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Role of *Ureaplasma* Respiratory Tract Colonization in BPD Pathogenesis: Current Concepts and Update

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SYNOPSIS

Respiratory tract colonization with the genital mycoplasma species *Ureaplasma parvum* and *U. urealyticum* in preterm infants is a significant risk factor for BPD. Recent studies of the ureaplasma genome, animal infection models, and human infants have provided a better understanding of specific virulence factors, pathogen-host interactions, and variability in genetic susceptibility that contribute to chronic infection, inflammation, and altered lung development. This review will provide an update on the current evidence supporting a causal role of *Ureaplasma* infection in BPD pathogenesis. The current status of antibiotic trials to prevent BPD in *Ureaplasma*-infected preterm infants is also reviewed.

Keywords

Ureaplasma parvum; *Ureaplasma urealyticum*; prematurity; bronchopulmonary dysplasia; macrolide antibiotics

INTRODUCTION

The mycoplasma species *Ureaplasma parvum* and *U. urealyticum* are genitourinary tract commensals in adults, but are associated with adverse pregnancy outcomes¹ and neonatal morbidities of prematurity including bronchopulmonary dysplasia (BPD),² necrotizing enterocolitis,³ and severe intraventricular hemorrhage.⁴ These organisms are the most commonly isolated organisms from infected placentas and amniotic fluid.^{1, 5} They have been detected in cord blood,^{4, 6} cerebrospinal fluid,⁴ respiratory secretions,^{2, 7} gastric aspirates,⁸ and brain⁹ and lung tissue¹⁰ of preterm infants. This review will focus on the epidemiological and experimental evidence for a causal role of *Ureaplasma* species in BPD pathogenesis and implications for therapeutic interventions.

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UREAPLASMA SPECIES

The 14 *Ureaplasma* serovars are grouped in 2 species, *Ureaplasma parvum* (serovars 1, 3, 6, and 14) and *U. urealyticum* (serovars 2, 4, 5 and 7–13) (Box 1). These organisms are among the smallest free-living, self-replicating cells. They lack cell walls, hydrolyze urea to generate ATP, have limited biosynthetic functions, and adhere to human mucosal surfaces of the genitourinary tract in adults and respiratory tract in newborns.¹¹ Our group and others have recently demonstrated that most *Ureaplasma* isolates from neonatal and adult clinical specimens as well as American Type Culture Collection (ATCC) reference strains have the capacity to form biofilms *in vitro*.^{12, 13} If biofilm formation is confirmed *in vivo*, it may be another mechanism by which *Ureaplasmas* evade the host immune response and increase resistance to antibiotics. *U. parvum* serovars are the most common serovars detected in neonatal respiratory samples at all gestational ages. In a prospective preterm cohort in a single institution, *U. parvum* was detected in 63% respiratory isolates.⁷ Serovars 3 and 6 alone and in combination accounted for 96% *U. parvum* respiratory isolates in this cohort.⁷ *U. urealyticum* isolates were commonly a mixture of multiple serovars with serovar 11 alone or combined with other serovars (59%) as the most common serovar. Most studies have not observed differences in prevalence of either species or specific serovars between infants with and without BPD.^{7, 14} Biofilm-forming capacity of clinical isolates also did not differ between infants with and without BPD.¹³ For clinical diagnostic purposes, it is not necessary to determine the species or serovar.

Box 1

Characteristics of genital mycoplasmas isolated from preterm infants

- 2 species
 - *U. parvum* (serovar 1, 3, 6, and 14)
 - *U. urealyticum* (serovars 2, 4, 5, 7–13)
- Small genomes (limited biosynthetic abilities)
- Lack cell walls (susceptible to dessication and heat)
- Hydrolyze urea to generate ATP
- Biofilm-forming capacity demonstrated *in vitro* and *in vivo* ^{12, 13, 95}
- *U. parvum* serovars most common in clinical specimens (70%) ⁷
- Require special transport and culture media to support growth
- Virulence Factors
 - Urease production of ammonia
 - IgA protease (degrading mucosal IgA)
 - Hydrogen peroxide (membrane peroxidation)
 - Phospholipases A and C (membrane phospholipid degradation)

