Neonatal Listeriosis Presentation and Outcome: A Prospective Study of 189 Cases

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Summary of the main point: We prospectively assessed the presentation and outcome of neonatal listeriosis and evidenced that maternal antibiotic treatment administered at least one day before delivery is associated with a significant reduction of neonatal severity.

ABSTRACT

Context – Listeriosis is caused by the foodborne pathogen *Listeria monocytogenes*. It can present as a maternal-neonatal infection. We implemented the nationwide prospective cohort MONALISA and analyzed the features of neonatal listeriosis.

Methods – We studied all neonates born alive from mothers with microbiologically-proven maternal-neonatal listeriosis enrolled from November 2009 to December 2017. We analyzed presentation, neonatal outcome at discharge and predictors of severe presentation and outcome. The study is registered at clinicaltrials.gov (NCT01520597).

Results – We studied 189 infants. 133/189 (70%) had abnormal clinical status at birth, including acute respiratory distress in 106/189 (56%). 132/189 (70%) infants developed early-onset listeriosis and 12/189 (6%) late onset listeriosis who all presented with acute meningitis. 17/189 (9%) had major adverse outcomes: 3% death (5/189), 6% (12/189) severe brain injury, 2% (3/189) severe bronchopulmonary dysplasia, 15/17 in infants born < 34 weeks of gestation ($p < 0.0001$ versus infants born ≥ 34 weeks of gestation). Maternal antimicrobial treatment ≥1 day before delivery was associated with a significant decrease of infants' severity (resulting in significantly less inotropic drugs, fluid resuscitation, or mechanical ventilation requirement), OR 0.23 [95% confidence interval CI 0.09-0.51], $p < 0.0001$).

Conclusion– Antenatal maternal antimicrobial treatment is associated with reduced neonatal listeriosis severity, justifying the prescription of preemptive maternal antimicrobial therapy when maternal-fetal listeriosis is suspected. Neonatal outcome is better than reported earlier, and its major determinant is gestational age at birth.

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INTRODUCTION

Listeriosis is a rare and severe foodborne infection, caused by *Listeria monocytogenes* (*Lm*), which manifests as bacteremia, neurolisteriosis and maternal-neonatal infections [1]. Maternal-neonatal listeriosis accounts for 10-20% of listeriosis cases across Europe and North America [2, 3] but can represent up to 50% of invasive cases, as reported in the South African outbreak, the largest so far [4]. It is associated with major adverse outcomes including fetal death, preterm birth and/or early or late-onset neonatal listeriosis in more than 80% of cases [5]. Public health agencies require for maternal-neonatal listeriosis diagnosis that *Lm* is isolated in any sample of maternal (blood, products of conception) or neonatal origin, including non-sterile sites [2, 6, 7]. This definition is broader than other maternal-fetal infections and reflects the pathophysiological steps leading to maternal-fetal listeriosis after consumption of contaminated food: (*i*) *Lm* crossing of the intestinal barrier, (*ii*) maternal bacteremia, (*iii*) hematogenous placental seeding and placental barrier crossing resulting in (*iv*) fetal/neonatal infection [8, 9]. Considering these steps and the fact that *Lm* is not a member of the maternal vaginal flora [10], its presence in any non-sterile neonatal sample is unlikely to reflect ascending colonization, but rather invasive infection following hematogenous placentitis [11]. Whereas these pathophysiological events are well characterized [9, 12-14], data regarding the features of neonatal listeriosis are scarce and limited to retrospective studies that captured heterogenous timeframes and areas [15, 16].

Listeriosis is a notifiable disease in France, with mandatory reporting of culture-confirmed cases and submission of isolates to its National Reference Center for Listeria (NRCL). The exhaustiveness of the French national surveillance system is estimated 85-88%, with isolates submitted to the NRCL in 98% of reported cases [17]. We implemented in 2009 the prospective Multicentric Observational NAtional Study on LISteriosis and ListeriA (MONALISA), an ongoing nationwide cohort study [5]. While data on maternal presentation have been previously reported, infants' clinical data have not [5]. Here we prospectively studied the cohort of infants born in the context of maternal-neonatal listeriosis.

PATIENTS AND METHODS

Study design – MONALISA is a prospective national observational cohort study. All eligible cases declared to the NRCL between 3rd November 2009 and 31th December 2017 with microbiologically proven listeriosis for which informed consent was obtained were enrolled, as part of the mandatory reporting [5]. Maternal-neonatal infection was defined as isolation of *Lm* from a pregnant woman, a fetus, or a newborn (below one month) [18]. When *Lm* was isolated from both the mother and her fetus/neonate, the event was counted as a single case. MONALISA is registered at clinicaltrials.gov (NCT01520597).

Definitions – Preterm birth was defined as birth before 37 weeks of gestation (WG), and classified as extreme (24-27 WG), very (28-31WG), moderate (32 and 33WG) or late (34-36WG) preterm birth [19].

Acute respiratory distress was defined by the presence of ≥ 1 following symptoms: tachypnea > 60/min, grunting, apnea, hypoxemia or cyanosis, or chest indrawing [19].

Cardiovascular impairment was defined by the presence ≥ 1 following symptoms: tachycardia > 160/min, bradycardia < 80/min, hypotension or poor peripheral perfusion including prolonged capillary refill [19].

Systemic samples included neonatal blood, cerebrospinal fluid or urine samples [20]. Other samples were classified as non-systemic : gastric fluid aspirate, pharynx, deep external auditory canal and anus swab.

Severe intraventricular hemorrhage (IVH) was defined as an IVH with ventricular dilatation or with intraparenchymal hemorrhages (large unilateral parenchymal hyperdensity by ultrasound imaging, and/or large unilateral porencephalic cyst) [21]. Cystic periventricular leukomalacia was defined as periventricular white matter echolucencies at ultrasonography [21]. Severe bronchopulmonary dysplasia was defined as administration of oxygen for ≥ 28 days plus need for > 30% oxygen and/or mechanical ventilation support or continuous positive airway pressure at 36 weeks postmenstrual age [22]. Other outcomes included necrotizing enterocolitis and retinopathy of prematurity.

Infections diagnosed between birth and six days of life were classified as early-onset infections, and infections diagnosed between seven and 28 days as late-onset infections [2, 6, 7].

Initial severity was defined as any condition requiring inotropic drugs and/or fluid resuscitation and/or mechanical ventilation. Major adverse outcome was defined by death or survival with severe brain injury (severe IVH or periventricular leukomalacia) and/or severe bronchopulmonary dysplasia, both of which are associated with severe morbidity [23].

Data collection – Data on maternal history, maternal treatment, infant clinical presentation at birth, management and in-hospital outcome were prospectively collected.

Ethics – All mothers provided written informed consent. In accordance with French legislation, the MONALISA Study received institutional review board approval by a local ethics committee (Comité de protection des personnes, Ile de France -3, Nov 6 2009).

Procedures – *L. monocytogenes* isolates were identified by with API Listeria (bioMérieux, France) until 2016 and then by MALDI-TOF mass spectrometry [24]. DNA extraction was performed using the DNeasy blood and tissue extraction kit (Qiagen, Aarhus, Denmark) from 5 mL of liquid cultures grown overnight at 35°C in brain–heart infusion medium under aerobic conditions following manufacturer's protocol. Library preparation was carried out using the Nextera XT DNA sample kit and whole genome sequencing was performed on the NextSeq 500 platform (Illumina, San Diego, CA, USA) using 2×150 bp runs and at a minimum coverage of 40x. Paired-end reads were trimmed and assembled as previously described [25]. *In silico* typing was performed using BIGSdb-*Lm* (https://bigsdb.pasteur.fr/listeria/) [26].

Statistical analysis –All tests were performed with R software (version 3.4.0). All tests were 2-tailed and p values < 0.05 (calculated by χ^2 test, Fisher exact test, Kruskall-Wallis or Student t test) were considered significant.

RESULTS

Cohort – Of the 245 maternal-neonatal listeriosis cases included in MONALISA from 3rd November 2009 to 31st December 2017, 56 fetal losses were recorded, and 189 liveborn infants were included in the study, including 2 pairs of twins. Maternal epidemiological data are presented in Table 1. Altogether, 108/189 (57%) infants were born prematurely, including 42/189 (22%) cases of extreme (9/189, 5%) or very preterm birth (33/189, 17%).

