-mortem* examinations, birds affected with the septicemic form showed degeneration and necrosis of the myocardium and liver, as well as a case of serofibrinous pericarditis [31,36].

In its encephalic form, histopathological examinations indicate brain injuries such as the formation of microabscesses and extensive thrombosis, associated with areas of necrosis. When injured tissues are subjected to Gram staining, typical gram-positive bacteria can be

observed. Immunohistochemistry, PCR, or isolation and biochemical identification of the microorganisms can be performed to confirm the diagnosis. Differential diagnoses may include diseases accompanying neurological and septicemic cases, such as colibacillosis, pasteurellosis, Marek's disease, and Newcastle disease [31].

4. *Listeria monocytogenes*, Aviculture, and Chicken Meat Processing

The poultry industry is consolidated as one of the most important sources of animal protein for the world's population. Data from the United States Department of Agriculture [37] corroborate this assertion, describing that, in 2014, the total production corresponded to 2.9 billion Euros. Meat production worldwide should increase by 1.6%. A projection for the year 2020 indicates that pork will no longer be the most widely produced meat in the world, and the population will thus likely be supplied by chicken meat, which has shown a significant evolution over the years. In this same period, there will also be an increment of 2.4 kg in meat consumption per habitant, which will consist mostly of chicken (around 72%) [38].

To meet the growing demand for chicken meat, food industries must ensure quality through their processes. In this regard, the concern regarding *L. monocytogenes* is present in industrial environments, since these places provide favorable conditions, like temperature and humidity, to its growth, especially on floors, drains, countertops, utensils, and equipment [5,39], and so the bacterium can remain for months, or even years in the industrial environment, leading to recurrent contamination of foods [40].

The persistence of the bacterium in the industry is a result of the ability of *L. monocytogenes* to form biofilms on many different materials in response to adverse

situations [7,41], making its elimination in the slaughterhouse a difficult task, with the consequent possibility of food cross-contamination. Biofilm formation as an important survival strategy of the bacterium against the action of detergents, disinfectants, and antimicrobials; however, biofilm formation in industrial food environments, just like its preventive measures, depends on the strain, the location where it was isolated, the levels of nutrients present in the medium, the temperature of the environment, and the hydrophobicity of adhesion surfaces and cells [42].

Cross-contamination may take place during all stages of chicken meat processing [32,43], mainly during the pre-chilling of carcasses, when water and ice become important contamination carriers, in addition to being an environment that allows the multiplication of psychrotrophic microorganisms if no strict control is carried out [44].

Although *L. monocytogenes* can be present in meat packing plants and companies endeavor to reduce this hazard, the search for the bacterium is not mandatorily required in *in natura* chilled or frozen chicken meat in several countries, with this procedure being applied only for ready-to-consume chicken products. This is probably hampering the generation of data about the prevalence of the bacterium in *in natura* meats, as well as the association between listeriosis cases in humans and chicken consumption, since there has been only one reported case of listeriosis attributed to the consumption of chilled chicken meat in a supermarket in the United Kingdom, involving a pregnant woman [45].

The occurrence of *L. monocytogenes* in chicken and poultry has been reported in the Spain [47], Brazil [32,48], Italy [49], Jordan [50], Iran [51], Malaysia [52], and Chile [43]. In these studies, the occurrence of this bacterium in chicken products *in natura* ranged from 2,9 to 38% of the evaluated samples (Table 2).

Table 2. Reported of *L. monocytogenes* in chicken and poultry

Year	Country	Occurrence	Vehicle	Reference
2001	Spain	32% (32/100)	fresh chicken carcasses	[47]
2002	France	38% (100/263)	swabbing samples in the environment or on the equipment during activity in poultry plants	[53]
2004	Finland, Iceland, Norway, and Sweden	20.6% to 24.1% 22.2%	poultryplants poultry	[54]
2005	Brazil	14,3% (3/21) 11,8% (4/37) 2,9% (1/35)	Carcass at packaging Hands and gloves cloacalswabs	[48]
2009	Brazil	11,7% (15/128) 33,3% (15/45)	abattoirsamples chilled chicken from retailers	[32]
2010	Italy	24,5 % (13/53)	freshchicken carcasses	[49]
2011	Jordan	18 % (45/280)	raw chicken and raw chicken and ready-to-eat	[50]
2012	Iran	7% (7/100)	cloacalsamples	[51]
2013	Malaysia	26,39% (57/216)	chickenoffalsamples	[52]
2015	Chile	19% (223/1196)	poultry	[43]

The frequency of *L. monocytogenes* has been estimated in slaughterhouses in France [53], Finland, Iceland, Norway, and Sweden [54], and Brazil [44,48] with frequency ranging between 11.7 and 38.9% of the monitored slaughterhouses.

