Advanced Journal of Microbiology Research Vol. 2015
Available online at http://internationalscholarsjournals.org/journal/ajmr

Full Length Research Paper

Adherence and damage to epithelial cells of human lung by *Ureaplasma urealyticum* strains biotype 1 and 2

A. Torres-Morquecho[1, 2], A. Rivera-Tapia[3], F. González-Velazquez[4], J. Torres[1], B. Chávez-Munguia[5], L. Cedillo-Ramírez[3] and S. Giono-Cerezo[2]*

[1]Unidad de Investigación Médica en Enfermedades Infecciosas, Hospital de Pediatría, Instituto Mexicano del Seguro Social, México.
[2]Depto. Microbiología, ENCB-IPN, México.
[3]Centro de Investigaciones Microbiológicas, Benemérita Universidad Autónoma de Puebla, México.
[4]División de Investigación, UMAE, Hospital de Especialidades # 14, IMSS, México.
[5]Departamento de Patología Experimental, CINVESTAV-IPN, México.

Accepted 3 December, 2014

***Ureaplasma* sp. is associated with chronic lung disease and it has been implicated in the morbidity and mortality of new born babies (human). Adherence is an important process for lung infection, and scarce information is available in this respect. This work shows its adhesion to epithelial cells from the respiratory tract in culture, and evaluates the *Ureaplasma* sp. references and clinical strains isolated from premature newborns. *Ureaplasma parvum and Ureaplasma urealyticum* serotype 8 and five clinical samples were evaluated, using the Bertholet assay. They were observed by scanning and transmission electron microscopy to A549 cell line. Adherence was abolished by pre-treatment of ureaplasmas with A549 cell extracts and inhibited by pretreatment of ureaplasmas with N-acetylneuraminic acid, trypsine, glucose and monoclonal antibodies (mAb 106.C anti-MB against *U. parvum* serotype 3). *Ureaplasma* sp. (biotypes 1 y 2) adherence had no significant difference (P = 0.127) . The clinical strains by scanning electron microscopy showed a similar damage. Both reference and clinical strains produced evident changes in cell morphology. Micro-colonies were observed on the cells. By transmission electron microscopy, ureaplasmas attached on the cell surface and into the cytoplasm were observed. The A549 cellular line is a good model for the study of adherence of *Ureaplasma* sp. The urease test by Bertholet assay is a good indicator of colonization. These data suggested that ureaplasma adhesins are proteinaceous antigenic substance. Inhibition assays with neuraminidase and glucose showed binding to sialic acid residues and suggested as possible adhesin to MB (multi banded antigen).**

Key words: *Ureaplasma urealyticum*, adherence, epithelial cells, damage.

INTRODUCTION

Ureaplasma sp. causes pneumonia in premature new born babies (human) and is associated to bronchopulmonar dysplasia (BPD); furthermore it is associated with a high morbidity and mortality in the perinatal period (Alpha et al., 1995; Brus and Van Warde, 1991; Cassell et al., 1991; Cassell, 1993; Waites et al., 2005; Faye-Petersen, 2008). *Ureaplasma urealyticum* is subtyped in - to two biovars and 14 serovars (Cassell, 1993) based on significant genotypic differences between the two biovars, it has been proposed to separate the species previously known as *U. urealyticum* (or *Ureaplasma* sp. human) into two new species, *Ureaplasma parvum* (biovar 1 containing serovars 1, 3, 6 and 14) and *U. urealyticum* (biovar 24 containing serovars 2, 4, 5 and 7 to 13) (Cassell, 1993; Harasawa et al., 1991; Kang et al., 2000; Lee et al., 1991). Biovar 1 (*U. parvum*) is the more common of the two biovars isolated from clinical samples, but both species may occur simultaneously in some people. Some serovars have been implicated with disease more commonly than others (Abele-Horn et al., 1997; Naessens

*Corresponding author. E-mail: sgiono@yahoo.com.

et al., 1998; Zheng et al., 1992) but any differences in pathogenicity among serovars are unproven. All *Ureaplasma* sp. serovars contains an external surface protein named multi banded antigen (MB), which is considered a virulence factor. Actually, its biologic activity is unknown (Zheng et al., 1995). Previous reports have examined the relationship between ureaplasma colonization of the neonatal respiratory tract release of inflammatory mediators that may be involved in damage and pathogenesis of BPD.

Ureaplasma sp. in the lower respiratory tracts with progression to BPD has been published since 1998 (Cassell, 1993; Sanchez and Regan, 1988; Wang et al., 1988); and certainly BPD occurs in premature infant who re-ceived mechanical ventilation. Evaluation of fibroblast of mouse infected with ureaplasmas without oxygen showed that proinflammatory cytokines play an important role in mediating pathology in a variety of lung disease, including BPD (Stancombe et al., 1993). Apoptosis of pulmonary cells may also be related with development of BPD, the proliferation of neutrophils at the site of lung infection will lead to prolonged inflammation by cytokine production and release of proteases and oxygen free radicals. Li et al. (2002) using human macrophage and lung epithelial cell lines demonstrated that when these cells are stimulated with *Ureaplasma* antigen, apoptosis will occur "*in vitro*" as evidenced by morphological eva-luation and analysis of DNA fragmentation. Damage to lung epithelial cell is not necessarily due to ureaplasmas itself. Ronald et al. (1997) infected A549 cells with