- Inhibition of host cell antimicrobial peptide expression²²
- Serine/threonine kinase and protein phosphatase (cytotoxicity)²⁰
- Multiple-banded antigen, major pathogen-associated molecular pattern (PAMP) of *Ureaplasma* serovars recognized by host immune system: size variations evade immune detection^{24, 53, 96}

Ureaplasma spp. Virulence Factors

Prior to the advent of genomics, potential ureaplasma virulence factors including IgA protease and phospholipase A1, A2, and C were identified by functional and enzymatic assays.^{15, 16} However, no matches were found to known sequences for these proteins in any of the 14 ATCC reference ureaplasma serovar genomes (American Type Culture Collection)¹⁷ or clinical *U. parvum* serovar 3 genome.¹⁸ Although not confirmed experimentally to date, ammonia generated by urea hydrolysis may react with water in tissues to form ammonium hydroxide that may contribute to mucosal injury and inflammation.¹⁹ Recently, the sequence of a *U. parvum* serovar 3 clinical strain SV3F4 revealed a putative new virulence factor serine/threonine kinase and protein phosphatase (STK/STP).²⁰ In *Mycoplasma genitalium*, an STP protein is required for host cell cytotoxicity and mutant strains lacking the STP gene produce less hydrogen peroxide than wild-type strains.²¹ Recently, Xiao et al.²² demonstrated that *Ureaplasma* spp. suppress expression of antimicrobial peptides genes *DEFB1*, *DEFA5*, *DEFA6*, and *CAMP* *in vitro* that may be an additional mechanism by which these organisms avoid the host immune response.

The proposed major ureaplasma virulence factor is the multiple banded antigen (MBA), a surface lipoprotein that is the predominant pathogen-associated molecular pattern (PAMP) detected by the host immune system.²³ The organism may evade host recognition by varying the MBA size.^{19, 23} In the sheep intrauterine infection model, MBA protein/*mba* gene size variants were detected in infected amniotic fluid and fetal lung with increasing duration of gestation, suggesting that the size variants escaped eradication.¹⁹ However, MBA size variation did not correlate with chorioamnionitis severity in the sheep model, suggesting that difference in the host immune response may be important in ureaplasma pathogenicity.²⁴

Host Response to *Ureaplasma* Infection

Microbial recognition by innate immune systems can be mediated by a variety of germline-encoded receptors, including Toll-like receptors (TLRs), RIG-like receptors (RLRs), Nod-like receptors (NLRs), and cytosolic DNA sensors such as the HIN200 family member AIM2.²⁵ *Ureaplasma* sp. lack a gram-negative or gram-positive bacterial cell wall, thus are devoid of lipopolysaccharides or peptidoglycans – the microbial products that are potent activators of TR4 (lipopolysaccharide or LPS) and the TLR2 or NOD1/2 (peptidoglycan) pathways. Nevertheless, placental leukocytes or neonatal monocytes exposed *in vitro* to *Ureaplasma* spp. induce the release of inflammatory cytokines.^{26, 27} Shimizu et al.²⁸ showed that *U. parvum* lipoproteins, including the MBA activate nuclear factor-kappa B (NF-κB) in reporter cell lines via TLR1, TLR2 and TLR6 signaling. Peltier et al.²⁹ found that the

macrophage-stimulating activity from *U. urealyticum* is mainly due to lipoproteins, and signaling involves TLR2 or TLR4 receptors. Mechanisms of immune alterations induced by *Ureaplasma* spp. *in vivo* have not been identified. However, ureaplasmas do induce antibody production in both humans and animals.^{24, 30}

Ureaplasma clearance in the lung is dependent on local mediators of the host immune response. Surfactant protein A (SP-A) enhances ureaplasma phagocytosis and killing *in vitro*.³¹ Compared to wild type mice, SP-A deficient mice had delayed clearance of *Ureaplasma* from the lungs, increased of inflammatory cells and pro-inflammatory cytokine expression.³² This observation may be relevant to preterm fetuses and neonates who will have low levels of SP-A and other innate host defense factors in the lungs.

Observed differences in host susceptibility may be due, in part, to variants in genes regulating the innate immune response, thus altering risk for *Ureaplasma* spp. respiratory colonization and BPD in preterm infants. In a study of single nucleotide polymorphisms (SNP) of TLR genes in a cohort of preterm infants with known *Ureaplasma* respiratory colonization status, four SNPs in *TLR2* and *TLR6* were significantly associated with *Ureaplasma* respiratory tract colonization.³³ Interestingly, a *TLR6* SNP (rs5743827) was associated with both a decreased risk for *Ureaplasma* respiratory tract colonization and decreased risk for BPD (OR=0.54 [0.34–0.86] and OR=0.54 [0.31–0.95], respectively). This variant may alter susceptibility to *Ureaplasma* infection and the severity of the inflammatory response that contributes to the development of BPD.

