

Neonatal *Listeria*-meningitis in San Luis, Argentina: a three-case report

ANALÍA L. LACIAR^{1*}, MARÍA L. VACA RUIZ¹, ALBAN LE MONNIER²

¹Área Microbiología, Universidad Nacional de San Luis. Chacabuco y Pedernera (5700) San Luis, Argentina; ²Laboratoire des Listeria, Centre National de Référence des Listeria and World Health Organization Collaborating Centre for Foodborne Listeriosis, Institut Pasteur. 75724 Paris Cedex 15, Paris, France.

*Correspondence. E-mail: alaciar@unsl.edu.ar

ABSTRACT

Between November 1996 and December 2006, two cases of early-onset and one case of late-onset neonatal listeriosis were reported in San Luis, Argentina. This article describes clinical and laboratory findings as well as treatment and outcome for newborns treated for *Listeria monocytogenes* meningitis or septicaemia. In one of the newborns with early-onset listeriosis, meningitis led to important complications including hydrocephalus. The two other newborns showed complete recovery following adequate treatment. The *L. monocytogenes* isolates from two patients belonged to PCR group IVb (including serovar 4b strains) and to PCR group IIb (including serovar 1/2b strains) in the third patient. Listeriosis, especially the maternal-fetal presentation, is still rare in Argentina for unknown reasons. Our data can be used in the future as an epidemiological survey.

Key words: *Listeria monocytogenes*, neonatal meningitis, Argentina

RESUMEN

Meningitis neonatal por *Listeria monocytogenes* en San Luis, Argentina: análisis de tres casos. En el presente estudio se describen tres casos de infección neonatal por *Listeria monocytogenes*, dos de inicio temprano y uno tardío, diagnosticados en San Luis, Argentina, entre noviembre de 1996 y diciembre de 2006. En uno de los pacientes afectados por listeriosis temprana, la meningitis condujo a la hidrocefalia secundaria. En los otros recién nacidos, la evolución clínica fue favorable después de la administración de un rápido y adecuado tratamiento. Los aislamientos de *L. monocytogenes* de dos pacientes pertenecieron al grupo IVb (serovar 4b) y el del tercer paciente al grupo IIB (serovar 1/2b) según la técnica de PCR. La listeriosis es, por razones que se desconocen, una enfermedad rara en Argentina, especialmente la presentación materno-fetal. Los resultados presentados aquí podrán ser utilizados en un futuro con fines epidemiológicos.

Palabras clave: *Listeria monocytogenes*, meningitis neonatal, Argentina

Listeriosis during pregnancy leads to abortion, stillbirth or premature delivery of newborns (NB) with neonatal sepsis and meningitis. These presentations are associated with a high fatality rate especially related to the term of pregnancy. Neonatal listeriosis may present either early or late (4). In San Luis province, between 1996 and 2006, a total of 33 cases of suspected neonatal *Listeria* meningitis were registered. Out of a total of 103 samples analyzed, *Listeria monocytogenes* was identified in 5 samples (4.85%) occurring in the three mentioned patients from two adjacent district general hospitals. We describe here their clinical and epidemiological findings to highlight their variable presentation.

In case 1, the mother related an influenza-like syndrome 15 days prior to the onset. The NB, a girl, was born by vaginal route after a 37-week gestation. The Apgar scores were 0/6 (one and five minutes after birth, respectively). She showed respiratory distress and required type IV re-animation. She was transferred to the Neonatal Intensive

Care Unit of the San Luis Hospital in incubator with respiratory assistance and presenting symptoms compatible with precocious neonatal sepsis and petechial lesions on her face, trunk and abdomen, which disappeared after 24 h. Blood and cerebrospinal fluid (CSF) cultures were taken and she was immediately treated with cephalothin. After 72 h, all the cultures were positive for *L. monocytogenes*. Initially prescribed antibiotic therapy was changed to ampicillin i.v. (100 mg/kg/day) plus gentamicin i.v. (3 mg/kg/day). The NB showed evidence of hydrocephalus and she was shunt-operated.

