RESEARCH ARTICLE

Neuropathological Findings in 9 Cases of Listeria Monocytogenes Brain Stem Encephalitis

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Brain stem encephalitis is a particular manifestation of infection with the bacterium Listeria monocytogenes. Here, we present the neuropathological findings in 9 such cases. In the brain stem, the inflammatory infiltrates were located predominantly within nuclei and tracts of cranial nerves innervating the oropharynx. These findings support the hypothesis that the food-borne bacterium Listeria monocytogenes invades the brain stem along cranial nerves.

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INTRODUCTION

Listeriosis is a food-borne infection (3, 14, 16) caused by the facultatively intracellular bacterium *Listeria monocytogenes*. Infection mainly occurs as sepsis or meningitis (14, 20). Listeria brain stem encephalitis is a well-defined entity characterized by progressive brain stem dysfunction; this includes variable degrees and patterns of combined motor, sensory and cerebellar deficits, as well as disturbances of respiration, circulation and consciousness (5, 6). Fever and meningeal affection are variably present, and the condition may be difficult to diagnose both clinically and with laboratory tests (7, 20).

Brain stem encephalitis is the main manifestation of listeriosis in sheep (15). In this animal, observations of focal subacute inflammation and bacteria in the brain stem and cranial nerves gave rise to the hypothesis that after crossing the oral mucosa, food-borne Listeria bacteria enter the brain stem by retrograde axonal transport within cranial nerves (9, 18). Recent experimental evidence for bacterial transport following peripheral inoculation of *L. monocytogenes* supports this hypothesis (4).

In humans, brain stem encephalitis occurs in at least 10% of the listeriosis cases (5). However, few autopsy descriptions of Listeria brain stem encephalitis have been published (6, 21) and the precise localization of the inflammatory infiltrates in relation to different brain stem structures has not been defined.

Here, we present the neuropathological findings in 9 human cases of Listeria brain stem encephalitis.

MATERIALS AND METHODS

The 9 cases of brain stem listeriosis, from which brain autopsy material was available, included 7 cases identified in a recent retrospective study of listeriosis patients reported in Norway between 1977 and 2000 (5), and 2 additional cases of brain stem listeriosis that occurred in Norway after 2000. *Listeria monocytogenes* had been identified in blood or CSF in 8 of the patients; and by immunohistochemical examination of autopsy tissue in one.

From each of the 9 autopsy cases, at least 2 sections from the brain stem in addition to several sections from other parts of the CNS were available for histological examination.

The histological sections were stained with H&E in order to identify the localization of the inflammatory infiltrates. Parallel sections were immunostained for *L. monocytogenes* serotypes I and IV with 2 polyclonal rabbit anti-Listeria antisera (dilution 1:1280, DifCo Laboratories, Mich). To characterize the leukocyte infiltrates and microglial activation, sections were immunostained with markers for various CD antigens (CD 3, CD 4 and CD 8 [pan-T, T-helper and T-suppressor lymphocyte subsets, respectively], CD 20 [B-lymphocyte], CD 45 [pan-leukocyte] and CD 68 [macrophage] markers). An indirect biotin avidine system (Ventana Detection Kit, Ventana Medical Systems, Ariz) was used for visualization of the immunostainings.

In addition to the findings in the 9 autopsy cases, we present cerebral MRI images of brain stem lesions in a patient who was successfully treated for Listeria brain stem encephalitis. The MRI scan was performed subsequent to clinically diagnosed brain stem deficits and positive blood cultures for *Listeria monocytogenes*. Details of the clinical course of this patient are described elsewhere (2).

RESULTS

Localization of inflammatory infiltrates. Listeria brain stem encephalitis mainly involved the medulla oblongata (Table 1). Leukocyte infiltrations were mostly present in one or more of the nuclei and tracts of cranial nerves innervating the oropharynx; the Vth, VIIth, IXth, Xth, and XIIth cranial nerves (Table 1; Figure 1). In cases 1, 2 and 3 (Table 1), inflammation was located unilaterally in the nucleus and corresponding brain stem part of the XIIth cranial nerve (Figures 1, 2). In case 4, inflammation was present in segments of the XIIth nerve, within and outside the brain stem. In case 5, inflammation located to the dorsal parts of the medulla oblongata. In cases 7 to 9, inflammation was present in the area of the solitary tract, its nucleus and the spinal trigeminal nucleus (Table 1; Figure 1). Other parts of the CNS than the brain stem were mostly free of inflammation; exceptions are described in Table 1.

