Methods

Sample

At each site of 14 participating institutions in 11 cities in 5 states, enrollment and consent processes were approved by the local institutional review board. Mothers were approached for consent either upon antenatal admission or shortly after delivery, depending on clinical circumstance and institutional preference. Approximately 260 additional women were eligible, but were either missed or did not consent to participate.

Pregnancy disorders that led to preterm delivery (1)

The clinical circumstances that led to each maternal admission and ultimately to each preterm delivery were operationally defined using both data from the maternal interview and data abstracted from the medical record. Each mother/infant pair was assigned to the category that described the primary reason for the preterm delivery. Preterm labor was defined as progressive cervical dilation with regular contractions and intact membranes. The diagnosis of preterm premature rupture of fetal membranes (pPROM) was defined as the presence of vaginal pooling with either documented nitrazine positive testing or ferning prior to regular uterine activity. Preeclampsia was defined as new onset hypertension and proteinuria of sufficient severity to warrant delivery for either a maternal or fetal indication. For a diagnosis of cervical insufficiency, a woman had to present with cervical dilation of greater than two centimeters, in the absence of membrane rupture and detected or perceived uterine activity. Placental abruption was defined as presentation with significant amount of vaginal bleeding (either documented in the medical record or a post-partum hematocrit <24%) and a clinical diagnosis of placental abruption in the absence of cervical change. Presentations under the category of fetal indication/IUGR included severe intrauterine growth restriction based on antepartum ultrasound examination, non-reassuring fetal testing, oligohydramnious, and Doppler abnormalities of umbilical cord blood flow.

Ultrasound scans (2-4)

All ultrasound scans were read independently by two readers who were unaware of all clinical information. When the two readers differed in their recognition of a lesion, the films were sent to a third (tie-breaking) reader who did not know what the first two readers reported.

Placenta microbiology (5, 6)

Eighty-two percent of the placentas were sampled within 1 hour of delivery. The microorganism recovery rate was not influenced by the interval between birth and sampling.

An instructional video and individual training were provided to ensure sterile technique. After lifting an area of amnion at the midpoint of the longest distance between the cord insertion and the edge of the disk, the underlying tissue was removed for culture under sterile conditions and then placed in liquid nitrogen until transfer to a –80°C freezer.

The frozen samples were allowed to thaw at room temperature, a portion approximately 1 cm squared was removed and weighed, then diluted 1:10 with sterile phosphate buffered saline (PBS), homogenized and aliquots plated on selective and non-selective media, including pre-reduced brucella-base agar with 5% sheep blood enriched with hemin and vitamin K_1, tryptic soy agar with 5% sheep blood, chocolate agar, and A-7 agar. After incubation, the various colony types were enumerated, isolated and identified by established criteria (7).

Placenta histology (8, 9)

Histologic examination of the placenta was performed following College of American Pathologists guidelines. (10) Two representative full thickness sections were taken from the center and a paracentral zone of the placenta disc as well as from all abnormal areas. Two sections each were taken from the central umbilical cord and a membrane roll from the fetal membranes away from the placenta disc.

At the chorionic plate, inflammation was assigned a stage and grade with Stage 3 representing neutrophils in the amnionic epithelium and grade 3 requiring more than 20 neutrophils/20x. Grade 3 inflammation in the amnion, and the chorion/decidua of the outer free membranes required numerous large foci or confluent foci of neutrophils. Grade 3 inflammation in the umbilical cord required neutrophils in perivascular Wharton's jelly.

Inter-villous fibrin, fetal stem vessel thrombosis, and decidual hemorrhage and fibrin deposition consistent with abruption were coded as present or absent. Syncytial knots were coded as either increased or not.

Data analysis

Potential confounders were sought by examining the relationship of candidate variables with each exposure of interest (*i.e.*, organism recovery or histologic lesion), as well as with each outcome of interest (*i.e.*, ultrasound lesion or cerebral palsy diagnosis). Variables associated with an exposure and an outcome (usually at $p < 0.3$) were considered candidates for logistic regression analyses (32).

