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Review

Pathogen, host and environmental factors contributing to the pathogenesis of listeriosis

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Abstract. Listeriosis is a severe human and animal disease caused by two species of pathogenic bacteria from the genus *Listeria*, *L. monocytogenes* and *L. ivanovii.* In humans, listeriosis is overwhelmingly a foodborne disease, yet much remains to be learned regarding the transmission dynamics of pathogenic *Listeria* from the environment, through food, to humans. Similarly, our understanding of the various host, pathogen and environmental factors that impact the pathogenesis of listeriosis at the cellular and molecular level is incomplete. This review will summarize what is currently known about animal and human listeriosis, detail the pathogen, host and environmental factors that contribute to pathogenesis and, finally, examine the interactions among those factors that influence the occurrence of human infection.

Key words. Listeriosis; *Listeria monocytogenes*; *Listeria ivanovii*; foodborne disease; pathogenesis; virulence.

Introduction

Listeriosis is an animal and foodborne human disease that is caused by pathogenic bacteria of the genus *Listeria.* While there are seven species within the genus *Listeria* (*L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. grayii* and *L. murrayi*), only two, *L. monocytogenes* and *L. ivanovii*, are pathogenic, the former causing disease in both humans and animals, and the latter causing disease predominantly in sheep. Human listeriosis is a significant health threat primarily to immunosuppressed individuals and is overwhelmingly a foodborne disease; Mead et al. estimated that 99% of all human listeriosis cases are caused by consumption of contaminated food products [1]. *Listeria* species are Gram-positive, non-spore-forming rods that are common in the environment and can grow over a wide range of pH's (4.3–9.6), temperatures (1–45 °C) and salt concentrations (up to 10%) [2, 3]. This ability to survive and multiply under conditions frequently used for food preservation makes *Listeria* particularly problematic to the food industry; *L. monocytogenes* is a common food contaminant and a major cause of food recalls due to bacterial contamination, particularly in developed countries and possibly worldwide [4]. The frequent occurrence of *L. monocytogenes* in food, coupled with a high mortality rate of 20–30% in those developing listeriosis, make *L. monocytogenes* and listeriosis serious public health concerns [1, 4]. Listeriosis in animals, furthermore, represents not only a financial burden for the livestock industry but also a possible link between *Listeria* in the environment and human disease. While the connections between the presence of pathogenic *Listeria* in animals, the environment, foods and humans are not clear, the transmission and pathogenesis of listeriosis remain dependent on the ability of *Listeria* to survive in each of these environments. This review will summarize what is currently known about animal and human listeriosis, detail the pathogen, host and environmental factors that contribute to pathogenesis and, finally, examine the interactions

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among those factors that influence the occurrence of human infection.

Animal listeriosis

L. monocytogenes can infect a wide variety of animal species, including mammals and birds, and has also been isolated from fish and crustaceans [5, 6]. The animals most commonly infected, however, include ruminants such as cattle, sheep and goats [6, 7]. Adult swine are easily infected but rarely develop disease, and birds are often asymtomatic carriers [6, 8, 9]. It is estimated that between 80 and 90% of reported animal listeriosis cases in North America occur in cattle, while most remaining cases occur in sheep [6, 8–10]. Several reviews describe the pathogenesis, epidemiology and clinics of animal listeriosis in detail [5, 6, 11].

Listeriosis in animals can manifest in an encephalitic or in a septicemic/abortive form. Although the pathogenesis of encephalitic listeriosis in animals is still controversial [12, 13], there is some evidence that *L. monocytogenes* can enter nerve endings through abrasions in the oral cavity, lips, nostrils, conjuctiva or teeth of sheep, goats and mice, and then migrate centripetally to the brain stem to cause inflammation and central nervous system infection [6, 7, 12]. The incubation period for encephalitic listeriosis is usually 2–3 weeks, and the course of the disease is short: approximately 1–4 days. Symptoms of encephalitic listeriosis include depression, anorexia, decreased milk production and a transient fever that may be followed by incoordination, hemiparesis and circling [7]. Other evidence argues that encephalitic listeriosis develops from a hematogenous route of infection; this evidence includes the development of encephalitis in sheep following intracarotid inoculation and the location of brain lesions near capillaries [12]. In contrast, the route of entry for *Listeria* resulting in septicemic/abortive listeriosis is thought to be the intestinal mucosa following oral ingestion [7]. The incubation period can be as short as 1 day, and initial symptoms are similar to those for the encephalitic form: depression, anorexia, decreased milk production and fever. The animal may also exhibit bloody diarrhea. If a female is pregnant, bacteria may colonize the placenta and cause abortion [6]. Often, infected animals are able to limit a primary septicemic infection but become latent carriers, shedding *Listeria* in their feces and milk [6, 7].

Animal listeriosis is predominantly a feedborne disease, and the source of feed contamination is usually environmental [14, 15]. *Listeria* are common in soil, plants and decaying vegetation, and may persist in these materials to infect animals exposed to them. Feed also may be contaminated by wild mammals and birds that shed *Listeria* in their feces [7]. Latent carrier animals on a farm also may shed *Listeria* in their feces, thereby continually contaminating the farm environment. Thus, the farm may serve as a reservoir for *Listeria* [6]. Silage, however, is the most frequently attributed source of feedborne animal listeriosis infection [6, 16, 17]. Specifically, pathogenic *Listeria* naturally present on the plant material used for silage multiply when it is not properly fermented to a pH below ~4.5 [17]. Several outbreaks of listeriosis in animals have been attributed to the feeding of poor-quality silage [6, 17, 18].