Pat. no/sex/age	P = Predisposing condition for listeriosis B = Brain stem deficits and symptoms of infection described in medical record LM = Sample in which *Listeria monocytogenes* was identified DT = Clinical diagnosis and treatment	Affected brain stem structures* according to available clinical information	D = Death, day after admission H = Histology; inflammatory infiltrations in the brain stem I = Immunohistochemical staining for *Listeria monocytogenes*
1/F/67	P: Rheumatoid arthritis treated with prednisolone and methotrexate. B: Fever. Gaze paresis, parestesias in left trigeminal region, left facial paresis, dysphagia. Ataxia, nausea, vertigo. Progression to somnolence and respiratory failure. MRI was performed after brain stem deficits were diagnosed by clinical examination and revealed a lesion in the medulla oblongata. LM: Blood and CSF. DT: Listeria brain stem encephalitis treated with ampicillin. Hydrocephalus treated with external ventricle drainage.	V, VII, IX/X/XII, C, R	D: 7 H: Subacute inflammation with numerous macrophages located in dorsal brain stem including the course and nuclei of IXth, Xth and XIIth nerves. I: Serotype 4.
2/F/54	P: Lymphoma treated with prednisolone. B: Fever. Horizontal gaze deviation, paresthesias in right trigeminal region, right facial paresis, dysphagia, paretic soft palate, dysarthria. Coma, bilateral plantar inversion. LM: Blood, positive culture on day of death. DT: Basal cranial metastases treated with total brain irradiation and dexamethasone.	V, VII, IX, X, XII, R, gaze center	D: 18 H: Subacute inflammation with abscess formation in the intramedullar course and nuclei of IXth, Xth and XIIth nerves. (Also abscesses in the right putamen and in the region of the left basal ganglia/ thalamus.) I: Serotype 1.
3/F/80	B: Headache, nausea, fever and hypertension. Dilated left pupil, unresponsive to light. Paretic extremities. Increased tendon reflexes. Cheynes Stokes' respiration. Reduced consciousness. Coma. LM: Autopsy tissue (immunohistochemistry). DT: Hydrocephalus treated with ventriculoperitoneal shunting day 5. No clinical effect.	III, M, R	D: 10 H: Subacute inflammation with small necrotic foci and microglial nodules in the lower dorsal brain stem including the course and nuclei of IXth, Xth and XIIth nerves. (Also meningitis, ventriculitis and secondary hydrocephalus.) I: Serotype 1.
4/M/55	B: Fever. Spastic extremities. Coma, respiratory failure. Multiorgan failure. LM: Blood and CSF. DT: Gram-positive peritonitis with sepsis, treated with ampicillin.	M, R	D: 3 H: Subacute inflammation in the dorsal medulla oblongata (the XIIth nerve and its nucleus) and in the proximal part of the XIIth nerve. (Also inflammatory infiltrations in the temporal lobe.) I: Serotype 1.
5/F/14	B: 7 days of fever and headache. Gaze paresis and facial paresis. Nystagmus. Coma, respiratory failure. LM: Blood, positive culture post mortem. DT: Viral meningitis treated with dexamethasone.	VI, VII, C, R	D: 8 H: Bilateral subacute inflammation in the intramedullary course of the Vth and Xth nerves, laterally to the solitary tract nuclei, Vth spinal nerve nucleus, along the olivocerebellar tract and focally in the cerebellum. (Also involvement in left thalamus and hippocampus.) I: Serotype 4.
6/M/74	P: Prednisolone treatment for COPD. Excessive alcohol consumption. B: Acute confusion and dysphagia. Fever. LM: Blood. DT: Sepsis treated with ampicillin.	IX, X, R	D: 4 H: Subacute inflammation in both solitary tract nuclei. I: Serotype 4.
7/M/76	P: Prednisolone treatment for COPD. Diabetes mellitus type II. B: Fever, respiratory distress and reduced consciousness. LM: Blood. DT: Bilateral Listeria pneumonia and meningitis treated with penicillin and aminoglycoside. B: Sudden death during reconvalescense phase.	No information	D: 11 H: Microglial nodules in one solitary tract nucleus. (Also macrophages and lymphocytes in the meninges.) I: No bacteria.
8/F/77	P: Prednisolone treatment for suspected temporal arteritis. LM: CSF. DT: Listeria meningitis treated with ampicillin. B: Sudden respiratory distress during reconvalescence phase, lung edema.	R	D: 17 H: Microglial nodules in one spinal trigeminal nucleus and the ipsilateral solitary tract nucleus. (Also recent hemorrhagic infarction in the cerebellar cortex.) I: No bacteria.
9/M/73	P: Diabetes mellitus, ulcerative colitis, rheumatoid arthritis. Methotrexate and azathioprine. B: Fluctuating fever, headache. Vertigo, progressively reduced consciousness to coma. LM: Blood. DT: Listeria sepsis treated with vancomycin, netilmycin and trimetoprim-sulphametoxazole.	VIII/ C, R	D: 21 H: Microglial nodules in one solitary tract nucleus. I: No bacteria.