Another factor that may explain variability in pathogenicity is immune modulation. Chronic intra-amniotic *Ureaplasma* infection in sheep profoundly diminished responses to intra-amniotic lipopolysaccharide (LPS) in the preterm fetal sheep.³⁴ A benefit of endotoxin tolerance is decreased inflammation in the host and therefore decreased organ injury. However, endotoxin tolerance also can increase susceptibility to infections due to suppression of innate immune responses. Thus a number of potential microbial factors may play in role in variability of host responses but none are known to play a role in clinical infection.

***Ureaplasma* spp. as Perinatal Pathogens Causing Preterm Birth**

Intrauterine inflammation is a common cause of preterm labor³⁵ and *Ureaplasma* spp. are the most common organisms isolated in the amniotic fluid either alone or as a part of a polymicrobial flora.^{36, 37} Presence of *Ureaplasma* spp. in the amniotic fluid is associated with shorter time from the amniocentesis to delivery time compared to cases where *Ureaplasma* was not isolated.³⁸ At the time of genetic amniocentesis at 15–17 weeks' gestation *Ureaplasma* species were identified in 11% pregnancies with subsequent preterm labor in 59% of *Ureaplasma*-positive women compared with 4% *Ureaplasma*-negative women.³⁹ Intra-amniotic inoculation of Rhesus macaques with *U. parvum* or the related organism *Mycoplasma hominis* induced chorioamnionitis, fetal inflammation and preterm labor.⁴⁰ Thus perinatal infections from *Ureaplasma* spp. are an important cause of preterm labor and delivery. These data also demonstrate the difficulties of reversing *Ureaplasma* induced pathologies by postnatal treatment, since many preterm infants may have been exposed to these organisms antenatally often for prolonged durations.

EPIDEMIOLOGIC EVIDENCE FOR ROLE OF *UREAPLASMA* SPP. IN BPD PATHOGENESIS

Characteristics of Infants with *Ureaplasma* Respiratory Tract Colonization

The reported incidence of *Ureaplasma* respiratory tract colonization rate in infants <1501 gm birthweight ranges from 28–33%.⁴¹ In a prospective study, we observed that the risk of *Ureaplasma* spp. respiratory colonization decreased with increasing gestational age (OR, 0.821; CI, 0.720–0.934).⁷ Sixty-five percent of infants <26 weeks gestation were culture or PCR-positive one or more times during the first month of life compared with 31% infants ≥26 weeks gestational age. As summarized in Box 2, infants with respiratory colonization are more likely to be born extremely preterm by vaginal delivery to women with pregnancies complicated by chorioamnionitis and preterm labor or preterm premature rupture of membranes.^{42–44} The vertical transmission rate increases with longer duration of membrane rupture.⁴⁵ Infants delivered for maternal indications have the lowest rate of respiratory tract colonization. Postnatally, *Ureaplasma*-colonized infants frequently exhibit peripheral blood leukocytosis,^{46,47} and less severe RDS,⁴⁷ but early radiographic emphysematous changes of BPD.^{42, 48} In archived autopsy specimens of colonized infants, we observed early lung fibrosis, increased number of myofibroblasts, disordered elastin accumulation, and increased number of immunoreactive cells expressing inflammatory mediators tumor necrosis factor- α (TNF α) and transforming growth factor β 1 (TGF β 1).^{49, 50}

Box 2

Characteristics of Infants with *Ureaplasma* spp. Respiratory Tract Colonization

- Extreme prematurity
 - Respiratory colonization rate is inversely related to gestational age²
 - Respiratory colonization rate 65% <26 weeks vs 31% ≥26 weeks gestational age⁷
 - Immature Innate Immune Responses
 - ◆ Surfactant protein A deficiency^{31, 32}
 - ◆ Low expression of relevant TLRs?⁹⁷
- PPROM⁵
 - Increased vertical transmission with longer duration ROM⁴⁵
- Evidence for Fetal Inflammatory Response Syndrome (FIRS)
 - Histological chorioamnionitis and fetal vasculitis^{43, 44}
 - Elevated admission white blood cell count⁹⁸
- Less severe RDS⁴⁷
- Early Radiographic BPD Changes^{48, 99}