Mycoplasma hominis and *U. urealyticum* serotype 8 and evaluated the interleukins effect; IL-8 levels were higher in A549 cells infected with *M. hominis* than A549 cells infected with *U. urealyticum* serotype 8. These results suggested that *M. hominis* and *U. urealyticum* serotype 8 participated for inflammatory response that Chronic Lung Disease (CLD) preceded directly for cytokines productions by cells type II stimulation. Although, ureaplasmas causes respiratory tract infection of preterm, neonates suggest that lung disease could be associated with these organisms and stimulation of proinflammatory cytokines (TNF- , IL-10 and IL-8) or blockage of counter-regulatory cytokines (IL-6 and 1L-10) (Waites et al., 2005; Shimizu et al., 2008). A juvenile mouse model of *Ureaplasma* pneumonia showed a persistent focal loss of airway ciliated epithelium and a mild increase in interstitial cellularity. *Ureaplasma* infected mice increased TNF- at

14 days and increased granulocyte-macrophage colony stimulating factor (GM-CSF) and IL-10 at 28 days. These data suggest that *Ureaplasma* alone may cause limited inflammation and minimal tissue injury in the early phase of infection, but may promote a mild chronic inflammatory response in the later phase of infection (days 14 to 28), similar to the process that occurs in human newborns (Viscardi et al., 2002; Novy et al., 2009).

Ureaplasma sp. is a very tiny microorganism, which cannot be observed by using conventional stains or light microscope; therefore, it is more difficult to study how the binding between the microorganism and the host cell is realized. The studies about adherence of this microorganism are few, using ureaplasmas of animal species and different cell lines through immunofluorescence; the adherence was not definitive (McGarrity et al., 1986). Adherence of mycoplasmas on the culture cells has been controversial to demonstrate (Mernaugh et al., 1993), however, other mycoplasmas showed adherence by immunocytochemistry and scanning electron microscopy techniques (Basolo et al., 1984; Diaz-Garcia et al., 2006; Jennsen et al., 1994; Robinson et al., 1991; Winner et al., 2000), inclusive; these powerful techniques showed that the invasion of host cells occurs and the organisms reside intracellularly (Diaz-Garcia et al., 2006; Jennsen et al., 1994; Winner et al., 2000). Recently, *Ureaplasma* sp., adherence showed that adherence is realized but, this is controversial (Robertson, 1991; Saada et al., 1991; Smith et al., 1994).

Adherence is a virulence factor for lung infection. The aim of this study was to evaluate a type II respiratory epithelial cell line (A549 cells) as a model to study the *Ureaplasma* sp. of human adherence by transmission and scanning microscopy which had not been performed before, and to evaluate the variability of adhesion of some clinical samples of *U. parvum and U. urealyticum* serotype 5 isolated from premature newborns and how MB antigen could participate as a possible adhesin.

MATERIALS AND METHODS

Bacterial strains and growth conditions

Reference strains, *U. parvum* serovar 3 and *U. urealyticum* serovar 8 were obtained directly from the American Type Culture Collection (strains referred after as *U. parvum* ATCC700970 and *U. urealyticum* ATCC27618, respectively) (Kang et al., 2000) and also 5 clinical strains (3 strains *U. parvum* serotype 3 and 2 strains *U. urealyticum* serotype 5), isolated from premature newborn with low birth weight (2,500 g) and/ or < 36 weeks gestational, were obtained from endotracheal specimens from 1 to 7 years of age. Reference strains and clinical isolates were grown in 5 ml of 10B broth (Shepard, 1983) and incubated at 37°C/ 18 h (1×10^5 UCC/ml, Log phase). All ureaplasmas strains were transferred to 200 l of culture and frozen to - 70°C before adherence assays. Two different strains were used like adherence control; *Escherichia coli* 2348/69, negative urease microorganism (localized adherence on Hep-2 cells), and *Proteus* sp. positive urease microorganism (clinical strain). Both strains were grown in BHI broth at 37°C/ 18 h. For the adherence assays cultures were centrifuged at 2,500 rpm for 5 min and pellets were washed three times with sterile phosphate-buffered saline (PBS). The pellets were mixed with PBS and diluted at 1.5×10^8 UFC/ml (0.5 MacFarland tube) concentration.

A549 human lung cells culture

A549 cells were obtained from the American Type Culture Collection, Rockville, Md. (CCL-185); this cell line was grown in Dulbecco Modified Eagle-Earle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Hyclone Inc., Logan, Utah) with and without antibiotics. A549 cells were incubated at 37°C in 5% CO_2.

Polyclonal and monoclonal antibodies

U. parvum serotype 3 and *U. urealyticum* serotype 8 were grown in 1000 ml of 10B broth (Shepard, 1983) and incubated at 37°C/ 48 h. When we observed a dark red color, this culture was centrifuged at 16,000 rpm for 2 h, the supernatant was removed careful and transferred to an Eppedorff tube, and this pellet was washed three times with sterile PBS (centrifuged at 16,000 rpm for 30 min). The ureaplasma's whole protein was suspended with PBS and diluted at 40 g/500 ul (to change by 80 µg/ml) protein concentration. Rabbits were injected subcutaneously with Complete Freund's Adjuvant (CFA, Sigma F-5506) and ureaplasma's whole protein (Inoculum's first day), and after 15 days same conditions previously mentioned, the rabbits were immunized with Incomplete Freund's Adjuvant (IFA) and ureaplasma's whole protein. 30 to 32 days, the rabbits were injected subcutaneously with ureaplasma's whole protein and saline solution. At 39 day, 100 ml of blood was drawn from each rabbit. At least 40 ml of serum was then obtained, which was then utilized at a dilution from 1:1,000 to 1:10,000. MAbs 10C6.6 directed to *U. parvum* serotype 3 were obtained of the Multipurpose Arthritis Center at the University of Alabama at Birmingham, previously described (Watson et al., 1988).