There are several factors that may predispose animals to infection with pathogenic *Listeria.* It has been shown in sheep and goats that abrupt changes in feed, concurrent disease, changes in dentition, damage to the epithelial lining of the digestive tract, overcrowding, heavy rains or extreme cold, as well as the addition of new animals to a herd may predispose to *L. monocytogenes* infection [6]. Most likely, these predisposing factors act by weakening the animal's physical and immunological defenses. Physical damage to the mouth, teeth and gastrointestinal tract may make it easier for pathogenic *Listeria* to invade the body, while stress caused by inclement weather or changes in herd routine may weaken an animal's immune response. In addition, pregnancy, parturition, lactation and extended transportation are believed to lower resistance to listeriosis in cattle. Poor general management practices, poor nutrition and particularly the feeding of poor-quality silage may also predispose a farm animal to listeriosis [6].

The connections between animal and human listeriosis are not completely understood. There may be a risk of zoonotic transmission of listeriosis through infected materials such as feces, milk, birth fluids, placenta and fetuses, although such cases, which usually manifest as non-life-threatening cutaneous lesions, are rare [7, 19]. Foodborne transmission is the most common means of contracting listeriosis [1, 20]. While most food-processing procedures kill *Listeria* in raw foods, postprocessing contamination of final food products often occurs. Several studies using molecular subtyping methods to track *L. monocytogenes* in food production facilities have demonstrated that such contamination of final food products often comes from the processing environment [5, 21, 22]. Accordingly, the World Health Organization concluded that listeriosis is predominantly transmitted by non-zoonotic means and that the primary route of transmission to humans is food contaminated with *L. monocytogenes* during production [20].

Human listeriosis

Several excellent reviews describing the epidemiology and pathogenesis of listeriosis in humans are available [4, 12, 23–25]. In brief, listeriosis in humans is caused by *L. monocytogenes* and occurs predominantly in two

forms: as a mild noninvasive gastrointestinal illness or as an invasive disease. The noninvasive gastrointestinal form of listeriosis occurs in otherwise healthy adults, although the infectious dose as well as host and bacterial characteristics that limit the severity of this form remain unclear. Symptoms are typical of gastroenteritis and include fever, diarrhea and vomiting, and the mean incubation time before the appearance of symptoms is ~18–20 h. The frequency of noninvasive gastrointestinal listeriosis is difficult to establish conclusively because humans can be asymptomatic carriers [23]. Several recent outbreaks of gastrointestinal listeriosis in the United States and a large 1997 outbreak in Italy suggest an attack rate of up to 72% [26, 27].

In contrast, there are ~2500 cases of invasive listeriosis in the United States per year, and from 2 to 15 cases per million population in developed countries [1, 4]. Similar to listeriosis in animals, human invasive listeriosis can manifest itself as septicemia or as a neuropathic disease. Symptoms of septicemic listeriosis in humans are usually fever, malaise, fatigue and abdominal pain, while those of neuropathic listeriosis can include fever, malaise, ataxia, seizures, altered mental status, meningitis and encephalitis. Invasive listeriosis in humans most often occurs in adults with underlying immunosuppression. These include the elderly, pregnant women, cancer patients, organ transplant recipients, patients with acquired immunodeficiency syndrome (AIDS) or anyone receiving immunosuppressive therapy. Neonates are also at particular risk of acquiring invasive listeriosis from an infected mother. Listeriosis in neonates can occur as early or late onset [23]. Most neonatal listeriosis (between 45 and 70%) occurs as early onset [28, 29]. In this form, the fetus is infected in utero by transplacental transmission of bacteria from a septic mother. A surviving neonate shows clinical manifestations at birth or within the first week of life [23, 30]. In contrast, a neonate with late-onset listeriosis will become ill from one to several weeks after birth and will usually present with meningitis rather than sepsis [30, 31]. The route of transmission for late-onset neonatal listeriosis remains unclear; however, there is some evidence supporting nosocomial or environmental transmission [23]. In both early- and late-onset neonatal listeriosis, the mortality rate ranges from 20 to 30% [29, 32].

Treatment and prevention

Although most pathogenic *Listeria* strains are susceptible to antibiotics such as ampicillin, amoxicillin and penicillin G, treatment of listeriosis in both humans and animals is difficult and often unsuccessful [23, 33, 34]. Antibiotic treatment in animals is often initiated too late due to the frequent absence of clinical signs early in infection, misdiagnosis and rapid death following onset of clinical signs [13]. In adult humans, once a diagnosis of listeriosis is made by the isolation of *L. monocytogenes* from a normally sterile body site such as blood or cerebral spinal fluid, treatment usually consists of a 3–6-week course of ampicillin or penicillin [35]. Again, however, treatment is difficult, as patients may be slow to respond or the infection may persist despite therapy [23].

Due to the high failure rate of treatment, prevention of listeriosis remains the best means of reducing morbidity and mortality. Although the United States maintains a zero-tolerance policy, and many European countries have well-developed risk-based standards for the presence of *L. monocytogenes* in ready-to-eat foods, human illness due to listeriosis continues to occur, indicating that current preventative programs and policies can be further improved [25, 36, 37]. Changing food production practices, such as the increased centralization of food production facilities, as well as a growing population of at-risk individuals, such as the elderly and AIDS patients, make it likely that listeriosis will continue to be a major human health threat [38, 39]. To reduce the incidence of listeriosis, efforts must focus on controlling food contamination by pathogenic *Listeria* species at all stages of food production and processing, beginning with a reduction of pathogenic *Listeria* on the farm. Farm animal listeriosis can be reduced by minimizing known risk factors such as overcrowding and the feeding of poor-quality silage. Comprehensive and quantitative risk assessments, such as the draft assessment recently published by the United States Food and Drug Administration (FDA), provide a systematic approach to scientifically evaluating the human health threat from *L. monocytogenes*, reviewing the effectiveness of current programs and regulations, and identifying areas necessitating further research [40–42]. As recognized in the FDA risk assessment, the food-processing environment represents a key point of food contamination; therefore, concentrated efforts to control *L. monocytogenes* there must be made, beginning with good manufacturing practices, standard sanitation operating procedures and implementation of properly devised Hazard Analysis and Critical Control Point (HACCP) programs [40, 43]. The continued development of surveillance programs, such as the Center for Disease Control and Prevention's Foodborne Diseases Active Surveillance Network (FoodNet), that monitor the public health impact of *L. monocytogenes* also play a critical role in controlling this foodborne disease. The risk of human listeriosis can be further reduced by education programs aimed to teach those in high-risk groups to avoid consumption of foods most likely to be contaminated with *L. monocytogenes.* Finally, further research is needed to understand the persistence of *L. monocytogenes* in food-processing environments and elucidate the transmission dynamics of this pathogen from farm and food-processing environments to final food products [23, 40].