Clinical presentation – Infant and maternal clinical presentations are detailed in Table 2 and S1. 133/189 (70%) infants presented with abnormal physical examination, including 12/12 (100%) infants with late-onset listeriosis. Symptoms arose either at birth/within 24 hours (early onset listeriosis) or within 7-22 days after birth (late-onset listeriosis). Neurological symptoms (lethargy and/or altered consciousness and/or seizures) were reported in 42/187 (23%) cases, including 7/9 (78%) infants with early onset meningitis and 4/12 (33%) infants with late onset infection, who all exhibited meningitis. No focal neurological sign was evidenced. Median APGAR score at one and 5 minutes were 7 [4-10] and 9 [8-10]. 36/189 (19%) infants presented with an APGAR score < 7 at 5 minutes. Acute respiratory distress was reported in 106/189 (56%). 77/189 (41%) infants required continuous positive airway pressure ventilation for a median of 2 days [Interquartile range IQ 25-75: 1-5], 67/189 (35%) required mechanical ventilation for a median of 2 days [IQ 25-75: 1-7] and 54/189 (29%) infants required nasal oxygen therapy for a median of 3 days [IQ 25-75: 1-6]. Fever was reported in 38/189 (20%) infants, 28/81 (35%) born at term and 10/108 (9%) born prematurely. Cardiocirculatory symptoms were reported in 39/189 (21%): 23/189 (12%) required fluid resuscitation and 16/189 (8%) inotropic therapy. Skin lesions were reported in 19/173 (11%), and consisted in macular and/or papular rash (n=9), vesicular and/or pustular skin lesions (n=5) and purpura (n=5). Hypospadias and cleft palate were reported in one case each.

Microbiological presentation –Microbiological features are detailed in Table 3. 144/189 (76%) infants had samples from which *Lm* was identified, classifying them as neonatal listeriosis cases: 132/189 (70%) with early-onset listeriosis and 12/189 (6%) with late-onset listeriosis. 57/189 (30%) infants had positive systemic samples, and 75/189 infants (40%) had positive non-systemic samples. The distribution of culture positive samples is shown in Figure 1. In 73/94 (78%), placenta evidenced signs of infection with *Lm* in culture and/or macroscopic or microscopic abscesses. Among the 114 infants in whom lumbar puncture was performed, 21 (18%) had *Lm* evidenced by cerebrospinal fluid (CSF) culture or PCR, including 9/46 (20%)

with early onset listeriosis based on systemic sample and 12/12 (100%) with late onset listeriosis. Of these 21 infants, 20 had available CSF biochemical and cellular data, reported in Table S2. Infants with early-onset and late-onset listeriosis exhibited similar CSF features, except for median nucleated cells count, which was significantly higher in the latter group (3,350/mm^3 [IQ 25-75: 1,040-5,055] versus 125/mm^3 [IQ 25-75: 40-945], p = 0.01). *Lm* belonging to hypervirulent clones (clonal complexes 1, 2, 4 and 6) was isolated in 115/184 (63%) of cases. Distribution of clonal complexes and sublineages are presented in Figure S1 and Table S3.

Antimicrobial treatment and outcome – Treatments and outcomes are detailed in Table 4. 38/189 (20%) mothers received active anti-*Lm* antimicrobial therapy before delivery. 163/189 (86%) infants received antimicrobial therapy based on amoxicillin (163/163, 100%), combined with aminoglycoside in 141 cases (86.5%). 26/189 (14%) infants did not receive any antibiotic (26/189, 14%), and another 8/189 (4%) had them tapered off within 72 hours. These 26 infants all exhibited normal clinical status and laboratory features with negative microbiological samples (except for 2 with only placenta positive culture); all exhibited good outcome.
IVH was reported in 25 preterm infants (25/101, 25%), 12 of which (48%) were severe (grade 3 or more), including one with concomitant cystic periventricular leukomalacia. Severe IVHs were mostly reported in the context of extreme (4/9, 44%) or very preterm birth (6/33, 18%). Three infants born between 25 and 27 WG developed severe bronchopulmonary dysplasia. No necrotizing enterocolitis nor retinopathy of prematurity was reported.
Median hospital stay was 16 days [IQ 25-75: 8-25]. Five neonatal deaths were recorded during the 8-year study period (5/189, 3%), all in infants born between 27 and 29 WG. Seventeen infants (17/189, 9%) had major adverse outcome (death or survival with severe brain injury and/or severe bronchopulmonary dysplasia): all exhibited positive samples at birth (17/132, 13%). Median gestational age for this subset of infants was 29 WG [IQ25-75: 27-29]. Outcome appeared strongly dependent on gestational age at birth, as major adverse outcome was reported in 7/9 (78%) and in 8/33 (24%) of infants born extremely preterm and very preterm, respectively, but only in 2/66 (3%) infants born moderately or late preterm and in none of the 81 infants born full term ($p < 0.0001$).

Severity by sample type – Infants could be classified according to the type of samples from which *Lm* was isolated. Infants with positive systemic samples (blood or CSF) at birth exhibited a more severe presentation than those with only non-systemic positive samples, and also than

those in whom listeriosis was only diagnosed from a maternal sample: they almost all presented with abnormal physical examination at birth (56/57 (98%), 58/75 (77%) and 7/45 (16%), respectively (Table 2)), with significantly more frequent neurological, cardiovascular and respiratory impairment. They also exhibited higher C-reactive protein and procalcitonin levels (Table 2). They had significantly longer antimicrobial therapy (median duration of 14 days [IQ 25-75: 10-21], 10 days [IQ 25-75: 8-14] and 6 days [IQ 25-75: 3-10], respectively (Table 4)). Infants with positive systemic samples at birth exhibited poorer outcome, with higher rate of severe IVHs (8/39 (21%), 4/58 (7%) and 0/11 (0%), respectively) ($p = 0.003$), longer hospital stay ($p < 0.0001$), more frequent intensive care requirement ($p < 0.0001$), and more frequent adverse outcome than other groups: 10/57 (18%), 6/75 (8%) and 1/45 (2%), respectively ($p = 0.03$) (Table 4).

Evidence for a protective effect of maternal treatment before delivery on newborn presentation – We studied determinants of initial severity in the 177 infants without late onset infection. Neither maternal background (ethnicity or immunosuppression) nor strain clonal complex was found associated with infant outcome (data not shown).

Mothers of infants without positive samples exhibited distinctive features from mothers of infants with positive systemic/ non-systemic samples: more frequent fever and influenza-like symptoms ($p<0.01$) but less obstetrical signs ($p<0.0001$).

Importantly, there was a significant association between the absence of antenatal anti-*Lm* maternal treatment and the occurrence of neonatal infection (Table 4): only 2/57 (4%) mothers of infants with systemic samples positive had received anti-*Lm* antibiotic before delivery, *versus* 22/26 (85%) mothers of infants classified as uninfected ($p = 0.008$). Antenatal anti-*Lm* maternal therapy (initiated at least one day before delivery) was associated with an OR of 0.05 [95% confidence interval (CI): 0.006-0.21], $p < 0.0001$) of positive systemic infant sample and with an odds ratio of 0.06 [95% CI: 0.02-0.14], $p < 0.0001$) of any infant positive sample. Moreover, antenatal anti-*Lm* maternal treatment was associated with reduced neonatal initial severity, defined as requirement for inotropic drugs, and/or fluid resuscitation, and/or mechanical ventilation at birth, with an odds ratio of 0.23 [95% CI: 0.09-0.51], $p < 0.0001$).

DISCUSSION

Here we report the results of the first prospective study on neonatal listeriosis. It included 189 cases and constitutes the largest series reported so far [15, 27-29]. We detailed initial presentation, defined neonatal patterns and assessed severity. We evidenced a drastic improvement of in-hospital outcome as compared to earlier studies, and for the first time the benefits of antenatal *Lm*-maternal treatment on neonatal presentation and severity. The main strength of the study is its prospective and nationwide design. The quasi-exhaustiveness of the French surveillance system for listeriosis provided the unique opportunity to collect a near-complete dataset on a large scale in space and time [5]. The main limitation lies in its observational nature, even though most infants benefited from the same work-up procedures.

Important conclusions can be drawn. First, clinical neonatal presentation is usually severe, with 70% of infants exhibiting abnormal clinical features at diagnosis, 22% with extreme or very preterm birth, and many requiring prolonged hospital care. This is consistent with retrospective studies that reported abnormal clinical presentation in > 75% of cases [15, 16]. The prospective nature of the study allowed to delineate four distinct neonatal patterns (Figure 2).

A first group consisted in the 132 infants (70%) with early-onset listeriosis. Acute respiratory distress was the prominent presentation, which reflects either non-specific signs of sepsis [30], and/or respiratory distress syndrome in prematurely born infants [23], and/or *Lm*-pulmonary involvement. It was associated with neurological and/or cardiocirculatory impairment as a consequence of sepsis and/or central nervous system infection (that was reported altogether in 10% (9/99). Vesicular/pustular rash was reported in 5 infants, 4 with bacteremia: this presentation is evocative of *granulomatosis infantiseptica,* a rare neonatal listeriosis condition characterized by multifocal granulomas [31]. Among these 132 infants with early-onset listeriosis, 2 subsets were identified. 57 infants had early-onset listeriosis diagnosed as bacteremia, associated with CSF (20%) or urine (4%) infection: they exhibited the most severe presentation. The rate of meningitis in these bacteremic infants was in the range of previous reports (25- 41% [15, 16, 32]), and of other pathogens involved in early-onset neonatal sepsis, such as group B streptococcus (7-16% [33, 34]). The other 75 had early-onset listeriosis diagnosed only from non-systemic samples; they exhibited less severe presentation, with 2-3 times less cardiocirculatory and neurological symptoms.

