

Listeria monocytogenes at the interface between ruminants and humans: A comparative pathology and pathogenesis review

Veterinary Pathology
1-25
© The Author(s) 2021
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/03009858211052659
journals.sagepub.com/home/vet



Stefano Bagatella¹, Leticia Tavares-Gomes¹,
and Anna Oevermann¹ 

Abstract

The bacterium *Listeria monocytogenes* (*Lm*) is widely distributed in the environment as a saprophyte, but may turn into a lethal intracellular pathogen upon ingestion. Invasive infections occur in numerous species worldwide, but most commonly in humans and farmed ruminants, and manifest as distinct forms. Of those, neuroinfection is remarkably threatening due to its high mortality. *Lm* is widely studied not only as a pathogen but also as an essential model for intracellular infections and host-pathogen interactions. Many aspects of its ecology and pathogenesis, however, remain unclear and are rarely addressed in its natural hosts. This review highlights the heterogeneity and adaptability of *Lm* by summarizing its association with the environment, farm animals, and disease. It also provides current knowledge on key features of the pathology and (molecular) pathogenesis of various listeriosis forms in naturally susceptible species with a special focus on ruminants and on the neuroinvasive form of the disease. Moreover, knowledge gaps on pathomechanisms of listerial infections and relevant unexplored topics in *Lm* pathogenesis research are highlighted.

Keywords

brain diseases, foodborne diseases, listeriosis, cattle, sheep, goats, bacterial infections, zoonosis, One Health

Listeria monocytogenes (*Lm*) is a globally distributed bacterial pathogen with zoonotic potential, able to cause disease (generally termed “listeriosis”) in a wide variety of domestic and wild mammalian and non-mammalian species,^{94,203} including humans, cattle, sheep, goats, dogs, cats, horses, pigs, South American camelids, farmed deer, and poultry.^{125,180,306} Species other than humans and ruminants, however, are only sporadically affected. As an animal pathogen, *Lm* is a cause of concern not only in terms of public health and food safety but also as a significant cause of economic losses when livestock and their offspring are affected.

First described in the 1920s in a human patient,⁸³ then a few years later in laboratory animals^{202,232} and in the following decade in ruminants,¹²⁵ the pathogenic nature of *Lm* has long been recognized. However, the bacterium has been perceived as an important and deadly human foodborne pathogen only following major outbreaks in the 1980s, even though its oral infection route was known decades prior in farm animals.^{60,260,261} Since then, surveillance in various countries has continually ranked *Lm* among the most fatal foodborne pathogens despite the low prevalence, as the mortality rate in listeriosis is high.^{85,260} In spite of rigorous, albeit unharmonized, food safety regulations adopted by different countries,^{90,287,288} major listeriosis outbreaks continue to occur worldwide today^{134,280} with an increasing incidence rate reported in many

countries,^{45,91,120} causing a noticeable burden on global public health.⁶⁰

Lm is an intensely studied pathogen that has been used for decades as a model for bacterial cell invasion, adaptation to and subversion of the host-cell molecular machinery,^{136,233} and for innate and adaptive immune responses toward bacterial infection.^{219,276} Noticeable features of *Lm* are its high resilience and versatility allowing it to reside as a saprophyte in various environmental habitats²⁹⁶ and its rapid switch into a dangerous opportunistic and intracellular pathogen once it is in contact with the host.^{103,281} Intriguingly, the bacterium causes various disease manifestations in susceptible hosts,^{91,250} of which central nervous system (CNS) infection, known as neurolisteriosis, is particularly threatening and occurs frequently in ruminants.^{63,198,309}

Despite the advances in our understanding of *Lm* infection at the cellular level, its environmental and farm life cycle as well

¹Division of Neurological Sciences, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Corresponding Author:

Anna Oevermann, Division of Neurological Sciences, Vetsuisse Faculty, University of Bern, Bremgartenstrasse 109a, CH-3012 Bern, Switzerland.
Email: anna.oevermann@vetsuisse.unibe.ch

as organ targeting mechanisms during listeriosis, in particular CNS infection, remain to be fully deciphered. This review aims to summarize the current knowledge and to map research gaps regarding *Lm* epidemiology and infection in ruminants with a focus on its peculiar neuroinvasive phenotype.

***Lm*, a Heterogeneous and Multi-Skilled Bacterium**

Lm is a member of the genus *Listeria*, which currently comprises 21 recognized species of Gram-positive nonsporulating coccobacillary bacteria.^{68,171,207,214,241} Among them, *Lm* is by far the most relevant member in terms of virulence, although *Listeria ivanovii* is also regularly associated with disease, but exclusively in domestic ruminants. Other species (*L. innocua*, *L. seeligeri*, *L. grayi*, among others) are only sporadically identified in human and animal infections.^{6,131,226,243,244,250,252,274,297} *Lm* is a particularly versatile bacterium being facultative intracellular, facultative anaerobic, and notably osmo- and halotolerant, able to replicate in media containing up to 10% NaCl. Furthermore, it grows in a wide range of temperatures (between 1 °C and 45 °C) and pH (5–9).^{51,95} This remarkable resistance to environmental stressors allows the bacterium to survive in a wide variety of ecological habitats and within the host.²⁹⁴

***Lm* Strain Heterogeneity**

Epidemiological and experimental studies suggest that the variability in environmental distribution, virulence, and clinical manifestations between different hosts are linked to genetic heterogeneity of *Lm*.^{17,190,192,199,213,221} However, the underlying bacterial determinants and mechanisms driving the variability and niche adaptation of *Lm* are not yet clear, and the investigation of such determinants is currently one focus of research.⁶⁶ Characterization of bacterial subtypes and their association with particular niches and virulence has been attempted through various techniques.^{54,177} Classically, serotyping methods based on specific antisera allowed for the distinction of *Lm* into 13 serotypes.²⁷¹ Despite its low discriminatory power,^{54,177,266} serotyping has been employed for decades as the standard subtyping technique in epidemiological investigations and provided first evidence that *Lm* subtypes are differentially distributed between environment and clinical disease.²¹³ Among the 13 serovars, 1/2a, 1/2b, and 4b are the most commonly identified in human and animal clinical isolates, with a noticeable preponderance of serotype 4b in major listeriosis outbreaks^{157,266,278} and ruminant neuroinvasive cases.^{155,164,251,308} All 3 serotypes, apart from being implicated in disease, were additionally isolated from food, food processing and farm environments, and animal feces.^{26,89,102,152,211,234,277}

More recently, molecular typing methods such as pulsed field gel electrophoresis (PFGE),^{36,106,123} multilocus sequence typing (MLST),¹¹⁴ and whole-genome sequencing (WGS)¹⁶¹ (reviewed in Datta and Burall⁵⁴ and Datta et al⁵⁵) have been employed to link clinical, food, and environmental isolates in epidemiological investigations during outbreaks (PFGE), to

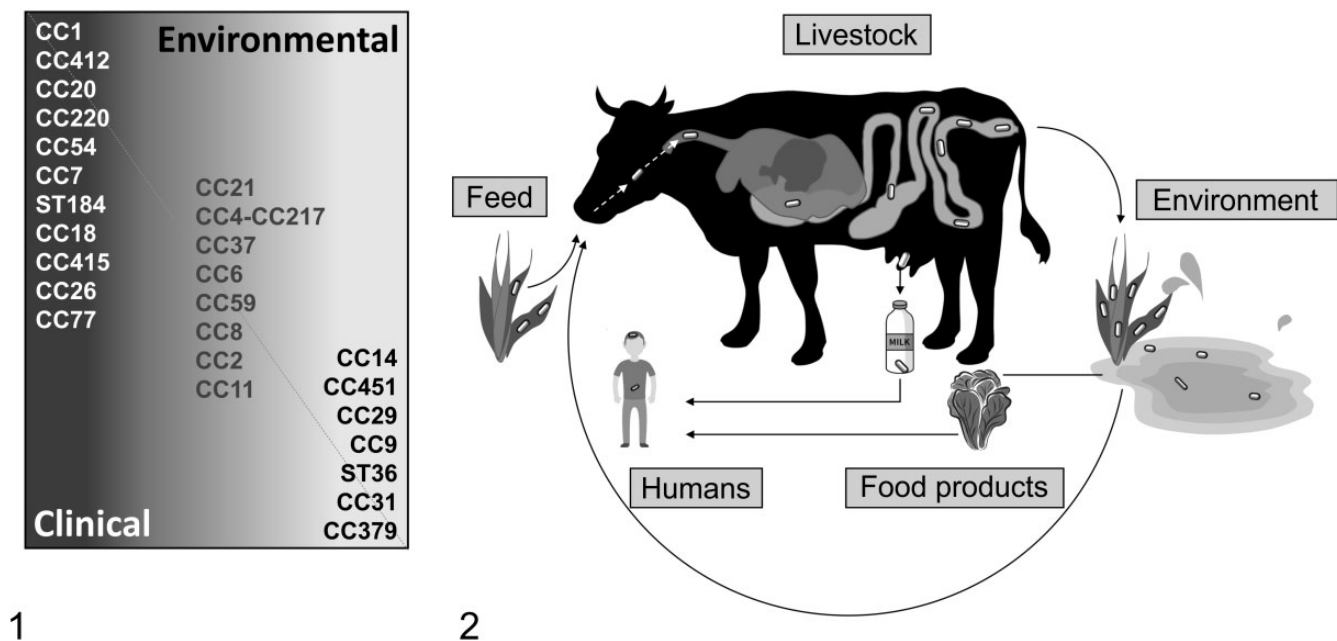
study genetic relatedness in *Lm* populations (MLST), or both (WGS). Phylogenetic analyses performed with MLST and WGS identified 4 distinct lineages (I–IV), further subdivided into clonal complexes (CCs) and sequence types (STs), or sublineages (SLs) and core genome MLST types (CTs), respectively. *Lm* clusters into 2 major lineages (I, II) that are frequently isolated from diverse sources, and 2 minor lineages (III, IV) that are only sporadically isolated from animal infections.²¹³ Of the major lineages, lineage I is the genetically most homogeneous and overrepresented in human clinical isolates and ruminant neuroinvasive cases.^{79,192,213,221,251} In contrast, lineage II is genetically heterogeneous and includes predominantly food- and environment-associated strains, but to a lesser extent also strains associated with clinical disease in humans and ruminants.^{151,213,234}

Prevalence of Hypervirulent Versus Hypovirulent *Lm* Clonal Complexes in Disease

Various CCs from lineage I belonging to serotype 4b (such as CC1, CC2, CC4, and CC6) are significantly linked to clinical cases in humans and have been shown to be hypervirulent in experimental models. Additionally, they are better adapted to host colonization than clones overrepresented in food and the environment (such as CC9 and CC121).^{190,192,199,312} In ruminants, lineage I and, in particular, CC1 and CC4 are significantly overrepresented in clinical isolates and notably in isolates from neuroinvasive when compared with other clinical listeriosis syndromes such as abortion, mastitis, or gastroenteritis. However, other CCs from lineage I (CC2, CC217, CC6, CC191, CC59) and lineage II (CC7, CC11, CC14, CC37, CC204, CC412) are also regularly isolated from diseased animals and their environment. In contrast, other strains, such as CC9, are predominantly detected in food processing facilities and the environment (Fig. 1).^{22,79,220,221,277} However, the cause for the epidemiologically evident predominance of a limited number of subtypes in clinical cases of both humans and ruminants is not exactly understood. Host-associated hypervirulent clones have been shown to possess conserved virulence genes,^{17,149,192,199} whereas environmental clones may harbor inactivating mutations of such genes, making them hypovirulent and potentially accounting for their sporadic association with disease. Expression of more stress resistance genes, such as benzalkonium chloride resistance genes in CC9 and CC121 strains, together with negative regulation of transcriptional factors for virulence genes may favor their environmental fitness.^{149,190–192,253,279} Yet, many hypovirulent clones express virulence genes crucial for host invasion and may cause severe disease in specific circumstances, for example, in immunosuppressed patients.^{157,313}

Global Distribution of Major *Lm* Clonal Complexes

Hypervirulent strains (eg, CC1) that are overrepresented in clinical isolates from major outbreaks appear to be distributed worldwide^{17,43} and, notably, often appear to share a similar distribution between humans and ruminants.^{79,190,192,221} Major



Figures 1–2. Prevalent clonal complexes (CC) and farm-host cycle of *Listeria monocytogenes* (*Lm*). **Figure 1.** The most frequently isolated *Lm* CCs from clinical infection in ruminants and the farm environment (from Papić et al^{220,221}). CCs strongly associated with clinical cases are represented in white (left), strains variably associated with disease and the environment are represented in gray (middle), while environment-associated strains are represented in black (right). Notice that no *Lm* strain is exclusively environmental or clinical, hence the gray gradient background. **Figure 2.** *Lm* host-environmental cycle. Bacteria are taken up by ruminants through contaminated feed and may colonize the gut. *Lm* is shed to the environment through feces, potentially contaminating crops and water resources, and is also spread in milk. Contaminated vegetation and water may be taken up by ruminants, thus perpetrating the on-farm infection cycle, while contaminated vegetables and animal products may pose a risk to human consumers.

Lm CCs have also been shown to spread globally over time, causing historically relevant outbreaks. Moreover, within a given geographic region, predominant lineages might shift over time.¹⁹⁹ For instance, hypervirulent (eg, CC6)^{17,192} and hypovirulent (eg, CC9, CC121)^{17,190} clones are emerging in the 21st century in various continents. This is speculated to occur due to genetic diversification, possibly as a result of fitness adaptation or global dispersion through human travel, animal, or food trade.^{17,199} CC1 constitutes a notable example in these regards, as it is speculated to have spread globally from North America through cattle trade.²⁰⁰ Given the high ability of *Lm* to adapt to disparate environments and hosts, as well as its relevance for public and animal health worldwide, it is important to map and understand the distribution and pathogenic potential of frequently isolated strains to make surveillance and control more efficient. Moreover, given the potential role of ruminants as carriers of strains pathogenic for humans, further comparative genomic studies are essential to identify strains causing disease in both species, as well as to understand their distinctive features of virulence and inter-host transmission.

Reservoirs of *Lm* in the Environment

Lm is considered to be a ubiquitously distributed bacterium, remarkably adaptable to a wide range of natural and

anthropized habitats (such as agricultural and food-associated ones), but knowledge of its environmental dynamics and ecology is only fragmentary.^{204,258,298} Importantly, the dynamics of *Lm* transmission between the natural environment and ruminant and human hosts remain unclear. In nature, *Lm* has been isolated from various sources, including soil, water, and vegetation, as well as from feces of numerous wild birds and mammals.^{150,204,296,305,314} Bacteria can be generally isolated from natural sources in low numbers and with low prevalence. However, the presence of animals and moisture (in the form of bodies of water and precipitation) has been proposed to favor growth and dispersion of *Lm* in the environment.^{147,176,258} Interestingly, serotypes prevalent in human and ruminant infections (specifically 1/2a and 4b) were commonly isolated from wild birds, red deer, wild boars, and black bears, suggesting that wild animals might constitute a reservoir for pathogenic strains in the natural environment.^{138,222,304,314} Invertebrates (eg, slugs) and free-living protozoans have also been shown to be capable of supporting bacterial growth, indicating their potential role as environmental vectors or reservoirs.^{117,296} Amoebae, in particular, have been speculated to constitute a niche for *Lm*, as they have been shown to phagocytose and host bacteria,^{169,183,316} while other studies reported them to be bactericidal (reviewed in Schuppler²⁶⁷). It has therefore been speculated that key *Lm* virulence genes have emerged during

its co-evolution alongside environmental phagocytic unicellular eukaryotes to enhance its survival upon predation.²⁶⁷ Given the similarities of amoebas with macrophages, such an adaptation to amoeba could potentially function as a “training ground” for macrophage infection, explaining the ability of *Lm* to invade and survive inside cells of higher vertebrates.¹⁸³ The ability to successfully adopt a saprophytic lifestyle in the environment and switch to an opportunistic pathogen in the host by reprogramming its gene expression raises serious concerns regarding food safety and animal health. Considering that no definitive reservoir host has been identified in nature, the possibilities of intervention and prevention of its spread into the agriculture and farm industry remain currently limited.

Epidemiology of *Lm* in the Farm Environment

How ruminants become exposed to *Lm* in the farm environment is not exactly understood. Contamination of feed, water, or pasture appears to be the most likely route through which *Lm* is transmitted to the livestock host (Fig. 2),^{101,204} but conditions enabling enrichment of the pathogen in these sources have not been fully elucidated. The *Lm* infectious dose remains currently undefined, estimated to be as low as 10^4 CFU (colony-forming unit) in susceptible humans and as high as 10^9 CFU for healthy individuals. Nevertheless, it is believed that infection generally requires high bacterial numbers or repeated consumption of food sources contaminated with low bacterial levels.^{32,96,235} Therefore, sources that allow high bacterial replication are also most likely to be involved in *Lm* infection of ruminants.

Sources of Farm Contamination

Interestingly, moist soil, decaying plant matter, and bodies of water, although shown to provide an appropriate environment for bacterial survival, do not favor extensive bacterial growth. Moreover, it remains unclear whether they are environmental reservoirs without the presence of shedding animals.^{148,176,258} Fecal shedding from wild animals and bacterial persistence in invertebrates and protozoal carriers, as previously discussed, may promote bacterial introduction into the agricultural environment,^{189,273,282,291} but a direct link between strains isolated from the pristine natural environment, farm environment, and ruminant host remains to be shown.

Fecal-Oral *Lm* Enrichment Cycles in the Farm Environment?

Persistent in-farm transmission cycles and outbreaks have been increasingly linked to a contaminated animal environment,^{38,80,197,204} possibly indicating a role for ruminants themselves in sustaining bacterial persistence in their environment. Fecal shedding may facilitate bacterial contamination and persistence in the farm ecosystem. Agricultural fertilizers based on sewage sludge and manure if left untreated can provide means for significant contamination of pastures or crops. Sheep

manure has been the source for the major coleslaw-associated outbreak in 1981 during which the foodborne origin of human *Lm* infection was discovered.^{59,111,261}

Lm shedding has been observed in healthy ruminants,^{89,141,204,286} and cattle have been reported to shed *Lm* more frequently and at higher bacterial titers than small ruminants, suggesting a potential role for cattle as significant reservoirs for *Lm* in the context of an animal-farm cycle of transmission.^{80,89,204,205} Various studies also indicate that farm management practices may contribute to bacterial contamination of and persistence in the farm environment.^{38,141,197,205} These observations point to the relevance of a fecal-oral route in the maintenance of *Lm* in the agricultural-farm environment, through fecal contamination of plant-derived food or feed (Fig. 2).

However, animal-farm transmission dynamics and their relevance remain currently rather unclear,²⁹⁸ hampering the possibilities of adopting effective measures to prevent animal infection. The presence of unidentified asymptomatic ruminant shedders has the potential to significantly contribute to bacterial spread into the food industry via fecal contamination of milk or meat (Fig. 2).^{38,100,135,141} The numerous outbreaks linked to contaminated fresh produce and ready to eat foods^{37,110} highlight the importance of following a farm-to-fork strategy by restricting bacterial contamination at the farm/farm animal level, in order to ultimately prevent foodborne illness in consumers through the application of a One Health approach.

Silage: The Culprit?

In ruminants, poorly acidified silage has long been implicated as the main source of bacterial contamination, in which *Lm* is indeed able to replicate abundantly.^{99,124,291} Ruminants fed with high quantities of silage have been reported to excrete bacteria in their feces more frequently and in higher numbers, and to develop clinical disease more frequently.^{14,100,181,205,309} Silage feeding during hibernal indoor housing has also been linked to the seasonality of clinical listeriosis cases in ruminants in northern hemispheres increasing during winter and peaking in spring.^{47,118,172,193,286} However, different epidemiological studies failed to link silage feeding to listeriosis outbreaks, challenging the common perspective that silage constitutes the exclusive source of infection.^{33,155,187,289,308} Moreover, studies in the southern hemisphere frequently report listeriosis cases in ruminants unrelated to silage feeding and occurring during the warmest months of the year or during the transition from rainy to dry season.^{237,247,248,256} As listeriosis cases can occur all year round in both hemispheres and with different diets, it appears likely that additional factors contribute to infection.

