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Listeriosis in pregnancy: a secular trend in a tertiary referral hospital in Barcelona

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Abstract The purpose of this study was to describe the variation in listeriosis infection incidence during pregnancy over a 25-year period based on salient clinical and laboratory features compiled in a tertiary referral hospital and to depict the clinical characteristics of these cases. A cohort was created of all cases of listeriosis in pregnant women or their neonates (early-onset form) diagnosed between 1985 and 2010. Forty-three cases of perinatal listeriosis were diagnosed among the 82,320 hospitalised pregnant women (incidence 0.5‰). Whereas the incidence remained almost constant at 0.24‰ until 2000, an increasing incidence was observed from then on, reaching 0.86‰ during the last years of the study period. A four-fold increase in listeriosis rate during pregnancy has occurred in recent years, with poorer outcome for those cases occurring before 28 weeks and for those in which early antibiotic treatment was not provided. These results should raise the awareness of the agencies and professionals involved in prenatal care.

Introduction

Among foodborne infections in the United States, listeria has the third highest mortality rate and accounts for approximately 19% of all deaths owing to these diseases [1]. Secondary to a zero tolerance policy in ready-to-eat (RTE) food, the incidence of listeriosis decreased by 24% from 1996 to 2003 [2]. On the contrary, in Europe, the annual report of communicable disease epidemiology shows a growing trend of cases of listeriosis from 1998 (0.14 cases per 100,000) to 2008 (0.31 cases per 100,000). Most cases occurred in people aged over 65, followed by children aged under 5, generally infected peripartum [3]. In Spain, the incidence in 2005 was 0.19/100,000 inhabitants in the general population [4].

Pregnant women, especially during the second and third trimester, have an increased susceptibility to certain intracellular pathogens because the immunity dependent on T cells is depressed in order to tolerate fetal antigens [5, 6]. Indeed, pregnant women are almost 20 times more susceptible to contract listeriosis than the general population [2, 7]. Listeriosis in pregnancy can cause a wide range of complications: miscarriage, stillbirth, chorioamnionitis, preterm delivery and maternal and neonatal sepsis [8]. Reported mortality among infants with neonatal listeriosis ranges

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from 25% to 50% [5–7]. Neonatal sepsis has either an early (<7 days) or late (≥ 7 days) onset form. The early-onset form is more common in preterm babies and is mostly due to transplacental infection. The clinical manifestation is more likely to be a sepsis-like illness with pneumonia. The late-onset form is less common and is acquired during labour after vaginal colonisation, meningitis being its more frequent clinical symptom [9]. However, cases of nosocomial transmission have also been reported, some even leading to outbreaks.

No previous study carried out in South-Europe has addressed the question of whether this increasing incidence of listeriosis is also occurring during pregnancy. This study aims to describe the changes in listeriosis infection incidence during pregnancy over the last 25 years and to depict the clinical characteristics of these cases.

Methods

A retrospective cohort was created of all cases of perinatal listeriosis diagnosed between November 1985 and October 2010 in a tertiary referral hospital in Barcelona (Spain), which covers an inner-city catchment area of about 600,000 inhabitants.

Perinatal listeriosis was defined as an episode of the infection diagnosed in a pregnant woman and/or in a neonate within the first 7 days of life, which was always confirmed by a positive culture obtained from a normally sterile site (maternal or neonatal blood, amniotic fluid, placenta, fetal pharynx at necropsy, neonatal pharynx/ear, or neonatal cerebrospinal fluid). Cases of concomitant positive results for listeria in maternal and neonatal samples were considered as a single case for this study.

Clinical characteristics of maternal and perinatal listeriosis, laboratory findings, therapy response and outcome were retrieved from medical records. In all the surviving newborns a neurodevelopmental follow-up until 2 years of life was performed including electroencephalograms, brain ultrasound examinations, brainstem potential evoked tests and psychomotor development tests (Brunet-Lezine [10]).