Another group (n = 12) presented with late-onset listeriosis (6%). They all developed a meningitis within 7-17 days after birth, with 8% bacteremia. This unique pattern probably reflects its distinctive invasion mechanism, with putative ingestion of *Lm* during passage through the birth canal [35].

A group of 26 infants (14%) appeared non-infected with normal clinical features, samples' cultures negative, and good outcome without antibiotic therapy (for these infants, diagnosis was made through maternal samples). Previous retrospective studies have suggested that 7-23% bacteremic pregnant mothers deliver uninfected infants, and this was confirmed here on a large scale by a combined clinical/biological approach [29, 36].

A last group of 19 infants (10%) with negative culture samples exhibited only non-specific signs likely attributable to prematurity.

Importantly, despite the overall severe initial presentation, infants' outcome appeared more favorable that previously reported, as only 9% of the cases exhibited major complications (including 3% mortality). Major adverse outcome (death, severe brain injury, severe bronchopulmonary dysplasia) was mostly observed in the context of extreme or very preterm birth (before 34WG), reflecting the vulnerability of this population and the likely contribution of coincident diagnoses/comorbidities such as severe IVH [23]. We however evidenced a much lower rate of complications than reported in earlier studies and/or in other areas: mortality reached 25% in a review of 100 cases from the 1980-2000 literature, and 33% in a compilation of 133 British cases between 1967 and 1985 [15, 16]. Neonatal mortality reached 18% (12/65) in a large study conducted in Israel from 1999 to 2007 [27]. This is in line with the major improvements achieved in neonatal intensive care management in the past decades, and provides reassuring new data for infants born in the context of listeriosis after 34 WG (78% of our cohort) [27, 37]. Long-term consequences of neonatal listeriosis remain largely unknown, and are currently under investigation.

Besides gestational age at birth, another critical parameter influenced neonatal severity, with major implications for the management of maternal listeriosis. Indeed, maternal anti-*Lm* treatment administered before delivery was associated with a significant decrease of severity at birth (OR 0.23 of inotropic drugs, and/or fluid resuscitation, and/or mechanical ventilation requirement), and with a major reduction of the frequency of neonatal infection (OR 0.06 of any positive infant sample). This strongly supports the empirical administration of ampicillin/amoxicillin in undifferentiated maternal fever, in line with recommendations and guidelines that such treatment should be prescribed especially after consumption of *Lm*-contaminated food [38, 39] or in listeriosis outbreaks [28, 40]. Mothers of infants without positive samples exhibited more signs evocative of listeriosis (fever, influenza-like symptoms) but less obstetrical signs reflecting/requiring urgent delivery than mothers of infants with positive samples, highlighting that maternal symptoms of listeriosis should prompt early antimicrobial therapy.

Finally, these results are in line with recent large reports that revealed non-existent to extremely low rates of mortality with early-onset infection in term infants [41, 42]. Such reduction in mortality could neither be attributed to improved therapies nor decreased pathogens virulence, but rather to the frequency of intrapartum antibiotic prophylaxis. Together, these data underline the critical impact of effective maternal management to improve neonatal infection-related outcomes, and the pivotal importance of antenatal anti-*Lm* antimicrobial therapy in febrile pregnant mothers.

FUNDING

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CONFLICT OF INTEREST

None.

FIGURE LEGENDS

Figure 1. Distribution of culture positive samples in the 177 infants without late onset listeriosis (blue, infant cerebrospinal fluid; pink, infant blood sample; yellow, other positive infant sample (gastric fluid, ear, skin, amnionic fluid or placenta); hatched pink, maternal blood culture). Maternal data are missing for 4 mothers (for detailed maternal microbiological features, see Table S1).

Figure 2. Distribution of the 189 infants of the cohort according to their clinical and biological presentation.

Table 1. Epidemiological characteristics of the study population

	Cohort N=189	Infants with *Lm*-positive systemic sample(s) (S) N=57	Infants with *Lm*-positive non-systemic sample(s) (NS) N=75	Infants with only maternal *Lm*-positive samples (M)* N=45	Infants with late onset infection N=12†	*P* value S *vs.* NS *vs.* M¶	*P value S vs. SC*‖
Sex ratio							
Male	108/189 (57%)	33/57 (58%)	45/75 (60%)	25/45 (56%)	5/12 (42%)	0.69	0.8
Female	81/189 (43%)	25/57 (42%)	30/75 (40%)	20/45 (44%)	7/12 (58%)	0.69	0.66
Maternal origin‡							
France	99/187 (53%)	29/56 (52%)	33/74 (45%)	26/45 (58%)	10/12 (83%)	0.57	0.43
Europe	13/187 (7%)	6/56 (11%)	5/74 (7%)	2/45 (4%)	0/12	0.47	0.43
Africa	51/187 (27%)	17/56 (30%)	17/74 (23%)	17/45 (40%)	1/12 (8%)	0.28	0.35
Other	14/187 (3%)	4/56 (7%)	0/75	0/45	1/12 (8%)	-	-
Maternal immunosuppressive comorbidity‡§	17/187 (9%)	2/56 (4%)	4/74 (5%)	7/45 (16%)	0/12	0.72	0.61
Median gestational age at birth (IQ 25-75)	36 WG (33-39)	34 WG (31-37)	34 WG (30-36)	38 WG (37-39)	39 WG (38-40)	< 0.0001	0.24
Premature delivery < 37 WG	108/189 (57%)	39/57 (68%)	58/75 (77%)	11/45 (24%)	0/12	< 0.0001	0.25
Extremely preterm birth 24-27 WG	9/189 (5%)	4/57 (7%)	4/75 (5%)	1/45 (2%)	-	0.54	0.69
Very preterm birth 28-31 WG	33/189 (17%)	11/57 (19%)	21/75 (28%)	1/45 (2%)	-	0.002	0.24
Moderate preterm birth 32-33 WG	25/189 (13%)	10/57 (18%)	11/75 (15%)	4/45 (9%)	-	0.43	0.62
Late preterm birth 34-36 WG	41/189 (22%)	14/57 (25%)	22/75 (30%)	5/45 (11%)	-	0.07	0.54

Systemic samples were neonatal blood, cerebrospinal fluid or urine samples. Other neonatal samples were classified as non- systemic. Maternal positive samples were blood, vaginal or urine samples.

Lm refers to *Listeria monocytogenes.*

* Maternal positive samples were blood cultures in 44/45 cases and CSF with PCR documentation in one case.

† Range 7-22 days after birth

‡ There were 2 twin pregnancies, hence 187 mothers. One mother gave birth to one infant with S and one infant with NS positive samples, while the other mother gave birth to 2 infants with NS positive samples.

§ Maternal immunosuppressive comorbidities included HIV infection (n=5), rheumatoid arthritis (n=2), solid organ cancer (n=1), inflammatory bowel disease under oral corticosteroids + infliximab, infliximab, or azathioprine (n=1, each), congenital immunodeficiency (n=1), spondylarthritis (n=1), lupus erythematosus (n=2), oral corticosteroids therapy n=2).

¶ χ^2 test was used for qualitative variables, Kruskall-Wallis test was used for quantitative variables.

‖ χ^2 and Fisher tests were used for qualitative variables, Student *t* test was used for quantitative variables.