In the entire ELGAN Study sample, both microorganism recovery and histologic characteristics of the placenta varied with gestational age at delivery (6, 8). In early sets of analyses, we adjusted for gestational age in two ways, by both week of gestation (23, 24, 25, 26, 27), and by groups of weeks (23-24, 25-26, 27). As the results were almost identical, we present only those with the groups-of-weeks adjustment.

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Table S1. Odds ratios (and 95% CI) of **ventriculomegaly** associated with each placental organism listed on the left in the total sample, and in the vaginal and Cesarean section sub-samples. The only adjustment is for gestational age (23-24, 25-26, and 27 weeks).

Microorganism	**All**	**Vaginal**	**Cesarean**
Actinomyces sp	2.6 (1.5, 4.5)	2.3 (1.2, 4.4)	2.8 (1.1, 7.2)
Prevotella bivia	0.7 (0.3, 1.7)	0.7 (0.3, 1.9)	0.4 (0, 2.7)
Corynebacterium sp	1.9 (1.2, 3.2)	1.8 (1.01, 3.3)	0.8 (0.1, 6.6)
Escherichia coli	0.9 (0.4, 1.9)	0.2 (0.1, 0.96)	2.2 (0.9, 5.3)
Lactobacillus sp	0.7 (0.3, 1.5)	0.4 (0.2, 1.3)	1.1 (0.4, 3.3)
Peptostrep magnus	0.9 (0.4, 2.0)	0.7 (0.3, 1.7)	0.8 (0.1, 6.4)
Propionibacterium sp	1.2 (0.7, 2.3)	0.5 (0.1, 2.2)	1.8 (0.9, 3.6)
Staphylococcus sp*	1.6 (0.99, 2.5)	1.4 (0.8, 2.5)	1.4 (0.6, 3.4)
Group B Strep	1.7 (0.9, 3.4)	2.2 (0.9, 5.2)	0.7 (0.2, 3.2)
Group D Strep	1.1 (0.5, 2.4)	1.2 (0.5, 2.8)	0.8 (0.2, 3.2)
α hemolytic Strep	0.8 (0.4, 1.8)	0.7 (0.3, 1.9)	0.9 (0.3, 2.9)
Anaerobic Strep	1.5 (0.7, 2.9)	1.5 (0.7, 3.5)	1.1 0(.3, 3.9)
Gardnerella vaginalis	1.7 (0.8, 3.3)	1.9 (0.8, 4.5)	1.1 (0.3, 3.9)
Mycoplasma sp	0.6 (0.3, 1.4)	0.8 (0.3, 1.9)	0.2 (0, 1.7)
U urealyticum	1.3 (0.6, 2.6)	1.0 (0.4, 2.6)	1.5 (0.5, 4.4)
Sample at risk	1246	441	805

* Excludes S aureus

Table S2. Odds ratios (and 95% CI) of **an echolucent lesion** associated with each placental organism listed on the left in the total sample, and in the vaginal and Cesarean section sub-samples. The only adjustment is for gestational age (23-24, 25-26, and 27 weeks). (N=1246)