Predisposing Factors for *Lm* Infection

While in humans clinical listeriosis has a clear predilection for defined risk groups collectively known as YOPI (young, old, pregnant, immunosuppressed),^{45,104,119,278} predisposing factors in farmed ruminants have yet to be clearly identified.

Concomitant stressing factors, such as late pregnancy or overcrowding, have been proposed to constitute predisposing factors for listeriosis,^{51,125,180,205,306} but the extent and mechanisms through which they contribute to disease onset have not been systematically explored. Season and spoiled wet feed due to climatic changes, particularly heavy rains, together with stressing factors including overcrowding and inadequate animal management have also been speculated to increase the incidence of listeriosis.^{196,245,289,301}

Molecular Mechanisms of *Lm* Intracellular Lifestyle

The pathogenic potential of *Lm* relies on the intracellular survival and replication of this microbe. For the intracellular life cycle of *Lm* (Fig. 3), PrfA (positive regulatory factor A), the transcriptional factor initiating the transcriptional switch from the saprophytic (extra-host) to the intra-host infectious stage, is essential. The expression of PrfA itself is thermo-regulated and becomes efficient at mammalian body temperature (37 °C).²⁹² PrfA induces the transcription of virulence factors located on the crucial *Listeria* pathogenicity island (LIPI-1) (eg, *hly* [LLO], *actA*, *plcA*, *plcB*, and *mlp*), and additionally virulence factors outside LIPI-1 (eg, *inlA*, *inlB*, *inlC*, and *hpt*) that altogether are essential for the intracellular infection cycle.^{58,165,290}

While *Lm* enters phagocytes via phagocytosis, it initiates internalization in non-phagocytic cells through a process called receptor-mediated endocytosis,¹⁹ primarily via 2 virulence factors, internalin A (InlA) and B (InlB).^{70,108} Both internalins bind to eukaryotic cell membrane receptors, InlA to E-cadherin and InlB to Met, gC1QR, and proteoglycans, respectively.¹⁹

Following internalization, *Lm* is temporarily confined to a primary vacuole, from which it escapes prior to phagolysosomal fusion via membranous pores that are formed by listeriolysin O (LLO). This process is facilitated by the 2 phospholipases PlcA (phospholipase A) and PlcB (phospholipase B). Vacuolar escape enables *Lm* to avoid phagosomal degradation, which is essential for *Lm* virulence, as shown by the strong attenuation of mutants in which LLO is deleted. Once in the cytosol, the bacterium starts rapidly replicating using nutrients acquired from the host cell. Actin assembly-inducing protein (ActA) promotes intracellular bacterial motility and cell-to-cell spread by hijacking and polymerizing actin from the host cytoskeleton.¹⁵⁹ Polymerized actin can be identified as “actin clouds,” surrounding *Lm*, or as polar filaments, termed “actin tails,” which enable *Lm* movement within the cytosol (Fig. 4). By propelling forward, *Lm* may arrive into a neighbor cell within a double-membrane vacuole (named a secondary vacuole) and reinitiates a new cycle escaping the vacuole. The dogma of *Lm*'s canonical intracytosolic infection cycle was recently challenged by several studies that discovered various intravacuolar infection stages associated with bacterial survival and persistence in different phagocytic and non-phagocytic cell types.^{21,163,225} It has been shown in phagocytes²¹ and more recently in epithelial cells²²⁵ that bacteria-

secreting reduced amounts of LLO at early stages of infection remain entrapped in large vacuolar compartments named spacious *Listeria*-containing phagosomes (SLAPs), where they can replicate at a slow pace.²¹ Another mechanism of intravacuolar persistence associated with slow replication (*Listeria*-containing vacuoles, LisCV) occurs subsequently to downregulation of bacterial ActA expression in the cytosol during long-term infections (2–3 days post-infection) of epithelial cells.¹⁶³

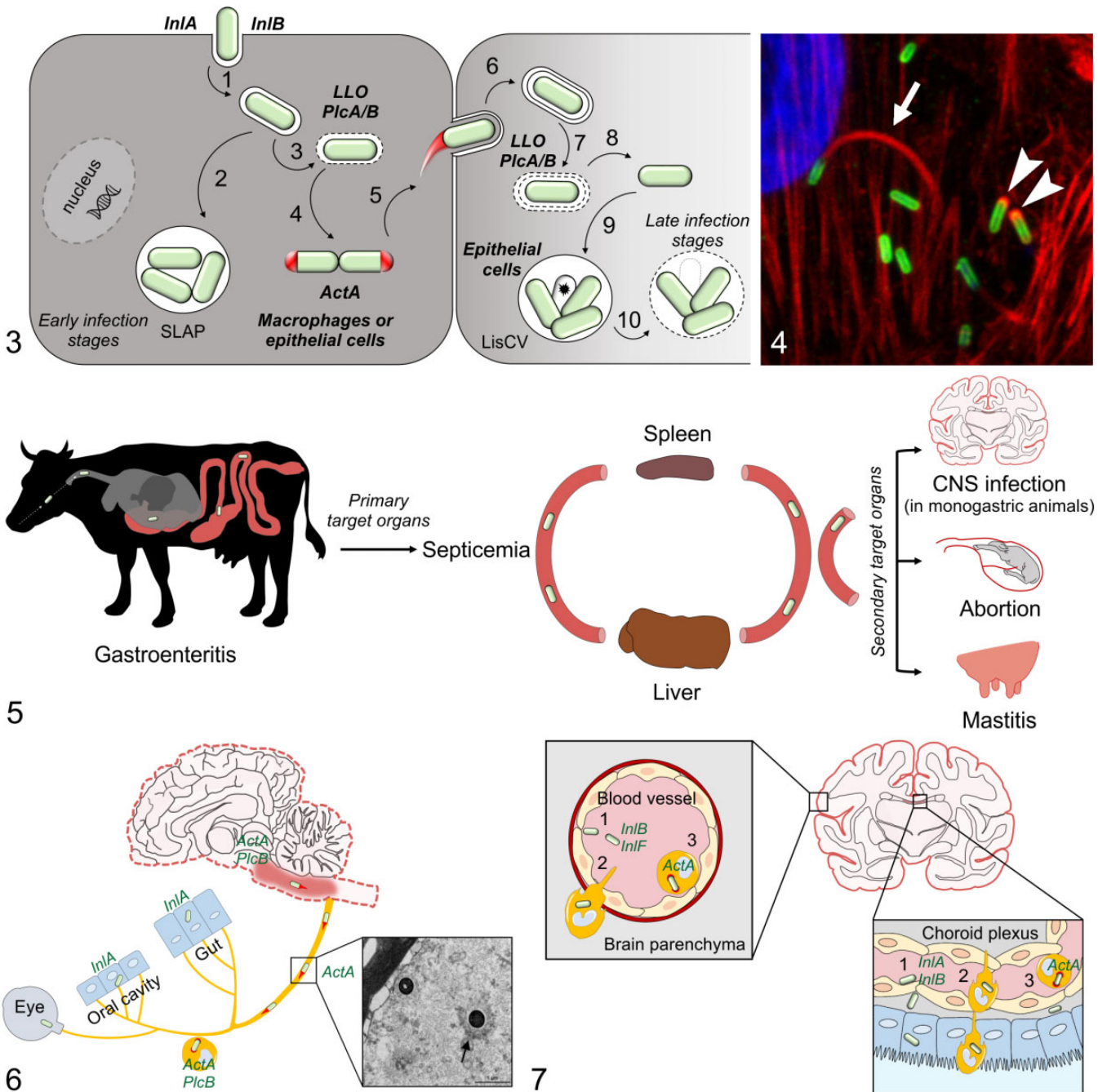
The previously mentioned virulence factors are also essential for host infection, as knock-out mutants for key virulence genes (eg, *prfA*, *hly*, *actA*, internalins-encoding genes)^{20,31,191,215,253} are almost completely attenuated in vivo. However, it remains open for exploration whether they are involved in different invasion routes and organotropism of *Lm*. The vast arsenal of additional genes in the *Lm* genome provides appealing candidates to potentially explain the versatility of *Lm* to exploit different entry routes and reach various organs. However, the impact on host infection of most genes is currently unknown, and the function of others that have been recently associated with enhanced virulence (eg, LIPI-4)¹⁹² requires further investigation.

Listeriosis: Clinical Disease, Pathology, and Pathogenesis

Listeriosis occurs most commonly upon oral infection, but can rarely result from local bacterial implantation at body surfaces (keratoconjunctivitis, dermatitis) or from ascending infection of the genital tract.^{125,236,262,294} Following oral infection, *Lm* may colonize the gastrointestinal (GI) tract and may either be shed from subclinical carriers or cause self-limiting enteritis, although *Lm* frequently crosses the GI barrier and causes invasive disease. Current research indicates that bacterial crossing may happen at the intestinal or oral cavity level, depending on the host and clinical form. The main forms of invasive listeriosis include septicemia, fetomaternal/perinatal infection, and CNS disease (Fig. 5), which tend to occur separately and are rarely concomitant in affected individuals or in the same herd or flock.^{180,181} The reason why different listeriosis forms seldom overlap remains unknown. As the neurologic manifestation of the disease (neuroinfection) is especially relevant in ruminants, it will be discussed in this review more extensively than other listeriosis forms.

Enteric Listeriosis

Lm can colonize the GI tract following ingestion via food or feed, but underlying pathomechanisms are yet to be fully elucidated. Survival in the inhospitable GI environment requires resistance against gastric and biliary acids, which is provided by a complex interplay of genes that are mainly coordinated by the stress-responsive sigma factor SigB (σ^B) and PrfA, among others (reviewed in Davis et al⁵⁶, and Gahan and Hill¹⁰⁷). Additionally, *Lm* needs to escape from the control mechanisms of the commensal microbial community.



Figures 3–7. *Lm* intracellular lifestyle and host infection pathways. **Figure 3.** *Lm* intracellular lifecycle. Bacterial internalization in non-phagocytic cells is mediated by internalin A (InlA) and internalin B (InlB) (1), following which *Lm* is enveloped in a single-membrane primary vacuole. In macrophages and epithelial cells, *Lm* may persist and multiply in non-acidified vacuoles (spacious *Listeria*-containing vacuoles, SLAPs) (2). Alternatively, in the canonical intracytosolic lifecycle, *Lm* escapes from the primary vacuole by secreting listeriolysin O (LLO) and PlcA/B (3). Free bacteria in the cytosol multiply and polymerize actin through actin assembly-inducing protein (ActA) in order to avoid autophagy (4) and spread to neighboring cells (5). In the new cell, *Lm* is enveloped in a double-membrane secondary vacuole (6), which is again lysed by LLO and PlcA/B (7) allowing for vacuolar escape into the cytoplasm (8). Following this phase, *Lm* can be recaptured in acidic vacuoles (*Listeria*-containing vacuoles, LisCVs) through xenophagy-like processes in epithelial cells (9). A subpopulation of intravacuolar bacteria may resist degradation and slowly multiply, while few others degenerate (asterisk). *Lm* can then escape from these vacuoles and re-initiate an infectious cycle. **Figure 4.** *Lm* (green) infection in an epithelial cell line (Caco-2 cells, blue: DAPI-stained nucleus). Intracytosolic bacteria polymerize actin (red) as polar "actin clouds" (arrowheads) or propulsive "actin tails" (arrow). Fluorescence microscopy. **Figure 5.** Schematic *Lm* infectious cycle in the host. Bacteria access the host through ingestion and transit across the GI tract, potentially causing gastroenteritis. Once *Lm* crosses the GI barrier it spreads hematogenously to its primary target organs (liver and spleen). If the infection is not cleared in these sites, septicemic spread to secondary target organs results in meningoencephalitis (in monogastric animals), fetoplacental infection, and mastitis.

Molecular pathogenesis of *Lm* enteritis in mouse models. In orally infected mice, enteric colonization is facilitated by the bacteriocin listeriolysin S (LLS), expressed by a subset of lineage I strains.²⁴⁰ Whether luminal colonization is sufficient to cause isolated enteritis or whether enteritis requires invasion and/or crossing of intestinal epithelium is not fully clear. Similarly, bacterial factors involved in enteritis remain to be identified. Factors enhancing or restricting bacterial invasion of the intestinal barrier are not entirely known. Experimental models provide evidence that *Lm* translocates the intestinal epithelium (via interaction of the major *Lm* internalins, InlA and InlB, with their respective cell receptors) without causing significant intestinal inflammation and damage to the intestinal barrier.^{173,283} InlA and InlB bind to their cell receptors with variable affinity in different species (reviewed in D’Orazio⁵², Drolia and Bhunia⁸¹, and Hoelzer et al¹⁴²), with ruminants and humans allowing for both InlA- and InlB-mediated cell entrance. On the other hand, mice are quite resistant to GI crossing due to a single amino-acid polymorphism in their E-cadherin, which impairs its affinity for InlA.¹⁷² In this species, *Lm* was shown to primarily enter M cells residing in Peyer’s patches by either InlB-mediated endocytosis or macropinocytosis.⁴⁴ In mice expressing “humanized” E-cadherin, bacterial InlA binds luminally expressed E-cadherin on intestinal goblet cells, allowing for bacterial crossing of the intestinal epithelium by exocytosis into the lamina propria at the villus level.²⁰⁶ It has been proposed that *Lm* additionally accesses luminal E-cadherin exposed at villus tips during epithelial renewal.²²⁴ Last, LAP (*Listeria* adhesion protein) was shown to cause the opening of cellular junctions and bacterial crossing of the intestinal barrier upon binding to its receptor Hsp60 on intestinal epithelial cells in mice, independently of InlA/E-cadherin interaction.⁸² Intestinal crossing into the lamina propria is relatively silent in terms of inflammation,²⁸³ while bacterial entry into the Peyer’s patches triggers a strong inflammatory response¹⁷³ and proliferation of intestinal epithelium with resulting loss of goblet cells and decrease of the mucus layer thickness.⁶⁴

Enteritis in natural hosts. Mechanisms of bacterial interaction with the ruminant GI tract, on the other hand, are completely unknown. Clinically associated strains, however, often display marked lysozyme resistance together with enhanced

invasiveness.^{22,302} It has therefore been speculated that these strains might better survive abomasal passage and are more efficient in invading conjunctival and caruncular epithelial tissues.

In ruminants, the enteric location of *Lm* is most commonly associated with prolonged fecal shedding in asymptomatic animals. However, acute enteric listeriosis has been reported in sheep and cattle of different age.^{47,93,94,109,317} Affected animals may show lethargy, anorexia, hyperthermia, and diarrhea. Clinical enteric listeriosis is associated with abomasitis and enteritis consisting of multifocal neutrophilic infiltrations, which are strikingly centered on the muscularis mucosae where bacteria reside inside myocytes.^{47,93,94,109} The cause and mechanisms of muscular targeting are not known. Associated fibrinosuppurative mesenteric lymphadenitis with intralesional bacteria further supports bacterial crossing of the GI barrier during enteritis.^{93,94,109} Moreover, small parenchymal pyogranulomas, mononuclear periportal infiltration, or foci of coagulative necrosis were observed in the liver following enteritis.^{93,94,109} Orally infected sheep, however, carried bacteria in the spleen, liver, and lymphoid organs in the absence of clinical signs,³¹⁷ indicating that *Lm* intestinal infection and translocation to visceral organs may occur asymptotically. As asymptomatic fecal shedders are relatively common and overt disease is infrequently identified, clinically evident enteric listeriosis in ruminants seems to constitute a fairly exceptional event.

In humans, GI colonization can also be asymptomatic with bacterial shedding in feces.^{129,201} Alternatively, it can lead to self-limiting gastroenteritis with acute clinical manifestation characterized by fever, diarrhea, and arthromyalgia. These signs can be prodromic to invasive infection in predisposed patients.²¹² Pathological data, unlike in ruminants, are lacking, possibly due to the self-limiting nature of the disease. Therefore, cellular targets of *Lm* in human gastroenteritis remain unknown.

Listerial Septicemia

Following intestinal breaching, *Lm* enters a bacteremic phase in which it spreads hematogenously to the viscera, mainly liver and spleen.^{125,186,317} It is assumed that hepatic and splenic infection can be either temporary in asymptomatic animals or provide a replication niche for further septicemic spread if the

Figures 3–7 (Continued). Ocular and cutaneous listeriosis resulting from direct *Lm* implantation also occur and are not depicted in the diagram. **Figure 6.** Proposed neural invasion route in rhombencephalitis. Bacteria access nerves following penetration through mucocutaneous barriers. Centripetal migration from the periphery to the brainstem occurs intraaxonally and is mediated by actin polymerization (inset: transmission electron microscopy image showing intraaxonal *Lm*, one of which is surrounded by polymerized actin [arrow]). Following access to the brainstem, *Lm* spreads within the brain causing rhombencephalitis. Virulence factors putatively involved in the neural invasion route are indicated in green (see main text for further details). **Figure 7.** Proposed hematogeneous routes in blood-borne neuroinfection. Bacteria access the brain by breaching the blood-brain barrier (BBB) (left) or the blood-cerebrospinal fluid barrier (B-CSFB) (right) in 3 possible ways. Blood-borne extracellular *Lm* are directly internalized in endothelial cells of the BBB or the B-CSFB, respectively, and from there access the meningeal or neuroparenchymal space or the choroid plexus (CP) epithelium and then the CSF compartment by cell-to-cell spread (1). Alternatively, infected leukocytes cross the BBB or B-CSFB carrying *Lm* into the neuroparenchyma or CSF compartment (2). Last, leukocytes may carry peripherally phagocytosed *Lm* to the cerebral or CP endothelium and interact with the endothelium allowing *Lm* spread from the phagocyte to endothelial cells (3). Virulence factors putatively involved in hematogeneous brain invasion are indicated in green (see main text for further details).

bacterium overcomes local innate immunity.^{262,317} In the latter case, systemic spread and colonization of other organs, notably placenta and brain, can occur (Fig. 5).²⁹⁴ Pathomechanisms of listerial septicemia are largely unknown. Recent evidence in experimental models suggests that hypervirulent strains are apt to thrive for a longer time in spleen and liver of infected mice, thus increasing the chance of secondary bacteremia with infection of target organs,²⁹⁵ which might explain the propensity of such strains to cause invasive disease.

Septicemic listeriosis is best known in humans, affecting about one third of patients with invasive disease. It is potentially associated with fatal complications such as disseminated intravascular coagulation and multi-organ failure.^{67,262} Focal infections after septicemic events include myocarditis and valvular endocarditis, hepatitis and cholecystitis, splenic abscessation, peritonitis, osteomyelitis, arthritis, pneumonia, and endophthalmitis.^{67,260,262} In non-ruminant mammals, septicemic listeriosis occurs more frequently than other forms of the disease. In ruminants, septicemia occurs mainly in perinatal or juvenile infections and manifests with hyperthermia, anorexia, and diarrhea.^{125,180,181,306} Although clinically evident septicemia is uncommon in adult ruminants, placentitis often occurs as a frequent sequela of systemic bacteremia.

Fetomaternal Listeriosis

Lm manifests a particular tropism for the pregnant uterus, which is rapidly colonized.^{30,125,180,294,306} In ruminants, fetal infection can develop from transplacental hematogenous transmission and inhalation of contaminated amniotic fluid, usually leading to stillbirths, while in humans it has also been proposed to develop from ascending infection from the maternal lower reproductive tract.^{180,236,306} It is unknown if the latter pathway also occurs in ruminants. Mechanisms of listerial invasion of the pregnant uterus are not yet fully understood.²⁹³ Studies have commonly relied on in vitro models of placental trophoblasts, placental explants, or in vivo infection of pregnant animals (most commonly rodents).^{39,182,236} These studies identified a variable role for InlA- and InlB-mediated cell entrance, depending on species-specific permissiveness, and a role for LLO and ActA in placental replication and placental-fetal spread, respectively.^{39,182} Epidemiological data support InlA's role in placental invasion of pregnant women, as clinical isolates from abortions invariably express nontruncated InlA.¹⁴⁹ Additionally, InlP, a recently identified *Lm* virulence factor, appears to be essential for placental invasion in pregnant rodents, and its deletion significantly attenuates bacterial growth in human placental organ cultures.⁹⁸ InlP interacts with the cytosolic protein Afadin, involved in cell-cell junctions, on the basal face of polarized epithelial layers, promoting bacterial transcytosis through the formation of actin-rich protrusions.⁹⁷ The role of the previously mentioned virulence factors in ruminants has not been characterized, as in vitro models of ruminant placental infection are only recently being developed.^{22,249,253,254} As hypervirulent strains (CC1, CC4-CC217, CC6,²²¹ CC14,²⁷⁷ CC59²²) are associated with abortions,

knowledge on pathomechanisms of fetoplacental infection in ruminants might benefit from investigation of such strains in these in vitro systems.