The occurrence of *L. monocytogenes* in different food matrices was also studied in Chile between the years 2008 and 2012. On the occasion, *L. monocytogenes* was isolated from 10% of a total of 2647 samples, with greatest occurrence in beef (23%) and poultry (19%) [43]. This significant

presence of *L. monocytogenes* in raw chicken meat and derivatives evidences the possibility of cross-contaminations during the preparation of ready-to-eat foods.

5. Diagnostic Methods

The detection of *L. monocytogenes* in food becomes difficult due to the presence of competitive microflora,

interferences by food inhibitor compounds and low levels counts of this pathogen [55]. In an attempt to solve this, new advances have been made in recent years, such as the development of selective culture media, and the development of new serological and molecular methods [20].

The main regulatory agencies require that isolation methods must be able to detect *Listeria* spp. in 25g of food. Therefore, the use of antimicrobial agents are necessary in order to inhibit microbiota growth, such as other Gram-positive and Gram-negative bacteria and fungi [56]. According to the nature of the sample, a certain method may be more appropriate than others. From the available methods, the most used are the ISO (International Organization for Standardization) 11290 method, from the Bacterial Analytical Manual (BAM), recommended by the Food and Drug Administration (FDA) for *Listeria* spp. isolation from dairy products, seafood and vegetables, and the United States Department of Agriculture (USDA / FSIS) method proposed for meat, poultry, egg and environmental samples. These methods are based on an enrichment step, followed by selective plating and identification/differentiation of the isolated microorganism. Differentiation of *Listeria* species is carried out by means of biochemical and phenotypic characteristics observed in sugar fermentation and hemolysis production tests [57].

Another alternative for the diagnosis of *L. monocytogenes* are immunological assays. These tests are based on specific *Listeria* spp. Antibodies, obtained as commercial kits. However, few are specific for the identification of *L. monocytogenes* [56]. The immunoassay enzyme (Enzyme-linked immunosorbent assay - ELISA) is an antigen-antibody reaction widely used for the detection of foodborne pathogens. This method produces rapid results (30-50 hours) and is applicable for use in more complex food matrices [58]. These tests target the specific identification of *L. monocytogenes* using monoclonal antibodies, which recognize the p60 protein, an active protein in cell invasion [59]. The disadvantage of this method is its low sensitivity, of approximately 10^5 - 10^6 CFU/mL. In order to meet this disadvantage, PCR (Polymerase chain reaction by the action of polymerase) has been widely used, as it is a sensitive and specific method, able to detect up to one cell of this microorganism [60]. In recent years a large number of tests based on this technique have been described for the detection of *L. monocytogenes* in foods [61,62,63]. Developed reactions seek to identify pathogens that target virulence factors, and the most used are *hly*, *iap*, *inlAB* and the p60 protein [64,65].

6. Preventive Measures

Efficient prevention against *Listeria monocytogenes* in foods should focus on the risks of the production chain in a holistic manner, from production to the end consumer [66]. To this end, it is paramount to obtain knowledge regarding the dispersion of this pathogen in food-processing plants and in residences of a given country so action strategies can be developed, given that the use of data from other regions may lead to the elaboration of ineffective control plants. The combination of assorted

measures can minimize the entry of *L. monocytogenes* in an industrial environment, e.g. effective self-control plans, sterilization of packaging material, and effective sanitation of the environment [67].

The best way to avoid contamination would be to prevent biofilm formation in food industries by frequently disinfecting and cleaning surfaces [42]. This procedure would be sufficient to remove cells not yet strongly adhered, although it may fail and not remove mature biofilms. To fulfill this purpose, the use of appropriate disinfectants is essential, and other strategies have been tested with this aim. Some examples are the use of ozone or acidic water, usually considered eco-friendly biocides, as they do not leave chemical residuals [68]. Besides these products, natural compounds extracted from bacteria or aromatic plants cultures and some GRAS (generally recognized as safe) ingredients have also been evaluated to eradicate biofilms [69,70].

As a means to prevent foodborne listeriosis, good food-manufacturing practices must be adopted, so as to avoid cross-contamination to ready-made foods when contact with chicken meat, cuts, or giblets is involved.

7. Conclusions

Poultry may be asymptomatic carriers of *L. monocytogenes* and introduce contamination in slaughterhouses.

L. monocytogenes can become a persistent problem in poultry slaughterhouses due to its ability to form biofilms on several different materials, causing cross-contamination to chicken meat and its derivatives.

Chilled or frozen chicken carcasses, cuts, or giblets are sources of contamination of *L. monocytogenes* and can transmit listeriosis to humans.

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Author Contributions

All authors actively participated in all construction stages of this manuscript.

Conflict of Interest Statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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