Processing and bacterial identification were performed according to standard methodology, that is, Gram stain and blood agar culture. Agglutination was performed with specific antiserums against serotypes 1/2 and 4 (DifcoTM Listeria O Antiserum, Becton, Dickinson and Company, Sparks, Maryland, USA).

Results

During the study period a total of 82,320 pregnant women were hospitalised at our institution. Among them, 43 cases
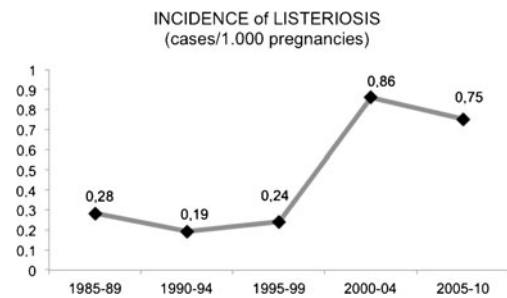

Fig. 1 Listeriosis incidence during pregnancy from 1985 until 2010

of perinatal listeriosis occurred throughout the follow-up period, giving an overall incidence of 0.5‰. All cases were sporadic infections, with no epidemiological and microbiological criteria of outbreak.

Figure 1 shows the incidence of cases during the study period. Whereas the incidence remained almost constant at 0.24‰ until 2000, an increasing incidence occurred from then, reaching 0.86‰ during the final years of the study period (chi-squared test 15.1; $p<0.001$). Seasonal distribution showed a higher frequency in summer ($n=18$ cases) (Fig. 2).

Figure 3 shows the distribution of cases and the outcome of the pregnancies according to the gestational age at the onset of the infection. There were 13 cases (30.2%) of pregnancy loss overall in various gestational ages, including eight miscarriages, three stillbirths and two neonatal deaths, being significantly more frequent when the infection occurred before 28 weeks (68.8% vs. 7.4%; $p<0.001$).

Table 1 shows the clinical characteristics of the 16 cases (37%) occurring before 28 weeks. There were eight miscarriages (50%), three stillbirths (22, 22 and 25 weeks of gestation; 19%), two cases of chorioamnionitis with a preterm delivery (25 and 27 weeks of gestation; 12%) and three pregnancies where early treatment with antibiotics resulted in a subsequent normal gestational course (19%). All mothers had a fever at the initial examination. Maternal leukocytosis with neutrophilia was detected in almost all cases (81%) and a left shift was also observed in 50%. C-
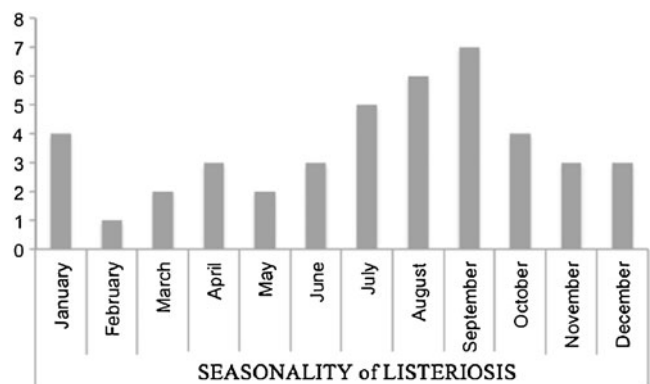