Table 1. Clinical information of nine patients with Listeria monocytogenes brain stem encephalitis.
* I-XII, deficits originating from cranial nerve and/or its nucleus; M, motor system deficits (paresis and/or spasticity and/or extensor plantar response); R, abnormal respiration and/ or consciousness; C, cerebellar system deficits (cerebellar ataxia and/ or nystagmus, dysdiadochokinesia and/ or deficits at finger-nose or knee-heel test).

Composition of inflammatory infiltrates. In 6 cases (Table 1; cases 1-6), the lesions consisted of bacteria, polymorphonuclear and mononuclear leukocytes and occasional microabscesses (Figure 3A, B). Occasional necrotic neurons and neuronophagia were also observed within the lesions. In case 1 (Table 1), the lesions were dominated by macrophages. In case 3 (Table 1), the inflammatory infiltrates consisted of acute inflammatory infiltrates with microabscess formation as well as microglial nodules and accumulations of lymphocytes. In cases 7 to 9 (Table 1) only light chronic inflammation with scattered lymphocytes, microglial activation and a few microglial nodules (Figure 4A) were observed in the dorsal brain stem. The microglial nodules were devoid of bacteria and composed of microglial cells and T-lymphocytes (Figure 4B). Most of the T-lymphocytes were CD8-positive (not shown).

Cerebral MRI findings in Listeria brain stem encephalitis. Contrast enhancement was visible along the intraparenchymal passage of the trigeminal nerve (Figure 5A) and in the area that includes the right spinal trigeminal nucleus, solitary tract and hypoglossal nerve nucleus (Figure 5B) of the additional patient who survived the infection (2).

DISCUSSION

We have described the neuropathological findings in 9 cases of human Listeria brain stem encephalitis. The inflammatory infiltrates were predominantly present within nuclei, tracts and intraparenchymal parts of cranial nerves innervating the oropharynx (V^{th}, VII^{th}, IX^{th}, X^{th} and XII^{th} nerves) (Figure 1). Findings on cerebral MRI in a tenth patient who survived the infection (Figure 5) indicated a similar location of inflammation in the brain stem. As shown elsewhere (5) the recorded neurological deficits tend to correspond to the localization of the brain stem lesions at autopsy.