Table 2. Clinical and laboratory features of the study population

	Cohort N=189	Infants with *Lm*-positive systemic samples (S) N=57	Infants with *Lm*-positive non-systemic samples (NS) N=75	Infants with only maternal *Lm*-positive samples (M) N=45	Infants with late onset infection N=12[*]	*P value S vs. NS vs. M*[¶¶]	*P value S vs. NS*[‖‖]
Clinical features							
Any clinical sign	133/189 (70%)	56/57 (98%)	58/75 (77%)	7/45 (16%)	12/12 (100%)	< 0.0001	< 0.0001
Temperature > 38°C	38/189 (20%)	15/57 (26%)	9/75 (12%)	3/45 (7%)	11/12 (92%)	0.01	0.03
Acute respiratory distress symptoms[†]	106/189 (56%)	52/57 (91%)	51/75 (68%)	3/45 (7%)	2/12 (17%)	< 0.0001	0.001
Cardiocirculatory symptoms[‡]	39/189 (21%)	26/57 (46%)	13/75 (17%)	0/45	0/12	< 0.0001	0.0004
Neurological symptoms[§]	42/187 (22%)	24/56 (43%)	13/74 (18%)	2/45 (4%)	3/12 (25%)	< 0.0001	0.002
Seizures	5/187 (3%)	3/56 (5%)	1/74 (1%)	0/45	1/12 (8%)	-	-
Median APGAR 1min score (IQ 25-75)	7 (4-10)	5 (2-8)	5 (2-9)	10 (9-10)	10 (10-10)	-	-
Median APGAR 5min score (IQ 25-75)	9 (8-10)	8 (7-9)	8 (6-10)	10 (10-10)	10 (10-10)	-	-
APGAR 5 min score < 7	36/189 (19%)	11/57 (19%)	23/75 (31%)	2/45 (4%)	0/12	0.002	0.14
Skin lesion[¶]	19/186 (10%)	13/56 (23%)	6/73 (8%)	0/45	0/12	< 0.0001	0.01
Macular and/or papular rash	9/186 (5%)	5/56 (9%)	4/73 (5%)	-	-	-	-
Purpura	5/186 (3%)	4/56 (7%)	1/73 (1%)	-	-	-	-
Vesicular and/or pustular	5/186 (3%)	4/56 (7%)	1/73 (1%)	-	-	-	-
Blood chemical tests							
Median C-reactive protein (mg/L) (IQ 25-75)[‖]	49 (11-96)	89 (53-127)	47.5 (23-97)	3 (1.75-6)	10 (4-24)	< 0.0001	< 0.001
C-reactive protein < 10 mg/L	42/171 (25%)	2/57 (4%)	11/74 (15%)	23/28 (82%)	6/12 (50%)	< 0.0001	0.0002
Median serum procalcitonin (ng/mL) (IQ 25-75)	1 (0.21-13)	23 (5-44)	4 (1-19)	0.12 (0.03-0.17)	0.27 (0.18-0.4)	< 0.0001	0.11
Serum procalcitonin < 0.5 ng/mL[**]	16/39 (41%)	0/9	2/14 (14%)	6/6 (100%)	8/10 (80%)	< 0.0001	0.50
Blood count							
Median leucocytes count (cells per µL) (IQ 25-75) [††]	10,635 (6,450-16,825)	7,790 (4,82(-11,750)	11,000 (6,800-15,400)	14,300 (9,035-18,480)	21,980 (18,150-22,625)	< 0.0001	0.04
Median polymorphonuclear cells (cells per µL) (IQ 25-75) [‡‡]	5,490 (3,168-10,038)	3,900 (1,470-6,600)	5,520 (3,214-8,725)	6,670 (4,290-18,480)	3,655 (1,750-5,500)	0.0001	0.04
Monocytopenia[§§]	11/110 (10%)	3/32 (9%)	7/53 (13%)	0/19	0/6	0.40	0.73

Systemic samples were neonatal blood, cerebrospinal fluid or urine samples. Other neonatal samples were classified as non- systemic. Maternal positive samples were blood, vaginal or urine samples

Lm refers to *Listeria monocytogenes*

[*] Range 7-22 days after birth

[†] Respiratory symptoms included: tachypnea > 60/min, grunting, apnea, cyanosis, or chest indrawing

[‡] Cardiocirculatory symptoms included: tachycardia > 160/min, bradycardia < 120/min, hypotension and poor peripheral perfusion

[§] Neurological symptoms included lethargy, altered consciousness and/or seizures. Information was available for 187 patients, including 56 with positive systemic samples, 74 with non-systemic positive samples and 45 with only maternal samples positive

[¶] Information was available for 186 patients, including 56 with positive systemic samples, 73 with non-systemic positive samples and 45 with only maternal samples positive

[‖] Information was available for 171 patients, including 57 with positive systemic samples, 74 with non-systemic positive samples 28 with only maternal samples positive and 12 with late onset infection

[**] Information was available for 39 patients, including 9 with positive systemic samples, 14 with non-systemic positive samples 6 with only maternal samples positive and 10 with late onset infection

[††] Information was available for 168 patients, including 56 with positive systemic samples, 73 with non-systemic positive samples, 27 with only maternal samples positive and 12 with late onset infection

[‡‡] Information was available for 155 patients, including 49 with positive systemic samples, 69 with non-systemic positive samples, 25 with only maternal samples positive and 12 with late onset infection

[§§] Information was available for 110 patients, including 32 with positive systemic samples, 53 with non-systemic positive samples, 19 with only maternal samples positive and 6 with late onset infection

[¶¶] χ^2 test was used for qualitative variables, Kruskall-Wallis test was used for quantitative variables.

[‖‖] $\chi^2$and Fisher tests were used for qualitative variables, Student *t* test was used for quantitative variables.

Table 3. Microbiological features of the neonatal study population

	Cohort N=189	Infants with *Lm*-positive systemic samples (S) N=57	Infants with *Lm*-positive non-systemic samples (NS) N=75	Infants with only maternal *Lm*-positive samples (M) N=45	Infants with late onset infection N=12[*]	*P value* *S vs. NS vs. M*[‡‡]	*P value* *S vs. NS*[§§]
Blood sample							
Blood culture	57/164 (35%)	56/57 (98%)	0/75 (0%)	0/45 (16%)	1/12 (8%)	< 0.0001	< 0.0001
Cerebrospinal fluid sample							
Positive culture	18/114 (16%)	6/46 (13%)	0/53 (0)	0/3 (0)	12/12 (100%)	0.02	0.007
Positive PCR	3/10 (30%)	3/6 (50%)[†]	0/3 (0)	-	0/1 (0)	-	-
Any positive microbiological CSF sample	21/114 (18%)	9/46 (20%)	0/53 (0)	0/3 (0)	12/12 (100%)	0.003	< 0.0001
Peripheral samples[‡]							
Any positive peripheral sample culture	117/189 (62%)	51/57 (89%)	66/75 (88%)	0/45 (0)	0/12 (0)	< 0.0001	0.79
Gastric aspirate	110/151 (73%)	48/50 (96%)	62/68 (92%)	0/32 (0)	0/1 (0)	< 0.0001	0.30
External auditory canal	60/82 (73%)	18/21 (86%)	42/46 (91%)	0/14 (0)	0/1 (0)	< 0.0001	0.49
Anus	29/45 (64%)	13/15 (87%)	16/21 (76%)	0/8 (0)	0/1 (0)	< 0.0001	0.43
Pharynx	20/38 (53%)	6/12 (50%)	14/19 (74%)	0/7 (0)	-	0.004	0.18
Amniotic fluid							
Any positive amniotic fluid culture	12/25 (48%)	6/8 (75%)	6/7 (86%)	0/9 (0)	0/1 (0)	< 0.001	0.60
Placenta sample							
Any placenta sample evocative of listeriosis (culture and/or histology)	73/94 (78%)	26/27 (93%)	47/48 (98%)	0/18 (0)	0/1 (0)	< 0.0001	0.29
Placenta culture positive	68/93 (73%)	26/27 (93%)	42/47 (89%)	0/18 (0)	0/1 (0)	< 0.0001	0.29
Macroscopic or microscopic abscesses [§]	14/18 (78%)	5/5 (100%)	9/10 (90%)[**]	0/3 (0)	-	0.002	0.44
Urine sample							
Any positive urine culture	2/5 (40%)[††]	2/3 (66%) [† † ‖]	-	-	0/2 (0)	-	-

Systemic samples were neonatal blood, cerebrospinal fluid or urine samples. Other neonatal samples were classified as non- systemic. Maternal positive samples were blood, vaginal or urine samples.
Lm refers to *Listeria monocytogenes.*

* Range 7-22 days after birth
† All samples with PCR evidence of *L. monocytogenes* had concomitant negative CSF culture.
‡ No meconium sample was collected, nor any tracheal aspirate.
§ Among the 13 placenta samples with abscesses, four had concomitant negative placenta culture (in all cases maternal treatment had been started before sampling (several hours to 24 hours).
¶ Maternal samples consisted in blood cultures or vaginal samples {Charlier, 2017 #10700}.
‖ All positive urine samples were collected among infants with concomitant positive blood cultures.
** Of the 9 infants with histological evidence of placental infection, 5 had negative placenta culture but all had other *Lm*-positive non systemic samples.
†† All infants with positive urine culture also had positive blood cultures.
‡‡ χ^2 test was used for qualitative variables, Kruskall-Wallis test was used for quantitative variables.
§§ $\chi^2$and Fisher tests were used for qualitative variables, Student *t* test was used for quantitative variables.