Abortion in natural hosts. Although experimental inoculation of *Lm* in pregnant ruminants has been shown to be capable of causing abortion regardless of the gestational stage,^{125,142} it is not known whether and how frequent early embryonic death occurs in invasive infection.²³⁶ Abortions in ruminants typically ensue during the third trimester of pregnancy, either sporadically or as outbreaks.^{125,181,306} Infection at early third trimester may cause fetal death and placental retention with minor maternal sequelae, while near term infection potentially causes serious complications for pregnant dams including dystocia, severe metritis, and septicemia.^{30,180,259,306} The affected placenta shows multifocal cotyledonary necrosis and exudative intercotyledonary placentitis, while aborted fetuses are usually autolytic and manifest miliary foci of necrosis in various organs, especially in liver and spleen, and severe necrotizing enteritis.^{180,259} Fetal lesions are indicative of oral infection, as they reflect the distribution observed in listerial septicemia. Perinatal listeriosis in animals typically develops within 2 weeks from birth, following in utero infection, and usually manifests with multiple necrotic foci in the liver, spleen, and other organs.^{137,180,272,306} Occasionally, it can manifest as neurolisteriosis characterized by fibrinosuppurative meningoencephalitis with vasculitis, thrombosis, perivascular cuffs, and microabscesses, the latter predominating in the brainstem.^{137,272} Of note, *L. ivanovii* can also be responsible for fetomaternal infection with a similar clinicopathological presentation in ruminants, albeit less frequently than *Lm*,^{6,274} but has not been associated with neurolisteriosis.

In humans, maternal infection commonly presents with mild and unspecific signs of malaise, chorioamnionitis and preterm delivery, miscarriage, stillbirths, or fetal death.^{185,236} Neonatal listeriosis results in septicemia and encephalitis in infants, with typical widespread multifocal granulomas (granulomatosis infantiseptica) occasionally seen in early-onset cases.^{185,236}

Listerial Mastitis

Mastitis caused by *Lm* has been reported exclusively in ruminants, ranging from subclinical chronic interstitial inflammation to severe suppurative inflammation.^{27,118,284,311} These infections are thought to arise hematogenously or through local invasion via the teat canal. It is currently not known whether it also occurs in other species, and molecular mechanisms underlying mastitis and *Lm* interaction with the mammary epithelium remain completely unexplored. Infected animals respond poorly to treatment. In latent infections transient bacterial excretion in milk may occur for prolonged periods.^{118,307} Subclinical bacterial shedding in milk has been reported in cows, ewes, and goats in both natural and experimental infections, and its association with contaminated milk products has been proposed in numerous studies as a source for human infection.^{2,105,145,220,231,265,284} Hypervirulent strains belonging

to CC2, CC4, and CC11, in particular, were found to be significantly associated with subclinical mastitis in dairy cattle.²²⁰ Hypervirulent CC1 strains, however, were isolated most commonly from dairy products and were more efficient in colonizing the GI tract in infected mice.¹⁹⁰ Hence, further studies on mastitis- and milk-associated clones are required to clarify whether dairy products are possible relevant vectors between “farm” and “fork.”

Cutaneous and Ocular Listeriosis

Cutaneous and ocular listeriosis are rare forms and occur following direct bacterial implantation in the absence of enteric infection and bacteremia. Cutaneous listeriosis has been observed in humans exposed to infected abortive material from ruminants and presents as mostly self-limiting papulo-pustular dermatitis, cellulitis, or skin abscesses. It may also occur in immunosuppressed and elderly patients unrelated to any contact with animal sources. Moreover, *Lm* has been sporadically isolated from “pox-like” skin lesions in pigs and from a dog with papulo-pustular dermatitis.^{125,178,194,230}

Listerial keratoconjunctivitis and uveitis is rather common in ruminants (with a reported farm prevalence of up to 8.6%),^{87,88} especially in cattle, as outbreaks or sporadic cases.^{87,167,170} Sporadic cases have also been rarely reported in horses.^{92,246} The infection most likely arises from direct conjunctival implantation of contaminated material during feeding and is strongly associated with big bale silage and ring feeding (hence the name “silage eye”).⁸⁸ Listerial keratoconjunctivitis is only rarely described in immunocompetent human patients, most frequently following exposure to farm environment, suggesting silage or infected animals as the origin for the infection.¹⁴³ Barely anything is known about pathogenetic mechanisms of listerial invasion into ocular tissues. Interestingly, a frequent involvement of the oculomotor nucleus in ruminant neuroinfection cases suggests that conjunctival tissue might provide a bacterial port of entry in neuroinfection,²⁰⁹ although experimental conjunctival instillation of *Lm* in various animal species could only rarely produce encephalitis.¹²⁵

Neuroinfection

CNS infection has been reported in many species and is a significant problem in humans and domestic ruminants. Despite its low incidence, averaging 1 to 11 cases/million persons annually, listeriosis accounts for the highest fatality rate among all food acquired illnesses in humans.^{60,195,275} Neuroinfection, which occurs in up to 79% of non-perinatal and 19% of perinatal cases, respectively, considerably contributes to mortality, being associated with fatality rates of 17% to 30% in spite of antimicrobial treatment.^{29,40,60,203,275} Unfortunately, similar data for ruminant listeriosis are unavailable, as large-scale surveillance studies are currently lacking. Moreover, in contrast to humans, animal listeriosis is not included among notifiable diseases in reporting systems of many countries,

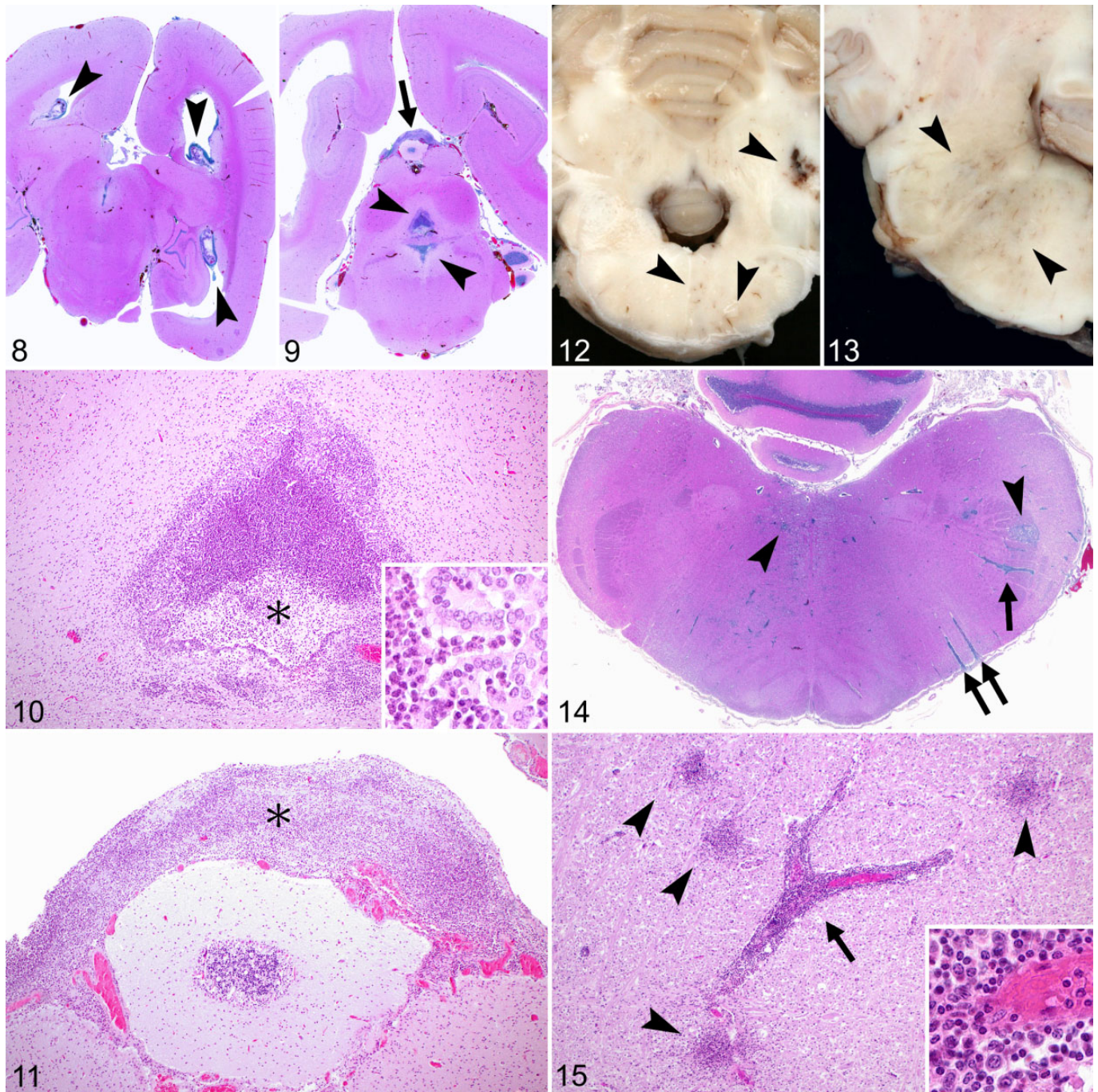
which severely hampers the possibility of accurately estimating its incidence.

Neuroinfection manifests in pathologically distinct forms, which is clearly indicative of different neuroinvasive pathomechanisms among species (Figs. 6, 7). In humans and monogastric animals 3 forms can be distinguished, of which meningitis/meningoencephalitis is the most frequent manifestation (Figs. 8–11), while brain abscessation and brainstem encephalitis (rhombencephalitis) occur less commonly.^{15,125,306} By contrast, rhombencephalitis is clearly the most common phenotype in ruminants (Figs. 12–39).²¹⁰ As the pathogenesis of the various neuroinfection forms is largely reflected in the pathological phenotype, the pathology will be discussed prior to the pathogenesis.

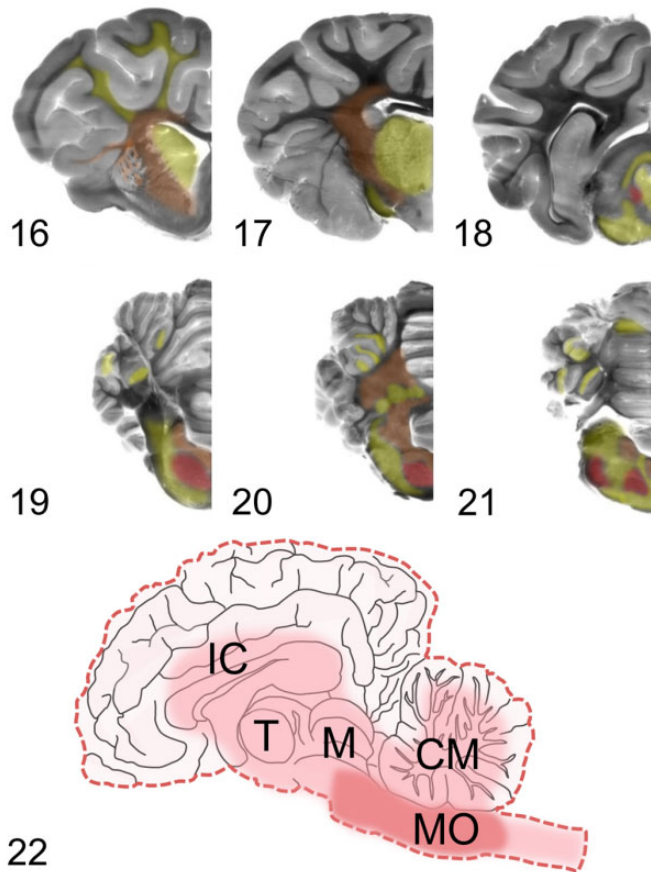
Rhombencephalitis: Incidence and Clinical Disease

Initially described as “circling disease” in sheep,¹¹⁶ rhombencephalitis occurs worldwide in farmed small ruminants and cattle, and accounts for the vast majority of invasive clinical infections caused by *Lm* in these species.^{63,198,309} Data from numerous retrospective studies and surveys of transmissible spongiform encephalopathies (TSE) rank neuroinfection among the most frequent neurological diseases affecting ruminants. The prevalence among CNS diseases ranges between 8% and 35.8%,^{3,115,146,174,193,208,282} and small ruminants appear to be more susceptible than cattle. The disease occurs either as single or multiple cases, including outbreaks (most frequently seen in sheep and goats), in a herd or flock.^{125,126,180,198,289,309} There appears to be no particular sex-, breed-, or age-related predisposition. Some authors speculate that most cases occur concomitantly with tooth loss and eruption,^{14,126,198} while other studies describe the highest prevalence of rhombencephalitis cases beyond teething.^{208,209}

Clinical manifestations of rhombencephalitis in ruminants. In wild, farmed, and other domesticated ruminant species, CNS infection follows the pattern of rhombencephalitis in adult animals and septicemic episodes in young animals.^{50,125,137,237,306} Clinical signs of rhombencephalitis are similar in all ruminant species and commonly appear unrelated to stressful conditions or any other clinical sign.^{198,210,289,309} Several studies claim a prolonged incubation period lasting between 2 and 6 weeks. However, neurological signs generally manifest acutely and progressing rapidly, more so in small ruminants than in cattle. Fever can be present during early phases of infection but is not constant.^{5,30,125,180,181} Typically, rhombencephalitis signs in ruminants manifest as unilateral or, less frequently, bilateral brainstem and cranial nerve deficits.^{28,30,180,198,268} Unilateral facial and tongue paralysis are common and result in ipsilateral drooping of ear, eyelid, lip, and muzzle, and hypersalivation, anorexia, and dehydration, respectively. Deficits of the oculomotor, facial, and trigeminal nerves can lead to loss of reflexes, strabismus, secondary exposure keratitis, and rarely blindness. Vestibular signs, consisting of head tilt and nystagmus, appear ipsilaterally, if the brainstem is involved, or contralaterally to



Figures 8–15. Neuroinflammation: principal patterns of meningitis and rhombencephalitis. **Figures 8–11.** Listeriosis, brain, cotton-top tamarin (*Saguinus oedipus*). **Figures 8–9.** Severe suppurative ventriculitis/ependymitis (Figs. 8–9, arrowheads) and meningitis (Fig. 9, arrow). Note the absence of neuroparenchymal lesions. Hematoxylin and eosin (HE). **Figure 10.** Suppurative ependymitis: neutrophils occupy the lumen of the cerebral aqueduct (asterisk), multifocally infiltrating the adjacent neuroparenchyma and causing ependymal erosion and hyperplasia (inset). HE. **Figure 11.** Suppurative meningitis: neutrophils are confined to the subarachnoid space (asterisk) without invading the underlying cerebellar neuroparenchyma. HE. **Figures 12–15.** Listeriosis, medulla oblongata, ruminants. **Figures 12–13.** Sheep. There are multifocal areas of hemorrhage and malacia (arrowheads). **Figure 14.** Sheep. Multifocal linear, deeply basophilic lesions corresponding to perivascular cuffs (arrows) and irregular, variably basophilic lesions corresponding to microabscesses (arrowheads) are present in the neuroparenchyma. HE. **Figure 15.** Cow. Cardinal lesions of rhombencephalitis: microabscesses, recognizable as scattered foci of phagocytes infiltrating the neuroparenchyma (arrowheads), and a perivascular cuff (arrow), predominantly consisting of mononuclear cells accumulating in the perivascular space (inset). HE. Microscopic features of microabscesses are illustrated in **Figures 23–26**.



Figures 16–21. Schematic topography of microabscess as investigated in 41 ruminants (cattle, goats, sheep) with neuroleptospirosis (from Henke et al¹³⁹). Brain areas are color-coded: red indicates areas affected by microabscesses in >50% of animals, orange indicates areas affected in 25% to 50% of animals, and yellow areas affected in <25% animals, respectively. **Figure 16.** Cerebral hemisphere and corpus striatum. Microabscesses are frequently located within white matter tracts, especially of the internal capsule (orange), and less frequently within the corona radiata and caudate nucleus (yellow). **Figure 17.** Cerebral hemisphere and thalamus. Microabscesses most frequently involve white matter tracts of the internal capsule (orange), but also affect the thalamic nuclei and optic tract (yellow). **Figure 18.** Cerebral hemisphere, midbrain, and hippocampus. The fasciculi tegmenti are heavily targeted (red), followed by various white matter tracts and nuclei with their associated fibers. The hippocampus and cerebrum are spared. **Figures 19–21.** Brainstem and cerebellum. The reticular formation (Fig. 19 [pons level], Figs. 20, 21 [rostral and caudal medulla oblongata]) and the spinal tract of cranial nerve (CN) V (Fig. 21) are frequently affected (red). Less frequently affected structures include the rostral cerebellar peduncle (Fig. 19), the medial longitudinal fasciculus (Figs. 19–21), nuclei of CN-VIII (Figs. 20, 21), and the corpus medullare of the cerebellum (Fig. 20, orange). Microabscesses may also be observed in other CN nuclei and associated tracts (Figs. 19–21), middle cerebellar peduncle (Figs. 19, 20), cerebellar nuclei (Fig. 20), and folia (Figs. 19, 20, yellow). **Figure 22.** Schematic representation of microabscess frequency in rhombencephalitis in the sagittal view. The brainstem (dark red) is targeted most extensively, most severely and more chronically than other brain areas (light red) (IC, internal capsule; T, thalamus; M, midbrain; CM, corpus medullare; MO, medulla oblongata).