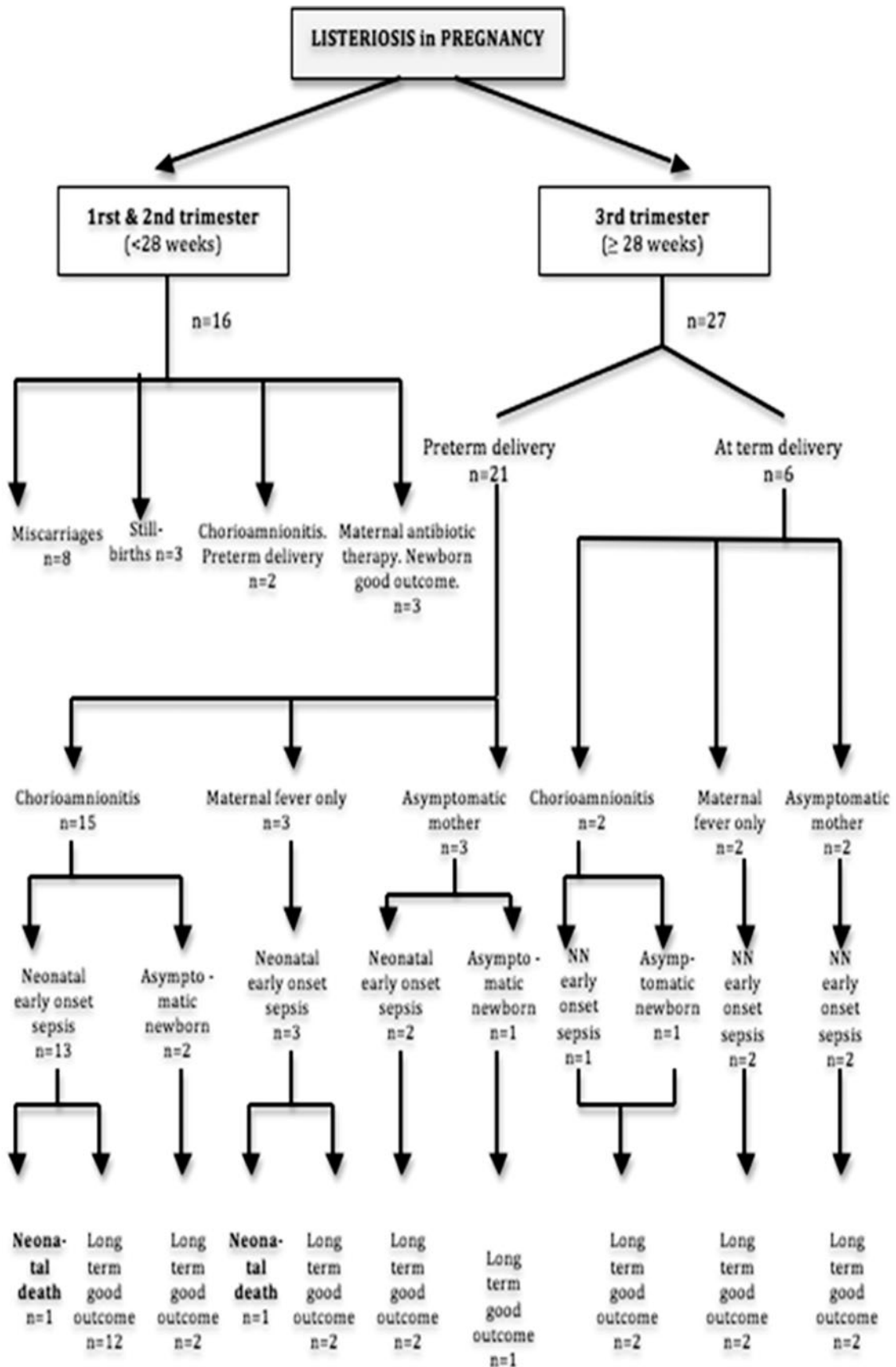**Fig. 2** Distribution of cases according to seasonality

Fig. 3 Salient features of listeriosis

Table 1 Materno-fetal listeriosis: Listeriosis in 1st and 2nd trimester (*n*=16)