Adherence assay

We used assay condition as a previous report (Smith et al., 1994) with the following changes: A549 cells were washed with PBS pre-incubated at 37°C and incubated with PBS/BSA 3% at 37°C in 5% CO_2 for 30 min, and A549 cells were washed with PBS pre-incubated at 37°C before adherence assays. Ureaplasmas inoculum of 4 ml of 10B culture with 1×10^5 UCC/ml was seeded onto six-well plastic plates (with different confluence 100%, 70% and 2.5 $\times 10^5$ cells per well) and incubated for 1, 2 and 3 h with 10B medium, DMEM-10% FBS, A549 cells. The culture of cells A549 plus *E. coli* 2349/68 and A549 plus *Proteus* sp. were used as controls. Adherence of *Ureaplasma* sp. (human) was evaluated by a colorimetric method and electron microscopy. All studies were performed in triplicate.

Adherence colorimetric assay

Adherence of *Ureaplasma* sp. reference and clinical strains of eukaryotic cell monolayers were quantified, using the Bertholet assay to monitor ammonia produced from urea by ureaplasma urease, just like previous reports (Smith et al., 1994; Thirkell et al., 1989).

Adherence abolitions

Adherence was abolished by pre-treatment of ureaplasmas with A549 cell extracts (1 mg/ml) and inhibited to different concentration by pretreatment of the ureaplasmas with N-acetylneuraminic acid (0.15 U/ml), glucose (100 mM), trypsine (250 g/ml y 500 g/ml), specific anti-sera (1:200, 1:500, 1:3000 and 1:5000 dilution) and monoclonal antibodies 10C6.6 (1:1000, 1:5000 and 1:10,000 dilution). They were incubated for 30 min at 20°C before abolitions assay. All studies were performed in triplicate.

Transmission electron microscopy

Cultures of cells A549 controls or from *Ureaplasma* sp. (human) adherence assays were fixed with 2.5% glutaraldehyde in 0.1 M, pH 7.2 cacodilate buffer at 37°C, and post-fixed with 1% osmium tetroxide in the same buffer. They were dehydrated by crescent concentrations of ethanol, and embedded in plastic resins; after polymerization at 60°C, longitudinal thin sections were obtained. Once contrasted with lead citrate and uranyl acetate, the thin sections were observed in a JEOL 100-SX transmission electron microscope (Chavez-Munguia et al., 1997; Dykstra, 1993).

Scanning electron microscopy

Plastic circles with the fixed samples were obtained from the culture dishes, dehydrated in ethanol, critical-point dried from CO_2 in a Samdri Tousimis apparatus and gold-coated in an ion sputtering device (JEOL JFC-100) as described previously (Chavez-Munguia et al., 1997; Dykstra, 1993). All samples were analyzed in a JEOL JSM-35 C scanning electron microscope.

Statistical analyses

Mann-Whitney U test was used to evaluate significance in the adherence inhibition and adherence assays, and differences were considered significant at the $P < 0.05$ (INSTAT version 2.0 Software).

RESULTS

Adherence assay conditions

First experiments were performed under the same conditions reported by Smith et al. (1994); however, adherence was not obtained. New assays were designed for A549 cell line, considering standardized conditions: cellular confluence, bacterial incubation time and Bertholet method (Smith et al., 1994).

A549 cell confluence of 2.5×10^5 cell/well infected with ureaplasmas showed the highest adherence (OD600 = 1.1) compared with 100% of A549 cell confluence, which showed an absorbance of 0.4 very similar to negative controls and adherence controls (*E. coli* and *Proteus*) (Figure 1). These assays were evaluated; with different confluences 100, 70% and 2.5×10^5 cell/well (40% approximately). Non-specific binding of ureaplasmas was observed in plastic ($P = 0.021$).

Adherence effect on A549 cell line of ureaplasmas at different times (1, 2 and 3 h) with 2.5×10^5 cell/well was done three times The optical density was decreased when the incubation time increased at 2 and 3 h (Figure 2).

Adherence abolition

A549 cell confluences of 2.5×10^5 cell/well and incubation time of 1 h were used for all abolition assays and they were evaluated by Bertholet method.

Pre- incubation of ureaplasmas with homologous polyclonal antibodies reduced the adherence to 44, 60, 88 and 91% (dilutions; 1:5000, 1:3000, 1:500 and 1:200 respectively) of the positive adherence control level. With 1:200 dilutions, the adherence was significantless ($P < 0.05$) than other dilution ($P > 0.05$) (Table 1).