Brain stem encephalitis occurs in at least 10% of human listeriosis cases (5, 7). Observations of bacteria within cranial nerves and their nuclei in sheep (9, 18) led to the hypothesis that food-borne *Listeria monocytogenes* enters the brain stem along neural pathways connecting the brain stem and the upper gastrointestinal tract. The hypothesis of retrograde, intraaxonal transport of Listeria has later been tested in mice (4, 11, 18). Five to 10 days after Listeria bacteria were inoculated unilaterally into facial or sciatic nerve, the animals developed ipsilateral central nervous deficits. On histological examination bacteria were present within axons of the inoculated nerve, and inflammatory infiltrates were seen in the inoculated nerve and its nucleus in the CNS. Section of the nerve proximally to the inoculation site prevented spread of the infection to the CNS (4).

Our observations in human autopsy material resemble those made in animals (1, 4, 8, 9, 18) and support the hypothesis that foodborne *Listeria monocytogenes* exploits an intraaxonal pathway within cranial nerves to enter the brain stem from the oral cavity. The distribution of inflammatory infiltrates in both motor and sensory nerves

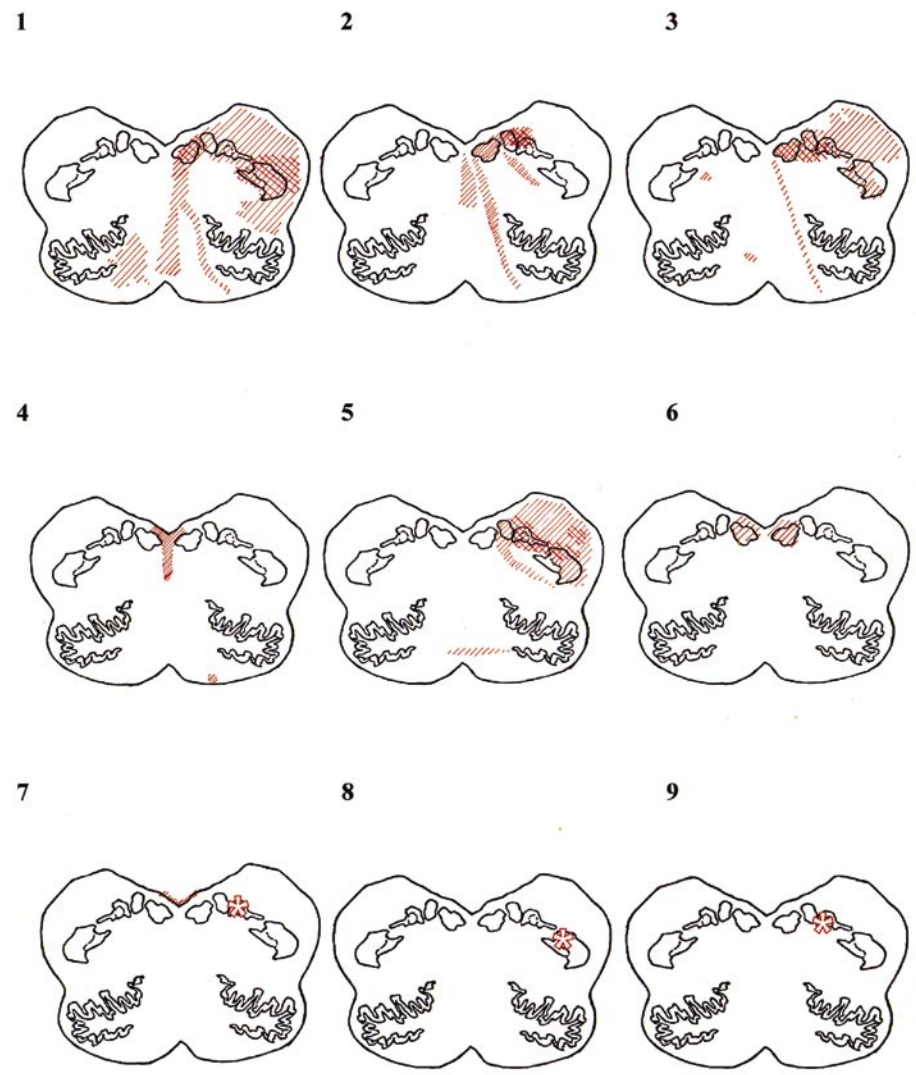

Figure 1. Sketch of the localization of inflammatory infiltrates in the medulla oblongata[1] of 9 patients with listeria brain stem encephalitis. Inflammation is mainly present in the regions of nuclei and pathways of cranial nerves innervating the mouth and pharynx (V^{th}, IX^{th}, X^{th}, XII^{th}).