Table 4. Antibiotic treatment and outcome of the study population

	Cohort N=189	Infants with *Lm*-positive systemic samples (S) N=57	Infants with *Lm*-positive non-systemic samples (NS) N=75	Infants with only maternal *Lm*-positive samples (M) N=45	Infants with late onset infection N=12[*]	*P value S vs. NS vs. M*[**]	*P value S vs. NS*[††]
Neonatal antibiotic treatment							
Amoxicillin (n=),	163/189 (86%)	57/57 (100%)	74/75 (99%)	20/45 (44%)	12/12 (100%)	< 0.0001	0.38
Median posology (mg/kg/d) (IQ 25-75) (n=)	164 (100-200) (n=170)	198 (103-200) (n=55)	164 (100-200) (n=62)	100 (96-142) (n=17)	181 (93-201) (n=12)	0.12	0.28
Median duration (days) (IQ 25-75) (n=)	12 (8-17) (n=173)	14 (10-21) (n=57)	10 (8-14) (n=74)	6 (3-10) (n=20)	21 (19.5-21)	< 0.0001	< 0.001
Aminoglycoside (n=),	141/189 (75%)[†]	51/57 (89%)	62/75 (83%)	18/20 (90%)	10/12 (83%)	0.46	0.27
Median duration (days) (IQ 25-75) (n=)	3 (2-5) (n=141)	3 (2-5) (n=51)	3 (2-5) (n=62)	2 (2-2)	8 (5-10)	0.01	0.20
Addition of a 3rd antibiotic active on *Lm*	7/189 (4%) [‡]	4/57 (7%)	2/75 (3%)	0/45 (0)	1/12 (8%)	0.14	0.23
No treatment	26/189 (14%)	0/57 (0)	1/75 (1%)	25/45 (56%)	0/12 (0)	< 0.0001	0.38
Treatment stopped at day 3	8/189 (4%)	0/57 (0)	2/75 (3%)	6/45 (13%)	0/12 (0)	0.003	0.21
Maternal antibiotic treatment							
Prescription of anti-*Listeria* antibiotic before birth	38/189 (20%)	2/57 (4%)	17/75 (23%)	33/45 (73%)	0/12 (0%)	< 0.0001	0.002
Median duration of anti-*Listeria* antibiotic before birth	0 (0-1)	8 (1-14)	1 (1-3)	56 (10-76)	-	< 0.0001	0.57
Outcome							
In-hospital death	5/189 (3%)	2/57 (4%)	3/75 (4%)	0/45 (0)	0/45 (0)	0.41	0.88
Intensive care unit management	94/189 (50%)	39/57 (68%)	40/75 (53%)	8/45 (18%)	4/12 (33%)	< 0.0001	0.11
Median hospital stay (days) (n=)	16 (8-25) (n=171)	21 (12-28) (n=55)	16 (10-30) (n=71)	6 (4-10) (n=35)	21 (17-22) (n=12)	< 0.0001	0.67
Intraventricular hemorrhage (n/prematurely born infants)	25/108 (23%)	12/39 (31%)	13/58 (22%)	0/11 (0)	-	0.1	0.35
Severe intraventricular hemorrhage (SIVH) [§]	12/25 (48%) [‖]	8/39 (21%) [‖]	4/58 (7%)	0/11 (0)	-	0.003	0.04
Severe bronchopulmonary dysplasia (SBPD) (n/prematurely born infants) [¶]	3/189 (2%)	1/57 (2%)	1/75 (1%)	1/45 (2%)	-	0.93	0.84
Necrotizing enterocolitis (n/prematurely born infants)	0/189	-	-	-	-	-	-

Major adverse outcome (death and/or severe brain injury and/or SBDP)	17/189 (9%)	10/57 (18%)	6/75 (8%)	1/45 (2%)	-	0.03	0.11

Systemic samples were neonatal blood, cerebrospinal fluid or urine samples. Other neonatal samples were classified as non- systemic. Maternal positive samples were blood, vaginal or urine samples.
Lm refers to *Listeria monocytogenes.*
[*] Range 7-22 days after birth
[†] Aminoglycosides included gentamicin in 93 cases (31 with C, 46 with NC, 12 with M and 4 with late onset infection), amikacin in 44 cases (16 with C and NC, each and 6 with M and late onset infection, each), tobramycin in 3 C cases and netilmicin in 1 C case.
[‡] Antibiotics added to amoxicillin eventually combined to aminoglycoside included vancomycin in 5 cases (3 with C, 1 with NC and with late onset infection, each) and rifampicin in 2 cases (1 with C and 1 with NC, each).
[§] Severe intraventricular hemorrhage (IVH) was defined as IVH with ventricular dilatation or as IVH with intraparenchymal hemorrhages (large unilateral parenchymal hyper density by ultrasound imaging, large unilateral porencephalic cyst) .
[¶] Severe bronchopulmonary dysplasia was defined as administration of oxygen for >=28 days plus need for >30% oxygen and/or mechanical ventilation support or continuous positive airway pressure at 36 weeks postmenstrual age [22].
[‖] One infant with SVIH also exhibited periventricular leukomalacia.
[**] χ^2 test was used for qualitative variables, Kruskall-Wallis test was used for quantitative variables.
[††] $\chi^2$and Fisher tests were used for qualitative variables, Student *t* test was used for quantitative variables.

REFERENCES

1. Allerberger F, Wagner M. Listeriosis: a resurgent foodborne infection. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases **2010**; 16(1): 16-23.
2. de Valk H, Tourdjman, M., Leclercq, A., Maury, M., Moura, A., Laurent, E., Chenal-Francisque, V., Goulet, V., Brisse, S., and Lecuit, M. . Changes in epidemiology and surveillance of listeriosis in France. In: International Symposium On Problems of Listeriosis. Paris: Institut Pasteur, 2016.
3. Silk BJ, Date KA, Jackson KA, et al. Invasive listeriosis in the Foodborne Diseases Active Surveillance Network (FoodNet), 2004-2009: further targeted prevention needed for higher-risk groups. Clin Infect Dis **2012**; 54 Suppl 5: S396-404.
4. 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): 632-43.
5. Charlier C, Perrodeau E, Leclercq A, et al. Clinical features and prognostic factors of listeriosis: the MONALISA national prospective cohort study. Lancet Infect Dis **2017**; 17(5): 510-9.
6. Center for Diseases Control. Listeriosis case- definition. 2019. https://wwwn.cdc.gov/nndss/conditions/listeriosis/case-definition/2019/. (Last accessed 05/16/2020).
7. European Center for Diseases Control. On the communicable diseases and related special health issues to be covered by epidemiological surveillance as well as relevant case definitions. 2012. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018D0945&from=EN#page=28. (Last accessed 05/16/2020).
8. Lecuit M, Nelson DM, Smith SD, et al. Targeting and crossing of the human maternofetal barrier by Listeria monocytogenes: role of internalin interaction with trophoblast E-cadherin. Proc Natl Acad Sci U S A **2004**; 101(16): 6152-7.
9. Disson O, Grayo S, Huillet E, et al. Conjugated action of two species-specific invasion proteins for fetoplacental listeriosis. Nature **2008**; 455(7216): 1114-8.
10. Ma B, Forney LJ, Ravel J. Vaginal microbiome: rethinking health and disease. Annu Rev Microbiol **2012**; 66: 371-89.
11. Charlier C, Disson O, Lecuit M. Maternal-neonatal listeriosis. Virulence **2020**; 11(1): 391-7.

12. Lecuit M, Dramsi S, Gottardi C, Fedor-Chaiken M, Gumbiner B, Cossart P. A single amino acid in E-cadherin responsible for host specificity towards the human pathogen Listeria monocytogenes. Embo J **1999**; 18(14): 3956-63.
13. Lecuit M, Vandormael-Pournin S, Lefort J, et al. A transgenic model for listeriosis: role of internalin in crossing the intestinal barrier. Science **2001**; 292(5522): 1722-5.
14. Lecuit M. Listeria monocytogenes, a model in infection biology. Cell Microbiol **2020**; 22(4): e13186.
15. Mylonakis E, Paliou M, Hohmann EL, Calderwood SB, Wing EJ. Listeriosis during pregnancy: a case series and review of 222 cases. Medicine **2002**; 81(4): 260-9.
16. McLauchlin J. Human listeriosis in Britain, 1967-85, a summary of 722 cases. 1. Listeriosis during pregnancy and in the newborn. Epidemiol Infect **1990**; 104(2): 181-9.
17. A. Leclercq, Charlier C, M.M. Maury and M. Lecuit. 2019. Rapport annuel d'activité du Centre national de Référence des Listeria – Année 2018. https://www.pasteur.fr/fr/file/32374/download. Paris, France 2019. Last accessed 12/31/2020.
18. Charlier C, Perrodeau E, Leclercq A, et al. Clinical features and prognostic factors of listeriosis: the MONALISA national prospective cohort study. The Lancet Infectious diseases **2017**; 17(5): 510-9.
19. Stoll BJ, Hansen NI, Bell EF, et al. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. Pediatrics **2010**; 126(3): 443-56.
20. Wynn JL, Wong HR, Shanley TP, Bizzarro MJ, Saiman L, Polin RA. Time for a neonatal-specific consensus definition for sepsis. Pediatr Crit Care Med **2014**; 15(6): 523-8.
21. Volpe JJ. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. Lancet Neurol **2009**; 8(1): 110-24.
22. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med **2001**; 163(7): 1723-9.
23. Ancel PY, Goffinet F, Group E-W, et al. Survival and morbidity of preterm children born at 22 through 34 weeks' gestation in France in 2011: results of the EPIPAGE-2 cohort study. JAMA Pediatr **2015**; 169(3): 230-8.
24. Thouvenot P, Vales G, Bracq-Dieye H, et al. MALDI-TOF mass spectrometry-based identification of Listeria species in surveillance: A prospective study. J Microbiol Methods **2018**; 144: 29-32.