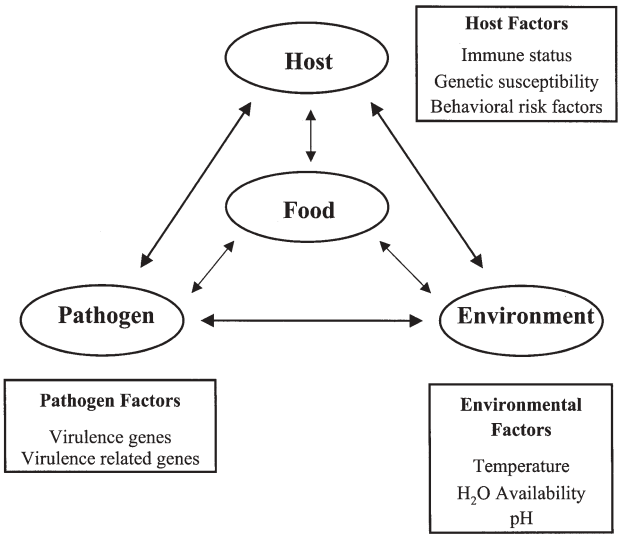

Figure 1. The central role of food in transmission of pathogenic *Listeria* and factors affecting the pathogenesis of listeriosis.

Pathogenesis of foodborne listeriosis

The pathogenesis of any foodborne disease depends on the ability of the pathogen to survive and be transmitted to humans from its animal or environmental origin through food. Thus, food plays a central role in the pathogenesis of a foodborne disease such as listeriosis, and inherent characteristics of the food such as pH and water activity, as well as variables such as how the food is processed, stored and prepared will affect the ability of a foodborne pathogen to cause disease. Additionally, a pathogen must interact with its environment, be that a food, natural or host environment, in such a manner as to ensure its survival and propagation. Factors specific to the pathogen, host and environment will influence each stage of the infectious process. Ultimately, however, it is the interactions of these factors that define the pathogenesis of a foodborne disease such as listeriosis most holistically (see fig. 1).

Pathogen factors

There are several pathogen-specific factors that influence the outcome of human and animal infection with a pathogenic *Listeria* strain. These can be grouped into the general categories of (i) virulence genes, or those genes that are essential for pathogenesis, and (ii) virulence-related genes, or those genes that are not essential for pathogenesis, but can enhance it. A virulence gene can be broadly defined according to 'molecular Koch's postulates'. These postulates require that true virulence genes fulfill the following criteria: first, the gene must be present in pathogenic strains and absent (or at least mutated or not expressed) in nonpathogenic strains; second, disrupting the gene should reduce its virulence and third, the gene should be expressed when the pathogen is in the host environment [44]. Virulence-related genes, in contrast, might be common to both pathogenic and nonpathogenic strains, redundant in function and expressed outside a host, but still assist in the infectious process. A comparison of relevant characteristics of *inlB* and *svpA*, which we have classified as virulence and virulence-related genes, respectively, may illustrate how these criteria can be applied. Although disrupting *inlB* has not always resulted in reduced virulence in currently available animal models, we have classified *inlB* as a virulence gene because (i) it is present in pathogenic *L. monocytogenes*, but absent in nonpathogenic *L. innocua* [45], (ii) has a clearly demonstrated role in internalization into human brain microvascular endothelial cells [46] and hepatocytes [47], arguing that it is required for virulence, and (iii) it is under the transcriptional control of positive regulatory factor A (PrfA), which specifically activates gene expression during infection [48, 49]. In contrast, although an SvpA-deficient strain was virulence attenuated in an intravenous (i.v.) mouse model of infection [50], it is not clear that the attenuation is not the result of a generalized growth defect brought about by an impaired stress response. The *svpA* gene is present in both *L. monocytogenes* and *L. innocua* (*lmo2185* and *lin2289*) [45], and while many *L. monocytogenes* virulence genes that are expressed during infection are regulated by PrfA, *svpA* transcription is PrfA independent [50]. This in conjunction with the generalized growth defect in an *svpA* mutant suggests that *svpA* is also expressed outside a host.

Virulence genes

L. monocytogenes and *L. ivanovii* have six virulence genes clustered on an 8.2-kb pathogenicity island (fig. 2 and table 1) [51]. Along with additional virulence genes, these genes function to carry out the intracellular infectious cycle, which occurs in the following stages: (i) internal-

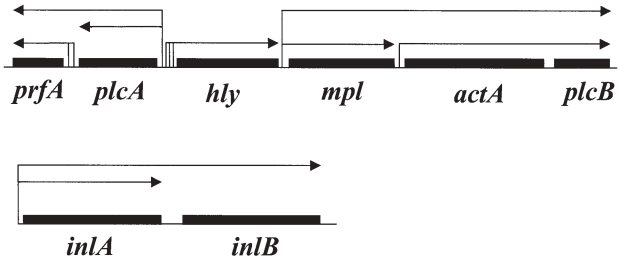

Figure 2. A schematic representation of the pathogenicity island of *L. monocytogenes* (not drawn to scale). Vertical lines represent location of promoters, and horizontal arrows indicate direction of transcription.