Characteristics	Number of cases (%)	Mean	SD/Quartiles
Gestational age mean (weeks)		19+2	5+0
Outcome			
Miscarriages	8/16 (50%)		
Stillbirths	3/16 (19%)		
Chorioamnionitis-preterm delivery	2/16 (12%)		
Good outcome with antibiotics (delivery at term)	3/16 (19%)		
Maternal clinical features			
Fever	16/16 (100%)		
Abdominal pain	10/16 (63%)		
Acute gastroenteritis	3/16 (19%)		
Vaginal bleeding	2/16 (13%)		
Maternal blood analysis data			
Leukocytosis (>12,000c/mm3)	13/16 (81%)	16,660	8,154–25,166
Leukopenia (<4,000c/mm3)	2/16 (13 %)	3,555	-
Neutrophilia (>70% neutrophils)	13/16 (81%)	80	74–85
Left shift (% of young neutrophil forms)	8/16 (50%)	8	2.7–12.5
CRP increased (>0.8 mg/dl)	16/16 (100%)	17.4	10.14–24.66
Microbiological data			
Gram staining amniotic fluid (+)	5/7 (71%)		
Samples (+) for listeria[a]			
Placenta	10/10 (100%)		
Fetal pharynx/ear	8/8 (100%)		
Amniotic fluid	7/7 (100%)		
Maternal blood.	9/12 (75%)		
Lower genital tract	2/6 (33%)		
Fetal autopsy	3/3 (100%)		
Fetal blood	1/1 (100%)		
Serotype isolated			
4	8/13 (62%)		
1/2	5/13 (38%)		

[a] All microbiological samples were not collected in all cases

reactive protein (CRP) was processed in all maternal samples showing an increased level (> 0.8 mg/dl) in all cases. An amniocentesis was performed on seven of these patients but the Gram staining was positive for Gram-positive bacilli in only 5 (71%). The final diagnosis of the infection was obtained by a positive culture in all cases. There is data on the type of strain in 16 cases, of which strain 4 was isolated in eight, and strain 1/2 in five. With regard to maternal complications, one case of septic shock secondary to septic miscarriage required admission to an intensive care unit for supportive care.

Table 2 shows the clinical characteristics of the 27 cases (63%) occurring after 28 weeks. In 21 cases maternal infection occurred before 37 weeks of pregnancy and in six at term. Maternal fever was reported in 22 cases (81%). Information on amniotic fluid features provided by medical records was available in 23 cases, with meconium-stained fluid in 18 (78%). Chorioamnionitis was the first clinical sign in 17 (62%) pregnancies (in one of them with an acute

maternal gastroenteritis), maternal fever was the only sign in five cases (19%) and an unexpected neonatal listeriosis was diagnosed in five infants from an asymptomatic mother (19%). Laboratory data was available for 21 patients and showed maternal leukocytosis in 81% and increased CRP levels in all of them. An amniocentesis to rule out intra-amniotic infection was performed in eight cases and the Gram staining was again positive for Gram-positive rod-shaped bacteria in only 5 (63%). Among the 18 cases where listeria strain was identified, the most prevalent was also the 4 strain (94%). In three placentas characteristic microabscesses were described on the surface. Although 60% of maternal blood cultures obtained were positive, no severe maternal complications were reported.

Among the 32 born-alive babies, 29 were born from a mother infected at the time of delivery and three from a mother infected and treated before 24 weeks with a good pregnancy outcome. In 25 infants early neonatal sepsis was the clinical presentation (86%) and among them only 12

Table 2 Materno-fetal listeriosis: Listeriosis in 3rd trimester (*n*=27)

Characteristics	Number of cases (%)	Mean	SD/Quartiles
Gestational age mean (weeks + days)		33w+2 d	3w+6 d
Preterm delivery (<37 weeks + days)	21/27 (78%)	31w+8 d	2w+6 d
Delivery			
Vaginal delivery	15/27 (56%)		
Caesarean section	12/27 (44%)		
Maternal clinical features			
Maternal fever	22/27 (81%)		
Maternal fever only	5/27 (19%)		
Meconium	18/23 (78%)		
Chorioamnionitis	17/27 (62%)		
Asymptomatic mother	5/27 (19%)		
Maternal acute gastroenteritis	1/27 (4%)		
Maternal blood analysis data			
Leukocytosis (>10,000c/mm^3)	17/21 (81%)	15,276	8,421–22,131
Leukopenia (<4,000c/mm3)	0/21 (0%)	-	-
Neutrophilia (>70% neutrophils)	13/21 (62%)	73	63–83
Left shift (% of young neutrophils forms)	12/21 (57%)	7.6	0.6–14.6
CRP increased (>0.8 mg/dl)	21/21 (100%)	11.6	4.10–19.18
Microbiological data			
Gram staining amniotic fluid (+)	5/8 (63%)		
Samples (+) for listeria[a]			
Placenta	12/12 (100%)		
Amniotic fluid	8/8 (100%)		
Neonatal pharynx/ear	18/19 (95%)		
Neonatal blood	13/20 (62%)		
Maternal blood	6/10 (60%)		
Maternal lower genital tract	2/9 (22%)		
Neonatal cerebrospinal fluid	1/19 (5%)		
Serotype isolated			
4	17/18 (94%)		
1/2	1/18 (6%)		