- Early lung fibrosis and disordered elastin distribution^{49, 50, 60}

Association of *Ureaplasma* Respiratory Tract Colonization and BPD

As summarized in Table 1, three meta-analyses that include data from over 4,000 infants and more than 40 individual studies have been published to date at approximately 10 year intervals that have assessed the association of *Ureaplasma* respiratory tract colonization and BPD. In the first meta-analysis in 1995, BPD at 28 days (BPD28) was the outcome for all studies and only 4 of 17 studies were done after exogenous surfactant was available.⁵¹ In 2005, Schelonka et al,⁵² demonstrated a significant association between *Ureaplasma* respiratory tract colonization and both BPD28 and 36 weeks postmenstrual age (BPD36), but noted significant heterogeneity among the studies primarily due to the inclusion of studies with small sample size. Lowe et al.² extended the analyses in 2014 to more recent studies and demonstrated persistence of the *Ureaplasma*-BPD association over time and no significant effect of differences in gestational ages between colonized and non-colonized infants on the strength of the association. Interestingly, the studies included in the latest meta-analysis were published over a 25 year span of considerable changes in neonatal care, but there has been no decrease in odds over time in the *Ureaplasma*-BPD association. However, no study to date has used physiologic BPD as an outcome.

Ureaplasma Species and Lung Inflammation in the Developing Lung – Animal Studies

Animal studies lend support to the hypothesis that *Ureaplasma* colonization can lead to lung injury similar to bronchopulmonary dysplasia. Intra-amniotic injection of *Ureaplasma* in early gestation sheep resulted in efficient colonization with persistent infection for 3 months to term with very little overt adverse effects in the ewe, consistent with a commensal-like host response²⁴ (Figure 1). The severity of chorioamnionitis following intraamniotic injection of *Ureaplasma* was variable with 10% of sheep not demonstrating any chorioamnionitis despite efficient colonization.⁵³ These experiments illustrate the complexities in understanding the host response to a *Ureaplasma* exposure.

Recruitment to and activation of inflammatory cells in the fetal lung could be detected as early as 3d after intra-amniotic injection of live *Ureaplasma* in sheep⁵⁴ (Figure 2a). Both monocytes and neutrophils increased, and MHCII expression in the monocytes increased 14d after intra-amniotic *Ureaplasma* injection, consistent with maturation of the monocytic cells.⁵⁴ The inflammatory cell infiltration was focal in nature and no areas of consolidation or “pneumonia” were detected. The inflammatory infiltrate was accompanied by modest increases in the pulmonary expression of the pro-inflammatory cytokines/chemokines IL-1 β , IL-6 and IL-8 within 1 week that persisted for at least 6 weeks.^{55, 56}

The modest lung inflammation was followed by the counter-intuitive observation of significant increases in lung gas volumes and surfactant lipids in the preterm fetal sheep. This early lung maturation was first detected in the preterm fetal lungs 3 weeks after intra-amniotic *Ureaplasma* injection and persisted for 10 weeks despite continuous exposures⁵⁷ (Figure 2b). Although these striking effects on lung physiology are consistent with clinical “lung maturation”, they probably represent “dysmaturation” since the improved lung

physiology was accompanied by evidence of impaired lung development.⁵⁴ Fourteen days after intra-amniotic *Ureaplasma* injection, preterm fetal sheep delivered at 80% gestation had decreased elastic foci and increased smooth muscle around bronchioles and pulmonary artery/arterioles.⁵⁴ These changes in elastin and smooth muscle are similar to those reported for infants with BPD and those dying with *Ureaplasma* infection.^{49,50,58} Overall, the sheep studies lend support to the clinical observation of less respiratory distress syndrome but more BPD after *Ureaplasma* exposure.