Table 1. Pathogen factors: Virulence genes.

Gene		Function	References
prfA	positive regulatory factor A	transcriptional activator	136–138
plcA	phosphatidylinositol-specific phospholipase C	aids in escape from vacuoles	54, 139
plcB	phosphatidylcholine-specific phospholipase C	aids in escape from vacuoles	5, 104, 139–141
hlyA	listeriolysin O	escape from vacuoles	104, 139, 142–144
mpl	zinc-dependent metalloprotease	maturation of PlcB	55, 57, 58, 140, 145
actA	actin-polymerizing protein	cell-to-cell spread	57, 58, 146, 147
inlA	internalin A	internalization	100, 148, 149
inlB	internalin B	internalization	47, 101–103, 149–151

ization of the bacterium into the host cell; (ii) bacterial escape from the host cell vacuole; (iii) intracytoplasmic multiplication and movement via bacterial-induced polymerization of host cell actin; (iv) bacterial spread to neighboring cells through pseudopod-like extensions of the host cell membrane and (v) bacterial escape from the resulting double-membrane vacuoles [52]. The master regulator for expression of genes in the virulence gene island is PrfA. The *prfA* gene acts as a transcriptional activator for all the other genes on the island, and is thus absolutely essential for *L. monocytogenes* virulence [53]. Adjacent to *prfA* is *plcA*, which codes for phosphatidylinositol phospholipase C and aids the bacterial cell in escape from the single- and double-membrane-bound vacuoles [54, 55]. Downstream from *plcA* is the key virulence factor *hlyA*, which encodes listeriolysin O. This hemolysin is absolutely essential for escape from the vacuoles, and mutants lacking *hlyA* are avirulent in a mouse model of infection [56]. Adjacent to *hlyA* is the *mpl* gene, which codes for a zinc-dependent metalloprotease. Mpl is responsible for cleaving phosphatidylcholine-specific phospholipase C (PlcB) into its mature and active form [55]. Downstream from *mpl* is another key virulence gene, *actA*, which codes for an actin-polymerizing protein. ActA is responsible for accumulating and polymerizing host cell actin into an 'actin tail' at one bacterial pole that serves to propel the bacterium into neighboring cells via pseudopod-like extensions of the host cell membrane. Thus, ActA plays a primary role in the cell-to-cell spread of *L. monocytogenes* and, like *prfA* and *hlyA* mutants, *actA* mutants are avirulent [57, 58]. Finally, downstream from *actA* lies *plcB*. Like PlcA, PlcB aids in bacterial escape from the vacuoles [55]. Two other well-characterized virulence genes are *inlA* and *inlB*. These genes code for internalins and are located on a separate operon under the partial control of PrfA [48, 49]. Both are present in *L. monocytogenes*, but absent in *L. ivanovii*, and are involved in attachment and internalization to different host cell types [51]. Each of the virulence genes and its role in pathogenesis are reviewed in greater detail by Sheehan et al. [49] and Vásquez-Boland et al. [12].

Virulence-related genes

There are several other genes in pathogenic *Listeria* that, although not contributing to virulence directly, are still important for survival within a host. These genes are often housekeeping genes necessary for saprophytic life, and many are components of general stress-response systems (for review see [12]). Several new genes that play a role in virulence have been identified using transposon mutagenesis [59, 60]. While the numbers and functions of the gene products are vast, many, such as p60 and SvpA, are surface proteins implicated in adherence and invasion [50, 60, 61]. Others, including Sigma B, LisRK and the Clp family, are involved in general, heat, ethanol and acid stress resistance [62–65]. Another example of virulence-related genes are those that play a role in DNA replication or repair such as Dfp and RecQ [59, 60]. Finally, some genes, such as those in the internalin family, can be considered virulence related because they are homologous to known virulence genes, but have as yet unknown functions or are not essential for virulence in current model systems. Some selected virulence-related genes and the mechanisms by which they contribute to pathogenesis are summarized in table 2.

Host factors

The host immune status represents the most important host component in the pathogenesis of listeriosis, as supported by the fact that most human listeriosis cases occur in immunocompromised individuals [4]. Additional host contributions to the pathogenesis of listeriosis may include genetic susceptibility and behavioral risk factors. While a detailed examination of host immunity to listeriosis is beyond the scope of this review, we will summarize some main themes. For complete reviews see North and Conlan [66], Shen et al. [67] and Parham [68].

Table 2. Pathogen factors: Selected virulence-related genes.

Gene		Function	References
iap	invasion-associated protein (p60)	murein hydrolase	61, 152
sigB	sigma B	general stress response alternative sigma factor	62, 64, 65
hpt	Hpt permease	glucose-6-phosphate translocase	153
dfp	flavoprotein	DNA synthesis	59, 60
clp family	ClpC, ClpE, ClpP, CtsR	heat shock, general stress	120, 154–157
lisRK	two-component system	acid, ethanol stress	63
svpA	surface virulence-associated protein	intracellular survival	50
recQ	DNA helicase	DNA repair	59
inl family	internalins	internalization	12, 51
srtA	sortase	linkage of LPXTG motif-containing proteins to cell wall	158

Immunity

The long-held view in the literature has been that immunity to listeriosis is effected primarily via the cell-mediated branch of the immune system with little to no involvement of a humoral response. Most studies on immunity to listeriosis, however, have relied on i.v. or intraperitoneal (i.p.) inoculation of *L. monocytogenes* into mice: models that may not adequately represent the immune responses that follow an oral infection. Recent evidence suggests that antibodies may play a previously unrecognized role. For example, Manohar et al. demonstrated a role for mucosal and serum antibodies using germ-free mice inoculated with a *L. monocytogenes actA*-negative mutant. They specifically observed that gut colonization by the bacteria was followed by an increase in total and specific immunoglobulin (Ig)A levels in the small intestine, as well as an increase in specific IgA and IgG1 levels in the serum [69]. It remains to be determined, however, whether mucosal antibodies are present in humans colonized by wild-type *L. monocytogenes* and, if present, whether they play a protective role against oral challenge. While it is known that humans can carry serum antibodies to several listerial proteins, the mechanisms of generation and putative protective role of serum antibodies against *L. monocytogenes* remain unclear [69, 70].