Types of inflammatory infiltrates:

Microabscesses consisting of polymorphonuclear granulocytes and listeria bacteria.

Inflammatory infiltrates with abscess formation and necrosis.

Microglial nodules.

[1] For simplicity, the right side of the sections has been chosen as a reference for the sketches in all 9 cases.

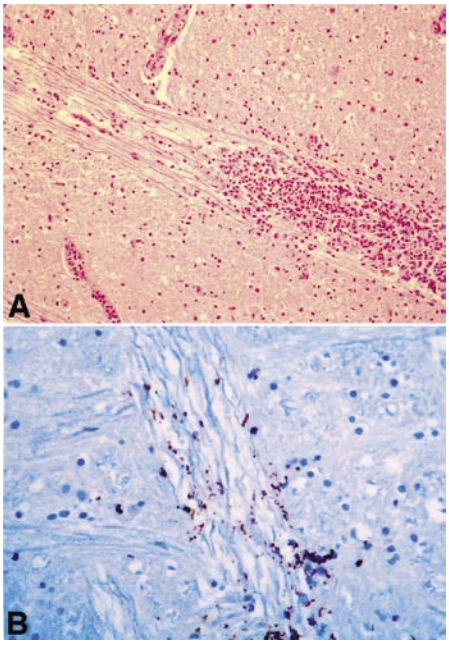

Figure 2. A. Inflammatory infiltrates along the pathway of the XIIth cranial nerve in case 3 (H&E staining, 4×). **B.** Bacteria along the pathway of the XIIth cranial nerve in case 2 (immunohistochemical staining for *Listeria monocytogenes*, serotype I, 20×).

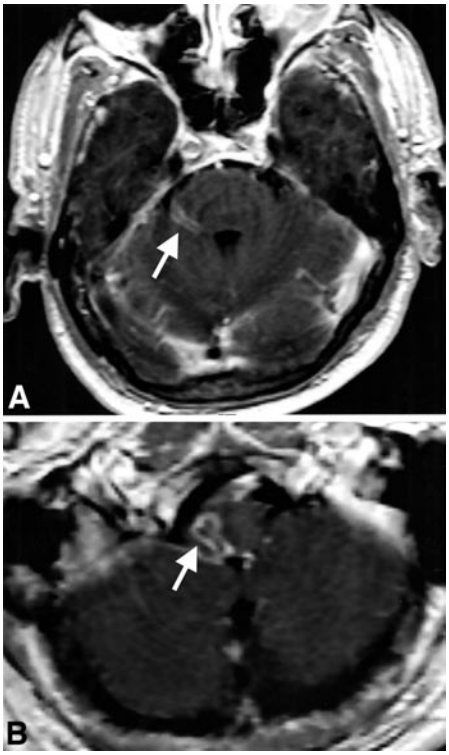

Figure 5. Additional patient successfully treated for Listeria brain stem encephalitis (2). T1-weighted Gadolinium–enhanced MRI. **A.** Transverse section showing contrast enhancement along the pathway of right Vth nerve. **B.** Transverse section showing contrast enhancement in the region of the 12th cranial nerve nucleus, solitary tract and spinal trigeminal nucleus in the right medulla oblongata.