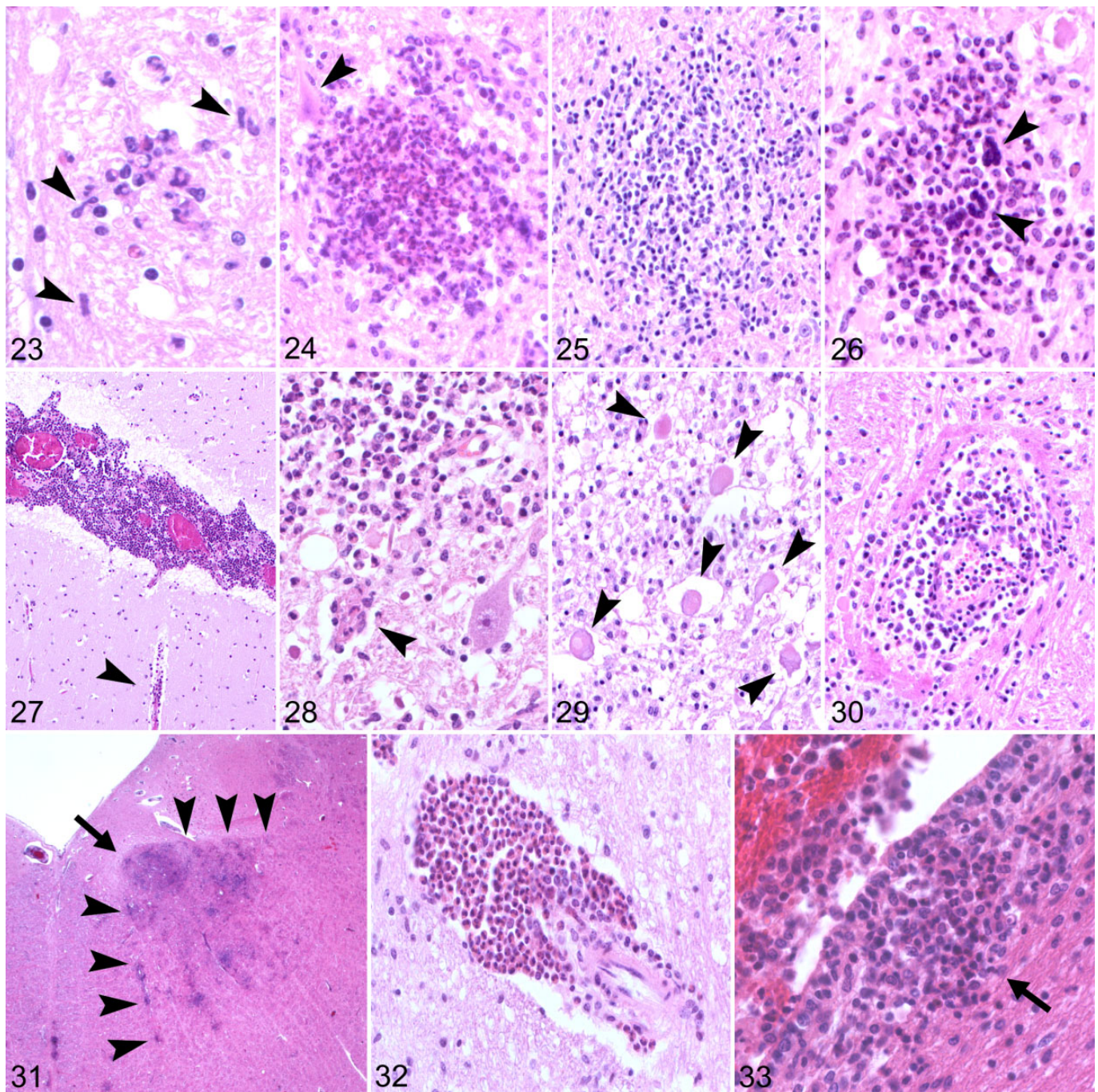
the side of the lesion if cerebellar peduncles are affected. The topography of the brainstem and cranial nerve lesions (V–XII) is usually responsible for the variability in clinical manifestations (Table 1). Delayed proprioception is common, superficial sensitivity is generally reduced, and spinal reflexes can be weak or absent. Small ruminants generally display more severe signs than cattle. If able to stand, they are ataxic and can manifest circling movements (hence the name “circling disease”). In later stages of disease, they often show depression and recumbency, and death can occur within 48 hours. On the other hand, cattle show milder clinical signs (typically head tilt) and tend to succumb later to the disease. Fatality rates are high in spite of treatment, especially if not instituted early.^{30,125,198,309} Rarely, myelitis without brainstem involvement has been reported in sheep, with variable clinical signs ranging from limb weakness to quadriplegia and death.^{112,270} Cerebrospinal fluid (CSF) features can be quite variable, showing either mononuclear or neutrophilic pleocytosis, and *Lm* isolation from CSF fails in up to 90% of cases.^{35,229,269}

Rhombencephalitis in non-ruminant species. In non-ruminant animals, the brainstem can be targeted in meningoencephalomyelitis during septicemia,^{125,306} and rhombencephalitis without generalized brain involvement has only been reported in a cat²⁴² and a horse.²⁵⁵

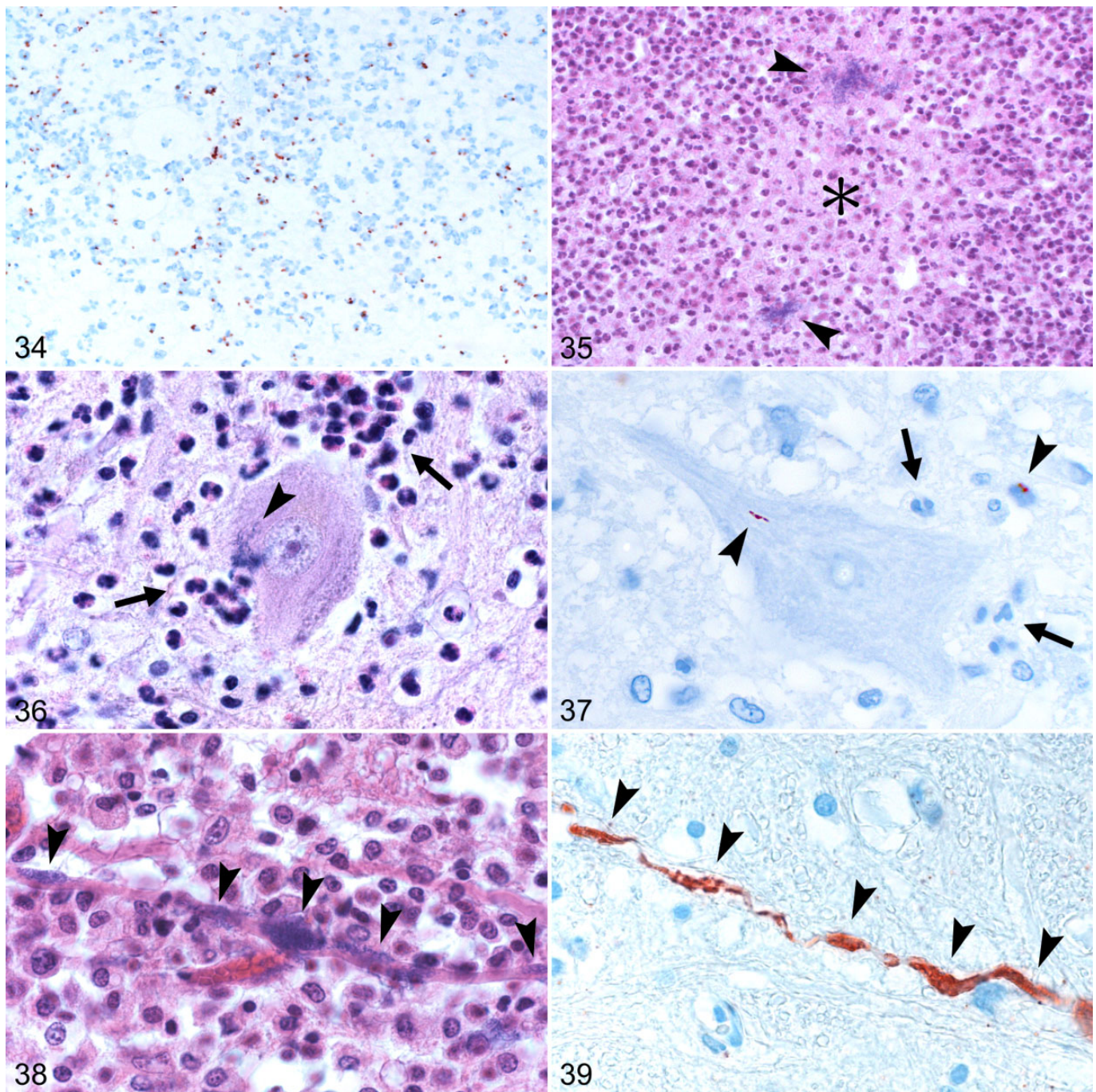
Rhombencephalitis is uncommon in humans, accounting for only 1% to 24% of all neuroleptospirosis manifestations.^{12,15,203,285} Interestingly, several studies report that similar to ruminants and in contrast to meningitis/meningoencephalitis, the vast majority of rhombencephalitis cases occur in individuals without underlying clinical conditions. Clinical signs tend to present in a peculiar biphasic fashion, with prodromic unspecific signs including fever, malaise, headache, nausea, and vomiting lasting up to 2 weeks, followed by rapidly appearing neurological signs indicative of brainstem involvement, unilateral facial palsy being the most frequently recognized.^{12,15,285} Such a biphasic pattern has not been reported in ruminant rhombencephalitis.

Neuropathology of Rhombencephalitis

The pathology of rhombencephalitis is rather peculiar, particularly regarding the topography and nature of its inflammatory lesions. The brainstem is specifically targeted, which is quite unusual for encephalitis. Moreover, cardinal lesions consist of a combination of suppurative/granulomatous foci (so-called microabscesses) alongside mononuclear perivascular cuffs (Fig. 15), which is fairly uncommon for bacterial encephalitis, but consistent with an intracellular microorganism. Lesions in ruminant rhombencephalitis are generally quite massive. They can span from the medulla oblongata and pons to regions localized caudally (cervical spinal cord) and rostrally (cerebellum, midbrain, thalamus, exceptionally hippocampus, basal nuclei, and cerebral cortex) in the affected animal.^{5,42,49,209,216} Notably, lesions appear to be continuous along the neuraxis, with



Figures 23–33. Listeriosis, brain, ruminants. Histopathological features of rhombencephalitis. Hematoxylin and eosin (HE). **Figures 23–26.** Temporal evolution of microabscesses. **Figure 23.** Midbrain, cow. Very early lesion consisting of a small aggregate of neutrophils and microglial cells (arrowheads). **Figure 24.** Midbrain, sheep. Acute microabscesses mainly contain neutrophils and can be associated with necrotic neurons (arrowhead). **Figure 25.** Medulla oblongata, cow. Subacute to chronic microabscesses are characterized by an increasing number of macrophages and lymphocytes and marked reduction in neutrophils. **Figure 26.** Medulla oblongata, cow. Chronic microabscesses may contain multinucleated giant cells (arrowheads). **Figure 27.** Cerebellum, cow. Mononuclear meningitis. Note the perivascular cuff in proximity to the subarachnoid space (arrowhead). **Figures 28–29.** Midbrain, cow. Neuronal damage in rhombencephalitis. **Figure 28.** Neurons in proximity to microabscesses can undergo neuronophagia (arrowhead). **Figure 29.** Axonal spheroids are often seen in close association with leukocytes (arrowheads). **Figure 30.** Midbrain, cow. Vascular damage in rhombencephalitis indicated by perivascular infiltration of mononuclear cells and fibrin deposition. **Figure 31.** Medulla oblongata, sheep. Microabscesses involve the hypoglossal nucleus (arrow) and the intraparenchymal roots of the hypoglossal nerve (arrowheads). **Figure 32.** Medulla oblongata, cow. Perivascular cuff composed almost exclusively of eosinophils. **Figure 33.** Third ventricle, goat. Subependymal abscess with neutrophils infiltrating the neuroparenchyma (arrow).



Figures 34–39. Listeriosis, medulla oblongata, sheep. *Lm* association with lesions in rhombencephalitis. **Figure 34.** The majority of bacteria are located within a microabscess and in close association with phagocytes. Immunohistochemistry (IHC) for *Listeria*. **Figure 35.** Extracellular bacterial colonies (arrowheads) in the necrotic center of an acute microabscess (asterisk). HE. **Figure 36.** Bacteria inside a neuron (arrowhead). Notice the perineuronal inflammatory infiltrate composed primarily of neutrophils (arrows). **Figure 37.** Bacteria within a neuron (left arrowhead) and a phagocyte (right arrowhead). Neutrophils (arrows) are near the infected neuron. IHC for *Listeria*. **Figures 38–39.** Bacterial colonies inside an axon (arrowheads). HE (Fig. 38) and IHC for *Listeria* (Fig. 39).

generally higher severity and chronicity in the pontomedullary area than in other brain regions (Figs. 16–22).^{42,209}

Microabscesses can range from small, delineated lesions to large coalescing areas, occurring both in gray and white matter. Frequently, they manifest a peculiar pattern in which they

appear to follow the direction of axonal fibers or single cranial nerve nuclei, and rarely lesions affecting both a cranial nerve and its fibers can be observed (Fig. 31). In the brainstem, microabscesses frequently affect the trigeminal spinal, facial, hypoglossal, and oculomotor nuclei and their tracts.^{139,209} The

Table 1. Affected cranial nerve (CN) nuclei and associated clinical signs of *Listeria monocytogenes* infection in ruminants (from Walland et al²⁹⁸).

Affected CN nuclei	Clinical signs
Trigeminal nerve (V)	Chewing difficulties Reduced palpebral and menace reflex Dropped jaw
Facial nerve (VII)	Reduced sensitivity of the head to touch Drooping eyelid, ear, and lip Reduced palpebral and menace reflex
Vestibulocochlear nerve (VIII)	Nystagmus
Glossopharyngeal nerve (IX)	Swallowing difficulties
Vagus nerve (X)	Swallowing difficulties
Hypoglossal nerve (XII)	Tongue paralysis
Vestibular system	Circling, head tilt, leaning toward one side

composition of microabscesses varies with chronicity: early lesions appear as small agglomerations of neutrophils and microglia (Fig. 23), progressing to almost purely neutrophilic microabscesses (Fig. 24) followed by a mixture of neutrophils and macrophages (Fig. 25), and finally by chronic granulomatous lesions where macrophages predominate and in which multinucleated giant cells may be occasionally seen (Fig. 26).^{39,46} Large coalescing microabscesses may have an extensive central necrotic core (Fig. 35), appearing similar to an abscess but devoid of a fibrous capsule, and can progress to frank malacia with infiltration of numerous gitter cells.^{42,49} Associated features include prominent perivascular cuffs predominantly composed of mononuclear cells, gliosis, focal edema, and vascular damage characterized by fibrinoid necrosis of the vessel wall, perivascular exudation of proteinaceous fluid, and hemorrhages (Fig. 30).²⁰⁹ Axonal spheroids are frequently located in proximity to microabscesses (Fig. 29), but it is unclear whether axonal destruction occurs due to direct bacterial axonal damage or as collateral damage due to local secretion of toxic/inflammatory mediators. Neuronal necrosis is also seen in the neighborhood of microabscesses (Fig. 28), and it is unknown whether it is elicited by intraneuronal *Lm*, by neutrophils attacking neurons, or by some other mechanism. Meningitis is also frequently present, often as an extension of local perivascular cuffs or microabscesses (Fig. 27), but mild meningeal lymphocytic infiltrates may occur in sites distant from parenchymal lesions.²⁰⁹ Moreover, cranial nerve ganglioneuritis, especially of the trigeminal nerve, can often be seen ipsilaterally to the parenchymal lesions.^{4,14,42,216} Rarely, ependymitis is observed when microabscesses breach the ependymal barrier (Fig. 33).²⁰⁹

Bacteria in varying numbers are generally associated with phagocytes in microabscesses (Fig. 34) but can also be seen extracellularly, occasionally forming colonies (Fig. 35) and inside neurons, axons, and neuropil, particularly during early stages (Figs. 36–39).^{139,209,216}

Perivascular cuffs and microabscesses tend to be more severe in small ruminants, in which a neutrophilic component of the latter predominates (Figs. 24, 35), which is compatible

with the more fulminant clinical course of the disease in these species.²⁰⁹ Microabscesses containing predominantly macrophages are instead more common in cattle, in which multinucleated giant cells and perivascular eosinophils can be occasionally seen (Figs. 26, 32).²⁰⁹

Despite the inflammation being severe on microscopic examination, gross pathology can be unrewarding, as macroscopic lesions are frequently absent or subtle on cut section. In a number of cases, areas of malacia, presenting as brownish discoloration with or without tissue loss, and multifocal hemorrhages, can be observed (Figs. 12, 13). Rarely, frank abscessation can occur. In contrast, severe inflammation was found to be consistently detected on magnetic resonance imaging (MRI) scans of infected small ruminants, in which the MRI lesion distribution was rather specific for neurolisteriosis.²³⁹ However, despite the diagnostic value of MRI for *in vivo* diagnosis in ruminants, associated costs impede its wide use in farm animals.

The overall pattern and distribution of rhombencephalitic lesions in human patients closely resemble ruminant rhombencephalitis, indicating similar pathogenetic mechanisms in both hosts.^{11,210}

Pathogenesis of Rhombencephalitis

Route of invasion. The mechanisms through which *Lm* selectively targets the brainstem in naturally infected hosts, especially ruminants, have been the object of speculation for decades. Very little is known about the pathogenesis of rhombencephalitis, partially due to the lack of adequate experimental animal models, although several rhombencephalitis models have been proposed over the years.^{5,8,7,23,154,273} Nevertheless, pathological specimens provide strong evidence that the infection route differs from septicemic listeriosis and meningitis/meningoencephalitis. Some authors have suggested a hematogenous origin for the infection, given the distribution of the inflammatory infiltrate and the association of early microglial foci with bacteria in the vicinity of parenchymal microvasculature.⁴⁹ The nature, distribution, and evolution of lesions in rhombencephalitis, however, is highly suggestive of a localized *Lm* centripetal intraaxonal spread from peripheral sites to the brainstem (Fig. 6).^{5,11,14,43,209,216,303} Lesions are characterized by brainstem targeting with a distinct pattern of involvement of cranial nerve nuclei and their root fibers,^{11,209} a feature that would not be expected from a hematogenous invasion route during septicemia, which generally causes random lesions throughout the whole brain. Additionally, dating of lesions reveals a consistent pattern with most chronic lesions in the brainstem and more acute lesions in the rostral brain, indicating that the infectious process originates in the brainstem and subsequently spreads to remote areas. Marked widespread meningitis and ependymitis, typically seen in blood-borne neurolisteriosis (Figs. 8–11), is lacking in rhombencephalitis (Fig. 14). Moreover, cranial nerve ganglioneuritis, especially of the trigeminal nerve, frequently occurs ipsilaterally to the CNS lesion and is often more chronic, which is not suggestive of

hematogenous spread.^{5,14,42,216} Bacteria may be observed unilaterally in cranial nerve axons and ganglia of naturally infected animals and humans, as well as their intra-encephalic fibers and nuclei, once again suggesting localized instead of hematogenous spread.^{11,42,209,216} Taken together, the absence of concomitant systemic infection, lack of generalized brain and cranial nerve involvement, and the presence of bacteria in intact axons strongly indicates that the infection does not arise hematogenously or spread peripherally to the nerves from infectious foci within the brain.^{5,14,42,216} Experimental modeling of rhombencephalitis in animals has been attempted through various routes,²¹⁰ but could only be replicated via feeding of bacteria-soaked abrasive foodstuffs or bacterial injection in the lip, conjunctival sac, dental pulp, or snout in small ruminants and mice, indicating that *Lm* can access nerve endings after breaching of mucocutaneous barriers.^{5,10,14,154} Additionally, mice injected with *Lm* in the triceps surae, or sciatic nerve developed ascending unilateral myelitis unless nerve resection was performed, further indicating that bacteria can efficiently spread to the CNS via intra-axonal migration.^{10,154}

Mechanisms of *Lm* entrance into nerves. Although it is rather clear that bacteria can access the brainstem via nerve fibers, it remains uncertain how *Lm* enters the nerve fibers themselves and whether invasion of the neuraxonal compartment involves direct interaction with neuronal receptors or receptor-independent cell-to-cell spread. While intraneuronal and intra-axonal bacteria can be observed in natural cases,^{41,42,139,184,209} *Lm* does not appear to be particularly neurotropic in vitro. Infection of ruminant and rodent dissociated brain cell cultures and organotypic brain slices indicates that neurons are not heavily targeted in contrast to other cells (notably, microglia) and are variably infected depending on their anatomical origin and culturing methods.^{69,71,133,227,228} An InlA-dependent mechanism of invasion of cranial nerves has been proposed in ruminants, in which *Lm* may initially invade E-cadherin expressing oral epithelium or myelinating Schwann cells and subsequently spread to neighboring axons.¹⁸⁴ Phagocytes have also been hypothesized to provide a source for axonal infection by ActA-dependent bacterial cell-to-cell spread.⁷¹ Moreover, experimental infections in mice suggested a role for PlcB-dependent spread from peripheral macrophages to the trigeminal nerve.^{153,154} Whether the mechanisms mentioned above are also relevant for naturally occurring infection in natural hosts is yet unknown.

Mechanisms of intra-axonal and intra-encephalic spread. Actin polymerizing bacteria have been observed inside axons of pathological specimens and ActA-dependent intraneuronal migration has been reported to occur in cultured bovine neuronal cells, indicating that actin-based motility plays a role in *Lm* intra-axonal spread.^{139,216} This is further supported by the observation of severely impaired neuroinvasion of *actA*-deletion mutants in the murine intranasal infection model.²¹⁸ This study also showed that LLO and, partially, ActA are required

for efficient breaching of the olfactory epithelium prior to the neuronal invasion, indicating their possible role in peripheral barrier breakdown, brain invasion, and spread.

Experimental and pathological evidence suggests that, after entry into the brain, *Lm* efficiently disseminates within the brain. Topographical distribution of microabscesses in naturally infected ruminants suggests that intra-encephalic bacteria spread between anatomically connected brain structures via white matter fiber tracts.¹³⁹ Intra-axonal migration is further supported by bacterial association with axons in naturally infected cases and ruminant organotypic brain slices.^{133,209} ActA-mediated actin polymerization has been observed in neurons in vitro and in situ,¹³⁹ suggesting its participation in intra-encephalic spread, but its role in vivo has only been partially explored.²¹⁸ PlcB-mediated cell-to-cell spread has also been implicated in intra-encephalic spread, as mice intracerebrally infected with a *plcB*-deletion mutant showed prolonged survival, lower bacterial load, and delayed intra-encephalic spread in comparison to a *inlA/B*-deletion mutant that retained a virulence comparable to the wild-type.²⁶³ These findings indicate that PlcB is relevant for neurovirulence in vivo, while InlA and InlB do not play a role during the intra-encephalic stage.