Cells of the innate immune system, including macrophages, neutrophils and natural killer (NK) cells, are most important at controlling a listerial infection in the early stages, while specific immunity effected primarily by CD8+ T cells plays a critical role in full resolution of the primary infection and providing immunity to secondary infection. Interestingly, dendritic cells are known to assist anti-*Listeria* immunity via antigen presentation, yet recent evidence indicates that dendritic cells beneath Peyer's patches of the intestine are early cellular targets of *L. monocytogenes* and facilitate bacterial spread to the blood and lymph [71, 72]. Upon accessing the blood and lymph, most bacteria are soon captured by resident macrophages (Kupffer cells) of the liver [73]. *Listeria* that survive macrophage killing in the liver spread into liver parenchymal cells and travel to phagocytic cells of the spleen, both of which are highly permissive environments for bacterial growth during the first 24 h of infection [66, 74]. As a listerial infection progresses, neutrophils accumulate around infectious foci in the liver and, there, function to lyse *Listeria*-infected hepatocytes [75, 76]. Neutrophils have also been shown to phagocytize and kill *L. monocytogenes* in vitro, and, therefore, assist liver macrophages in clearing bacteria [77, 78]. NK cells aid anti-*Listeria* defense via their ability to secrete interferon-γ (IFN-γ), which activates nearby macrophages [79]. Even though 60–80% of *Listeria* cells are effectively killed in the liver, those that survive will continue to proliferate in the liver and spleen, giving rise to an infection that will necessitate a specific cell-mediated response for complete resolution [75].

Cell-mediated immunity to listeriosis is mediated by CD8+ T cells, with CD4+ T cells seemingly playing little role [66]. CD8+ T cells can be primed, in a primary listerial infection, by both secreted and nonsecreted *Listeria*-derived antigens. Secreted antigens such as ActA and listeriolysin O are processed and presented to CD8+ T cells via the endogenous major histocompatibility (MHC) class I pathway in all infected cell types, while nonsecreted *Listeria*-derived antigens are processed and presented via the exogenous MHC class I pathway, but only in professional antigen-presenting cells. In the immune response to a secondary listerial infection, on the other hand, memory CD8+ T cells are only able to respond to antigens presented via the endogenous pathway [67]. The exact mechanism of CD8+ T-cell-effected protection is still debated and could be due either to their ability to lyse infected target cells or to their ability to secrete IFN-γ and, thus, activate macrophages [66]. Some researchers have called into question the essential importance of CD8+ T cells in immunity to *L. monocytogenes* infection based on the evidence that mice without CD4+ or CD8+ T cells are still capable of resolving infection, albeit much more slowly [80, 81]. This argues in support of a greater role for cells

of the innate immune system in susceptibility and immunity to listeriosis [66]. Finally, it should be noted that it is generally agreed that an intact cytokine system, which includes a healthy tumor necrosis factor (TNF) and IFN-γ response, is essential for resolving listeriosis [67, 82].

Genetic susceptibility and behavioral risk factors

Genetic variation between human hosts, as well as the presence or absence of certain behavioral risk factors, may result in differences in susceptibility to infection by pathogenic *Listeria* and to different disease outcomes. Such a relationship between host genetics and susceptibility to infection occurs in other infectious diseases, such as peptic ulcer disease caused by the gastric pathogen *Helicobacter pylori*; specifically, epidemiological and cross-sectional studies have demonstrated higher frequencies of the human O blood group and ABH antigen non-secretor phenotypes among patients colonized by *H. pylori* [83, 84]. Likewise, Rad et al. found that 91% of 141 antral biopsies were positive for the Lewis(b) blood group antigen, a cellular receptor for *H. pylori* [84, 85]. In the case of listeriosis, it is possible that genetic polymorphisms in the human epithelial cell surface receptor E-cadherin or other internalin receptors might render *L. monocytogenes* unable to bind effectively to initiate infection. Listeriosis research, furthermore, is just beginning to identify risk factors other than compromised host immune status and consumption of contaminated food that contribute to development of listeriosis. One such behavioral risk factor is the use of antacids and H_2 pump inhibitors. Epidemiological studies have suggested a link between antacid therapy and development of listeriosis in humans, and in vivo experiments using rodents have confirmed an association [86–88]. By decreasing the level of stomach acidity, antacid use likely increases the number of ingested *Listeria* that are available to infect the small intestine. Alcohol consumption may also be a risk factor, as demonstrated by the impairment of host cellular defense mechanisms after acute exposure to high levels of ethanol in rats [89]. Further research that aims to identify possible genetic and behavioral risk factors for listeriosis may prove useful in understanding what limits listeriosis to gastroenteritis in some individuals and expand our knowledge of the cellular and molecular mechanisms of listeriosis pathogenesis.