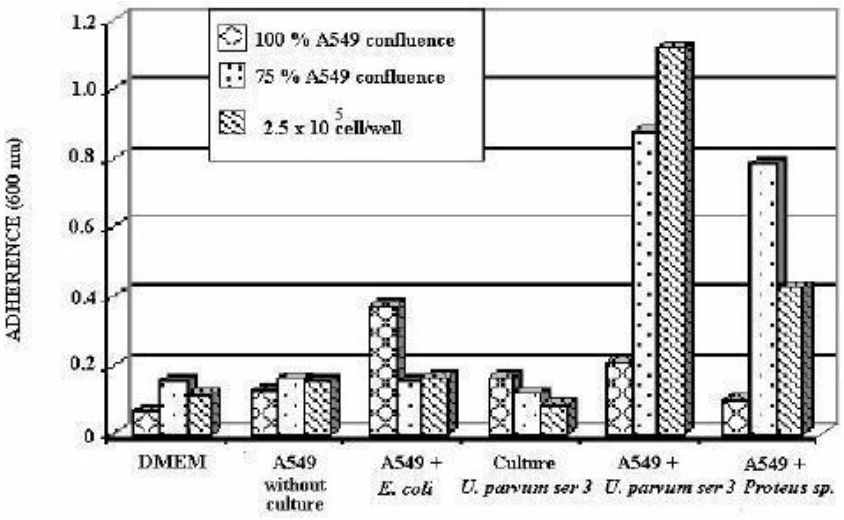


Figure 1. Adherence of *Ureaplasma parvum* serotype 3 to A549 cells with different confluences. The confluences used in adherence assays were 100%, 70% and 2.5 × 10^5 cell/well. *E. coli* 2348/69 was used like control positive of adherence but negative urease and *Proteus* sp. positive control of adherence but positive urease. All assays were performed in triplicate.

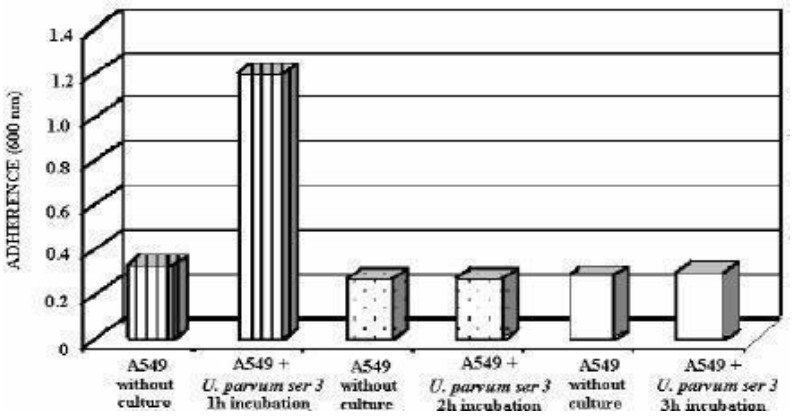


Figure 2. Adherence kinetic of *Ureaplasma parvum* serotype 3 to A549 cell. The adherence was done at different times 1, 2 and 3 h. All assays were performed in triplicate.

The specific binding of ureaplasmas to A549 cells was confirmed by pre- treatment of ureaplasmas with A549 cells extracts ($P < 0.05$), whose adherence was reduced to 66% compared with positive control (Table 1). Both assays demonstrate an antigen-specific recognition from *U. parvum and U. urealyticum* at the A549 cell. Pre-treatment of A549 cells with glucose and neuraminidase abolished adherence to 61 and 70% respectively, suggesting that a high proportion of the cell receptors terminate in sialic acid in these cells. Both of them were statistical significant ($P < 0.05$) (Table 1).

Pre-incubation of ureaplasmas with trypsin at 250 and 500 g/ml reduced the adherence to 26 and 55% respectively (Table 1). This abolition assay indicates that some of the *Ureaplasma*'s adhesions appear to be protease-sensitive.

Adherence abolition with monoclonal antibody 10C6.6 against multi-banded antigen (MB 10C6.6) of *U. parvum* reduced attachment to 25, 48 and 63% (1:10,000, 1:5000 and 1:1000 respectively) in a dose-dependent manner, suggesting ($P < 0.05$) that MB antigen could be an adhesin (Table1).

Scanning electron microscopy

Adherence changes correlated with damage observed on electron microscopy. Figure 3a showed an A549 cell

Table 1. Statistic analysis of *Ureaplasma parvum* adherence abolition to A549 cell.

Adherence Inhibitors	% Abolition adherence	P
A549+*U. parvum*	0	
Polyclonal Ab anti *U. parvum* 1:5000	44	0.513
Polyclonal Ab anti *U. parvum* 1:3000	60	0.513
Polyclonal Ab anti *U. parvum* 1:500	88	0.513
Polyclonal Ab anti *U. parvum* 1:200	91	< 0.05 *
A549 extract (1mg/ml)	66	< 0.05 *
Neuromanidase	70	< 0.05 *
Glucose 100 mM	61	< 0.05 *
Trypsin 250 g/ml	26	0.275
Trypsin 500 g/ml	55	< 0.05 *
Monoclonal Ab 10C6.6 dil. 1:10,000	25	0.275
Monoclonal Ab 10C6.6 dil. 1:5,000	48	< 0.05 *
Monoclonal Ab 10C6.6 dil. 1:1000	63	< 0.05 *