In Rhesus macaques, intra-amniotic injection of *Ureaplasma* caused chorioamnionitis, preterm labor and delivery within 15d and fetal pneumonia characterized by increased neutrophils and macrophages and alveolar type II cell proliferation indicating injury.⁴⁰ In preterm baboons delivered at 65% gestation 2–3 d after intra-amniotic inoculation with *Ureaplasma* and exposed to mechanical ventilation for 14d, half of the neonatal baboons cleared *Ureaplasma* from their airways, while the remaining half had persistent *Ureaplasma* colonization of the lungs.⁵⁹ The neonatal baboons with persistent *Ureaplasma* colonization demonstrated lung inflammation and fibrosis and worse lung function compared to those animals that cleared the *Ureaplasma* from the lungs.^{59, 60} In general, the lung pathology after intrauterine *Ureaplasma* exposure was more severe in non-human primates compared to sheep, suggesting species differences in susceptibility to *Ureaplasma*.

DIAGNOSTIC METHODS

Culture Methods

Although new diagnostic methods are currently available, culture remains the gold standard for *Ureaplasma* spp. detection (Table 2). Proper sample collection is essential to avoid false-negative results. Because the organisms lack a cell wall and are susceptible to drying and heat, tracheal or nasopharyngeal (NP) aspirates or NP swabs should be directly inoculated in 10B broth, Copan's Universal transport media, or routine Bacteriology Transport media for transport on ice to the laboratory. Once inoculated in urea containing media such as 10B broth, organism growth is indicated by color change from yellow to pink, indicating pH change due to urease activity in the absence of turbidity.⁶¹ The ureaplasma colonies are identified by their characteristic brown appearance in the presence of the CaCl₂ indicator in A8 agar. Although ureaplasmas can be detected after 24–48 h incubation, results may take up to 7 days and there are few laboratories with *Ureaplasma* culture expertise.

Colorimetric Assays

To facilitate *Ureaplasma* spp. detection, commercially available diagnostic kits have been developed that require less skilled personnel and more rapid detection time. The diagnostic kits listed in Table 2 have reported sensitivities and specificities comparable to culture and PCR methods.^{62–64} These kits also allow organism quantitation and antimicrobial susceptibility testing, but do not differentiate between species and only the Mycoplasma Duo Kit (Sanofi Diagnostics Pasteur, Marnes la Coquette, France) has been tested in preterm infants.⁶²

Molecular Diagnostic Methods

Both conventional gel-based PCR and real-time PCR methods have been developed to improve *Ureaplasma* detection. Real-time PCR can differentiate serovars and detects 15% more positive samples than culture and 6% more positive samples than conventional PCR.⁶⁵ PCR does not distinguish between viable and non-viable organisms. Although real-time PCR provides for rapid detection, often samples are batched for cost-effectiveness. Recently a DNA chip assay (STDetect® Chip, LabGenomics, Korea) has been developed that allows rapid simultaneous detection of 13 major genitourinary pathogens including *Ureaplasma* spp.⁶⁶ It has not been tested with infant respiratory samples.

THERAPEUTIC CONSIDERATIONS

Macrolide Antibiotics

Azithromycin, an azalide antibiotic and clarithromycin, a macrolide, have immunomodulatory properties,^{67–69} are preferentially concentrated in lung epithelial lining fluid and alveolar macrophages,^{70, 71} and have antimicrobial activity against *Ureaplasma* spp. *in vitro*^{72–74} and in *in vivo* experimental models.^{75–78} Treatment with these antibiotics may enhance *Ureaplasma* clearance in infected infants and inhibit the pulmonary inflammatory response possibly contributing to a decreased risk for BPD. A new fluoroketolide antimicrobial, Solithromycin has shown promising efficacy in sheep models.⁷⁹