Environmental factors

There are several factors in food or natural environments that play an important role in the survival and growth of pathogenic *Listeria* which include, but are not limited to, temperature, water availability and pH. *L. monocytogenes* can grow over a wide range of temperatures (1–45 °C) and can survive water activities as low as ~0.83 [3, 90]. Such flexibility in temperature and water requirements might contribute to the abundance of *Listeria* in nature. More important from a human health perspective, *L. monocytogenes* can survive and grow at refrigeration temperature (4 °C) and at the low water activities that are inhibitory to many other foodborne pathogens. For example, Junttila et al. tested 78 strains of *L. monocytogenes* for their ability to grow on tryptose soy agar at subrefrigeration temperatures and determined that their mean minimum growth temperature was 1.1 (± 0.3) °C [91]. Similarly, several studies have demonstrated extended survival of *L. monocytogenes* in low water activity foods such as hard salami and cheddar cheese [2, 92]. Also contributing to *L. monocytogenes*' survival in foods is its ability to tolerate low pH. For example, it is known to be able to grow at a pH as low as 4.3 and has been shown to better survive an acid shock of pH 3.0 following acid adaptive growth at pH 5.0 [93, 94]. This ability to adapt to acid has caused speculation that *Listeria* growing in acid-fermented food products might better survive passage through the human stomach, resulting in a reduced infectious dose required for development of listeriosis. Indeed, O'Driscoll et al. demonstrated that acid-adapted *L. monocytogenes* mutants are more virulent in mice [95]. Thus, the various temperatures, water activities and pH's that *L. monocytogenes* encounters in its various food and natural environments not only impact its ability to survive and grow, but may also contribute to pathogenicity.

Interactions among factors

In foodborne infectious diseases, the food environment plays the critical role in transmission of the pathogen between environment and host. Equally important to its survival, growth, and transmission, however, are the pathogen's interactions with its host and other nonhost, nonfood environments.

Host-pathogen interactions

The sum of all interactions at the host-pathogen interface, including initiation of the infectious process, propagation of infection and the mounting of a corresponding host immune response, ultimately determines the outcome of any foodborne infection. The infectious cycle of *L. monocytogenes* in the mammalian host is well understood, and several excellent reviews describe the process in detail [12, 49, 96]. The cycle begins with bacteria gaining entry into host tissues at the small intestine. Following oral ingestion of *L. monocytogenes*, bacteria that survive gastric passage access intestinal lymph and blood vessels by in-

vading the M cells of Peyer's patches and intestinal epithelial cells. There is evidence supporting both routes of entry, and the exact mechanisms used by *Listeria* to cross the intestine are still debated [12, 97, 98]. *L. monocytogenes* has many cell surface proteins used for attachment and entry, but among these, internalin A (InlA) and internalin B (InlB) are the two best characterized [12, 99]. It is known that *L. monocytogenes* can invade several host cell types, and the diversity of internalin proteins may contribute to this phenomenon. Mengaud et al. identified E-cadherin as the cellular receptor for internalin, which aids entry of *L. monocytogenes* into nonphagocytic cells such as epithelial cells [100]. Internalin B, on the other hand, facilitates entry into a wider variety of cell types, including some epithelial, fibroblast and endothelial cell lines and is necessary for entry into hepatocytes [47, 101]. The Met receptor and the C1q-binding protein, gC1q-R/p32, have been identified as cellular receptors for InlB [102, 103]. It is clear, therefore, that *L. monocytogenes* uses several cell surface proteins to facilitate its entry into a variety of cell types, and that the availability of host cell receptors will determine, in part, which host tissues *L. monocytogenes* can invade.

Once it has entered a host cell, *L. monocytogenes* is enclosed in a membrane-bound vacuole from which it must escape in order to multiply and spread to neighboring cells to propagate infection. At this stage, acidification of the vacuole by the host cell is one defense mechanism that can successfully eliminate invading *Listeria*. In response, the primary listerial protein used in escape from the vacuole, listeriolysin O, has evolved an acidic pH optimum [104]. Similarly, the zinc-dependent metalloprotease (Mpl) is dependent upon the acidic environment of the vacuole for its activity. Within 2 h after intracellular infection, *L. monocytogenes* escapes the vacuole and is located in the host cell cytoplasm. Upon entry into the cytoplasm, changes in bacterial gene expression occur, resulting from microenvironmental differences between the vacuole and the cytoplasm, to cause the bacterial cell to begin dividing and expressing ActA [52]. ActA has no known catalytic activity, but instead recruits host cell proteins such as the Arp2/3 complex, cofilin, profilin, VASP and G-actin, assembling them in a configuration that allows the formation of actin tails and actin-based motility [105]. The intracytoplasmic movement of *L. monocytogenes* results in migration of the bacteria toward the membrane and extrusion of pseudopod-like extensions, which are then phagocytized by neighboring cells. An infectious cycle is completed upon bacterial escape from the resulting double-membrane-bound vacuole.

Some reports have described mechanisms by which *Listeria* thwart the host's immune response to infection. For example, *Listeria* has been shown to interfere with antigen presentation and induce apoptosis in certain cell types. *L. monocytogenes*-infected antigen-presenting cells are able to irreversibly inactivate antigen-specific CD4+ T cells [106]. Apoptosis is induced in the hepatocytes and dendritic cells of neutropenic mice, although it is unclear whether this is a bacterial induced mechanism of pathogenesis or a host defense mechanism [67, 107, 108]. Some evidence suggests that *L. monocytogenes* inhibits apoptosis in macrophages [109]. Thus, *L. monocytogenes* has evolved a variety of mechanisms to avoid or delay destruction by the immune system.