In case 2, the NB, a male, was born by vaginal route at term gestation. He was discharged 48 h after delivery and readmitted to San Luis Hospital on day 15 because of fever, reticulated skin, weeping, diminished suction and high heart rate. Lumbar puncture yielded cloudy CSF with a leukocyte count of 1300 cells/mm³, a glucose concentration of 1.9 g/l, and a protein concentration of 3 g/l. CSF culture yielded *L. monocytogenes* after 48h of incubation,

Table 1. Characteristics of the *L. monocytogenes* isolates obtained in this study

Isolates	Origin	Patient	Date	MIC(μ g/ml)	Serotyping	
					Serovar ⁽¹⁾	PCR group
<i>L. monocytogenes</i> 1	CSF ⁽²⁾	1	1996/06/15	1.75	4b	IVb
<i>L. monocytogenes</i> 2	Blood	1	1996/06/07	1.75	4b	IVb
<i>L. monocytogenes</i> 3	Blood	1	1996/06/10	1.75	4b	IVb
<i>L. monocytogenes</i> 4	CSF	2	2001/11/11	1.25	1/2b	IIb
<i>L. monocytogenes</i> 5	CSF	3	2006/04/26	1.50	4b	IVb

⁽¹⁾Determined by the slide agglutination test; ⁽²⁾cerebro-spinal fluid.