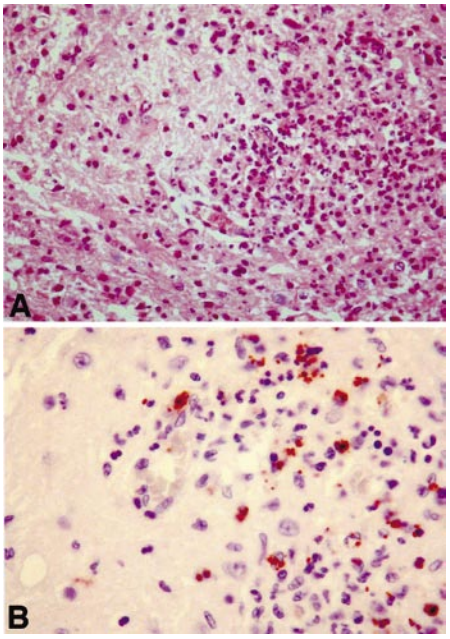

Figure 3. A. Subacute inflammation with microabscess formation in the nucleus of the XIIth cranial nerve in the medulla oblongata of case 2 (H&E staining, 20×). **B.** Bacteria within microabscess (immunohistochemical staining for *Listeria monocytogenes*, serotype I, 40×).

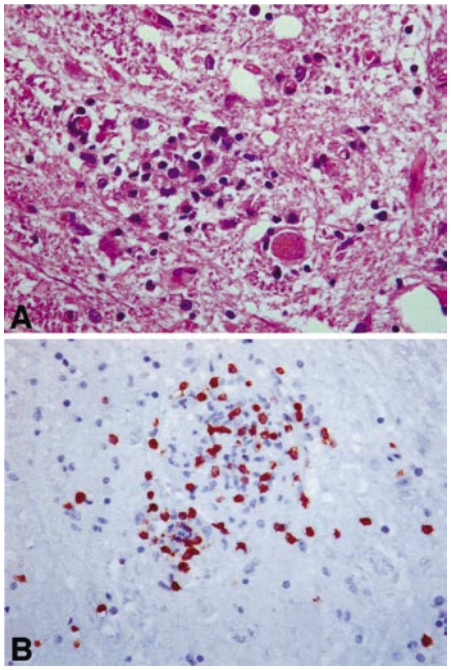

Figure 4. A. Microglial nodule localized in the solitary tract nucleus of case 8 (H&E, 20× (× 1.5). **B.** T-lymphocytes (CD 3+) and microglial cells (unstained) in the same microglial nodule (immunohistochemical staining for CD3 antigen, 20×).

and nuclei suggests that the bacterium may enter both retrogradely in motor nerves and transganglionically in sensory nerves.

How *Listeria monocytogenes* crosses epithelial barriers is poorly understood. Listeria brain stem encephalitis may arise in association with mucosal injury within the oral cavity, such as tooth loss in sheep (8, 15) and dental extractions in human cases (13, 17). Moreover, in mice, exposure of experimentally injured oral mucosa to Listeria bacteria may lead to the development of Listeria brain stem encephalitis (1, [Antal, unpublished data]). It has not been demonstrated that the organism is able to cross intact oral mucosa, but uptake of Listeria via M-cells in gastrointestinal lymphoid tissue has been suggested (12).

Few neurons are infected when exposed to Listeria bacteria in vitro, but neuronal entry appears to be facilitated if the cultures are enriched with bacteria-infected macrophages (10). How *Listeria monocytogenes* is able to enter axons of peripheral nerves in vivo remains unknown.

Intraaxonal transport along microtubules allows several viruses (and maybe prions) to invade the brain along peripheral nerves (22). Listeria monocytogenes may exploit a different intraaxonal transport mechanism. In cell culture, intracellular Listeria bacteria exhibit high-speed (0.15 μm/s), actin based motility (14, 19). It is interesting that *Burkholderia pseudomallei*—the other bacterium that also may give rise to brain stem encephalitis (with microabscesses and bacteria predominantly in the brain stem)—also demonstrates actin based intracellular motility in cell culture (23). It remains to be shown whether the intraaxonal transport of *L. monocytogenes* occurs along microtubules or is actin dependent.

In conclusion, we have identified inflammatory infiltrates and Listeria bacteria within cranial nerves innervating the oropharynx and their nuclei in nine human cases of Listeria brain stem encephalitis. We suggest that *L. monocytogenes* may invade the human brain stem along cranial nerves.

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REFERENCES

1. Akiyama Y, Asahi O, Hosoda T (1957) Studies on the mechanism of infection of the brain with Listeria monocytogenes. *Am J Vet Res* 18:147-157.