Host-environment interactions

The interactions of a host with its environment can affect the pathogenesis of *Listeria* infections. As host immune status is the most important determinant of listeriosis pathogenesis, any environmental factor that weakens the immune system will probably increase the likelihood of developing listeriosis. For example, exposure to radiation, malnutrition and stress are all known to depress immune function and might be likely to increase one's risk for listeriosis. Similarly, in animals, the stress associated with weather, transport or introduction of a new animal into a herd is a risk factor for listeriosis [6]. Also in animals, the consumption of silage is associated with cases of listeriosis, not only because of poor quality (contamination and high pH) but because the micronutrient composition of silage may predispose livestock to infection [6]. Specifically, it has been shown in sheep that imunosuppression characterized by a decrease in circulating lymphocytes and reduced total serum protein may result from silage consumption [110]. In humans, an abnormally high concentration of iron in the blood following transfusion has been identified as a risk factor for listeriosis [12]. Additionally, any interactions with the environment or human behaviors that increase exposure to pathogenic *Listeria* could theoretically increase the incidence of disease. For example, changes in food preparation practices designed to satisfy a growing demand for ready-to-eat and minimally processed products with extended shelf life may increase human exposure to pathogenic *Listeria*.

Environment-pathogen interactions

A pathogen's interactions with its environment may impact pathogenesis and the development of disease. In the case of a foodborne disease such as listeriosis, important environments include the natural soil, water and vegetation environments encountered by saphrophytic *Listeria*, as well as a variety of food environments following food contamination. In order for pathogenic *Listeria* to cause disease, it is essential that the bacteria be able to distinguish between the natural/food and the host environments. The mechanisms that *Listeria* use to sense and respond to

environmental or host signals are still not well understood, but may include the availability of specific sugars as well as certain stress conditions; furthermore, how this sensing of and response to environmental signals influences pathogenesis is a relatively new line of research that includes studies of biofilm and protozoan grown *L. monocytogenes*.

Sugars

L. monocytogenes is known to respond to certain fermentable sugars and their concentrations in its environment. Specifically, Park and Kroll demonstrated that the environmentally ubiquitous plant disaccharide cellobiose inhibits the expression of the virulence genes *hlyA* and *plcA* [111]. This inhibition occurs by a mechanism independent of *prfA* transcription [112]. Cellobiose is presumably abundant in the decaying vegetation where *Listeria* are commonly found and may be an important signal indicating residence in the environment. In contrast, the absence of cellobiose in the mammalian body may serve as an inducer of virulence gene expression [111]. Another sugar, glucose, has been shown to interfere with *prfA* expression, resulting in the reduction of *hlyA* expression [113]. However, it remains to be determined how *Listeria* uses glucose concentration as a signal of environmental or host intracellular location.

Stress survival

Survival in the environment, whether a natural environment of decaying vegetation or a food or food-processing environment, requires that *Listeria* be able to cope with a variety of stresses, including extremes of pH, temperature variation, high salt, starvation, and predation by protozoa. The changes in gene expression and resulting physiological changes that a bacterium undergoes to be able to survive the stresses encountered in its environment may enhance its ability to mount a successful infection in a mammalian host. Adaptation to environmental stress may enable *L. monocytogenes* to survive, via a general stress response, similar stresses encountered during infection. For example, before mounting a successful infection in humans, *L. monocytogenes* must survive passage through the acidic stomach and must be able to temporarily withstand the acid and oxidative stresses of the vacuole. *Listeria* adapted to these stresses by previous exposure to them in an acidic natural or food environment may be better equipped to infect a host. In fact, several in vitro studies provide evidence that acid-adapted *L. monocytogenes* survive acid challenge better than nonadapted cells [95, 114, 115]. Another mechanism by which adaptation to environmental stress may contribute to virulence is through the direct activation of virulence genes. For example, Sokolovich et al. demonstrated that heat stress results in the synthesis of the PrfA-dependent proteins and known virulence factors ActA, listeriolysin O, PlcA and PlcB [116].

Another requirement for bacterial stress survival is the sensing of stress conditions in the environment and the coordinated communication of these signals into an appropriate response. Such sensing and regulatory activities are often linked to virulence. Two-component signal transduction systems are frequently used by bacteria to sense and respond to stress [117]. Cotter et al. identified the two-component system LisRK in *L. monocytogenes* and showed that a deletion in the histidine kinase component negatively affected acid tolerance, ethanol tolerance and significantly reduced virulence in mice [63]. Similarly, three *L. monocytogenes* mutants in response regulators were virulence attenuated and had a decreased ability to grow in the spleens of infected mice [118]. Further evidence for a linkage between stress response and virulence is provided by the stress response mediator ClpC and the virulence protein PrfA; Ripio et al. demonstrated that PrfA negatively controls the transcription of ClpC, a chaperone protein important for stationary phase survival and resistance to low pH, oxidation, osmotic stress, high temperature and iron deprivation [119, 120].

Biofilms

L. monocytogenes is capable of forming biofilms, which likely increases the persistence of this pathogen in food production facilities and may enhance virulence. A biofilm can be defined as a sessile community of surface-attached bacteria surrounded by an extracellular polymeric matrix and exhibiting altered phenotypes and gene expression patterns [121]. Most work that has been done on *Listeria* in biofilms has focused either on comparing the ability of different *L. monocytogenes* strains to adhere and form biofilms or on comparing adherence and biofilm formation on different food-processing surfaces. Research suggests that *L. monocytogenes* strains differ intrinsically in adsorption characteristics. Specifically, reports by Norwood and Gilmour [122] and Lunden et al. [123] suggest that strains belonging to serotype 1/2c along with strains that had previously persisted in a food-processing environment are better able to adhere to stainless steel surfaces. Furthermore, Djordjevic et al. demonstrated that strains belonging to lineage I are better at forming biofilms on PVC microtiter plates than either lineage II or III strains and, therefore, hypothesized than an enhanced biofilm formation capability of lineage I strains might contribute to this lineage's higher prevalence among human cases [124]. Other work, however, has shown no relationship between processing environment persistence, strain source (food or clinical), and strain subtype (serotype or lineage) to adherence and biofilm formation [125]. Likewise, while work in other pathogens such as