Microorganism	All	Vaginal	Cesarean
Actinomyces sp	1.0 (0.5, 2.3)	0.9 (0.4, 2.2)	0.7 (0.1, 5.0)
Prevotella bivia	0.3 (0.1, 1.3)	0.2 (0, 1.2)	0.7 (0.1, 5.5)
Corynebacterium sp	1.2 (0.6, 2.4)	0.9 (0.4, 1.9)	----†
Escherichia coli	0.6 (0.2, 1.7)	----	2.1 (0.7, 6.2)
Lactobacillus sp	1.0 (0.5, 2.3)	0.9 (0.3, 2.4)	1.0 (0.2, 4.4)
Peptostrep magnus	1.5 (0.7, 3.5)	1.2 (0.5, 2.9)	1.7 (0.2, 14
Propionibacterium sp	1.5 (0.7, 3.0)	0.8 (0.2, 3.4)	**2.3 (1.02, 5.1)**
Staphylococcus sp*	1.6 (0.9, 2.7)	0.7 (0.3, 1.4)	**3.7 (1.6, 8.5)**
Group B Strep	1.1 (0.4, 2.4)	1.2 (0.4, 3.6)	0.7 (0.1, 5.4)
Group D Strep	1.7 (0.8, 3.9)	1.9 (0.8, 4.7)	0.6 (0.1, 4.7)
α hemolytic Strep	0.6 (0.2, 1.8)	0.9 (0.3, 2.6)	----
Anaerobic Strep	1.4 (0.6, 3.2)	1.5 (0.6, 3.9)	0.6 (0.1, 4.7)
Gardnerella vaginalis	1.3 (0.6, 3.2)	0.5 (0.1, 2.0)	**3.2 (1.05, 10)**
Mycoplasma sp	1.0 (0.4, 2.2)	0.8 (0.3, 2.2)	0.9 (0.2, 4.0)
U urealyticum	1.3 (0.5, 3.1)	0.5 (0.1, 2.2)	2.9 (0.97, 8.9)
Sample at risk	1246	441	805

* Excludes S aureus

† Empty cells prohibit calculating odd ratios

Table S3. Risk ratios (and 95% CI) of each cerebral palsy diagnosis in **the total sample** associated with each placental organism or group of organisms listed on the left. The only adjustment is for gestational age (23-24, 25-26, and 27 weeks). (N=899)

Microorganism	**Quadriparesis**	**Diparesis**	**Hemiparesis**
Actinomyces sp	1.8 (0.7, 4.9)	1.8 (0.5, 6.3)	1.1 (0.1, 8.2)
Prevotella bivia	0.9 (0.3, 3.0)	1.9 (0.6, 6.0)	1.0 (0.1, 7.7)
Corynebacterium sp	2.1 (0.9, 4.9)	**5.1 (2.2, 12)**	----†
Escherichia coli	0.5 (0.1, 2.2)	2.3 (0.8, 6.5)	----
Lactobacillus sp	1.1 (0.4, 3.3)	0.4 (0, 3.2)	----
Peptostrep magnus	2.0 (0.7, 5.6)	2.5 (0.8, 8.2)	----
Propionibacterium sp	0.5 (0.1, 2.0)	0.4 (0, 2.7)	0.9 (0.1, 6.1)
Staphylococcus sp*	1.9 (0.9, 3.9)	**2.8 (1.2, 6.5)**	----
Group B Strep	0.7 (0.2, 3.0)	1.1 (0.2, 5.0)	1.1 (0.1, 8.7)
Group D Strep	2.4 (0.97, 5.9)	0.9 (0.2, 4.0)	----
α hemolytic Strep	**2.7 (1.1, 6.4)**	1.8 (0.5, 6.1)	1.1 (0.1, 8.5)
Anaerobic Strep	**3.1 (1.2, 7.2)**	1.2 (0.3, 5.4)	2.7 (0.6, 12)
Gardnerella vaginalis	1.5 (0.4, 5.1)	0.8 (0.1, 5.9)	----
Mycoplasma sp	0.9 (0.3, 2.9)	2.5 (0.9, 7.0)	----
U urealyticum	0.8 (0.2, 3.4)	1.3 (0.3, 5.6)	1.3 (0.2, 9.9)

***** Excludes S aureus

† Empty cells prohibit calculating odd ratios

Table S4. Risk ratios (and 95% CI) of each cerebral palsy diagnosis in **the vaginal sub-sample** associated with each placental organism or group of organisms listed on the left. The only adjustment is for gestational age (23-24, 25-26, and 27 weeks). (N=304)