25. Moura A, Tourdjman M, Leclercq A, et al. Real-Time Whole-Genome Sequencing for Surveillance of Listeria monocytogenes, France. Emerg Infect Dis **2017**; 23(9): 1462-70.
26. Moura A, Criscuolo A, Pouseele H, et al. Whole genome-based population biology and epidemiological surveillance of Listeria monocytogenes. Nat Microbiol **2016**; 2: 16185.
27. Elinav H, Hershko-Klement A, Valinsky L, et al. Pregnancy-associated listeriosis: clinical characteristics and geospatial analysis of a 10-year period in Israel. Clin Infect Dis **2014**; 59(7): 953-61.
28. Benshushan A, Tsafrir A, Arbel R, Rahav G, Ariel I, Rojansky N. Listeria infection during pregnancy: a 10 year experience. Isr Med Assoc J **2002**; 4(10): 776-80.
29. Smith B, Kemp M, Ethelberg S, et al. Listeria monocytogenes: maternal-foetal infections in Denmark 1994-2005. Scand J Infect Dis **2009**; 41(1): 21-5.
30. Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD. Early-onset neonatal sepsis. Clin Microbiol Rev **2014**; 27(1): 21-47.
31. Schwarze R, Bauermeister CD, Ortel S, Wichmann G. Perinatal listeriosis in Dresden 1981-1986: clinical and microbiological findings in 18 cases. Infection **1989**; 17(3): 131-8.
32. Jackson KA, Iwamoto M, Swerdlow D. Pregnancy-associated listeriosis. Epidemiol Infect **2010**; 138(10): 1503-9.
33. May M, Daley AJ, Donath S, Isaacs D, Australasian Study Group for Neonatal I. Early onset neonatal meningitis in Australia and New Zealand, 1992-2002. Arch Dis Child Fetal Neonatal Ed **2005**; 90(4): F324-7.
34. Nanduri SA, Petit S, Smelser C, et al. Epidemiology of Invasive Early-Onset and Late-Onset Group B Streptococcal Disease in the United States, 2006 to 2015: Multistate Laboratory and Population-Based Surveillance. JAMA Pediatr **2019**; 173(3): 224-33.
35. Schuchat A, Swaminathan B, Broome CV. Epidemiology of human listeriosis. Clin Microbiol Rev **1991**; 4(2): 169-83.
36. Siegman-Igra Y, Levin R, Weinberger M, et al. Listeria monocytogenes infection in Israel and review of cases worldwide. Emerging infectious diseases **2002**; 8(3): 305-10.
37. Girard D, Leclercq A, Laurent E, Lecuit M, de Valk H, Goulet V. Pregnancy-related listeriosis in France, 1984 to 2011, with a focus on 606 cases from 1999 to 2011. Euro Surveill **2014**; 19(38).
38. Janakiraman V. Listeriosis in pregnancy: diagnosis, treatment, and prevention. Rev Obstet Gynecol **2008**; 1(4): 179-85.

39. Charlier C, Goffinet F, Azria E, Leclercq A, Lecuit M. Inadequate management of pregnancy-associated listeriosis: lessons from four case reports. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases **2013**.
40. Committee on Obstetric Practice ACoO, Gynecologists. Committee Opinion No. 614: Management of pregnant women with presumptive exposure to Listeria monocytogenes. Obstet Gynecol **2014**; 124(6): 1241-4.
41. Stoll BJ, Puopolo KM, Hansen NI, et al. Early-Onset Neonatal Sepsis 2015 to 2017, the Rise of Escherichia coli, and the Need for Novel Prevention Strategies. JAMA Pediatr **2020**; 174(7): e200593.
42. Polcwiartek LB, Smith PB, Benjamin DK, et al. Early-onset sepsis in term infants admitted to neonatal intensive care units (2011-2016). J Perinatol **2021**; 41(1): 157-63.

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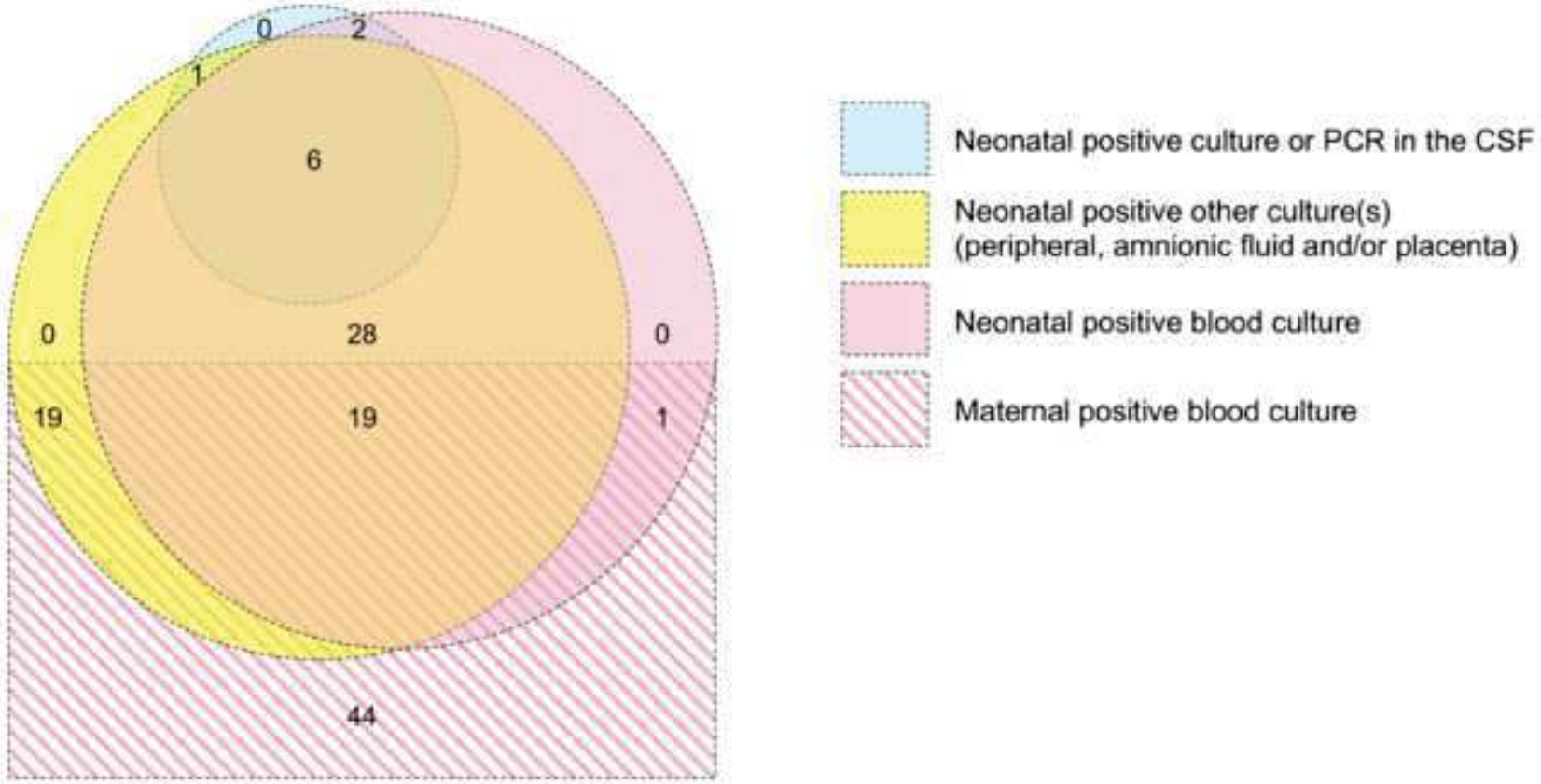
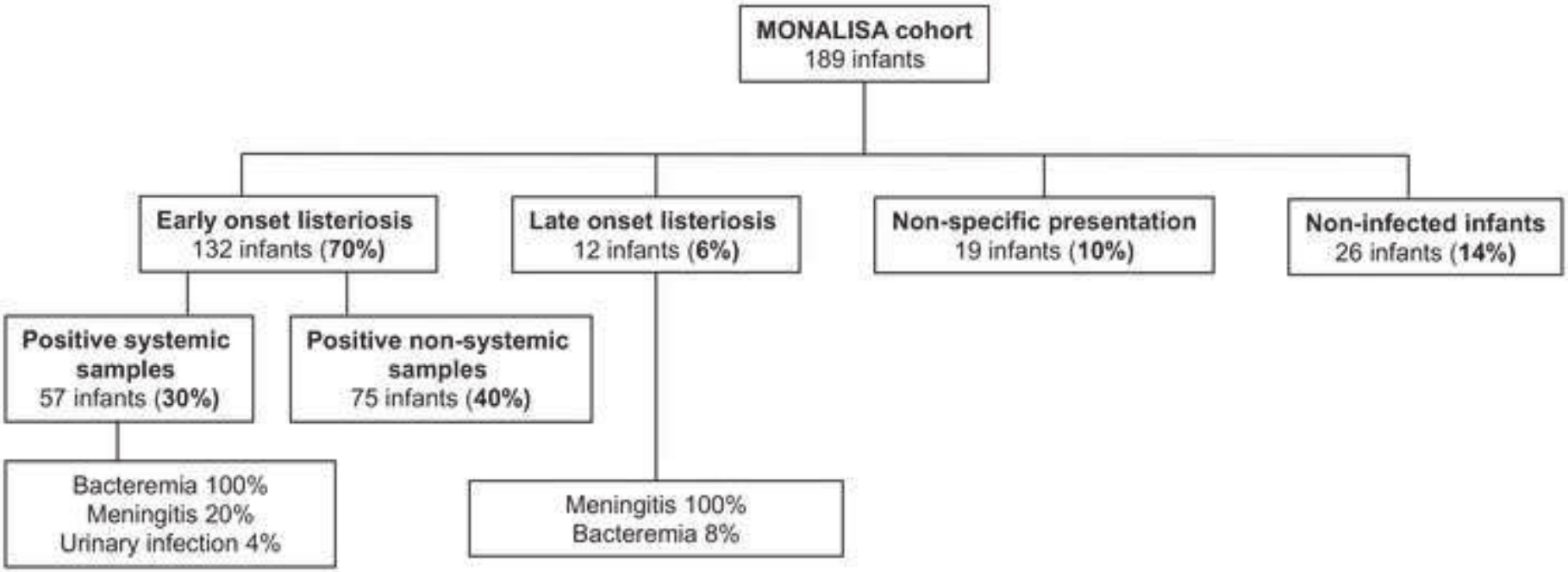