Salmonella and *Enterococcus* has suggested that growth in biofilms may increase virulence [126, 127], the relationship between growth in a biofilm and the virulence of *L. monocytogenes* remains unclear. One possible link between growth in biofilms and virulence may be the similar physiological adaptations that bacterial cells undergo in response to the similar stresses encountered in both environments. For example, one stress encountered in both biofilms and a mammalian host is nutrient limitation [128]. Taylor et al. demonstrated in *L. monocytogenes* a link between nitrogen starvation, biofilm formation and virulence by identifying two transposon mutants mapped to *hpt* and *relA* that were unable to synthesize (p)ppGpp and mount a stringent response to nitrogen starvation. Despite having the same ability as wild-type cells to adhere to microtiter wells, they were unable to grow and form biofilms, and importantly, these mutants were severely virulence attenuated when inoculated intravenously into mice [129]. In another experiment, Tremoulet et al. compared the proteomes of *L. monocytogenes* grown planktonically or in a biofilm and demonstrated that several of the proteins whose levels increased in biofilm-grown bacteria are known or putative stress-response proteins. These included superoxide dismutase, a homolog of the DNA repair and protection protein, RecO, and a homolog of the 30S ribosomal protein, YvyD, which is known to be induced under starvation, heat, ethanol and salt stress in *B. subtilis* [130]. Future research examining the levels of these proteins in intracellularly grown *L. monocytogenes* along with future work probing adherence and biofilm formation capability will allow us to further understand how adaptation via growth in a biofilm may facilitate *L. monocytogenes* infection.

Protozoa

Bacteria living in protozoans may represent an overlooked but important contributor to human pathogenesis by serving as reservoirs of pathogenic bacteria, providing novel routes of transmission and supplying a growth environment that may adapt a pathogen for survival in a mammalian host [131]. As has been demonstrated in *Legionella pneumophila*, the bacterial mechanisms for host recognition, cellular entrance and intracellular proliferation can be similar for both amoeba and mammalian cells [132]. Several groups have also shown that *L. pneumophila* growth in protozoan hosts results in phenotypic and behavioral changes that render the bacterium more invasive and increases virulence. Another advantage for bacteria existing in a symbiotic or parasitic relationship with protozoa is the ability to grow in otherwise inaccessible or inhospitable environmental niches. The anaerobic bacterium *Molibuncus curtisii*, for example, is able to inhabit aerobic environments by proliferating in amoeba [133]. Similarly, pathogenic bacteria that can survive within protozoa may proliferate in new environments and be transmitted to humans by previously unrecognized routes. Ly and Müller demonstrated in 1990 that *L. monocytogenes* is able to survive and multiply within the protozoans, *Tetrahymena pyriformis* and vegetative *Acanthamoeba* sp. [134]. They proposed that the virulence of *L. monocytogenes*, which normally lives as a saphrophyte in the environment, can be attributed to its ability to parasitize protozoa. Unfortunately, little work has been done since that time to probe the effect of protozoa parasitism by *L. monocytogenes* on its virulence or transmission dynamics. It is possible that protozoa may represent a reservoir of pathogenic *Listeria* and serve to maintain virulence genes during environmental survival. Further research is needed, though, to determine what role protozoa may play in *Listeria* pathogenesis and transmission.

Conclusions and future directions

In this review, we have highlighted some of the important pathogen, host and environmental factors that play a role in the pathogenesis of animal and human listeriosis, and have, furthermore, described how those factors can interact to influence the development and outcome of disease. A comprehensive understanding of the pathogenesis and transmission of listeriosis will require continued research to investigate all factors and interactions influencing disease. Much work has already been done to elucidate the molecular mechanisms of pathogenesis at the pathogen-host interface; for example, studies have identified several virulence genes of *L. monocytogenes* and have revealed the roles those genes play in the infectious cycle. Future work on the molecular virulence mechanisms of *Listeria* might focus on characterizing the various surface proteins and their host cell receptors to gain insight into the mechanisms of host cell specificity and tissue tropism. Additionally, the molecular characteristics that distinguish encephalitic from septicemic/abortive listeriosis in animals remain to be determined. Also, what, if any, molecular or cellular factors related to host or pathogen affect the occurrence of gastrointestinal or invasive listeriosis in humans? Finally, much remains to be learned about the contributions to pathogenesis of virulence-related genes such as those involved in acid, oxidative and general stress response.

In comparison to our level of understanding of the interactions occurring at the pathogen-host interface, very little is known about the molecular and cellular interactions between pathogen and the environment, between host and the environment, and about how these interactions influence the pathogenesis of listeriosis. The emergence of new approaches to study molecular and cellular biology (genomics, proteomics, metanomics) provides unique op-

portunities for the discovery of predisposing genetic risk factors, such as polymorphisms in host cell surface receptors. The availability of the complete genome sequences for both pathogen and human host clearly also facilitates these types of studies [45, 99]. Furthermore, the interactions of *Listeria* with its natural or food environment that influence human or animal pathogenesis are largely just beginning to be studied. For example, while it has been shown that *L. monocytogenes* survives acid and ethanol stress better in vitro following preadaptation, it remains to be determined what effect preadaptation to pH, temperature, salt, starvation and oxidative stress in the environment has on survival to stresses encountered in vivo [95, 114, 115, 135]. The possibility that biofilm- or protozoan-grown *Listeria* are more pathogenic than their planktonic counterparts is intriguing and represents a line of research that warrants further attention due to its potential to shed light on the transmission of listeriosis from the environment through foods to humans, and to provide insight into the relationship between bacterial stress response and virulence. Ultimately, it is the combination and interactions of pathogen, host and environmental factors that cause the development of listeriosis, and thus a cellular and molecular systems research approach that addresses the complexities of each of these components of pathogenesis will be required to fully understand and control this deadly disease.

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