Microorganism	Quadriparesis	Diparesis	Hemiparesis
Actinomyces sp	1.4 (0.4, 5.0)	1.8 (0.5, 6.8)	1.3 (0.3, 23)
Prevotella bivia	0.8 (0.2, 4.0)	1.5 (0.4, 5.9)	1.0 (0.1, 9.2)
Corynebacterium sp	1.9 (0.7, 5.4)	**5.0 (1.8, 14)**	----†
Escherichia coli	----	1.5 (0.4, 5.9)	----
Lactobacillus sp	0.4 (0.1, 3.6)	----	----
Peptostrep magnus	2.6 (0.9, 7.8)	1.6 (0.4, 6.2)	----
Propionibacterium sp	----	1.0 (0.1, 8.1)	----
Staphylococcus sp*	1.7 (0.6, 4.4)	2.1 (0.8, 6.0)	----
Group B Strep	0.5 (0.1, 3.8)	0.5 (0.1, 4.2)	1.2 (0.1, 11)
Group D Strep	2.9 (0.9, 9.3)	0.5 (0.1, 4.5)	----
α hemolytic Strep	2.9 (0.8, 9.7)	----	1.9 (0.2, 17)
Anaerobic Strep	2.1 (0.6, 8.0)	1.6 (0.3, 7.7)	4.5 (0.8, 25)
Gardnerella vaginalis	0.8 (0.1, 6.3)	0.9 (0.1, 7.8)	----
Mycoplasma sp	0.8 (0.2, 3.7)	1.0 (0.2, 4.6)	----
U urealyticum	0.7 (0.1, 5.3)	0.7 (0.1, 6.0)	----

***** Excludes S aureus

† Empty cells prohibit calculating odd ratios

Table S5. Risk ratios (and 95% CI) of each cerebral palsy diagnosis associated with each placental organism or group of organisms listed on the left. This sample is limited to infants **delivered by Cesarean section**, with adjustment for gestational age only (23-24, 25-26, and 27 weeks). (N=595)

Microorganism	Quadriparesis	Diparesis	Hemiparesis
Actinomyces sp	2.6 (0.5, 12)	----†	----
Prevotella bivia	1.0 (0.1, 7.8)	2.2 (0.2, 19)	----
Corynebacterium sp	2.8 (0.3, 24)	----	----
Escherichia coli	1.5 (0.3, 6.4)	3.4 (0.7, 17)	----
Lactobacillus sp	2.1 (0.6, 7.9)	1.4 (0.2, 12)	----
Peptostrep magnus	----	4.8 (0.5, 50)	----
Propionibacterium sp	0.7 (0.2, 3.1)	----	1.6 (0.2, 13)
Staphylococcus sp*	2.0 (0.5, 7.1)	2.7 (0.6, 13)	----
Group B Strep	1.3 (0.2, 11)	3.6 (0.4, 33)	----
Group D Strep	1.3 (0.3, 6.5)	1.3 (0.1, 11)	----
α hemolytic Strep	2.8 (0.8, 10)	**7.2 (1.7, 30)**	----
Anaerobic Strep	**4.1 (1.2, 14)**	----	----
Gardnerella vaginalis	2.4 (0.5, 12)	----	----
Mycoplasma sp	0.8 (0.1, 6.3)	**6.0 (1.5, 25)**	----
U urealyticum	1.0 (0.1, 7.6)	2.2 (0.3, 18)	3.2 (0.4, 27)

***** Excludes S aureus

† Empty cells prohibit calculating odd ratios

Table S6. Odds ratios (and 95% CI) of **ventriculomegaly and an echolucent white matter lesion** associated with each placenta histologic characteristic listed on the left. These data are from **the two components of the sample**. The only adjustment is for gestational age (23-24, 25-26, and 27 weeks).