Efficacy studies

Table 3 summarizes clinical studies of macrolides in eradicating *Ureaplasma* spp. from the respiratory tract and prevention of BPD in colonized infants. There are significant methodological issues with each study, such as appropriate controls, lack of blinding, low colonization rate, suggesting false-negatives included in comparison group, and lack of adequate pharmacokinetic data to inform dose selection. However, when post-dosing cultures were obtained, erythromycin therapy was effective in eradicating respiratory tract colonization in 3 of 4 studies,^{80–83} but did not alter BPD rates. In a single center study by Ballard et al,⁸⁴ 10 mg/kg/d azithromycin for 1 week followed by up to 5 weeks of 5 mg/kg/d was no different than placebo in eradication rates determined by PCR, but reduced the risk for BPD in colonized infants. The BPD36 rate was high (70–90%) so these results may not be replicated in centers with lower incidences of BPD. Ozdemir et al.,⁸⁵ who conducted a trial of a 10 d course of clarithromycin 20 mg/kg/d observed 68.5% eradication based on follow-up cultures 2d post last dose and greatly reduced BPD rate in colonized infants compared to the treatment group. However, infants <750 gm with the highest *Ureaplasma* colonization rate and risk for BPD were excluded, duration of mechanical ventilation was brief in all subjects, culture status was based on NP cultures so they did not distinguish between upper and lower respiratory tract infection, and organism clearance was not assessed in the placebo group. In most studies, treatment was started at a median age of 7 days, suggesting that antibiotic therapy in colonized infants needs to be started soon after birth to prevent BPD. However, treatment started on the day of birth in one randomized trial of erythromycin did not affect the BPD rate.⁸⁶ Since the sample size of this study was small and *Ureaplasma* colonization rate was lower than expected, repeat studies of adequate

sample size, with early treatment with appropriate antibiotic dose determined in initial dose-response studies should be conducted.

Pharmacokinetics studies

Only seven (16%) randomized clinical trials (RCTs) included in a systematic review of pharmacologic interventions to prevent BPD were preceded by early phase studies evaluating pharmacokinetics and/or safety and efficacy.⁸⁷ In addition, despite the large number of RCTs to prevent BPD, no drugs are currently FDA-labeled for the prevention of BPD. Under a FDA Investigational New Drug application, we have completed a Phase I open-label, pharmacokinetic (PK) study characterizing the population PK, safety, tolerability, and bacterial clearance of a single dose of 10 and 20 mg/kg IV azithromycin and multi-dose 20 mg/kg/d x3d in preterm neonates 24⁰–28⁶ wk gestation who are at high-risk for *Ureaplasma* spp. respiratory tract colonization and BPD.^{88–90} Inclusion of drug concentration data from the combined 40 subjects showed that the disposition of azithromycin in plasma was biphasic suggesting that azithromycin pharmacokinetics follows a two compartment model and the inclusion of body weight as a covariate for clearance and volume of the peripheral compartment improved the model fit to the data and explained some of the inter-subject variability. The single 10 mg/kg and 20 mg/kg dose regimens were safe, but did not completely eradicate *Ureaplasma* or suppress pulmonary inflammatory responses. The multi-dose eradicated respiratory colonization in all culture-positive subjects and decreased IL-17A, but not other tracheal aspirate cytokines (IL-1 β , IL-8, IL-6) associated with BPD.

BEST PRACTICES: CURRENT RECOMMENDATIONS AND THE FUTURE

Azithromycin is increasingly used in NICUs in the United States and Europe,^{91, 92} but its safety in the preterm population has not been adequately assessed. The FDA added a warning in 2013 to the drug label concerning its' pro-arrhythmic potential. Azithromycin has been associated with increased risk for cardiovascular death in older adults, primarily those with co-existing risk factors.^{41, 93, 94} We are currently conducting a Phase IIb, placebo-controlled, randomized trial of the multi-dose azithromycin regimen in a larger sample of preterm infants (clinicaltrials.gov NCT01778634) that will include assessments of long-term pulmonary and neurodevelopmental outcomes.

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KEY POINTS

1. Meta-analyses of clinical studies over the past 30 years have confirmed *Ureaplasma* respiratory colonization as an independent risk factor for BPD, but have not established causality.
2. Experimental infection models in sheep and non-human primates have demonstrated that *Ureaplasma* can establish a chronic infection with inflammation in the intrauterine compartment and alter fetal lung development.
3. Although *U. parvum* serovars are the most commonly isolated serovars from clinical samples, no specific serovar or virulence factor has been identified in association with BPD.
4. There is currently insufficient data concerning the benefit/risk ratio of antibiotic therapy to recommend treatment guidelines to prevent BPD in preterm infants at-risk or with confirmed *Ureaplasma* infection.

Figure 1. Persistent colonization of Ureaplasma in the sheep amniotic compartment

Pregnant ewes (n=20) were given intraamniotic injection of *Ureaplasma Parvum* (UP) (2×10^4 CFU) at 55d gestational age (term=150d). Amniocentesis was done at regular intervals until term gestation and the amniotic fluid titers of Ureaplasma were determined. There was a rapid growth of UP and the titers persisted till term gestation demonstrating poor clearance of UP.