Phagocytes as local bacterial amplifiers? Additional factors involved in intra-encephalic *Lm* dissemination are currently unknown, but a role for phagocytes has been proposed based on observations in natural infections.¹³⁹ Neutrophils and, less frequently, macrophages were shown to access the axonal space and phagocytose intra-axonal bacteria, while a high bacterial load was found inside adaxonal microabscesses, as previously reported in other studies.^{62,139,209} It was therefore suggested that *Lm* could replicate locally in microabscess-associated phagocytes and subsequently reenter the neuraxonal compartment, further propagating the infection. This view is in line with studies in mice and ruminants showing that the innate immune response is inefficient in providing sterilizing immunity in listeriosis, and resolution of infection appears to be dependent on the adaptive immune response.^{18,62,168,257} The ability of *Lm* to survive inside phagocytic cells further supports this view,^{57,158} but the protective role of the immune response or its impact in ruminant neuroinfection remain severely underinvestigated in comparison to murine experimental models in which the immune responses toward *Lm* have been extensively dissected.^{53,189,219} Overall, additional studies are required to unravel the role of phagocytes in bacterial persistence and potential intra-encephalic spread.

Bacterial strains in rhombencephalitis. Further relevant yet unclear aspects of ruminant neuroinfection are the bacterial dose required for establishing infection and potential predisposing factors.²¹⁰ Despite common bacterial exposure, as indicated by widespread/high prevalence of LLO antibodies in the bovine population,²⁴ and frequent asymptomatic fecal shedding,^{89,204,286} only a limited number of animals develop brain disease. It has been suggested that in such cases immune responses mounted against orally acquired bacteria are

inefficient in preventing neuroinvasion.¹⁸⁰ Inefficiency of pre-existing immunity might be partially dependent on the bacterial strain, but its involvement in neuroinvasion in ruminants remains unknown. Interestingly, immunization with a homologous strain induced a protective brain response in mice,²⁶⁴ while outbreak-isolated strains displayed neuroinvasiveness regardless of preexisting immunity toward a reference strain.¹¹⁴ The high prevalence of hypervirulent and hyperinvasive CCs, particularly CC1, in ruminant rhombencephalitis cases could be in line with such observations.^{13,79,132,221,251} Although genomic comparisons identified putative CC-specific neurovirulence factors,^{4,13} their role in hypervirulence was not confirmed.^{122,254} Thus, strain-related *Lm* neurovirulence factors in ruminants require further exploration.

Brain Abscessation

Rarely, *Lm* can cause single or multiple cerebral and cerebellar abscesses in human patients, especially if immunocompromised.^{1,46,48,61,84,203} However, they are not reported to occur with a similar pattern in ruminants. The distribution of brain abscesses strongly indicates hematogenous entry of *Lm* into the brain. However, abscesses in the deep white matter may be aligned along connected white matter fiber tracts suggesting that following hematogenous invasion *Lm* may enter and spread within axons from original foci of infection.^{25,144} This pattern parallels that seen in rhombencephalitis cases in ruminants,¹³⁹ indicating that, no matter how *Lm* enters the brain, it can spread within the brain along axonal pathways and that similar mechanisms of intra-encephalic *Lm* spread are shared between rhombencephalitis and brain abscesses.

Meningitis/Meningoencephalitis: Clinical Disease and Pathology

The most frequent neuroinfection manifestation in humans is meningitis/meningoencephalitis, which predominantly occurs in predisposed individuals (especially elderly and immunosuppressed patients) and is the consequence of hematogenous spread to meninges and brain during bacteremia.^{29,40,203,275} Clinical signs appear between 1 and 14 days following contaminated food consumption^{9,121} and variably include fever, headache, neck stiffness, altered sensation, seizures, and focal neurological signs.^{29,40,203} Severe long-term neurologic impairment persists after recovery and treatment in up to 60% of surviving patients, which can manifest altered consciousness, sensorimotor dysfunctions, or rarely cranial nerve palsies.^{29,40,61,203} Bacterial isolation from hematologic and CSF samples yields positive results in about 60% and 40% of cases, respectively, which is in contrast to the low rate of bacterial isolation in rhombencephalitis.^{29,40,203}

Lesions mainly consist of mild to severe multifocal or diffuse meningeal inflammation with focal cortical infiltration, diffuse ventriculitis with ependymal erosion and focal periventricular infiltration, meningeal and parenchymal vasculitis, thrombosis, and small parenchymal perivascular abscesses.⁸⁶

The inflammatory infiltrate is mostly composed of monocytes/macrophages and neutrophils, with frequent efferocytosis of phagocytes operated by macrophages, while bacteria can be seen intra- and extracellularly in the meninges, parenchymal abscesses, and ependyma.⁸⁶ Neuroinfection in monogastric mammals and birds is rarely encountered and, like in humans, prevalently manifests as meningoencephalomyelitis in the context of septicemia (Figs. 8–11), especially in juvenile animals or in association with concurrent predisposing conditions in adults.^{51,125,242,306} On the other hand, meningoencephalitis is extremely rare in ruminants and is usually observed during perinatal septicemia, in which patterns of hematogenous neuroinfection and rhombencephalitis are concurrently featured,^{137,272} possibly suggesting simultaneous hematogenous and ascending brain invasion.

Pathogenesis of Meningitis/Meningoencephalitis

Little is known about the pathomechanisms involved in blood-borne neuroinfection in naturally susceptible hosts and studies on *Lm* brain infection in monogastric animals other than rodents are virtually nonexistent. Surveys on human neuroinfection are rare,^{8,29,217,223} generally include small patient cohorts and bacterial typing has been rarely performed.^{161,175} Therefore, neuroinvasive strains remain poorly characterized.

Bacterial strains in meningitis/meningoencephalitis. Lineage I hypervirulent clonal complexes (CC1, CC2, CC4, CC6) were shown to be significantly associated with fetomaternal and, notably, CNS infection, possibly indicating their proclivity toward efficient breaching of placental and neural barriers.^{160,192} Experimental *in vivo* infections correlated the novel LIPI-4 gene-cluster (encoding a putative cellobiose-family phosphotransferase system) in CC4 strains to such enhanced invasiveness, but underlying mechanisms are yet unknown.¹⁹² Interestingly, other frequently isolated neurovirulent CCs (eg, CC1) do not possess LIPI-4, indicating that factors specifically involved in neuroinvasiveness remain to be discovered or might differ among hypervirulent strains. Emerging CC6 strains were increasingly found in cases of meningitis with poorer prognosis,^{160,161} possibly due to a plasmid conferring resistance to antibiotics and disinfectants.¹⁶⁶ However, they were less neurovirulent than CC1 strains in mice infected intracisternally and their neuroinvasive mechanisms were not assessed.¹⁶² Thus, further studies on listerial meningitis isolates are needed to characterize strain-specific factors involved in neuroinvasiveness and neurovirulence.

Route of invasion in meningitis/meningoencephalitis. Most of the knowledge concerning mechanisms of *Lm* brain invasion was derived from mouse infection models, in which artificial routes of inoculation (intravenous, intracerebral, intracisternal) are often used to bypass the GI phase.^{16,108,155,181,244,252} Despite proving useful for circumventing the species-specific barrier posed by the lack of affinity of InIA for murine E-cadherin,¹⁷² such approaches poorly translate to infection dynamics

observed in natural hosts. Despite these caveats, pathological and experimental findings indicate that CNS invasion in blood-borne listeriosis is achieved through breaching of the blood-brain barrier (BBB) or blood-cerebrospinal fluid barrier (B-CSFB; Fig. 7). Direct infection of brain endothelium or choroid plexus epithelial cells from bloodstream bacteria has been proposed as a likely route of hematogenous brain invasion following bacteremia.^{65,77} A second mechanism for *Lm* CNS entry is the so-called “Trojan horse” model, in which bacteria are transported to the brain as cargo in infected circulating phagocytes, efficiently sheltered from extracellular factors.^{65,73,77}

Direct invasion of the BBB/B-CSFB by extracellular bacteria. Direct bacterial invasion of brain endothelium is supported by clinical and pathological findings. *Lm* is frequently isolated from the blood of patients with meningitis,^{29,40,203} and bacteria have been identified in the cytosol and adhering to the vascular side of cerebral endothelial cells in meningoencephalitis cases.¹⁵⁸ Moreover, bacteremia appears to be a requirement in murine experimental brain invasion, especially following sublethal inocula, in which bacteria are initially cleared from the bloodstream, proliferate in the liver, and then undergo a subsequent secondary wave of hematogenous spread following proliferation in liver and spleen. This can provide means for bacterial invasion of brain endothelium and choroid plexus epithelial cells, with subsequent translocation across the BBB/B-CSFB.^{16,72,156} Experimental findings also indicate that *Lm* is capable of efficiently infecting human brain microvascular endothelial cells (HBMECs) in vitro.^{127,128} Mechanisms responsible for such invasion are still controversial, as some authors speculate that an InlB-dependent internalization is required,^{127,128} while others could not find any evidence for an InlA or InlB role in bacterial entrance into endothelial cells.³¹⁰ Recently, InlF has been shown to be involved in the invasion of various cells (including a human brain endothelial cell line) by interacting with surface-expressed vimentin and, concurrently, InlF deletion mutants were deficient in their ability to colonize the brain of intravenously infected mice.¹¹³ Mechanisms underlying this interaction, however, remain unknown. As an alternative route to BBB invasion, *Lm* has also been proposed to be capable of invading the B-CSFB in vivo,²³⁸ a process that was shown to require both InlA and InlB in vitro.¹³⁰ Another bacterial surface protein, the autolysin IspC expressed by serotype 4b strains, has been shown to promote bacterial attachment and invasion of choroid plexus epithelial cells and is involved in virulence factor regulation, positively contributing to brain invasion in intravenously infected mice.³⁰⁰

In addition to the previously mentioned ones, other *Lm* virulence factors can enhance brain invasion in the context of hematogenous infection. However, their precise mechanisms and interaction underlying brain invasion and their relevance in CNS infection of naturally susceptible hosts remain unexplored. The bacterial surface protein Vip, expressed in lineage I and II strains, has been shown to bind host cell gp96 and appears to be relevant for cell and brain invasion.³⁴ A gene

encoding a putative leucine-rich-repeat-containing protein (LMOh7858_0369) from a serotype 4b strain was also found to be relevant for systemic and brain invasion in an intravascular sepsis mouse model.³¹⁵

In spite of such observations, the importance of free bacteria in the bloodstream in establishing cerebral invasion has been called into question in the context of natural infection, as antibodies present in normal adult human serum strongly inhibit HBMECs invasion.¹⁴⁰ Moreover, mice systemically infused with gentamicin (an antibiotic that poorly diffuses intracellularly but rapidly inhibits or kills extracellular bacteria) show a bacterial brain burden similar to untreated mice, indicating that most bacteria that enter the brain are intracellular.^{76,188} Thus, additional mechanisms involving infected phagocytes sheltering and transporting intracellular *Lm* to the brain have been speculated to play a pivotal role in hematogenous neuroinfection.

Invasion via infected phagocytes (“Trojan horse” model). The “Trojan horse” model of listerial brain invasion claims that phagocytes containing intracellular bacteria enter the bloodstream from peripheral sites of infection and carry intracellular *Lm* to or across the BBB and B-CSFB.⁷⁷ Numerous in vivo studies have shown that peripherally infected monocytes can allow *Lm* survival following phagocytosis and greatly enhance bacterial brain invasion.^{72,75,156,188} It is currently unclear whether *Lm* invades the BBB/B-CSFB by spreading to the endothelium from lumenally adhering phagocytes or whether bacterial escape follows phagocyte transmigration across brain barriers. Nevertheless, cell-to-cell spread appears to be essential for bacterial dissemination through this infection route. Macrophages in human neuroinfection cases can host cytosolic *Lm* displaying actin polymerization,¹⁵⁸ indicating that bacteria have the potential to undergo intercellular spread following phagocytosis. Similar findings have also been reported in mice, in which *Lm* was shown to polymerize actin inside monocytes adhering to brain endothelial vessels.¹⁸⁸ In vitro studies, in which bacteria were able to efficiently spread from infected macrophages to HBMECs and rat spinal neurons in a PlcB- and ActA-dependent manner, respectively, further support the idea that intracellular *Lm* can spread from infected phagocytes to cells of both vascular and neural compartments.^{71,128}

It is not precisely known how infected phagocytes are targeted to the brain. Peripheral infection with *Lm* has been proposed to enhance monocyte recruitment to the brain even before neuroinvasion occurs. Indeed, proinflammatory cytokines released in the plasma (especially IFN- γ) were shown to activate monocyte-recruiting inflammatory pathways in the brain prior to detectable cerebral infection.^{74,78} Subcutaneous infection in mice also resulted in upregulation of adhesion molecules (ICAM-1, P-selectin) in cerebral endothelium days before brain infection was detected,¹⁷⁹ but it is not known whether the process is involved in enhanced influx of infected monocytes and adhesion to the cerebral vasculature. Moreover, it remains unknown whether bacterial spread from monocytes

to brain endothelial cells could enhance the recruitment process. *Lm* invasion of HBMECs has been shown to induce upregulation of adhesion molecules (E-selectin, ICAM-1) and chemoattractant cytokines (IL-8, IL-15),^{299,310} but it is unknown whether this process also occurs in vivo. Last, a significant upregulation of MCP-1 was shown to occur in infected mice's brains concomitantly with influx of infected monocytes,⁷⁵ but it is unclear whether MCP-1 initiates monocyte recruitment. An explanation for monocyte predisposition in carrying intracellular *Lm* to the brain was recently proposed.¹⁸⁸ *Lm* may prolong the intravascular survival of infected monocytes via InlB-mediated selective block of CD8⁺ T-cell mediated killing, thereby enhancing the chances of bacterial transmission to the brain. It is interesting to note that mononuclear phagocytes appear to play a central role in brain invasion independently of the neuroinfection form, despite being considered to be at least partially bactericidal.⁵⁷ Further studies focusing on their interaction with *Lm* in the context of neuroinfection, therefore, will prove crucial in establishing their definitive role in neuroinvasion and, possibly, unravel new mechanisms of listerial intraphagocytic persistence and cerebral spread.

Conclusion and Outlook

A clearer understanding of the pathogenesis of listeriosis and its molecular epidemiology at the interface between environment, ruminants, and humans will foster the development of efficient surveillance and control measures to prevent disease in ruminants and transmission to humans according to farm-to-fork and One Health concepts. However, despite the high death toll of listeriosis, the molecular pathogenesis of infection remains only partially known. This is particularly true for neuroinfection, the most devastating and pathomechanistically complex form of the disease. The striking versatility of *Lm* is reflected in its ability to swiftly switch from a saprophyte to an opportunistic pathogen that is able to survive in a wide range of host cells including members of the immune system and to reach the brain through different pathways. The investigation of mechanisms and factors involved in the various neuroinvasion routes and in intra-encephalic spread will contribute to the identification of new targets for neuroinfection therapy in affected species. In this context, ruminants might provide a valuable, yet so far underappreciated, animal model for the study of human infection.

Acknowledgements

We would like to thank all current and former lab members for their work and contribution to the understanding of ruminant neuroinfection. We also thank Dr Sohvi Blatter (Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Bern, Switzerland) for providing us with the case reported in Figures 8 to 11.


Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The writing of this review was supported by funding of the Charlotte und Ernst Frauchiger-Reyher-Stiftung.

ORCID iD

Anna Oevermann  <https://orcid.org/0000-0002-3569-8547>

References

1. Addas BM, Jan MS. Cerebellar abscess due to *Listeria monocytogenes*. *Saudi Med J*. 2002;**23**(2):226–228.
2. Addis MF, Cubeddu T, Pilicchi Y, et al. Chronic intramammary infection by *Listeria monocytogenes* in a clinically healthy goat—a case report. *BMC Vet Res*. 2019;**15**(1):229.
3. Agerholm JS, Tegtmeyer CL, Nielsen TK. Survey of laboratory findings in suspected cases of bovine spongiform encephalopathy in Denmark from 1990 to 2000. *APMIS*. 2002;**110**(1):54–60.
4. Aguilar-Bultet L, Nicholson P, Rychener L, et al. Genetic separation of *Listeria monocytogenes* causing central nervous system infections in animals. *Front Cell Infect Microbiol*. 2018;**8**:20.
5. Akiyama Y, Asahi O, Hosoda T. Studies on the mechanism of infection of the brain with *Listeria monocytogenes*. *Am J Vet Res*. 1957;**18**(6):147–157.
6. Alexander A, Walker R, Johnson B, et al. Bovine abortions attributable to *Listeria ivanovii*: four cases (1988–1990). *J Am Vet Med Assoc*. 1992;**200**(5):711–714.
7. Altimira J, Prats N, Lopez S, et al. Repeated oral dosing with *Listeria monocytogenes* in mice as a model of central nervous system listeriosis in man. *J Comp Pathol*. 1999;**121**(2):117–125.
8. Amaya-Villar R, García-Cabrera E, Sulleiro-Igual E, et al. Three-year multi-center surveillance of community-acquired *Listeria monocytogenes* meningitis in adults. *BMC Infect Dis*. 2010;**10**(1):324.
9. Angelo KM, Jackson KA, Wong KK, et al. Assessment of the incubation period for invasive listeriosis. *Clin Infect Dis*. 2016;**63**(11):1487–1489.
10. Antal EA, Løberg EM, Bracht P, et al. Evidence for intraaxonal spread of *Listeria monocytogenes* from the periphery to the central nervous system. *Brain Pathol*. 2001;**11**(4):432–438.
11. Antal EA, Løberg EM, Dietrichs E, et al. Neuropathological findings in 9 cases of *Listeria monocytogenes* brain stem encephalitis. *Brain Pathol*. 2005;**15**(3):187–191.
12. Armstrong RW, Fung PC. Brainstem encephalitis (rhombencephalitis) due to *Listeria monocytogenes*: case report and review. *Clin Infect Dis*. 1993;**16**(5):689–702.
13. Balandyté L, Brodard I, Frey J, et al. Ruminant rhombencephalitis-associated *Listeria monocytogenes* alleles linked to a multilocus variable-number tandem-repeat analysis complex. *Appl Environ Microbiol*. 2011;**77**(23):8325–8335.
14. Barlow R, McGorum B. Ovine listerial encephalitis: analysis, hypothesis and synthesis. *Vet Rec*. 1985;**116**(9):233–236.
15. Bartt R. *Listeria* and atypical presentations of *Listeria* in the central nervous system. *Semin Neurol*. 2000;**20**(03):361–374.
16. Berche P. Bacteremia is required for invasion of the murine central nervous system by *Listeria monocytogenes*. *Microb Pathog*. 1995;**18**(5):323–336.
17. Bergholz TM, Shah MK, Burall LS, et al. Genomic and phenotypic diversity of *Listeria monocytogenes* clonal complexes associated with human listeriosis. *Appl Microbiol Biotechnol*. 2018;**102**(8):3475–3485.
18. Bhardwaj V, Kanagawa O, Swanson PE, et al. Chronic *Listeria* infection in SCID mice: requirements for the carrier state and the dual role of T cells in transferring protection or suppression. *J Immunol*. 1998;**160**(1):376–384.
19. Bierne H, Cossart P. *Listeria monocytogenes* surface proteins: from genome predictions to function. *Microbiol Mol Biol Rev*. 2007;**71**(2):377–397.