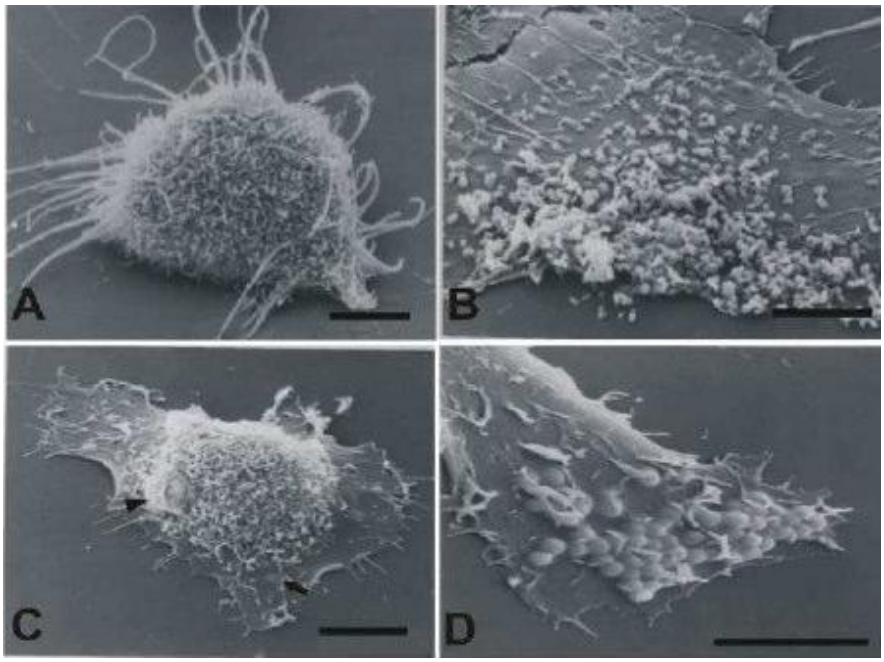


Figure 3. Scanning electron micrograph. (A) Uninfected A549 cell. (B) A549 cell showed several ureaplasmas adhered, the cell looks a plane form or distending and without microvillus. (C) A micro- colony of ureaplasmas was signaling with a head arrow and one ureaplasma covered by membrane cellular was signaling with an arrow. (D) Close up of A549 cell showed several ureaplasmas adhered, someone look covered by cellular- like membrane figure C. Size of the bar corresponding to 1 m.

uninfected as negative control; it looks whole with its microvillus and oval shape. Loss of microvillus was observed in an A549 cell infected with ureaplasmas (Figure 3B). Ureaplasmas micro-colonies or clumps were observed on the A549 cell (Figure 3c). An A549 cell infected with ureaplasmas showed someone covered apparently by cellular membrane (Figure 3D). An amplification of an A549 cell showed ureaplasma with structures similar as a pedestal (Figure 4a). Figure 4b showed an A549 cell infected with *E. coli*, it looks its microvillus and a little damage; it was used like a control adherence and different size compared with *U. parvum* serotype 3 and other bacteria.

An adherence kinetic was made at 15, 30, 45 min, 1 and 2 h. Scanning electron microscopy of *U. parvum* serotype 3 was observed bound to A549 cell at 15, 30 and 45 min of incubation period time with a little damage (Figures 5a, b and c). Severe damage was observed

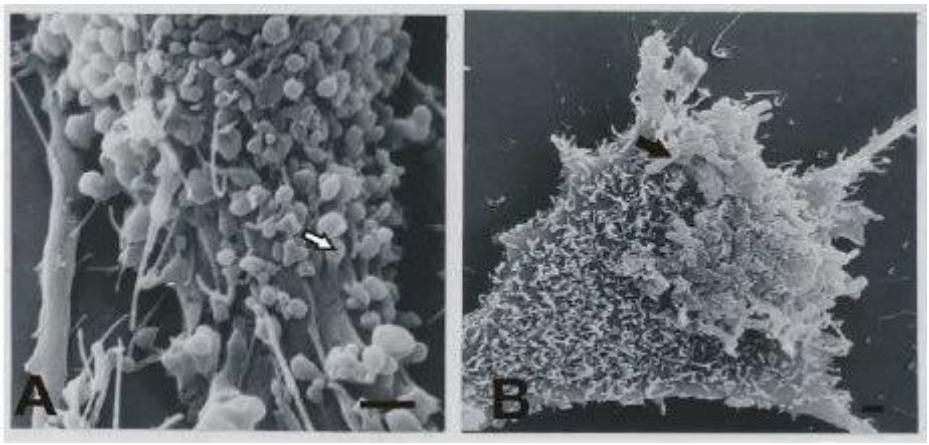


Figure 4. Amplification of A549 cells infected with *U. parvum* serotype 3 (A) and *E .coli* (B). One ureaplasma is on a structure like pedestal, it is signaling by a white arrow (A). The black arrow signaling an *E. coli* bacillus; we can differentiate the size between microorganisms. Size of the bar corresponding to 1 m.