Data from Dando SJ, Nitsos I, Kallapur SG, et al: The role of the multiple banded antigen of Ureaplasma parvum in intra-amniotic infection: major virulence factor or decoy? PLoS One 2012, 7:e29856

Figure 2. Lung inflammation and increased lung maturation after intraamniotic injection of *Ureaplasma Parvum* in sheep

Pregnant ewes were given intraamniotic injection of *Ureaplasma Parvum* (UP) 3d, 7d, 14d or 70d prior to delivery at 125 d gestation (term=150d). (A) Inflammatory cells (neutrophils + monocytes) in the broncho-alveolar lavage fluid (BALF) normalized to body weight. (B) Lung volumes measured at 40 cm H₂O pressure normalized to body weight. Intraamniotic injection of UP caused an initial lung inflammation followed by improved static compliance. (* $p < 0.05$ vs. controls)

Data from Kallapur SG, Kramer BW, Knox CL, et al: Chronic fetal exposure to *Ureaplasma parvum* suppresses innate immune responses in sheep. *J Immunol* 2011, 187:2688–95 and Collins JJ, Kallapur SG, Knox CL, et al: Inflammation in fetal sheep from intra-amniotic injection of *Ureaplasma parvum*. *Am J Physiol Lung Cell Mol Physiol* 2010, 299:L852–60

Table 1

Association of *Ureaplasma* spp. Respiratory Colonization and BPD at 28d (BPD28) and 36 weeks PMA (BPD36): Summary of Three Meta-analyses.

Meta-analysis	Years included in database search	Outcomes	Number of publications	Number of Subjects	RR (95% CI)	What Study Added
Wang (1995) ⁵¹	1988–1994	BPD28	17	1,479	1.75 (1.5–1.99)	Only 4/17 studies after surfactant available; none with BPD36 outcome.
		BPD36	0	N/A	N/A	
Schelonka (2005) ⁵²	1966–2004	BPD28	23	2,216	2.8 (2.3–3.5)	Includes 15 studies 1995–2004
		BPD36	8	751	1.6 (1.1–2.3)	Earlier publication year, sample size <100, reported surfactant use >90%, and endotracheal culture only diagnostic method were associated with higher reported odds of <i>Ureaplasma</i> -BPD association.
Lowe (2014) ²	1947–2013	BPD28	31	2,421	3.04 (2.4–3.8)	Used meta-regression to demonstrate that <i>Ureaplasma</i> -BPD28 association persists despite difference in gestational age of colonized and non-colonized infants.
		BPD36	17	1,599	2.2 (1.4–3.5)	Demonstrates persistence of <i>Ureaplasma</i> -BPD36 association over time. No studies reported testing for severity of BPD.

Table 2

Diagnostic tests for *Ureaplasma* spp. detection in clinical specimens.

Diagnostic Method	Test	Source	Detection Time	Advantages	Disadvantages
Culture ¹¹	10B broth A8 agar	Remel, Lenexa, KS	1–7 d, A8 agar morphology confirmation	Detects live organisms; quantitation; is gold standard	Requires special transport media; unable to speciate, Requires lab skilled in organism detection
Colorimetric Assays ^{62–64}	Mycoblast Revolution Mycoplasma Duo Kit Mycoview MycolST2	Elitech Diagnostic, Puteaux, France Sanofi Diagnostics Pasteur, Marnes la Coquette, France Fumouze Diagnostics, Cedex, France BioMérieux, Marcy l'Etoile, France	24–48h	Detects live organism >10 ³ CCU/ml), organism enumeration, antimicrobial susceptibility; does not require skilled personnel	Requires special transport media; unable to speciate, less sensitive than PCR
Molecular Assays ¹¹					
Gel-based conventional PCR	Targets sequences of 16S rRNA and 16S rRNA to 23S rRNA intergenic spacer regions, and the urease and <i>mba</i> genes		1–2 days	More sensitive than culture; provides speciation; detects non-viable organisms; does not require use of transport media	Does not distinguish between live and dead organisms; no antibiotic susceptibility
real-time PCR	Targets conserved sequences specific to either <i>U. parvum</i> or <i>U. urealyticum</i> ; urease gene subunits; 16S rRNA		Hours; but often samples batched for cost-effectiveness	More sensitive than culture; provides speciation; detects non-viable organisms; does not require use of transport media; provides quantitation of bacterial load	High cost of equipment and reagents
Multiplex PCR	See above		1–2 days	Detects multiple organisms in same specimen	
DNA chip assay ⁶⁶	STDetectChip	LabGenomics, Korea	6 h	Simultaneous detection of 13 GU pathogens; high sensitivity and specificity compared to multiplex PCR	Not trialed with infant samples; no antibiotic susceptibility testing