Figure 2 600ppi(.tif and .eps files only)



Neonatal listeriosis presentation and outcome: a prospective study of 189 cases from the MONALISA cohort

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Table S1. Features of the maternal population

	Cohort N=187*	Mothers of infants with *Lm*-positive systemic samples (S) N=56*	Mothers of infants with *Lm*-positive non-systemic samples (NS) N=74*	Mothers of infants with only maternal *Lm*-positive samples (M) N=45	Mothers of infants with late onset infection N=12†	*P value S vs. NS vs. M*§	*P value S vs. NS*§
Clinical features							
Any clinical sign	167/187 (89%)	50/56 (89%)	70/75 (93%)	45/45 (100%)	1/12 (8%)	0.08	0.4
Temperature > 37.7°C	129/187 (69%)	36/56 (64%)	49/74 (66%)	43/45 (96%)	1/12 (8%)	0.0004	0.81
Influenza-like symptoms	49/185 (26%)	13/56 (23%)	15/74 (20%)	21/43 (49%)	0/12	0.002	0.69
Diarrhea	10/187 (5%)	5/56 (9%)	1/74 (2%)	4/45 (9%)	0/12	0.1	0.04
Neurological symptoms‡	1/187 (1%)	0/56	0/74	1/45 (2%)	0/12	-	-
Systemic blood pressure < 90 mm Hg	3/187 (2%)	1/56 (2%)	0/74 (1%)	1/45 (2%)	1/12 (8%)	-	-
Septic shock	0/187	0/56	0/74	0/45	0/12	-	-
Obstetric signs (contractions, labor, abnormal fetal heart rate)	130/187 (70%)	48/56 (86%)	66/74 (89%)	15/45 (33%)	0/12 (0%)	<0.0001	0.55
Death	0/187	0/56	0/74	0/45	0/12	-	-
Positive maternal blood cultures		20/54 (37%)	19/47 (40%)	44/45 (98%)	-	<0.0001	0.84
Positive maternal CSF culture		0/54	0/47	0/45	-	-	-
Positive maternal CSF PCR		0/54	0/47	1/45 (2%)		-	-

Systemic samples were neonatal blood, cerebrospinal fluid or urine samples. Other neonatal samples were classified as non-systemic. Maternal positive samples were blood, vaginal or urine samples

Lm refers to *Listeria monocytogenes*

* There were 2 twin pregnancies, hence 187 mothers. One mother gave birth to one infant with S and one infant with NS positive samples, while the other mother gave birth to 2 infants with NS positive samples.

† Range 7-22 days after birth

‡ χ^2 and Fisher tests were used for qualitative variables

Table S2. Biochemical and cellular features of the cerebrospinal fluid in the 20 patients with *Listeria monocytogenes*
IQ denotes interquartiles.

	Infants with early onset infection *n*=8	Infants with late onset infection *n*=12[*]	*P*
Direct examination positive	1 (13%)	6 (50%)	*0.37*
Median nucleated cells count (cells /mm^3) (IQ 25-75)	125 (40-945)	3,350 (1,040-5,055)	*0.01*
Median polymorphonuclear to nucleated cells ratio (IQ 25-75)	0.55 (13-65)[†]	0.52 (38-70)	*0.67*
Median protein concentration (g/L) (IQ 25-75)	2.6 (1.7-5.05)[†]	2.5 (1.5-4.7)	*0.68*
Median cerebrospinal fluid to blood glucose ratio (IQ 25-75)	0.05 (0.03-0.1)[‡]	0.56 (0.35-0.57)	*0.67*

[*] Range 7-22 days after birth
[†] Based on *n*=6
[‡] Based on *n*=3

Table S3. Distribution of *L. monocytogenes* clonal complexes among samples collected from the study cohort

Data available for 184 isolates, including 56 isolates from infants with central positive samples, 75 infants with non-central positive samples including 2 twins, 43 infants with maternal positive samples only and 12 with late onset infection. In case of maternal and infant sampling or twin sampling (n=2), only one sample was analyzed.

MLST Clonal Complex	No. isolates (*N*=184)	Infants with Lm-positive central samples (C) *n*=56*	Infants with Lm-positive non-central samples (NC) *n*=73	Infants with only maternal samples positive (M) *n*=43†	Infants with late onset infection *n*=12‡
Lineage I					
CC1	48 (26%)	11 (20%)	17 (23%)	14 (33%)	6 (50%)
CC6	27 (15%)	11 (20%)	10 (14%)	3 (7%)	3 (25%)
CC4	25 (14%)	11 (20%)	12 (16%)	2 (5%)	-
CC2	15 (8%)	3 (5%)	10 (14%)	2 (5%)	-
CC5	13 (7%)	3 (5%)	6 (8%)	3 (7%)	1 (8%)
CC87	4 (2%)	-	3 (4%)	1 (2%)	-
CC3	3 (2%)	1 (2%)	2 (3%)	-	-
CC220	3 (2%)	-	1 (1%)	2 (5%)	-
CC224	3 (2%)	2 (4%)	-	1 (2%)	-
CC54	2 (1%)	1 (2%)	-	1 (2%)	-
CC59	2 (1%)	1 (2%)	1 (1%)	-	-
CC77	2 (1%)	-	1 (1%)	1 (2%)	-
CC183	2 (1%)	1 (2%)	1 (1%)	-	-
CC217	2 (1%)	-	2 (3%)	-	-
CC218	1 (1%)	-	-	1 (2%)	-
CC240	1 (1%)	-	-	1 (2%)	-
Lineage II					
CC7	4 (2%)	2 (4%)	1 (1%)	1 (2%)	-
CC101	4 (2%)	3 (5%)	-	1 (2%)	-
CC11	3 (2%)	1 (2%)	-	1 (2%)	1 (8%)
CC14	3 (2%)	1 (2%)	1 (1%)	1 (2%)	-
CC18	3 (2%)	1 (2%)	-	2 (5%)	-
CC37	3 (2%)		1 (1%)	2 (5%)	-
CC155	3 (2%)	1 (2%)	2 (3%)	-	-
CC121	2 (1%)	-	2 (3%)	-	-
CC8	1 (1%)	-	-	-	1 (8%)
CC20	1 (1%)	-	-	1 (2%)	-
CC29	1 (1%)	-	-	1 (2%)	-
CC403	1 (1%)	1 (2%)	-	-	-
CC412	1 (1%)	-	-	1 (2%)	-
CC475	1 (1%)	1 (2%)	-	-	-

* Central samples were neonatal blood, cerebrospinal fluid or urine cultures. Other neonatal samples were classified as non-central.