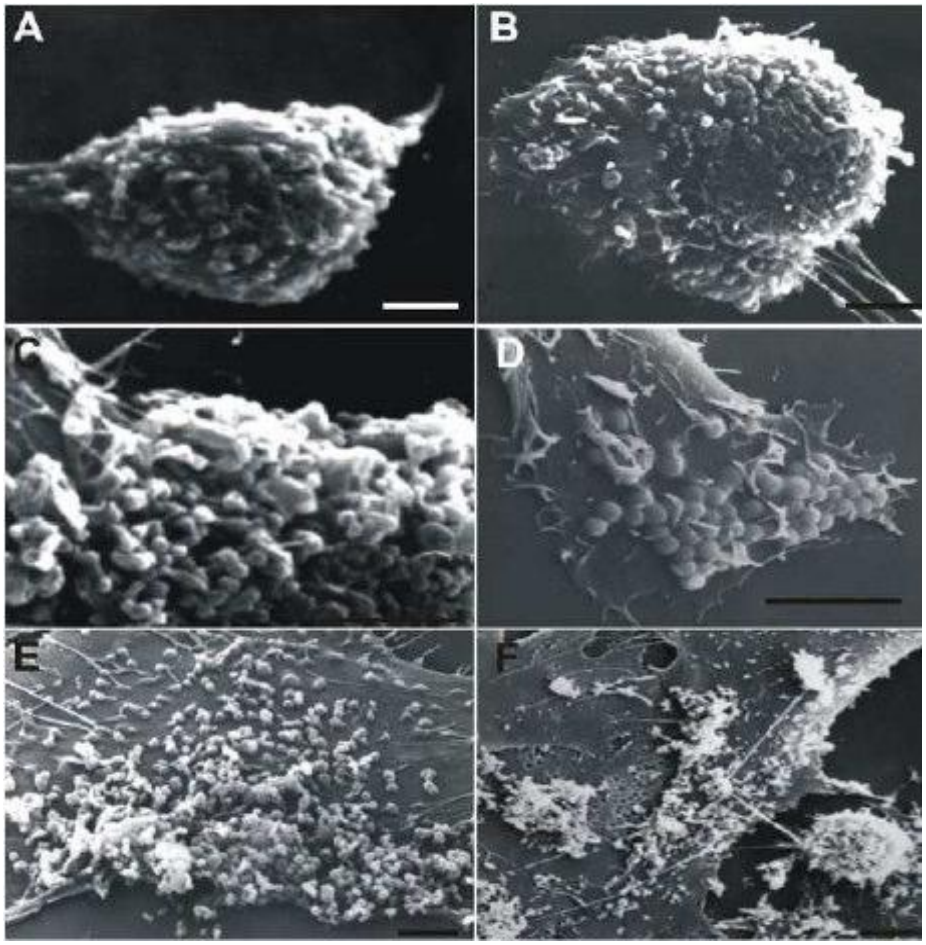


Figure 5. Kinetic of adherence of *Ureaplasma parvum* serotype 3 to A549 cell. (A) Incubation at 15 min showed a little damage. (B) Incubation at 30 min, and (C) Incubation at 45 min showed several ureaplasmas adhered and a little damage. (D) A549 showed several ureaplasmas and it presented severe damage at 1 h of incubation. (E) Incubation at 2 h and (F) at 3 h showed a little ureaplasmas adhered but with a severe damage. Size of the bar corresponding to 1 m.

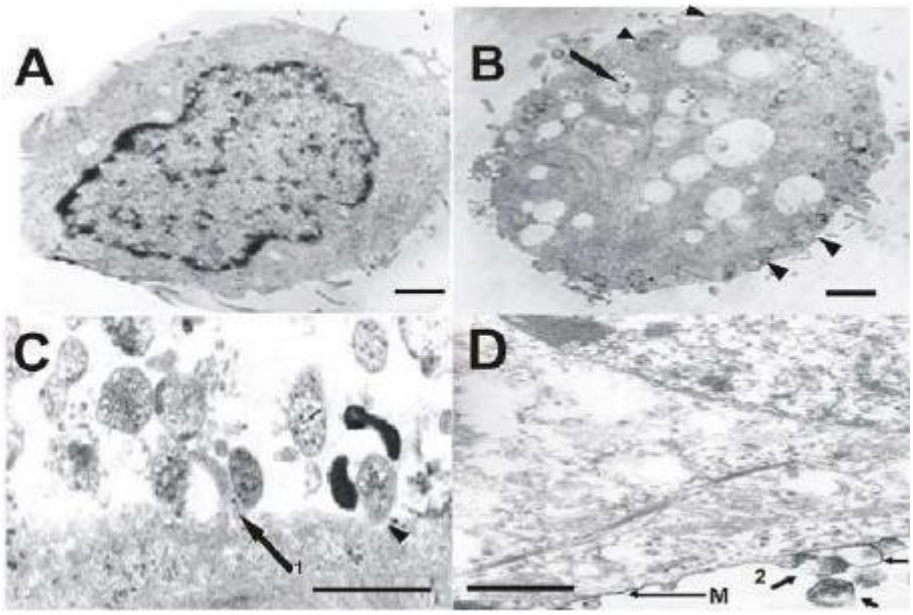


Figure 6. Transmission electron micrograph. (A) Longitudinal cut of uninfected A549 cell. (B) Longitudinal cut of A549 cell infected with ureaplasmas; arrows signaling ureaplasmas into the cell and into some vacuoles. (C) Close up of border A549 cell plus ureaplasmas, the arrow number 1 showed one ureaplasma entered to the cell. (D) Close up of border A549 cell plus ureaplasmas dyeing with red ruthenium and it highlight membranes of the A549 cell and *U. parvum* serotype 3, the arrow number 2 showed one ureaplasma with an structure like a "tip".

after 1 h of incubation (Figure 5d). A few ureaplasmas were observed after 2 or 3 h of incubation (Figures 5e and f).