Histologic characteristic	**Ventriculomegaly**		**Echolucent lesion**	
	Vaginal	**Cesarean**	**Vaginal**	**Cesarean**
Inflamm chorionic plate	1.6 (0.9, 2.8)	1.2 (0.6, 2.3)	1.5 (0.8, 2.9)	1.1 (0.5, 2.6)
Inflamm chorn/decidua	1.4 (0.8, 2.3)	1.3 (0.8, 2.1)	1.1 (0.6, 2.1)	1.1 (0.5, 2.1)
Neutrphls fetal vessels	1.4 (0.8, 2.4)	1.3 (0.7, 2.3)	1.4 (0.8, 2.6)	0.9 (0.4, 2.1)
Umbilicl cord vasculitis	1.6 (0.9, 2.9)	1.1 (0.5, 2.1)	0.9 (0.4, 1.9)	0.6 (0.2, 1.9)
Thrombsis fetal vessels	1.9 (0.8, 4.4)	2.1 (0.8, 5.3)	1.4 (0.5, 3.8)	1.6 (0.5, 5.4)
Infarct	0.6 (0.3, 1.5)	0.5 (0.2, 1.05)	1.6 (0.8, 3.5)	0.6 (0.3, 1.6)
Increasd syncytl knots	0.7 (0.3, 1.7)	0.8 (0.5, 1.5)	0.5 (0.1, 1.6)	1.4 (0.7, 2.7)
Decid hem/fibrn depstn	0.6 (0.3, 1.1)	1.4 (0.7, 2.6)	1.4 (0.7, 2.7)	1.6 (0.7, 3.6)

Table S7. The odds ratios (and 95% confidence intervals) of ventriculomegaly (VM) and an echolucent lesion (EL) among children whose placenta had the characteristics listed on the left relative to the risk among children whose placenta harbored no recoverable organism and had no histologic lesion considered indicative of inflammation. Adjusted for gestational age (23-24, 25-26, 27 weeks).

Organism	Placenta lesion*	Vaginal		Cesarean	
		VM	EL	VM	EL
+	+	**4.1 (1.02, 17)**	2.9 (0.7, 12)	1.7 (0.6, 5.2)	1.8 (0.3, 9.8)
+	—	0.9 (0.4, 2.2)	0.7 (0.3, 2.0)	1.3 (0.7, 2.2)	1.9 (0.9, 3.8)
—	+	0.4 (0.1, 1.3)	0.6 (0.2, 1.9)	0.7 (0.3, 1.8)	0.5 (0.1, 2.2)
—	—	1.0	1.0	1.0	1.0

***** Placenta lesions considered indicative of inflammation:
Inflammation chorionic plate stage 3 and severity 3
Inflammation chorion/decidua grades 3 and 4
Neut infiltratn fetal stem vessels
Umbilical cord vasculitis grades 3, 4 and 5

† Empty cells prohibit calculating odd ratios

Table S8. The odds ratios (and 95% confidence intervals) of each cerebral palsy diagnosis, among children whose placenta had the characteristics listed on the left relative to the risk among children whose placenta harbored no recoverable organism and had no histologic lesion considered indicative of inflammation. This is limited to the sub-sample of children and placentas delivered by **Cesarean section**. Adjusted for gestational age (23-24, 25-26, 27 weeks).

Organism	Placenta lesion*	Cerebral palsy		
		Quadriparesis	Diparesis	Hemiparesis
+	+	2.0 (0.3, 13)	0.6 (0.1, 5.9)	---†
+	—	1.4 (0.5, 3.5)	2.9 (0.6, 13)	0.4 (0, 3.3)
—	+	0.7 (0.2, 3.4)	3.3 (0.5, 21)	1.5 (0.3, 8.0)
—	—	1.0	1.0	1.0

***** Placenta lesions considered indicative of inflammation:
Inflammation chorionic plate stage 3 and severity 3
Inflammation chorion/decidua grades 3 and 4
Neut infiltratn fetal stem vessels
Umbilical cord vasculitis grades 3, 4 and 5

† Empty cells prohibit calculating odd ratios