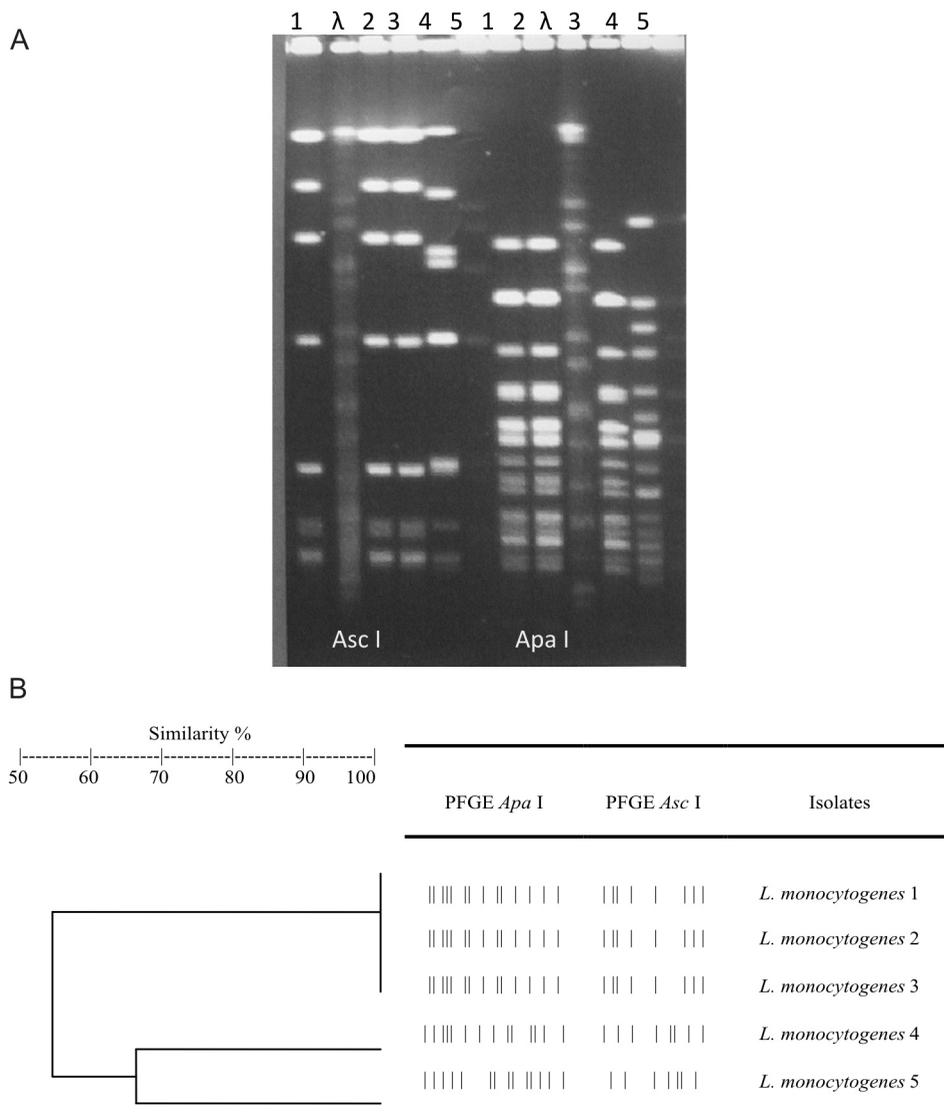


Figure 1 A. PFGE separation of *Asc*I (left) and *Apa*I (right) macrorestriction fragments of *L. monocytogenes* genomic DNA from three clinical cases. Lanes 1, 2 and 3: case 1, lane 4: case 3, lane 5: case 2, lanes λ : control corresponding to *Salmonella* serotype Braenderup (H9812) after DNA macrorestriction by *Xba*I, according to the internationally standardized protocol from PulseNet. **B.** PFGE of *L. monocytogenes* strains analyzed in this study. Schematic patterns and total dendrogram. The dendrograms were produced by the UPGMA algorithm based on a Dice similarity coefficient with a 1.5% band position tolerance.

and ceftriaxone initially prescribed was changed to ampicillin (100 mg/kg/day) plus gentamicin (5 mg/kg/day). The NB was discharged 16 days later.

Finally, case 3 was a male preterm NB. He was born by vaginal route in his home (Justo Daract town). He was immediately transferred to Villa Mercedes Hospital because of important signs of prematurity. On admittance, he showed respiratory distress and was therefore intubated for mechanical ventilation. He was febrile and presented signs of meningitis. The CSF analysis showed a mildly opalescent liquor with pleocytosis of 546 leucocytes/mm³ and polymorphonuclear cell predominance (> 70%), and increased protein concentration. The CSF culture yielded *L. monocytogenes* after 48h of incubation. Initial therapy consisted in ceftriaxone, and was rapidly switched to ampicillin (100 mg/kg/day) plus gentamicin (5 mg/kg/day). The patient was discharged 15 days later.

All evocative gram-positive bacilli isolated were identified by the API *Listeria* strip (bioMérieux Marcy l'Etoile, France) and haemolysin production in 5% horse-blood (Columbia agar base, Merck KGaA, Darmstadt, Germany) (6). *L. monocytogenes* CLIP 22762 was obtained from the *Listeria* Collection of the Pasteur Institute, Paris, and was included as reference strain. MIC values of ampicillin ($\leq 2 \mu\text{g/ml}$) were determined by the broth microdilution method (2) (Table 1). The isolates were stored at -80°C in brain-heart infusion broth (BHI) containing 15% glycerol for further characterization.

Serotyping of all isolates was performed by the classical method (9) and a multiplex PCR (3). The isolates from cases 1 and 3 belonged to PCR-group IVb/serovar 4b. The isolate from case 2 belonged to PCR-group IIb/serovar 1/2b (Table 1). All the isolates were characterized by pulsed-field gel electrophoresis (PFGE) using the Pulse Net protocol involving the restriction enzymes *Ascl* and *Apal* (1). PFGE of the DNAs of the 5 isolates digested with *Ascl* showed 7-8 fragments ranging from approximately 30 to 1130 kb in size, while 14 fragments of 30-510 kb were obtained following digestion using *Apal* (Figure 1A). The relationships among *L. monocytogenes* strains based on their combined *Apal* and *Ascl* PFGE profiles are shown in the dendrogram displayed in Figure 1B. The three isolates from case 1 showed indistinguishable PFGE types. Although the strains from cases 1 and 3 belonged to the same PCR-group, they were not similar according to the analysis of PFGE profiles (Figure 1A). The discrimination index (DI) value of PFGE was calculated by Simpson's diversity index (5). PFGE of

both *Ascl* and *Apal* macrorestriction fragments, differentiated the 5 isolates into 3 different patterns. The same DI value was obtained with *Apal* (0.70) and *Ascl* (0.70). The combination of patterns produced by both enzymes yielded 3 combined *Apal* and *Ascl* PFGE types but did not increase the discriminatory power.

In the present study, the differences observed in the clinical presentation of cases 1 and 3 of early-onset listeriosis may be related to the developmental stage of the neonates (7). Late-onset listeriosis is much rarer and linked to another situation: healthy full-term NB with no clinical signs for the mother and late onset of more than 7 days. Meningitis is more likely in late-onset infection as in the patient in case 2 (4). In case 1, delay in *L. monocytogenes* identification led to severe hydrocephalus because empirical treatment had only included a cephalosporin (8). The two other NB showed complete recovery following adequate treatment.

In Argentina, listeriosis is not a national notifiable disease but physicians should always consider *L. monocytogenes* as a possible etiologic agent of neonatal meningitis.

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