Transmission electron microscopy

Figure 6A showed a longitudinal cut of an A549 cell uninfected as a negative control. We observed several vacuoles forming into the A549 cell infected with *U. parvum* serotype 3 and someone too near to the border of the cell (Figure 6b). An amplification of the A549 border cell showed some ureaplasmas adhered and one showed a structure like a "Tip". It could be in an enter process (Figure 6c). Similar results were observed when we used red ruthenium, which highlighted membrane of the ureaplasmas and A549 cell; adherence of *U. parvum* serotype 3 to A549 cell and a structure forming like a "Tip" (Figure 6d).

Adherence of *Ureaplasma* sp. clinical strains

Five clinical isolates were evaluated by Bertholet assay and scanning electron microscopy. *U. parvum* serotype 3 and *U. urealyticum* serotype 8 were used like controls (Figures 7a and b).

Bertholet assay did not show statistical significance between *U. parvum* and *U. urealyticum* (Figure 7b). Clinical strains were less adherent than reference strains and they were statistical significant (P 0.05) (Figure 7b). Scanning electron microscopy showed similar images among all strains; A549 cells infected with clinical ureaplasma presented severe damage like a distending form or stretching and without microvillus (data not shown).

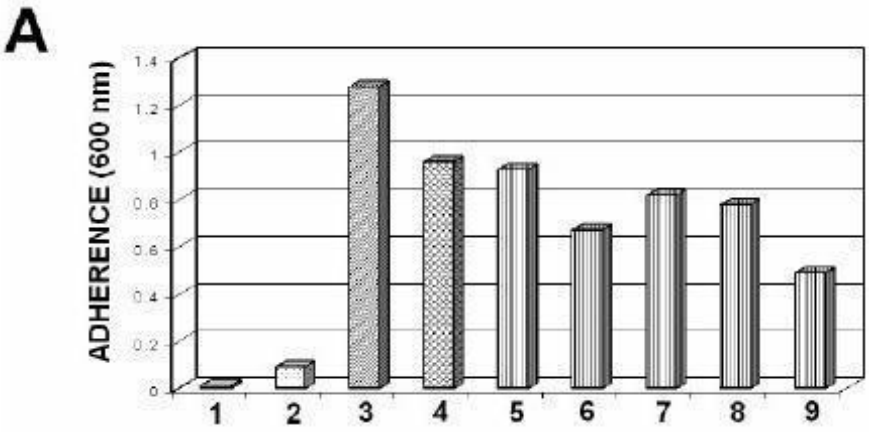
DISCUSSION

Ureaplasma sp. as human pathogen of adults and neonatal infections is supported by several reports (Cassell, 1993; Sanchez and Regan, 1988; Deguchi et al., 2004; Sanchez and Regan, 1990; Yoder and Albertine, 2008). However, there are many questions about its pathogenicity because there is a high prevalence in healthy persons (Cassell, 1993; Waites et al., 2005; Duncan et al., 1992).

Localization and attachment on host cell surface is important as virulence factor to *Ureaplasma* sp. to colonize and subsequently produce pathological lesions (Waites et al., 2005; Tarrant et al., 2009; Oue et al., 2009).

Previous reports have mentioned ureaplasma adherence to a variety of human cells including erythrocytes (Saada et al., 1991), spermatozoa (Basolo et al., 1984; Diaz-Garcia et al., 2006) and urethral epithelial cells (Smith et al., 1994). Principal goal of this work was to prove ureaplasma's adherence to human respiratory cells

A



B

Ureaplasma sp.	*U. parvum* ser 3 (biovar 1) vs	*U. urealyticum* ser 8 (biovar 2) vs
U. urealyticum ser 8 Sample 11	P=0.275 P=0.127	P=0.827
U. urealyticum ser 5 Sample 61	P=0.05*	P=0.275
U. parvum ser 3 Sample 74C	P=0.05*	P=0.513
U. urealyticum ser 5 Sample 85	P=0.127	P=0.275
U. parvum ser 3 Sample 121A	P=0.05*	P=0.05*
U. parvum ser 3		

Figure 7. Adherence of reference and clinical ureaplasmas strain to A549 cells. (A) Columns: 1, *Ureaplasma parvum* serotype 3 alone; 2, A549 cell alone; 3, A549 cells plus *Ureaplasma parvum* serotype 3; 4, A549 cells plus *Ureaplasma urealyticum* serotype 8. Columns 5 to 9, A549 cells plus clinical strains (61, 85 and 121A clinical strains corresponding to *Ureaplasma parvum* serotype 3; and 11 and 74C clinical strains belong to *Ureaplasma urealyticum* serotype 5). (B) Table of statistic analysis of adherence of reference and clinical ureaplasmas strain to A549 cells.

using clinical strains isolated from preterm with someone respiratory disease.

A549 cell line was a good adherence and invasive model for *Streptoccocus pneumoniae* (Bérube et al., 1999; Talbot et al., 1996). We evaluated the adherence of *Ureaplasma* sp. (human) to A549 cell line by Bertholet assay and electron microscopy.

A previous report on adherence of *U. urealyticum* serovar 8 to eukaryotic cell monolayers was quantified, using Bertholet assay to monitor ammonia produced from urea by ureaplasma urease; they used HeLa cells with 100% of confluence and 2×10^5 UCC/ml at 1 h of incubation and observed 20% approximately ureaplasmas adhered to monolayers. Using the same conditions from a previous report (Smith et al., 1994), we could not observe adherence. But A549 cells to 2.5×10^5 cell/well and 4×10^5 UCC/ml with 1 h of incubation, we had better adherence effects.