[a] All microbiological samples were not collected in all cases

(48%) were prenatally treated with antibiotic therapy (ampicillin and gentamicin). However, there were three infants delivered from infected mothers where the infection was not confirmed by neonatal clinical findings nor by culture results and all of them had been prenatally treated (ampicillin and gentamicin). Finally, there was a case with no proven infection in the newborn, where perinatal listeriosis was a fortuitous diagnosis through the culture of the placenta obtained at the finding of a foul smelling amniotic fluid.

Table 3 shows the perinatal outcome of these 29 infants born from an infected mother at the time of delivery: 23 were premature and 2 (29 and 30 weeks of gestation) died in the early neonatal period due to a severe sepsis without antibiotic treatment administered before birth. Laboratory data were available for 21 cases, with young neutrophil forms and an elevated CRP being the most common

disorders (95%). Neonatal complications were greatly influenced by prematurity, including intraventricular haemorrhage (34%), severe respiratory distress (24%), petechial rash (24%), meningitis (17%), seizures (14%), bronchopneumonia (10%), disseminated intravascular coagulation (10%), osteopenia of prematurity (7%) and retinopathy (3%). The average hospital stay of the infected newborns was 27 days (range 4–51 days). However, all infants treated early were discharged from the hospital in good health and without serious sequelae at pediatric follow-up. Among the 25 surviving children born in the third trimester, no neurological sequelae were found at follow-up. Among the two extremely premature survivors born before 28 weeks with severe stigmata of prematurity and with a subsequent intraventricular hemorrhage, the neurodevelopment follow-up was also normal, but one of them showed a severe retinopathy, which required laser therapy.

Table 3 Neonatal infection: perinatal outcome (*n*=29)

Characteristics	Number of cases (%)	Mean	SD/Range
Neonatal death	2/29 (7%)		
APGAR score			
1 minute		5	3–8
5 minutes		9	7–10
pH umbilical cord			
Artery		7.20	0.10
Vein		7.29	0.12
Antibiotic therapy before delivery	15/29 (52%)		
Early onset neonatal sepsis:	25/29 (86%)		
Blood analysis data			
Leukocytosis (>30,000c/mm3)	9/21 (43%)	32,936	13,520–52,352
Leukopenia (<4,000c/mm3)	1/21 (5%)	2,870	–
Neutrophilia (>70% neutrophils)	1/21 (5%)	84	–
Left shift (% of young neutrophil forms)	20/21 (95%)	7.4	1.5–13.3
CRP increased (>0.8 mg/dl)	19/21 (90%)	14.9	0–32.8
Neonatal resuscitation	20/29 (69%)		
Complications			
Intraventricular haemorrhage	10/29 (34%)		
Exanthematic rash	7/29 (24%)		
Severe respiratory distress	7/29 (24%)		
Meningitis	5/29 (17%)		
Seizures	4/29 (14%)		
Bronchopneumonia	3/29 (10%)		
DIC	3/29 (10%)		
Osteopenia of prematurity	2/29 (7%)		
Retinopathy of prematurity	1/29 (3%)		
Length of hospital stay (days)		27	4–51