Table 3
Impact of Macrolides on *Ureaplasma* respiratory colonization and BPD Prevention in Infected Preterm Infants: Summary of Clinical Studies

Source	Macrolide Study Design	Dose and Duration	N	Inclusion Criteria	Age Drug initiated	<i>Ureaplasma</i> detection method and colonization rate	Rate <i>Ureaplasma</i> clearance	BPD 36 wk rate
Waites et al (1994) ⁸⁰	Erythromycin open-label PK study	25 or 40 mg/kg/d IV x 10d	14	BW<1500g with TA* <i>Ureaplasma</i> -positive; no non-colonized comparisons	Median 7d (2–15d)	TA Culture only; Colonization rate not reported	9/10 (90%) post-treatment cultures negative	Not reported
Lyon et al (1998) ⁸⁶	Erythromycin randomized, no placebo; not blinded	45 mg/kg/d IV x 7 vs no therapy	75 (34 treated and 41 controls)	<30 wk mechanically ventilated	Day of birth	TA culture and conventional PCR; 9/60 (15%) positive pre-Rx [3/34 (9%) treatment group vs 6/41 (15%)]	Not reported	Controls 27% Erythromycin 38%
Jonsson et al (1998) ⁸¹	Erythromycin randomized, no placebo; not blinded	40 mg/kg/d oral or IV x 10d	28 (14 treated and 14 not-treated)	<30 wk mechanically ventilated, TA/NP culture-positive	Mean 7d	TA and NP culture; 29/155 (19%)	erythromycin-treated: 12/14 (86%) non-treated: 0/14 (0%)	<i>Ureaplasma</i> +/- non-treated (71%) <i>Ureaplasma</i> +/- treated (64%)
Bowman et al (1998) ⁸²	Erythromycin Comparison to non-colonized infants	20 mg/kg/d IV x 7 d	124 (22 UU-positive)	<1000 gm BW IMV>6h; TA culture positive	Median 7d (1–31d)	TA Culture only; (18%)	erythromycin-treated: 18/22 (82%)	<i>Ureaplasma</i> +/- Treated (32%) <i>Ureaplasma</i> -/- non-treated (48%)
Baier et al 2003 ⁸³	Erythromycin Dose duration comparison	40 mg/kg/d IV x 5d vs 10d	17	<1500 gm BW, mechanically ventilated	Median 7d (2–33 d)	TA Culture only; Colonization rate not reported	5d Rx: 25% 10d Rx: 57%	Not reported
Ballard et al (2011) ⁸⁴	Azithromycin RCT	10 mg/kg/d IV x7d & 5 mg/kg/d IV up to 5 wk	220 (111 AZM vs 109 placebo)	<1250 gm BW, mechanically ventilated	Within 72h birth and 12h start mechanical ventilation	TA conventional PCR only 31% AZI group 40% controls	5 subjects each group (AZI and placebo) still PCR positive 5 wks	<i>Ureaplasma</i> +/-AZI treated: 19/26 (73%) <i>Ureaplasma</i> +/- placebo: 33/35 (94%)
Ozdemir, et al 2011 ⁸⁵	Clarithromycin RCT Blinded?	20 mg/kg/d IV x 10d	74 (37 CLAR vs 37 placebo)	750–1250 gm BW; NP culture positive first 72h	1–5 d age?	NP swab culture only: 33% positive	Follow-up cultures once 2 days post last dose in clarithromycin-treated subjects: 68.5% eradication; no follow-up cultures on placebo group	<i>Ureaplasma</i> +/-CLAR-treated: 1/33 (3%) [‡] <i>Ureaplasma</i> +/- placebo: 12/33 (36.4%)

* Abbreviations: TA, tracheal aspirate; NP, nasopharyngeal; AZM, azithromycin; CLAR, clarithromycin

[‡] Physiologic BPD at 36 weeks by timed oxygen reduction test 100