2. Alstadhaug KB, Antal EA, Nielsen EW, Rusic Z, Mortensen L, Salvesen R (2005) Listeria rhombencephalitis - a case report. *Eur J Neurol* in press.

3. Altekruse SF, Cohen ML, Swerdlow DL (1997) Emerging foodborne diseases. *Emerg Infect Dis* 3:285-293.

4. Antal EA, Dietrichs E, Løberg EM, Melby KK, Mæhlen J (2005) Brain stem encephalitis in listeriosis. *Scand J Infect Dis* 37:190-194.

4. Antal EA, Løberg EM, Bracht P, Melby KK, Mæhlen J (2001) Evidence for intraaxonal spread of Listeria monocytogenes from the periphery to the central nervous system. *Brain Pathol* 11:432-438.

6. Armstrong RW, Fung PC (1993) Brainstem encephalitis (rhombencephalitis) due to Listeria monocytogenes: case report and review. *Clin Infect Dis* 16:689-702.

7. Bartt R (2000) Listeria and atypical presentations of Listeria in the central nervous system. *Semin Neurol* 20:361-373.

8. Barlow RM, McGorum B (1985) Ovine listerial encephalitis: Analysis, hypothesis and synthesis. *Vet Rec* 2:233-236.

9. Charlton KM, Garcia MM (1977) Spontaneous listeric encephalitis and neuritis in sheep. Light microscopic studies. *Vet Pathol* 14:297-313.

10. Dramsi S, Levi S, Triller A, Cossart P (1998) Entry of Listeria monocytogenes into neurons occurs by cell-to-cell spread: an in vitro study. *Infect Immun* 66:4461-4468.

11. Drevets DA, Leenen PJ, Greenfield RA (2004) Invasion of the central nervous system by intracellular bacteria. *Clin Microbiol Rev* 17:323-347.

12. Jensen VB, Harty JT, Jones BD (1998) Interactions of the invasive pathogens Salmonella typhimurium, Listeria monocytogenes, and Shigella flexneri with M cells and murine Peyer's patches. *Infect Immun* 66:3758-3766.

13. Kennard C, Howard AJ, Scholtz C, Swash M (1979) Infection of the brainstem by Listeria monocytogenes. *J Neurol Neurosurg Psychiatry* 42:931-933 .

14. Lorber B (1997) Listeriosis. *Clin Infect Dis* 24:1-9.

15. Low JC, Donachie W (1997) A review of Listeria monocytogenes and listeriosis. *Vet J* 153:9-29.

16. Malinverni R, Bille J, Perret C, Regli F, Tanner F, Glauser MP (1985) Epidemic listeriosis. Report of 25 cases in 15 months at the Vaud University Hospital Center. *Schweiz Med Wochenschr* 115:2-10.

17. Medina-Flores R, Germanwala A, Molina JT, Meltzer CC, Wiley CA (2004) October 2003: a 59-year-old woman with sudden onset of diplopia. Listerial rhombencephalitis. *Brain Pathol* 14:225-226 .

18. Otter A, Blakemore WF (1989) Observation on the presence of Listeria monocytogenes in axons. *Acta Microbiol Hung* 36:125-131.

19. Pizarro-Cerda J, Sousa S, Cossart P (2004) Exploitation of host cell cytoskeleton and signalling during Listeria monocytogenes entry into mammalian cells. *C R Biol* 327:523-531 .

20. Shuchat A, Broome V (2004) Infection caused by Listeria monocytogenes. In: *Harrison's Principles of Internal Medicine*, 16th edition, Kasper DL, E Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL, Isselbacher KJ (eds.), Chapter 123, McGraw-Hill, New York, USA .

21. Uldry PA, Kuntzer T, Bogousslavsky J et al (1993) Early symptoms and outcome of Listeria monocytogenes rhombencephalitis:14 adult cases. *J Neurol* 240:235-242.

22. Von Bartheld CS (2004) Axonal transport and neuronal transcytosis of trophic factors, tracers, and pathogens. *J Neurobiol* 58:295-314 .

23. White NJ (2003) Melioidosis. *Lancet* 361:1715-1722.