20. Bierne H, Sabet C, Personnic N, et al. Internalins: a complex family of leucine-rich repeat-containing proteins in *Listeria monocytogenes*. *Microb Infect*. 2007; **9**(10):1156–1166.
21. Birmingham CL, Canadien V, Kaniuk NA, et al. Listeriolysin O allows *Listeria monocytogenes* replication in macrophage vacuoles. *Nature*. 2008; **451**(7176):350–354.
22. Blanchard AM, Billenness R, Warren J, et al. Characterisation of *Listeria monocytogenes* isolates from cattle using a bovine caruncular epithelial cell model. *Heliyon*. 2020; **6**(7):e04476.
23. Blanot S, Joly MM, Vilde F, et al. A gerbil model for rhombencephalitis due to *Listeria monocytogenes*. *Microb Pathog*. 1997; **23**(1):39–48.
24. Boerlin P, Boerlin-Petzold F, Jemmi T. Use of listeriolysin O and internalin A in a seroepidemiological study of listeriosis in Swiss dairy cows. *J Clin Microbiol*. 2003; **41**(3):1055–1061.
25. Bojanowski MW, Seizeur R, Effendi K, et al. Spreading of multiple *Listeria monocytogenes* abscesses via central nervous system fiber tracts: case report. *J Neurosurg*. 2015; **123**(6):1593–1599.
26. Borucki MK, Gay CC, Reynolds J, et al. Genetic diversity of *Listeria monocytogenes* strains from a high-prevalence dairy farm. *Appl Environ Microbiol*. 2005; **71**(10):5893–5899.
27. Bourry A, Poutrel B, Rocourt J. Bovine mastitis caused by *Listeria monocytogenes*: characteristics of natural and experimental infections. *J Med Microbiol*. 1995; **43**(2):125–132.
28. Braun U, Stehle C, Ehrensperger E. Clinical findings and treatment of listeriosis in 67 sheep and goats. *Vet Rec*. 2002; **150**(2):38–42.
29. Brouwer MC, van de Beek D, Heckenberg SG, et al. Community-acquired *Listeria monocytogenes* meningitis in adults. *Clin Infect Dis*. 2006; **43**(10):1233–1238.
30. Brugère-Picoux J. Ovine listeriosis. *Small Ruminant Res*. 2008; **76**(1–2):12–20.
31. Brundage RA, Smith GA, Camilli A, et al. Expression and phosphorylation of the *Listeria monocytogenes* ActA protein in mammalian cells. *Proc Natl Acad Sci U S A*. 1993; **90**(24):11890–11894.
32. Buchanan RL, Gorris LGM, Hayman MM, et al. A review of *Listeria monocytogenes*: an update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food Control*. 2017; **75**:1–13.
33. Bundrant BN, Hutchins T, den Bakker HC, et al. Listeriosis outbreak in dairy cattle caused by an unusual *Listeria monocytogenes* serotype 4b strain. *J Vet Diagn Invest*. 2011; **23**(1):155–158.
34. Cabanes D, Sousa S, Cebriá A, et al. Gp96 is a receptor for a novel *Listeria monocytogenes* virulence factor, Vip, a surface protein. *EMBO J*. 2005; **24**(15):2827–2838.
35. Câmara ACL, Gonzaga MC, Ziober TM, et al. Cerebrospinal fluid analysis in 58 ruminants showing neurological disorders. *Pesq Vet Bras*. 2020; **40**(5):346–354.
36. Cantinelli T, Chenal-Francois V, Diancourt L, et al. “Epidemic clones” of *Listeria monocytogenes* are widespread and ancient clonal groups. *J Clin Microbiol*. 2013; **51**(11):3770–3779.
37. Cartwright EJ, Jackson KA, Johnson SD, et al. Listeriosis outbreaks and associated food vehicles, United States, 1998–2008. *Emerg Infect Dis*. 2013; **19**(1):1–9.
38. Castro H, Jaakkonen A, Hakkinen M, et al. Occurrence, persistence, and contamination routes of *Listeria monocytogenes* genotypes on three Finnish dairy cattle farms: a longitudinal study. *Appl Environ Microbiol*. 2018; **84**(4):e02000–e02017.
39. Charlier C, Disson O, Lecuit M. Maternal-neonatal listeriosis. *Virulence*. 2020; **11**(1):391–397.
40. Charlier C, Perrodeau É, Leclercq A, et al. Clinical features and prognostic factors of listeriosis: the MONALISA national prospective cohort study. *Lancet Infect Dis*. 2017; **17**(5):510–519.
41. Charlton KM. Spontaneous listeric encephalitis in sheep. Electron microscopic studies. *Vet Pathol*. 1977; **14**(5):429–434.
42. Charlton KM, Garcia MM. Spontaneous listeric encephalitis and neuritis in sheep. Light microscopic studies. *Vet Pathol*. 1977; **14**(4):297–313.
43. Chenal-Francois V, Lopez J, Cantinelli T, et al. Worldwide distribution of major clones of *Listeria monocytogenes*. *Emerg Infect Dis*. 2011; **17**(6):1110–1112.
44. Chiba S, Nagai T, Hayashi T, et al. Listerial invasion protein internalin B promotes entry into ileal Peyer’s patches in vivo. *Microbiol Immunol*. 2011; **55**(2):123–129.
45. Choi MH, Park YJ, Kim M, et al. Increasing incidence of Listeriosis and infection-associated clinical outcomes. *Ann Lab Med*. 2018; **38**(2):102–109.
46. Choudhury N, Khan A, Tzvetanov I, et al. Cerebellar abscess caused by *Listeria monocytogenes* in a liver transplant patient. *Transpl Infect Dis*. 2013; **15**(6):E224–E228.
47. Clark R, Gill J, Swanney S. *Listeria monocytogenes* gastroenteritis in sheep. *NZ Vet J*. 2004; **52**(1):46–47.
48. Cone LA, Leung MM, Byrd RG, et al. Multiple cerebral abscesses because of *Listeria monocytogenes*: three case reports and a literature review of supratentorial listerial brain abscess (es). *Surg Neurol*. 2003; **59**(4):320–328.
49. Cordy D, Osebold JW. The neuropathogenesis of *Listeria* encephalomyelitis in sheep and mice. *J Infect Dis*. 1959; **104**(2):164–173.
50. Cranfield M, Eckhaus M, Valentine B, et al. Listeriosis in Angolan giraffes. *J Am Vet Med Assoc*. 1985; **187**(11):1238–1240.
51. Czuprynski C, Kathariou S, Poulsen K. *Listeria*. In: Gyles CL, Prescott JF, Songer JG, eds. *Pathogenesis of Bacterial Infections in Animals*. 4th ed. Wiley-Blackwell; 2010:167–187.
52. D’Orazio SEF. Animal models for oral transmission of *Listeria monocytogenes*. *Front Cell Infect Microbiol*. 2014; **4**:15.
53. D’Orazio SEF. Innate and adaptive immune responses during *Listeria monocytogenes* infection. *Microbiol Spectr*. 2019; **7**(3). doi:10.1128/microbiolspec.GPP3-0065-2019
54. Datta AR, Burall LS. Serotype to genotype: the changing landscape of listeriosis outbreak investigations. *Food Microbiol*. 2018; **75**:18–27.
55. Datta AR, Laksanalamai P, Solomotis M. Recent developments in molecular sub-typing of *Listeria monocytogenes*. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. 2013; **30**(8):1437–1445.
56. Davis ML, Ricke SC, Donaldson JR. Establishment of *Listeria monocytogenes* in the gastrointestinal tract. *Microorganisms*. 2019; **7**(3):75.
57. de Chastellier C, Berche P. Fate of *Listeria monocytogenes* in murine macrophages: evidence for simultaneous killing and survival of intracellular bacteria. *Infect Immun*. 1994; **62**(2):543–553.
58. de las Heras A, Cain RJ, Bielecka MK, et al. Regulation of *Listeria* virulence: PrfA master and commander. *Curr Opin Microbiol*. 2011; **14**(2):118–127.
59. De Luca G, Zanetti F, Fateh-Moghadam P, et al. Occurrence of *Listeria monocytogenes* in sewage sludge. *Zentralbl Hyg Umweltmed*. 1998; **201**(3):269–277.
60. de Noordhout CM, Devleeschauwer B, Angulo FJ, et al. The global burden of listeriosis: a systematic review and meta-analysis. *Lancet Infect Dis*. 2014; **14**(11):1073–1082.
61. Dee RR, Lorber B. Brain abscess due to *Listeria monocytogenes*: case report and literature review. *Rev Infect Dis*. 1986; **8**(6):968–977.
62. Di Palma S, Brunetti B, Doherr MG, et al. Comparative spatiotemporal analysis of the intrathecal immune response in natural listeric rhombencephalitis of cattle and small ruminants. *Comp Immunol Microbiol Infect Dis*. 2012; **35**(5):429–441.
63. Disease presentations of *Listeria monocytogenes* in sheep. *Vet Rec*. 2018; **183**(21):649.
64. Disson O, Blériot C, Jacob JM, et al. Peyer’s patch myeloid cells infection by *Listeria* signals through gp38+ stromal cells and locks intestinal villus invasion. *J Exp Med*. 2018; **215**(11):2936–2954.
65. Disson O, Lecuit M. Targeting of the central nervous system by *Listeria monocytogenes*. *Virulence*. 2012; **3**(2):213–221.
66. Disson O, Moura A, Lecuit M. Making sense of the biodiversity and virulence of *Listeria monocytogenes*. *Trends Microbiol*. 2021; **29**(9):811–822
67. Doganay M. Listeriosis: clinical presentation. *FEMS Immunol Med Microbiol*. 2003; **35**(3):173–175.

68. Doijad SP, Poharkar KV, Kale SB, et al. *Listeria goeensis* sp. nov. *Int J Syst Evol Microbiol.* 2018;**68**(10):3285–3291.
69. Dons L, Weclawicz K, Jin Y, et al. Rat dorsal root ganglia neurons as a model for *Listeria monocytogenes* infections in culture. *Med Microbiol Immunol.* 1999;**188**(1):15–21.
70. Dramsi S, Biswas I, Maguin E, et al. Entry of *Listeria monocytogenes* into hepatocytes requires expression of InlB, a surface protein of the internalin multigene family. *Mol Microbiol.* 1995;**16**(2):251–261.
71. Dramsi S, Levi S, Triller A, et al. Entry of *Listeria monocytogenes* into neurons occurs by cell-to-cell spread: an in vitro study. *Infect Immun.* 1998;**66**(9):4461–4468.
72. Drevets DA. Dissemination of *Listeria monocytogenes* by infected phagocytes. *Infect Immun.* 1999;**67**(7):3512–3517.
73. Drevets DA, Bronze MS. *Listeria monocytogenes*: epidemiology, human disease, and mechanisms of brain invasion. *FEMS Immunol Med Microbiol.* 2008;**53**(2):151–165.
74. Drevets DA, Dillon MJ, Schawang JE, et al. IFN-gamma triggers CCR2-independent monocyte entry into the brain during systemic infection by virulent *Listeria monocytogenes*. *Brain Behav Immun.* 2010;**24**(6):919–929.
75. Drevets DA, Dillon MJ, Schawang JS, et al. The Ly-6Chigh monocyte subpopulation transports *Listeria monocytogenes* into the brain during systemic infection of mice. *J Immunol.* 2004;**172**(7):4418–4424.
76. Drevets DA, Jelinek TA, Freitag NE. *Listeria monocytogenes*-infected phagocytes can initiate central nervous system infection in mice. *Infect Immun.* 2001;**69**(3):1344–1350.
77. Drevets DA, Leenen PJ, Greenfield RA. Invasion of the central nervous system by intracellular bacteria. *Clin Microbiol Rev.* 2004;**17**(2):323–347.
78. Drevets DA, Schawang JE, Dillon MJ, et al. Innate responses to systemic infection by intracellular bacteria trigger recruitment of Ly-6Chigh monocytes to the brain. *J Immunol.* 2008;**181**(1):529–536.
79. Dreyer M, Aguilar-Bultet L, Rupp S, et al. *Listeria monocytogenes* sequence type 1 is predominant in ruminant rhombencephalitis. *Sci Rep.* 2016;**6**(1):36419.
80. Dreyer M, Thomann A, Böttcher S, et al. Outbreak investigation identifies a single *Listeria monocytogenes* strain in sheep with different clinical manifestations, soil and water. *Vet Microbiol.* 2015;**179**(1-2):69–75.
81. Drolia R, Bhunia AK. Crossing the intestinal barrier via *Listeria* adhesion protein and internalin A. *Trends Microbiol.* 2019;**27**(5):408–425.
82. Drolia R, Tenguria S, Durkes AC, et al. *Listeria* adhesion protein induces intestinal epithelial barrier dysfunction for bacterial translocation. *Cell Host Microbe.* 2018;**23**(4):470–484.
83. Dumont J, Cotoni L. Bacille semblable au bacille du rouget du porc rencontré dans le liquide céphalo-rachidien dun méningitique. *Ann Inst Pasteur.* 1921;**35**:625–633 [in French].
84. Eckburg PB, Montoya JG, Vosti KL. Brain abscess due to *Listeria monocytogenes*: five cases and a review of the literature. *Medicine.* 2001;**80**(4):223–235.
85. EFSA Panel on Biological Hazards; Ricci A, Allende A, et al. *Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU. *EFSA J.* 2018;**16**(1):e05134.
86. Engelen-Lee JY, Koopmans MM, Brouwer MC, et al. Histopathology of *Listeria* meningitis. *J Neuropathol Exp Neurol.* 2018;**77**(10):950–957.
87. Erdogan H, Cripps P, Morgan K, et al. Prevalence, incidence, signs and treatment of clinical listeriosis in dairy cattle in England. *Vet Rec.* 2001;**149**(10):289–293.
88. Erdogan HM. Listerial keratoconjunctivitis and uveitis (silage eye). *Vet Clin North Am Food Anim Pract.* 2010;**26**(3):505–510.
89. Esteban JI, Oporto B, Aduriz G, et al. Faecal shedding and strain diversity of *Listeria monocytogenes* in healthy ruminants and swine in Northern Spain. *BMC Vet Res.* 2009;**5**:2.
90. European Commission. Commission regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Off J Eur Union L.* 2005;**338**:1–26.
91. European Food Safety Authority and European Centre for Disease Prevention and Control. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA J.* 2018;**16**(12):e05500.
92. Evans K, Smith M, McDonough P, et al. Eye infections due to *Listeria monocytogenes* in three cows and one horse. *J Vet Diagn Invest.* 2004;**16**(5):464–469.
93. Fairley RA, Colson M. Enteric listeriosis in a 10-month-old calf. *N Z Vet J.* 2013;**61**(6):376–378.
94. Fairley R, Pesavento PA, Clark R. *Listeria monocytogenes* infection of the alimentary tract (enteric listeriosis) of sheep in New Zealand. *J Comp Pathol.* 2012;**146**(4):308–313.
95. Faleiro ML, Andrew P, Power D. Stress response of *Listeria monocytogenes* isolated from cheese and other foods. *Int J Food Microbiol.* 2003;**84**(2):207–216.
96. FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization). Risk assessment of *Listeria Monocytogenes* in ready-to-eat foods: technical report 2004. Accessed October 25, 2021. <https://apps.who.int/iris/bitstream/handle/10665/42875/9241562625.pdf?sequence=1&isAllowed=y>
97. Faralla C, Bastounis EE, Ortega FE, et al. *Listeria monocytogenes* InlP interacts with afadin and facilitates basement membrane crossing. *PLoS Pathog.* 2018;**14**(5):e1007094.
98. Faralla C, Rizzuto GA, Lowe DE, et al. InlP, a new virulence factor with strong placental tropism. *Infect Immun.* 2016;**84**(12):3584–3596.
99. Fenlon D. Rapid quantitative assessment of the distribution of *Listeria* in silage implicated in a suspected outbreak of listeriosis in calves. *Vet Rec.* 1986;**118**(9):240–242.
100. Fenlon DR, Wilson J, Donachie W. The incidence and level of *Listeria monocytogenes* contamination of food sources at primary production and initial processing. *J Appl Bacteriol.* 1996;**81**(6):641–650.
101. Fox E, O'Mahony T, Clancy M, et al. *Listeria monocytogenes* in the Irish dairy farm environment. *J Food Prot.* 2009;**72**(7):1450–1456.
102. Fox EM, deLappe N, Garvey P, et al. PFGE analysis of *Listeria monocytogenes* isolates of clinical, animal, food and environmental origin from Ireland. *J Med Microbiol.* 2012;**61**(Pt 4):540–547.
103. Freitag NE, Port GC, Miner MD. *Listeria monocytogenes*—from saprophyte to intracellular pathogen. *Nat Rev Microbiol.* 2009;**7**(9):623–628.
104. Friesema I, Kuiling S, van der Ende A, et al. Risk factors for sporadic listeriosis in the Netherlands, 2008 to 2013. *Euro Surveill.* 2015;**20**(31):21199.
105. Fthenakis G, Saratsis P, Tzora A, et al. Naturally occurring subclinical ovine mastitis associated with *Listeria monocytogenes*. *Small Rumin Res.* 1998;**31**(1):23–27.
106. Fugett EB, Schoonmaker-Bopp D, Dumas NB, et al. Pulsed-field gel electrophoresis (PFGE) analysis of temporally matched *Listeria monocytogenes* isolates from human clinical cases, foods, ruminant farms, and urban and natural environments reveals source-associated as well as widely distributed PFGE types. *J Clin Microbiol.* 2007;**45**(3):865–873.
107. Gahan CG, Hill C. *Listeria monocytogenes*: survival and adaptation in the gastrointestinal tract. *Front Cell Infect Microbiol.* 2014;**4**:9.
108. Gaillard JL, Berche P, Frehel C, et al. Entry of *L. monocytogenes* into cells is mediated by internalin, a repeat protein reminiscent of surface antigens from gram-positive cocci. *Cell.* 1991;**65**(7):1127–1141.
109. García JA, Micheloud JF, Campero CM, et al. Enteric listeriosis in grazing steers supplemented with spoiled silage. *J Vet Diagn Invest.* 2016;**28**(1):65–69.
110. Garner D, Kathariou S. Fresh produce-associated listeriosis outbreaks, sources of concern, teachable moments, and insights. *J Food Prot.* 2016;**79**(2):337–344.
111. Garrec N, Picard-Bonnaud F, Pouchet A. Occurrence of *Listeria* sp. and *L. monocytogenes* in sewage sludge used for land application: effect of dewatering, liming and storage in tank on survival of *Listeria* species. *FEMS Immunol Med Microbiol.* 2003;**35**(3):275–283.
112. Gates GA, Blendon DC, Kintner LD. Listeric myelitis in sheep. *J Am Vet Med Assoc.* 1967;**150**(2):200–204.