† Maternal positive samples were blood, vaginal or urine samples.

‡ Range 7-22 days after birth

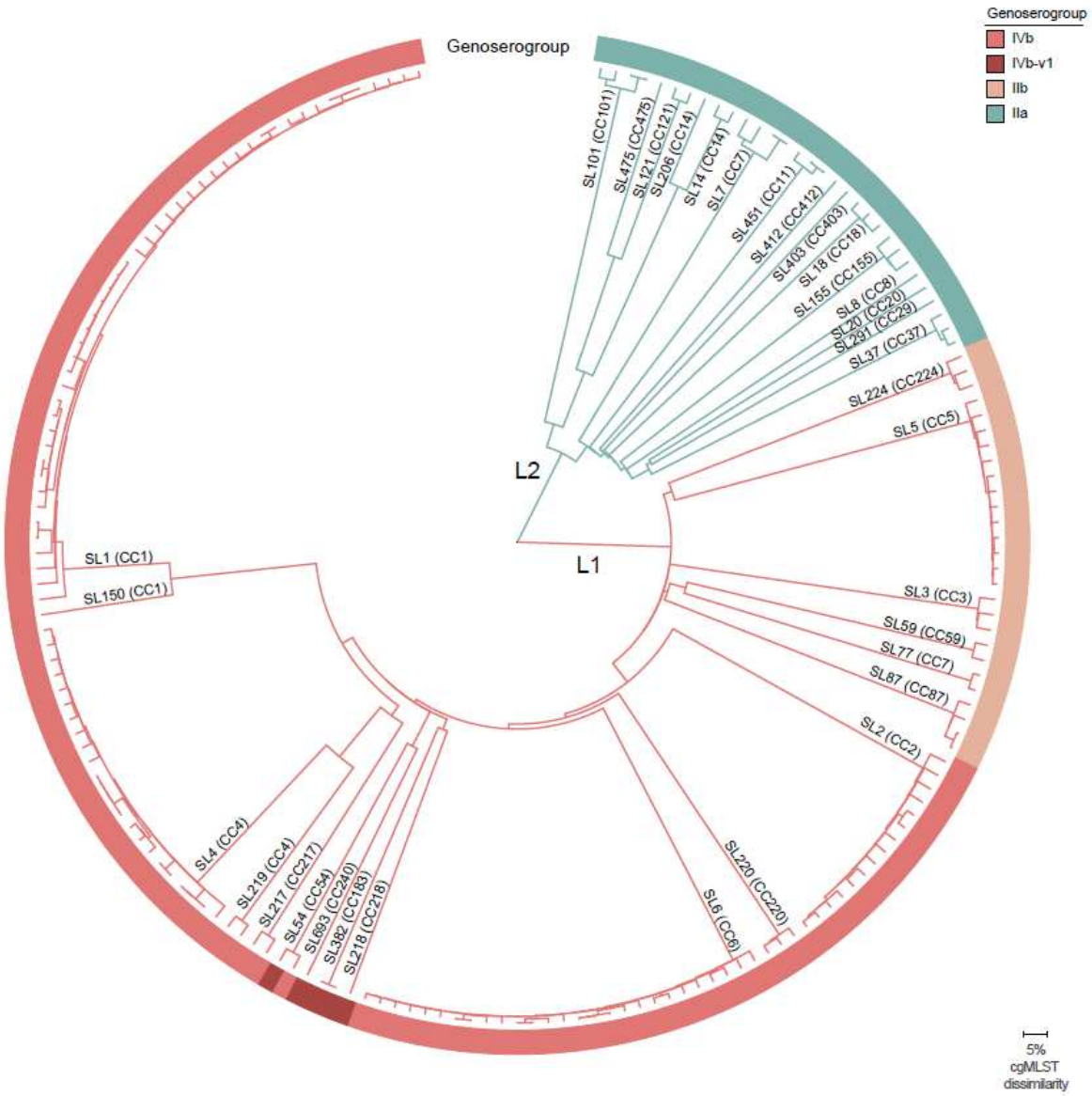

Figure S1. cgMLST analysis of *Listeria monocytogenes* isolates (data available for 184 isolates)

cgMLST allelic profiles were obtained using the Institut Pasteur scheme of 1748 loci (Moura *et al.*, 2016), implemented at BIGSdb-*Lm* (https://bigsdb.pasteur.fr/listeria/). The dendrogram was constructed using the single linkage algorithm implemented at BioNumerics v.7.6 (Applied-Maths, Belgium) and annotated with iTol v.4.2 (Letunic & Bork, 2016). Phylogenetic lineages (L#) and sublineages (SL#) are labelled in the branches. Corresponding clonal complexes (based on MLST scheme of 7 loci; Ragon *et al.*, 2008) are also indicated in brackets (CC#). The external ring represents the genoserogroups (based on Doumith *et al.*, 2004 and Leclercq *et al.*, 2011), colored as indicated in upper right panel.

Supplemental references

Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C. & Martin, P. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. J Clin Microbiol 2004 42 : 3819–3822.

Leclercq A, Chenal-Francisque V, Dieye H, Cantinelli T, Drali R, Brisse S, Lecuit M. Characterization of the novel *Listeria monocytogenes* PCR serogrouping profile IVb-v1. Int J Food Microbiol 2011 147:74–77.

Letunic, I. & Bork, P. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Res 2016 44 :W242-245.

Moura A, Criscuolo A, Pouseele H, Maury MM, Leclercq A, Tarr C, Björkman JT, Dallman T, Reimer A, Enouf V, Larsonneur E, Carleton H, Bracq-Dieye H, Katz LS, Jones L, Touchon M, Tourdjman M, Walker M, Stroika S, Cantinelli T, Chenal-Francisque V, Kucerova Z, Rocha EPC, Nadon C, Grant K, Nielsen EM, Pot B, Gerner-Smidt P, Lecuit M, Brisse S. Whole genome-based population biology and epidemiological surveillance of *Listeria monocytogenes*. Nat Microbiol 2016 2:16185.

Ragon, M. Wirth T, Hollandt F, Lavenir R, Lecuit M, Le Monnier A, Brisse S. A new perspective on *Listeria monocytogenes* evolution. PLoS Pathog 2008 4:e1000146.

Neonatal listeriosis presentation and outcome: a prospective study of 189 cases

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Running title: neonatal listeriosis

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Summary of the main point: We prospectively assessed the presentation and outcome of neonatal listeriosis and evidenced that maternal antibiotic treatment administered at least one day before delivery is associated with a significant reduction of neonatal severity.

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ABSTRACT

Context – Listeriosis is caused by the foodborne pathogen *Listeria monocytogenes*. It can present as a maternal-neonatal infection. We implemented the nationwide prospective cohort MONALISA and analyzed the features of neonatal listeriosis.

Methods – We studied all neonates born alive from mothers with microbiologically-proven maternal-neonatal listeriosis enrolled from November 2009 to December 2017. We analyzed presentation, neonatal outcome at discharge and predictors of severe presentation and outcome. The study is registered at clinicaltrials.gov (NCT01520597).

Results – We studied 189 infants. 133/189 (70%) had abnormal clinical status at birth, including acute respiratory distress in 106/189 (56%). 132/189 (70%) infants developed early-onset listeriosis and 12/189 (6%) late onset listeriosis who all presented with acute meningitis. 17/189 (9%) had major adverse outcomes: 3% death (5/189), 6% (12/189) severe brain injury, 2% (3/189) severe bronchopulmonary dysplasia, 15/17 in infants born < 34 weeks of gestation ($p < 0.0001$ versus infants born ≥ 34 weeks of gestation). Maternal antimicrobial treatment ≥1 day before delivery was associated with a significant decrease of infants' severity (resulting in significantly less inotropic drugs, fluid resuscitation, or mechanical ventilation requirement), OR 0.23 [95% confidence interval CI 0.09-0.51], $p < 0.0001$).

Conclusion– Antenatal maternal antimicrobial treatment is associated with reduced neonatal listeriosis severity, justifying the prescription of preemptive maternal antimicrobial therapy when maternal-fetal listeriosis is suspected. Neonatal outcome is better than reported earlier, and its major determinant is gestational age at birth.

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