DIC disseminated intravascular coagulation

Discussion

In recent years, an increased incidence of listeriosis has been reported in several countries, mainly in people aged over 65, but the incidence among pregnant women has remained stable or has even declined [11–15]. In contrast, the analysis of data related to the last 25 years shows a nearly four-fold increase in the incidence of listeriosis in our pregnant population over the last decade. This increase could not be attributed to a higher incidence of predisposing factors in our population. The inexistence of an official medical policy of listeriosis prevention in pregnant women in our region may have acted as a contributing factor to this increased rate. Moreover, in accordance with other studies [16], we have not found demographic differences that may explain the rise in listeria infection. It has been speculated that the increasing consumption of ready-to-eat food and the reduction in salt (recommended by health agencies) may have contributed to the growth of microorganisms, increasing the likelihood of infection in the most susceptible consumers [13]. Furthermore, a seasonal trend was observed with a higher number of cases reported in summer, which was documented

in most European countries [3]. It could be related to a transgression of expiry dates of freshly-cut fruit and vegetable products and storage of this type of food at inappropriate temperatures in refrigerators, which could contribute to the growth of listeria on ready-to-eat products, especially during the summer season [17]. As other studies have shown [8, 18, 19], there is an increased susceptibility for listeriosis infection in late pregnancy, which was confirmed in the present series, where the incidence of infection was two times higher after 28 weeks compared with the first trimesters of pregnancy.

Listeriosis in pregnancies less than 28 weeks can be devastating, and in the present series the final outcome was a miscarriage or a stillbirth in most cases. However, in our series, an early suspicion and antibiotic treatment given to three mothers resulted in a subsequent normal pregnancy course in all of them. This finding highlights the treatability of the condition, in accordance with previous reports [18, 20–22]. It then seems reasonable that at the suspicion of a maternal bacterial infection based on the onset of fever without an apparent focus, and an abnormal blood test with leukocytosis with a left shift and an increased CRP, appropriate antibiotic prophylaxis should be administered until

the results of the microbiological studies become available. Other clinical features that may help to define the diagnosis are flu-like symptoms, gastroenteritis and an abnormal liver profile with a cytolytic pattern [23]. However, microbiological sampling of a normally sterile sample-site is the conclusive diagnostic method.

Perinatal listeriosis in pregnancies over 28 weeks typically produces the classic triad: prematurity, meconium stain fluid and maternal fever. These three events were met in nearly 80% of the cases we have studied.

The most serious complications and the two neonatal deaths observed in our series occurred in preterm babies. However, the long-term neonatal outcome was good. All the infants who were discharged from the neonatal care unit did so in good health and without sequelae. Moreover, pediatric follow-up, psychomotor development and neurological maturation were within regular parameters in all cases.

Two surprising facts were found. On the one hand, neonatal listeriosis was diagnosed in five completely asymptomatic mothers. This leads us to suppose that there could be a transplacental transmission of the infection despite the fact that it does not produce a systemic inflammatory response in the mother. On the other hand, there were three cases of maternal listeriosis with clinical symptoms of acute chorioamnionitis without confirmation of a subsequent neonatal infection. The documentation of culture confirmed asymptomatic *Listeria monocytogenes* infections in mothers and in neonates widens our knowledge on listeriosis during pregnancy [24]. The mothers had all started prenatal antibiotic treatment for less than one day, so we assume that the treatment may have had an influence in the neonatal prognosis. However, 12 other women with active infection also treated prenatally with the same antibiotics (never for more than 3 days), gave birth to infected neonates, casting doubt on the efficacy of a short antibiotic treatment regimen in advanced stages of infection.

Conclusion

These results confirm an increase in listeriosis infection rates during pregnancy in recent years. Because of its potentially devastating consequences, maternal-fetal specialists and public health agencies must be aware of the risks this infection entails. Early suspicion and early treatment of pregnant women are likely to minimise the adverse outcome. Apart from personal precautions in the control of listeriosis, action from public health agencies and the food industry would be required.

Conflict of interest statement We declare that we have no conflict of interest.

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