113. Ghosh P, Halvorsen EM, Ammendolia DA, et al. Invasion of the brain by *Listeria monocytogenes* is mediated by InlF and host cell vimentin. *mBio*. 2018;**9**(1):e00160–00118.
114. Ghosh P, Zhou Y, Richardson Q, et al. Characterization of the pathogenesis and immune response to *Listeria monocytogenes* strains isolated from a sustained national outbreak. *Sci Rep*. 2019;**9**(1):19587.
115. Giles L, Orr J, Viora L, et al. Ruminant neurological disease: a retrospective cohort study. *Vet Rec*. 2017;**181**(14):372–373.
116. Gill DA. “Circling” disease of sheep in New Zealand. *Vet J*. 1931;**87**(2):60–74. doi:10.1016/S0372-5545(17)40605-5
117. Gismervik K, Aspholm M, Rørvik LM, et al. Invading slugs (*Arion vulgaris*) can be vectors for *Listeria monocytogenes*. *J Appl Microbiol*. 2015;**118**(4):809–816.
118. Gitter M, Bradley R, Blampied P. *Listeria monocytogenes* infection in bovine mastitis. *Vet Rec*. 1980;**107**(17):390–393.
119. Goulet V, Hebert M, Hedberg C, et al. Incidence of listeriosis and related mortality among groups at risk of acquiring listeriosis. *Clin Infect Dis*. 2011;**54**(5):652–660.
120. Goulet V, Hedberg C, Le Monnier A, et al. Increasing incidence of listeriosis in France and other European countries. *Emerg Infect Dis*. 2008;**14**(5):734–740.
121. Goulet V, King LA, Vaillant V, et al. What is the incubation period for listeriosis? *BMC Infect Dis*. 2013;**13**(1):11.
122. Gözel B, Monney C, Aguilar-Bultet L, et al. Hyperinvasiveness of *Listeria monocytogenes* sequence type 1 is independent of lineage I-specific genes encoding internalin-like proteins. *Microbiologyopen*. 2019;**8**(7):e790.
123. Graves LM, Swaminathan B. PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. *Int J Food Microbiol*. 2001;**65**(1–2):55–62.
124. Gray M. Isolation of *Listeria monocytogenes* from oat silage. *Science*. 1960;**132**(3441):1767–1768.
125. Gray ML, Killinger A. *Listeria monocytogenes* and listeric infections. *Bacteriol Rev*. 1966;**30**(2):309–382.
126. Green L, Morgan K. Descriptive epidemiology of listerial meningoencephalitis in housed lambs. *Prev Vet Med*. 1994;**18**(2):79–87.
127. Greiffenberg L, Goebel W, Kim KS, et al. Interaction of *Listeria monocytogenes* with human brain microvascular endothelial cells: an electron microscopic study. *Infect Immun*. 2000;**68**(6):3275–3279.
128. Greiffenberg L, Goebel W, Kim KS, et al. Interaction of *Listeria monocytogenes* with human brain microvascular endothelial cells: InlB-dependent invasion, long-term intracellular growth, and spread from macrophages to endothelial cells. *Infect Immun*. 1998;**66**(11):5260–5267.
129. Grif K, Patscheider G, Dierich M, et al. Incidence of fecal carriage of *Listeria monocytogenes* in three healthy volunteers: a one-year prospective stool survey. *Eur J Clin Microbiol Infect Dis*. 2003;**22**(1):16–20.
130. Gründler T, Quednau N, Stump C, et al. The surface proteins InlA and InlB are interdependently required for polar basolateral invasion by *Listeria monocytogenes* in a human model of the blood–cerebrospinal fluid barrier. *Microbes Infect*. 2013;**15**(4):291–301.
131. Guillet C, Join-Lambert O, Le Monnier A, et al. Human listeriosis caused by *Listeria ivanovii*. *Emerg Infect Dis*. 2010;**16**(1):136–138.
132. Guldimann C, Bärtschi M, Frey J, et al. Increased spread and replication efficiency of *Listeria monocytogenes* in organotypic brain-slices is related to multilocus variable number of tandem repeat analysis (MLVA) complex. *BMC Microbiol*. 2015;**15**(1):134.
133. Guldimann C, Lejeune B, Hofer S, et al. Ruminant organotypic brain-slice cultures as a model for the investigation of CNS listeriosis. *Int J Exp Pathol*. 2012;**93**(4):259–268.
134. Halbedel S, Wilking H, Holzer A, et al. Large nationwide outbreak of invasive listeriosis associated with blood sausage, Germany, 2018–2019. *Emerg Infect Dis*. 2020;**26**(7):1456–1464.
135. Haley BJ, Sonnier J, Schukken YH, et al. Diversity of *Listeria monocytogenes* within a US dairy herd, 2004–2010. *Foodborne Pathog Dis*. 2015;**12**(10):844–850.
136. Hamon M, Bierre H, Cossart P. *Listeria monocytogenes*: a multifaceted model. *Nat Rev Microbiol*. 2006;**4**(6):423–434.
137. Hawkins IK, Ilha M, Anis E, et al. Septicemia and meningoencephalitis caused by *Listeria monocytogenes* in two neonatal llamas. *J Vet Diagn Invest*. 2017;**29**(5):700–703.
138. Hellström S, Kiviniemi K, Autio T, et al. *Listeria monocytogenes* is common in wild birds in Helsinki region and genotypes are frequently similar with those found along the food chain. *J Appl Microbiol*. 2008;**104**(3):883–888.
139. Henke D, Rupp S, Gaschen V, et al. *Listeria monocytogenes* spreads within the brain by actin-based intra-axonal migration. *Infect Immun*. 2015;**83**(6):2409–2419.
140. Hertzog T, Weber M, Greiffenberg L, et al. Antibodies present in normal human serum inhibit invasion of human brain microvascular endothelial cells by *Listeria monocytogenes*. *Infect Immun*. 2003;**71**(1):95–100.
141. Ho A, Ivanek R, Gröhn Y, et al. *Listeria monocytogenes* fecal shedding in dairy cattle shows high levels of day-to-day variation and includes outbreaks and sporadic cases of shedding of specific *L. monocytogenes* subtypes. *Prev Vet Med*. 2007;**80**(4):287–305.
142. Hoelzer K, Pouillot R, Dennis S. Animal models of listeriosis: a comparative review of the current state of the art and lessons learned. *Vet Res*. 2012;**43**(1):18.
143. Hof H. *Listeria* infections of the eye. *Eur J Ophthalmol*. 2017;**27**(2):115–121.
144. Hsu CCT, Singh D, Watkins TW, et al. Serial magnetic resonance imaging findings of intracerebral spread of *Listeria* utilising subcortical U-fibres and the extreme capsule. *Neuroradiol J*. 2016;**29**(6):425–430.
145. Hunt K, Drummond N, Murphy M, et al. A case of bovine raw milk contamination with *Listeria monocytogenes*. *Ir Vet J*. 2012;**65**(1):13.
146. Iulini B, Maurella C, Pintore M, et al. Ten years of BSE surveillance in Italy: neuropathological findings in clinically suspected cases. *Res Vet Sci*. 2012;**93**(2):872–878.
147. Ivanek R, Gröhn Y, Wells M, et al. Modeling of spatially referenced environmental and meteorological factors influencing the probability of *Listeria* species isolation from natural environments. *Appl Environ Microbiol*. 2009;**75**(18):5893–5909.
148. Ivanek R, Gröhn Y, Wiedmann M. *Listeria monocytogenes* in multiple habitats and host populations: review of available data for mathematical modeling. *Foodborne Pathog Dis*. 2006;**3**(4):319–336.
149. Jacquet C, Doumith M, Gordon JI, et al. A molecular marker for evaluating the pathogenic potential of foodborne *Listeria monocytogenes*. *J Infect Dis*. 2004;**189**(11):2094–2100.
150. Jagtap U, Kolhe R, Deshpande P, et al. Isolation of *Listeria monocytogenes* from peridomestic birds and captive wild animals. *Curr Sci*. 2017;**113**(9):1783–1787.
151. Jeffers GT, Bruce JL, McDonough PL, et al. Comparative genetic characterization of *Listeria monocytogenes* isolates from human and animal listeriosis cases. *Microbiology (Reading)*. 2001;**147**(Pt 5):1095–1104.
152. Jensen NE, Aarestrup FM, Jensen J, et al. *Listeria monocytogenes* in bovine mastitis. Possible implication for human health. *Int J Food Microbiol*. 1996;**32**(1–2):209–216.
153. Jin Y, Dons L, Kristensson K, et al. Colony-stimulating factor 1-dependent cells protect against systemic infection with *Listeria monocytogenes* but facilitate neuroinvasion. *Infect Immun*. 2002;**70**(8):4682–4686.
154. Jin Y, Dons L, Kristensson K, et al. Neural route of cerebral *Listeria monocytogenes* murine infection: role of immune response mechanisms in controlling bacterial neuroinvasion. *Infect Immun*. 2001;**69**(2):1093–1100.
155. Johnson GC, Maddox CW, Fales WH, et al. Epidemiologic evaluation of encephalitic listeriosis in goats. *J Am Vet Med Assoc*. 1996;**208**(10):1695–1699.
156. Join-Lambert OF, Ezine S, Le Monnier A, et al. *Listeria monocytogenes*-infected bone marrow myeloid cells promote bacterial invasion of the central nervous system. *Cell Microbiol*. 2005;**7**(2):167–180.
157. Kathariou S. *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. *J Food Prot*. 2002;**65**(11):1811–1829.

158. Kirk J. Diagnostic ultrastructure of *Listeria monocytogenes* in human central nervous tissue. *Ultrastruct Pathol.* 1993;**17**(6):583–592.
159. Kocks C, Gouin E, Tabouret M, et al. *L. monocytogenes*-induced actin assembly requires the actA gene product, a surface protein. *Cell.* 1992;**68**(3):521–531.
160. Koopmans MM, Bijlsma MW, Brouwer MC, et al. *Listeria monocytogenes* meningitis in the Netherlands, 1985–2014: a nationwide surveillance study. *J Infect.* 2017;**75**(1):12–19.
161. Koopmans MM, Brouwer MC, Bijlsma MW, et al. *Listeria monocytogenes* sequence type 6 and increased rate of unfavorable outcome in meningitis: epidemiologic cohort study. *Clin Infect Dis.* 2013;**57**(2):247–253.
162. Koopmans MM, Engelen-Lee J, Brouwer MC, et al. Characterization of a *Listeria monocytogenes* meningitis mouse model. *J Neuroinflammation.* 2018;**15**(1):257.
163. Kortebe M, Milohanic E, Mitchell G, et al. *Listeria monocytogenes* switches from dissemination to persistence by adopting a vacuolar lifestyle in epithelial cells. *PLoS Pathog.* 2017;**13**(11):e1006734.
164. Kotzamanidis C, Papadopoulos T, Vafeas G, et al. Characterization of *Listeria monocytogenes* from encephalitis cases of small ruminants from different geographical regions, in Greece. *J Appl Microbiol.* 2019;**126**(5):1373–1382.
165. Kreft J, Vázquez-Boland JA. Regulation of virulence genes in *Listeria*. *Int J Med Microbiol.* 2001;**291**(2):145–157.
166. Kremer PHC, Lees JA, Koopmans MM, et al. Benzalkonium tolerance genes and outcome in *Listeria monocytogenes* meningitis. *Clin Microbiol Infect.* 2017;**23**(4):265.
167. Kummeneje K, Mikkelsen T. Isolation of *Listeria monocytogenes* type 04 from cases of keratoconjunctivitis in cattle and sheep. *Nord Vet Med.* 1975;**27**(3):144–149.
168. Ladel CH, Flesch IE, Arnoldi J, et al. Studies with MHC-deficient knock-out mice reveal impact of both MHC I- and MHC II-dependent T cell responses on *Listeria monocytogenes* infection. *J Immunol.* 1994;**153**(7):3116–3122.
169. Lambrecht E, Baré J, Chavatte N, et al. Protozoan cysts act as a survival niche and protective shelter for foodborne pathogenic bacteria. *Appl Environ Microbiol.* 2015;**81**(16):5604–5612.
170. Laven RA, Lawrence KR. An outbreak of iritis and uveitis in dairy cattle at pasture associated with the supplementary feeding of baleage. *N Z Vet J.* 2006;**54**(3):151–152.
171. Leclercq A, Moura A, Vales G, et al. *Listeria thailandensis* sp. nov. *Int J Syst Evol Microbiol.* 2018;**69**(1):74–81.
172. Lecuit M, Dramsi S, Gottardi C, et al. A single amino acid in E-cadherin responsible for host specificity towards the human pathogen *Listeria monocytogenes*. *EMBO J.* 1999;**18**(14):3956–3963.
173. Lecuit M, Sonnenburg JL, Cossart P, et al. Functional genomic studies of the intestinal response to a foodborne enteropathogen in a humanized gnotobiotic mouse model. *J Biol Chem.* 2007;**282**(20):15065–15072.
174. Ligios C, Viglietti A, Carta P, et al. Clinicopathological findings in sheep from Sardinia showing neurological signs of disease. *Vet Rec.* 2004;**154**(12):365–370.
175. Lim S, Chung DR, Kim YS, et al. Predictive risk factors for *Listeria monocytogenes* meningitis compared to pneumococcal meningitis: a multicenter case-control study. *Infection.* 2017;**45**(1):67–74.
176. Linke K, Rückerl I, Brugger K, et al. Reservoirs of *Listeria* species in three environmental ecosystems. *Appl Environ Microbiol.* 2014;**80**(18):5583–5592.
177. Liu D. Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. *J Med Microbiol.* 2006;**55**(Pt 6):645–659.
178. Loncarevic S, Artursson K, Johansson I. A case of canine cutaneous listeriosis. *Vet Dermatol.* 1999;**10**(1):69–71.
179. López S, Prats N, Marco AJ. Expression of E-selectin, P-selectin, and intercellular adhesion molecule-1 during experimental murine listeriosis. *Am J Pathol.* 1999;**155**(4):1391–1397.
180. Low J, Donachie W. A review of *Listeria monocytogenes* and listeriosis. *Vet J.* 1997;**153**(1):9–29.
181. Low J, Renton C. Septicaemia, encephalitis and abortions in a housed flock of sheep caused by *Listeria monocytogenes* type 1/2. *Vet Rec.* 1985;**116**(6):147–150.
182. Lowe DE, Robbins JR, Bakardjiev AI. Animal and human tissue models of vertical *Listeria monocytogenes* transmission and implications for other pregnancy-associated infections. *Infect Immun.* 2018;**86**(6):e00801–e00817.
183. Ly TMC, Müller H. Ingested *Listeria monocytogenes* survive and multiply in protozoa. *J Med Microbiol.* 1990;**33**(1):51–54.
184. Madarame H, Seuberlich T, Abril C, et al. The distribution of E-cadherin expression in listeric rhombencephalitis of ruminants indicates its involvement in *Listeria monocytogenes* neuroinvasion. *Neuropathol Appl Neurobiol.* 2011;**37**(7):753–767.
185. Madjunkov M, Chaudhry S, Ito S. Listeriosis during pregnancy. *Arch Gynecol Obstet.* 2017;**296**(2):143–152.
186. Marco A, Prats N, Ramos J, et al. A microbiological, histopathological and immunohistological study of the intragastric inoculation of *Listeria monocytogenes* in mice. *J Comp Pathol.* 1992;**107**(1):1–9.
187. Matto C, Varela G, Braga V, et al. Detection of *Listeria* spp. in cattle and environment of pasture-based dairy farms. *Pesqui Vet Bras.* 2018;**38**(9):1736–1741.
188. Maudet C, Kheloufi M, Levallois S, et al. Bacterial inhibition of CD8+ T-cells mediated cell death promotes neuroinvasion and within-host persistence. *bioRxiv.* Preprint. doi:10.1101/2020.11.20.384974
189. Maudet C, Levallois S, Disson O, et al. Innate immune responses to *Listeria* in vivo. *Curr Opin Microbiol.* 2021;**59**:95–101.
190. Maury MM, Bracq-Dieye H, Huang L, et al. Hypervirulent *Listeria monocytogenes* clones' adaption to mammalian gut accounts for their association with dairy products. *Nat Commun.* 2019;**10**(1):2488.
191. Maury MM, Chenal-Francisque V, Bracq-Dieye H, et al. Spontaneous loss of virulence in natural populations of *Listeria monocytogenes*. *Infect Immun.* 2017;**85**(11):e00541–17.
192. Maury MM, Tsai YH, Charlier C, et al. Uncovering *Listeria monocytogenes* hypervirulence by harnessing its biodiversity. *Nat Genet.* 2016;**48**(3):308–313.
193. McGill I, Wells G. Neuropathological findings in cattle with clinically suspect but histologically unconfirmed bovine spongiform encephalopathy (BSE). *J Comp Pathol.* 1993;**108**(3):241–260.
194. McLaughlin J, Low J. Primary cutaneous listeriosis in adults: an occupational disease of veterinarians and farmers. *Vet Rec.* 1994;**135**(26):615–617.
195. Mead PS, Slutsker L, Dietz V, et al. Food-related illness and death in the United States. *Emerg Infect Dis.* 1999;**5**(5):607–625.
196. Meredith CD, Schneider DJ. An outbreak of ovine listeriosis associated with poor flock management practices. *J S Afr Vet Assoc.* 1984;**55**(2):55–56.
197. Mohammed HO, Stipetic K, McDonough PL, et al. Identification of potential on-farm sources of *Listeria monocytogenes* in herds of dairy cattle. *Am J Vet Res.* 2009;**70**(3):383–388.
198. Morin DE. Brainstem and cranial nerve abnormalities: listeriosis, otitis media/interna, and pituitary abscess syndrome. *Vet Clin North Am Food Anim Pract.* 2004;**20**(2):243–273.
199. Moura A, Criscuolo A, Pouseele H, et al. Whole genome-based population biology and epidemiological surveillance of *Listeria monocytogenes*. *Nat Microbiol.* 2017;**2**(2):16185.
200. Moura A, Lefrancq N, Leclercq A, et al. Emergence and global spread of *Listeria monocytogenes* main clinical clonal complex. *bioRxiv.* Preprint. doi: 10.1101/2020.12.18.423387
201. Müller H. *Listeria* isolations from feces of patients with diarrhea and from healthy food handlers. *Infection.* 1990;**18**(2):97–99.
202. Murray E, Webb R, Swann MR. A disease of rabbits characterized by large mononuclear leucocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (nova sp.). *J Pathol Bacteriol.* 1926;**29**:407–439.
203. Mylonakis E, Hohmann EL, Calderwood SB. Central nervous system infection with *Listeria monocytogenes*. 33 years' experience at a general hospital and review of 776 episodes from the literature. *Medicine (Baltimore).* 1998;**77**(5):313–336.

204. Nightingale K, Schukken Y, Nightingale C, et al. Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. *Appl Environ Microbiol*. 2004;**70**(8):4458–4467.
205. Nightingale KK, Fortes ED, Ho AJ, et al. Evaluation of farm management practices as risk factors for clinical listeriosis and fecal shedding of *Listeria monocytogenes* in ruminants. *J Am Vet Med Assoc*. 2005;**227**(11):1808–1814.
206. Nikitas G, Deschamps C, Disson O, et al. Transcytosis of *Listeria monocytogenes* across the intestinal barrier upon specific targeting of goblet cell accessible E-cadherin. *J Exp Med*. 2011;**208**(11):2263–2277.
207. Núñez-Montero K, Leclercq A, Moura A, et al. *Listeria costaricensis* sp. nov. *Int J Syst Evol Microbiol*. 2018;**68**(3):844–850.
208. Oevermann A, Botteron C, Seuberlich T, et al. Neuropathological survey of fallen stock: active surveillance reveals high prevalence of encephalitic listeriosis in small ruminants. *Vet Microbiol*. 2008;**130**(3–4):320–329.
209. Oevermann A, Di Palma S, Doherr MG, et al. Neuropathogenesis of naturally occurring encephalitis caused by *Listeria monocytogenes* in ruminants. *Brain Pathol*. 2010;**20**(2):378–390.
210. Oevermann A, Zurbriggen A, Vandeveld M. Rhombencephalitis caused by *Listeria monocytogenes* in humans and ruminants: a zoonosis on the rise? *Interdiscip Perspect Infect Dis*. 2010;**2010**:632513.
211. Okwumabua O, O'Connor M, Shull E, et al. Characterization of *Listeria monocytogenes* isolates from food animal clinical cases: PFGE pattern similarity to strains from human listeriosis cases. *FEMS Microbiol Lett*. 2005;**249**(2):275–281.
212. Ooi ST, Lorber B. Gastroenteritis due to *Listeria monocytogenes*. *Clin Infect Dis*. 2005;**40**(9):1327–1332.
213. Orsi RH, den Bakker HC, Wiedmann M. *Listeria monocytogenes* lineages: genomics, evolution, ecology, and phenotypic characteristics. *Int J Med Microbiol*. 2011;**301**(2):79–96.
214. Orsi RH, Wiedmann M. Characteristics and distribution of *Listeria* spp., including *Listeria* species newly described since 2009. *Appl Microbiol Biotechnol*. 2016;**100**(12):5273–5287.
215. Osborne SE, Brummell JH. Listeriolysin O: from bazooka to Swiss army knife. *Philos Trans R Soc Lond B Biol Sci*. 2017;**372**(1726):20160222.
216. Otter A, Blakemore WF. Observation on the presence of *Listeria monocytogenes* in axons. *Acta Microbiol Hung*. 1989;**36**(2–3):125–131.
217. Paciorek M, Bieńkowski C, Bednarska A, et al. The clinical course and outcome of *Listeria monocytogenes* meningitis: a retrospective single center study. *Neuro Endocrinol Lett*. 2019;**40**(2):79–84.
218. Pägelow D, Chhatbar C, Beineke A, et al. The olfactory epithelium as a port of entry in neonatal neuroinfection. *Nat Commun*. 2018;**9**(1):4269.
219. Pamer EG. Immune responses to *Listeria monocytogenes*. *Nat Rev Immunol*. 2004;**4**(10):812–823.
220. Papić B, Golob M, Kušar D, et al. Source tracking on a dairy farm reveals a high occurrence of subclinical mastitis due to hypervirulent *Listeria monocytogenes* clonal complexes. *J Appl Microbiol*. 2019;**127**(5): 1349–1361.
221. Papić B, Pate M, Félix B, et al. Genetic diversity of *Listeria monocytogenes* strains in ruminant abortion and rhombencephalitis cases in comparison with the natural environment. *BMC Microbiol*. 2019;**19**(1):299.
222. Parsons C, Niedermeyer J, Gould N, et al. *Listeria monocytogenes* at the human–wildlife interface: black bears (*Ursus americanus*) as potential vehicles for *Listeria*. *Microb Biotechnol*. 2020;**13**(3):706–721.
223. Pelegrín I, Moragas M, Suárez C, et al. *Listeria monocytogenes* meningoencephalitis in adults: analysis of factors related to unfavourable outcome. *Infection*. 2014;**42**(5):817–827.
224. Pentecost M, Otto G, Theriot JA, et al. *Listeria monocytogenes* invades the epithelial junctions at sites of cell extrusion. *PLoS Pathog*. 2006;**2**(1):e3.
225. Peron-Cane C, Fernandez JC, Leblanc J, et al. Fluorescent secreted bacterial effectors reveal active intravacuolar proliferation of *Listeria monocytogenes* in epithelial cells. *PLoS Pathog*. 2020;**16**(10):e1009001.
226. Perrin M, Bemer M, Delamare C. Fatal case of *Listeria innocua* bacteremia. *J Clin Microbiol*. 2003;**41**(11):5308–5309.
227. Peters M, Hewicker-Trautwein M. Infection of murine fetal brain cell cultures with *Listeria monocytogenes*. *Vet Microbiol*. 1994;**41**(1–2):19–28.
228. Peters M, Hewicker-Trautwein M. Studies on the cell tropism of *Listeria monocytogenes* in ovine fetal brain cell cultures. *Vet Microbiol*. 1996;**49**(3–4):169–179.
229. Peters M, Pohlentz J, Jatou K, et al. Studies of the detection of *Listeria monocytogenes* by culture and PCR in cerebrospinal fluid samples from ruminants with listeric encephalitis. *Zentralbl Veterinarmed B*. 1995;**42**(1–10):84–88.
230. Pilms B, Leclercq A, Maury MM, et al. Cutaneous listeriosis, a case series of 16 consecutive patients over 25 years. *J Infect*. 2020;**80**(2): 232–254.
231. Pintado CM, Grant KA, Halford-Maw R, et al. Association between a case study of asymptomatic ovine listerial mastitis and the contamination of soft cheese and cheese processing environment with *Listeria monocytogenes* in Portugal. *Foodborne Pathog Dis*. 2009;**6**(5):569–575.
232. Pirie JH. A new disease of veld rodents “tiger river disease.” *Publ S Afr Inst Med Res*. 1927;**3**(13):163–187.
233. Pizarro-Cerdá J, Kühbacher A, Cossart P. Entry of *Listeria monocytogenes* in mammalian epithelial cells: an updated view. *Cold Spring Harb Perspect Med*. 2012;**2**(11):a010009.
234. Pohl MA, Wiedmann M, Nightingale KK. Associations among *Listeria monocytogenes* genotypes and distinct clinical manifestations of listeriosis in cattle. *Am J Vet Res*. 2006;**67**(4):616–626.
235. Pouillot R, Klontz KC, Chen Y, et al. Infectious dose of *Listeria monocytogenes* in outbreak linked to ice cream, United States, 2015. *Emerg Infect Dis*. 2016;**22**(12):2113–2119.
236. Poulsen KP, Czuprynski CJ. Pathogenesis of listeriosis during pregnancy. *Anim Health Res Rev*. 2013;**14**(1):30–39.
237. Prado RGS, Domiciano TAO, Paredes LJA, et al. Nervous form of listeriosis in buffaloes. *Pesqui Vet Bras*. 2019;**39**(5):299–303.
238. Prats N, Briones V, Blanco M, et al. Choroiditis and meningitis in experimental murine infection with *Listeria monocytogenes*. *Eur J Clin Microbiol Infect Dis*. 1992;**11**(8):744–747.
239. Precht C, Vermathen P, Henke D, et al. Correlative magnetic resonance imaging and histopathology in small ruminant listeria rhombencephalitis. *Front Neurol*. 2020;**11**:518697.
240. Queda JJ, Dussurget O, Nahori MA, et al. Bacteriocin from epidemic *Listeria* strains alters the host intestinal microbiota to favor infection. *Proc Natl Acad Sci U S A*. 2016;**113**(20):5706–5711.
241. Queda JJ, Leclercq A, Moura A, et al. *Listeria valentina* sp. nov., isolated from a water trough and the faeces of healthy sheep. *Int J Syst Evol Microbiol*. 2020;**70**(11):5868–5879.
242. Raith K, Müntener T, Vandeveld M, et al. Encephalomyelitis resembling human and ruminant rhombencephalitis caused by *Listeria monocytogenes* in a feline leukemia virus-infected cat. *J Vet Intern Med*. 2010;**24**(4): 983–985.
243. Ramage CP, Low JC, McLauchlin J, et al. Characterisation of *Listeria ivanovii* isolates from the UK using pulsed-field gel electrophoresis. *FEMS Microbiol Lett*. 1999;**170**(2):349–353.
244. Rapose A, Lick SD, Ismail N. *Listeria grayi* bacteremia in a heart transplant recipient. *Transpl Infect Dis*. 2008;**10**(6):434–436.
245. Reuter R, Bowden M, Palmer M. Ovine listeriosis in south coastal Western Australia. *Aust Vet J*. 1989;**66**(7):223–224.
246. Revold T, Abayneh T, Brun-Hansen H, et al. *Listeria monocytogenes* associated kerato-conjunctivitis in four horses in Norway. *Acta Vet Scand*. 2015;**57**(1):76.
247. Rissi DR, Kommers GD, Marcolongo-Pereira C, et al. Meningoencefalite por *Listeria monocytogenes* em ovinos. *Pesqui Vet Bras*. 2010;**30**(1):51–56 [in Portuguese].
248. Rissi DR, Rech RR, Barros RR, et al. Forma nervosa de listeriose em caprinos. *Pesqui Vet Bras*. 2006;**26**(1):14–20 [in Portuguese].
249. Rocha CE, Mol JP, Garcia LN, et al. Comparative experimental infection of *Listeria monocytogenes* and *Listeria ivanovii* in bovine trophoblasts. *PLoS One*. 2017;**12**(5):e0176911.
250. Rocha PRDA, Dalmaso A, Grattarola C, et al. Atypical cerebral listeriosis associated with *Listeria innocua* in a beef bull. *Res Vet Sci*. 2013;**94**(1): 111–114.

251. Rocha PRDA, Lomonaco S, Bottero MT, et al. Ruminant rhombencephalitis-associated *Listeria monocytogenes* strains constitute a genetically homogeneous group related to human outbreak strains. *Appl Environ Microbiol*. 2013;**79**(9):3059–3066.
252. Rocourt J, Hof H, Schrettenbrunner A, et al. Acute purulent *Listeria seeligeri* meningitis in an immunocompetent adult. *Schweiz Med Wochenschr*. 1986;**116**(8):248–251 [in French].
253. Rupp S, Aguilar-Bultet L, Jagannathan V, et al. A naturally occurring prfA truncation in a *Listeria monocytogenes* field strain contributes to reduced replication and cell-to-cell spread. *Vet Microbiol*. 2015;**179**(1–2):91–101.
254. Rupp S, Bärtschi M, Frey J, et al. Hyperinvasiveness and increased intercellular spread of *Listeria monocytogenes* sequence type I are independent of listeriolysin S, internalin F and internalin J1. *J Med Microbiol*. 2017;**66**(7):1053–1062.
255. Rütten M, Lehner A, Pospischil A, et al. Cerebral listeriosis in an adult Freiburger gelding. *J Comp Pathol*. 2006;**134**(2–3):249–253.
256. Sanches AWD, Langohr IM, Stigger AL, et al. Doenças do sistema nervoso central em bovinos no Sul do Brasil. *Pesqui Vet Bras*. 2000;**20**(3):113–118 [in Portuguese].
257. Sasaki T, Mieno M, Udono H, et al. Roles of CD4+ and CD8+ cells, and the effect of administration of recombinant murine interferon gamma in listerial infection. *J Exp Med*. 1990;**171**(4):1141–1154.
258. Sauders BD, Wiedmann M. Ecology of listeria species and *L. monocytogenes* in the natural environment. In: Ryser ET, Marth EH, eds. *Listeria, Listeriosis and Food Safety*. 3rd ed. Taylor & Francis; 2007:21–53.
259. Schlafer DH, Foster RA. Female genital system. In: Maxie G, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 6th ed. Elsevier Health Sciences; 2015:358–464.
260. Schlech WF 3rd. Foodborne listeriosis. *Clin Infect Dis*. 2000;**31**(3):770–775.
261. Schlech WF 3rd, Lavigne PM, Bortolussi RA, et al. Epidemic listeriosis—evidence for transmission by food. *N Engl J Med*. 1983;**308**(4):203–206.
262. Schlech WF. Epidemiology and clinical manifestations of *Listeria monocytogenes* infection. *Microbiol Spectrum*. 2019;**7**(3). doi:10.1128/microbiolspec.GPP3-0014-2018
263. Schlüter D, Domann E, Buck C, et al. Phosphatidylcholine-specific phospholipase C from *Listeria monocytogenes* is an important virulence factor in murine cerebral listeriosis. *Infect Immun*. 1998;**66**(12):5930–5938.
264. Schlüter D, Oprisiu SB, Chahoud S, et al. Systemic immunization induces protective CD4+ and CD8+ T cell-mediated immune responses in murine *Listeria monocytogenes* meningoencephalitis. *Eur J Immunol*. 1995;**25**(8):2384–2391.
265. Schoder D, Winter P, Kareem A, et al. A case of sporadic ovine mastitis caused by *Listeria monocytogenes* and its effect on contamination of raw milk and raw-milk cheeses produced in the on-farm dairy. *J Dairy Res*. 2003;**70**(4):395.
266. Schuchat A, Swaminathan B, Broome CV. Epidemiology of human listeriosis. *Clin Microbiol Rev*. 1991;**4**(2):169–183.
267. Schuppler M. How the interaction of *Listeria monocytogenes* and *Acanthamoeba* spp. affects growth and distribution of the food borne pathogen. *Appl Microbiol Biotechnol*. 2014;**98**(7):2907–2916.
268. Schweizer G, Ehrensperger F, Torgerson PR, et al. Clinical findings and treatment of 94 cattle presumptively diagnosed with listeriosis. *Vet Rec*. 2006;**158**(17):588–592.
269. Scott P. Cerebrospinal fluid collection and analysis in suspected sheep neurological disease. *Small Rumin Res*. 2010;**92**(1–3):96–103.
270. Seaman JT, Carter GI, Carrigan MJ, et al. An outbreak of listerial myelitis in sheep. *Aust Vet J*. 1990;**67**(4):142–143.
271. Seeliger HPR, Höhne K. Serotyping of *Listeria monocytogenes* and related species. *Methods Microbiol*. 1979;**13**(2):31–49.
272. Seimiya Y, Ohshima K, Itoh H, et al. Listeric septicemia with meningitis in a neonatal calf. *J Vet Med Sci*. 1992;**54**(6):1205–1207.
273. Senay TE, Ferrell JL, Garrett FG, et al. Neurotropic lineage III strains of *Listeria monocytogenes* disseminate to the brain without reaching high titer in the blood. *MSphere*. 2020;**5**(5):e00871–e00820.
274. Sergeant ES, Love SC, McInnes A. Abortions in sheep due to *Listeria ivanovii*. *Aust Vet J*. 1991;**68**(1):39.
275. Siegman-Igra Y, Levin R, Weinberger M, et al. *Listeria monocytogenes* infection in Israel and review of cases worldwide. *Emerg Infect Dis*. 2002;**8**(3):305–310.
276. Stavru F, Archambaud C, Cossart P. Cell biology and immunology of *Listeria monocytogenes* infections: novel insights. *Immunol Rev*. 2011;**240**(1):160–184.
277. Steckler AJ, Cardenas-Alvarez MX, Ramsett MKT, et al. Genetic characterization of *Listeria monocytogenes* from ruminant listeriosis from different geographical regions in the US. *Vet Microbiol*. 2018;**215**:93–97.
278. Swaminathan B, Gerner-Smidt P. The epidemiology of human listeriosis. *Microbes Infect*. 2007;**9**(10):1236–1243.
279. Témoin S, Roche S, Grepinet O, et al. Multiple point mutations in virulence genes explain the low virulence of *Listeria monocytogenes* field strains. *Microbiology (Reading)*. 2008;**154**(Pt 3):939–948.
280. Thomas J, Govender N, McCarthy KM, et al. Outbreak of listeriosis in South Africa associated with processed meat. *N Engl J Med*. 2020;**382**(7):632–643.
281. Tiensuu T, Guerreiro DN, Oliveira AH, et al. Flick of a switch: regulatory mechanisms allowing *Listeria monocytogenes* to transition from a saprophyte to a killer. *Microbiology (Reading)*. 2019;**165**(8):819–833.
282. Truchet L, Walland J, Wüthrich D, et al. Neuropathological survey reveals underestimation of the prevalence of neuroinfectious diseases in cattle in Switzerland. *Vet Microbiol*. 2017;**208**:137–145.
283. Tsai YH, Disson O, Bierne H, et al. Murinization of internalin extends its receptor repertoire, altering *Listeria monocytogenes* cell tropism and host responses. *PLoS Pathog*. 2013;**9**(5):e1003381.
284. Tzora A, Fthenakis GC, Linde K. The effects of inoculation of *Listeria monocytogenes* into the ovine mammary gland. *Vet Microbiol*. 1998;**59**(2–3):193–202.
285. Uldry PA, Kuntzer T, Bogousslavsky J, et al. Early symptoms and outcome of *Listeria monocytogenes* rhombencephalitis: 14 adult cases. *J Neurol*. 1993;**240**(4):235–242.
286. Unnerstad H, Romell A, Ericsson H, et al. *Listeria monocytogenes* in faeces from clinically healthy dairy cows in Sweden. *Acta Vet Scand*. 2000;**41**(2):167–171.
287. US Food and Drug Administration. Draft guidance for industry: control of *Listeria monocytogenes* in ready-to-eat foods. *Fed Regist*. 2017;**82**:4803–4805.
288. USDA Food Safety and Inspection Service. Control of *Listeria monocytogenes* in ready-to-eat meat and poultry products; final rule. *Fed Regist*. 2003;**68**:34208–34254.
289. Vandegraaff R, Borland N, Browning J. An outbreak of listerial meningoencephalitis in sheep. *Aust Vet J*. 1981;**57**(2):94–96.
290. Vasanthakrishnan RB, de Las Heras A, Scotti M, et al. PrfA regulation offsets the cost of *Listeria* virulence outside the host. *Environ Microbiol*. 2015;**17**(11):4566–4579.
291. Vazquez-Boland J, Dominguez L, Blanco M, et al. Epidemiologic investigation of a silage-associated epizootic of ovine listeric encephalitis, using a new *Listeria*-selective enumeration medium and phage typing. *Am J Vet Res*. 1992;**53**(3):368–371.
292. Vázquez-Boland JA, Domínguez-Bernal G, González-Zorn B, et al. Pathogenicity islands and virulence evolution in *Listeria*. *Microbes Infect*. 2001;**3**(7):571–584.
293. Vázquez-Boland JA, Kryptou E, Scotti M. *Listeria* placental infection. *mBio*. 2017;**8**(3):e00949–17.
294. Vázquez-Boland JA, Kuhn M, Berche P, et al. *Listeria* pathogenesis and molecular virulence determinants. *Clin Microbiol Rev*. 2001;**14**(3):584–640.

295. Vázquez-Boland JA, Wagner M, Scortti M. Why are some *Listeria monocytogenes* genotypes more likely to cause invasive (brain, placental) infection? *mBio*. 2020;**11**(6):e03126–20.
296. Vivant AL, Garmyn D, Piveteau P. *Listeria monocytogenes*, a down-to-earth pathogen. *Front Cell Infect Microbiol*. 2013;**3**:87.
297. Walker J, Morgan J, McLauchlin J, et al. *Listeria innocua* isolated from a case of ovine meningoencephalitis. *Vet Microbiol*. 1994;**42**(2–3):245–253.
298. Walland J, Lauper J, Frey J, et al. *Listeria monocytogenes* infection in ruminants: is there a link to the environment, food and human health? A review. *Schweiz Arch Tierheilk*. 2015;**157**(6):319–328.
299. Wang C, Chou CH, Tseng C, et al. Early gene response of human brain microvascular endothelial cells to *Listeria monocytogenes* infection. *Can J Microbiol*. 2011;**57**(5):441–446.
300. Wang L, Lin M. A novel cell wall-anchored peptidoglycan hydrolase (autolysin), IspC, essential for *Listeria monocytogenes* virulence: genetic and proteomic analysis. *Microbiology (Reading)*. 2008;**154**(Pt 7):1900–1913.
301. Wardrope D, MacLeod N. Outbreak of listeria meningoencephalitis in young lambs. *Vet Rec*. 1983;**113**(10):213–214.
302. Warren J, Owen AR, Glanvill A, et al. A new bovine conjunctiva model shows that *Listeria monocytogenes* invasion is associated with lysozyme resistance. *Vet Microbiol*. 2015;**179**(1–2):76–81.
303. Wei P, Bao R, Fan Y. Brainstem encephalitis caused by *Listeria monocytogenes*. *Pathogens*. 2020;**9**(9):715.
304. Weindl L, Frank E, Ullrich U, et al. *Listeria monocytogenes* in different specimens from healthy red deer and wild boars. *Foodborne Pathog Dis*. 2016;**13**(7):391–397.
305. Weis J, Seeliger H. Incidence of *Listeria monocytogenes* in nature. *Appl Microbiol*. 1975;**30**(1):29–32.
306. Wesley IV. Listeriosis in animals. In: Ryser E, Marth E, eds. *Listeria, Listeriosis, and Food Safety*. 3rd ed. Taylor & Francis; 2007:73–102.
307. Wesley IV, Bryner JH, Van Der Maaten MJ, et al. Effects of dexamethasone on shedding of *Listeria monocytogenes* in dairy cattle. *Am J Vet Res*. 1989;**50**(12):2009–2013.
308. Wiedmann M, Czajka J, Bsat N, et al. Diagnosis and epidemiological association of *Listeria monocytogenes* strains in two outbreaks of listerial encephalitis in small ruminants. *J Clin Microbiol*. 1994;**32**(4):991–996.
309. Wilesmith J, Gitter M. Epidemiology of ovine listeriosis in Great Britain. *Vet Rec*. 1986;**119**(19):467–470.
310. Wilson SL, Drevets DA. *Listeria monocytogenes* infection and activation of human brain microvascular endothelial cells. *J Infect Dis*. 1998;**178**(6):1658–1666.
311. Winter P, Schilcher F, Bago Z, et al. Clinical and histopathological aspects of naturally occurring mastitis caused by *Listeria monocytogenes* in cattle and ewes. *J Vet Med B Infect Dis Vet Public Health*. 2004;**51**(4):176–179.
312. Yin Y, Tan W, Wang G, et al. Geographical and longitudinal analysis of *Listeria monocytogenes* genetic diversity reveals its correlation with virulence and unique evolution. *Microbiol Res*. 2015;**175**:84–92.
313. Yin Y, Yao H, Doijad S, et al. A hybrid sub-lineage of *Listeria monocytogenes* comprising hypervirulent isolates. *Nat Commun*. 2019;**10**(1):4283.
314. Yoshida T, Sugimoto T, Sato M, et al. Incidence of *Listeria monocytogenes* in wild animals in Japan. *J Vet Med Sci*. 2000;**62**(6):673–675.
315. Zhang T, Bae D, Wang C. LMOh7858_0369, a gene encoding a putative leucine-rich repeat-containing protein, is required for virulence of *Listeria monocytogenes*. *FEMS Microbiol Lett*. 2016;**363**(9):fnw060.
316. Zhou X, Elmore J, Call DR. Interactions between the environmental pathogen *Listeria monocytogenes* and a free-living protozoan (*Acanthamoeba castellanii*). *Environ Microbiol*. 2007;**9**(4):913–922.
317. Zundel E, Bernard S. *Listeria monocytogenes* translocates throughout the digestive tract in asymptomatic sheep. *J Med Microbiol*. 2006;**55**(Pt 12):1717–1723.