Pathogenic bacteria have selected receptors on the host cells and specific ones were recognized. Adherence patterns as *E. coli* (Donnerberg et al., 1997), *Proteus* sp. on the A549 cells showed minimal adherence than *U. parvum* serotype 3 by Bertholet assay. *U. parvum* serotype 3 is a respiratory tract pathogen and the adherence results compared with *Proteus* sp. suggested that A549 line cell is a selective good model adherence of *Ureaplasma* sp. human strains isolated of the tract respiratory from newborn.

Saada et al. (1991) evaluated the adherence of *U. urealyticum* serotype 8 to human erythrocytes using [35S] methionine labeling; they observed decreased adherence when the incubation time was increased. These results agreed when the time incubation was 2 and 3 h with Bertholet method; the optical density decreased too.

Abolition adherence assay monitored by Bertholet assay showed that specific receptors could be involved on ureaplasmas adherence to A549 cells when there is pre-incubation of ureaplasmas with A549 cells extracts; this abolition assay supports the specific adherence to A549 cells and it was similar to previous report with HeLa cells extracts (Smith et al., 1994).

Adherence of *U. parvum* serotype 3 to A549 cells was reduced being dose-dependent, with polyclonal antiserum; this suggested that a specific-antigen was recognized from *U. parvum* serotype 3 to A549 cell.

Bacteria-host relationships involve many interactions and they are biologically important. Attachment to carbohydrates is an important interaction with host cell to bacteria protein, as lectins and adherence abolition assay with carbohydrates and neuraminidase are used to block receptors with mono or oligosaccharides (Kahane and Jacobs, 1995). Pretreatment of A549 cells with neuraminidase or ureaplasmas suspensions with glucose resulted in statistically significant reduction in adherence to 78%; similar value was reported by Saada et al. (1991). Both results were higher than 13% reported by Smith et al. (1994). These results confirmed the importance of sialic acid residues in the recognition cellular process and agreed with previous ureaplasma reports. They are also similar to others observed with *Mycoplasma* sp. and *M. pneumoniae* (Waites et al., 2005; Razin et al., 1998; Thirkell et al., 1989).

A dose- dependent manner adherence reduced more to 50% with pre-treatment of ureaplasmas suspension with trypsin, suggesting that they had adhesins proteinaceous moiety; this is similar to previous reports (Saada et al., 1991; Smith et al., 1994).

The lack of a cell wall or other appendix in mycoplasmas may facilitate the direct contact of the mycoplasma's membrane to its eukaryotic host. Like other mycoplasmas they possess variable surface proteins (Kahane and Jacobs, 1995; Baseman et al., 1997; Waites and Talkington, 2004; Watson et al., 1988; Shimizu et al., 2008). The multiple banded (MB) protein shows a striking variability of its molecular weight. This is caused by changes of the number of C-terminal repeating units (Zheng et al., 1995; Chavez-Munguia et al., 1997). *Mycoplasma bovis* has a membrane protein (pMB67) with spontaneous changes and variable surface lipoproteins (Vsps) that play an important role like adherence mediators (Beehrens et al., 1996; Sachse et al., 2000). The MB antigen of *Ureaplasma* sp. was identified as highly immunogenic outer membrane protein of serum human in clinical sample (Zheng et al., 1995; Monecke et al., 2003). Adherence abolition with mAbs 10C6 against multi-banded antigen (MB) reduced attachment of dose-dependent manner, suggesting that the MB antigen could be an adhesin. The size of *Ureaplasma* sp. is 330 nm; that is the principal reason why ureaplasmas are difficult to see with light microscopy (Duncan et al., 1992). A methodology for the ureaplasmas adherence studies needs an easy technique to evaluate attachment. Bertholet assay facilitated our work as a previous report (Smith et al., 1994). However; we used electron microscopy techniques as a support of adherence mediated by colorimetric method.

The scanning electron microscopy showed that the A549 cells lost all microvillus, and they looked like a plane form or stretched when *Ureaplasma* sp. was adhered to the cell. This damage is similar to attachment and effacing (A/E) produced by *E. coli* EPEC (Donnenberg et al., 1997).

Several pathogenic bacteria produced severe changes altering the host cytoskeleton architecture as manifested by dramatic rearrangement of microtubule and microfilament proteins (Rosenshine et al., 1992; Scharamm and Wyrick, 1995; Young et al., 1992). This damage has been observed with *Mycoplasma penetrans* (Giron et al., 1996). An amplification of A549 cell showed one ureaplasma on structure like a pedestal.

Micro- colonies of ureaplasmas were observed on A549 cell. It is known that *Ureaplasma* sp. cultured in 10B broth or agar (A7 or A8) would produce "tiny" colonies and because it reproduces by binary fusion (Shepard, 1983). Micro-colonies have been observed with other mycoplasmas too. By Giemsa staining micro-colonies of *M. penetrans* on Hep-2 cells were observed and confirmed by immunofluorescence microscopy (Giron et al., 1996).

Kinetic adherence of *U. parvum* serotype 3 to A549 cell by scanning electron microscopy showed ureaplasmas binding to A549 cell at 15 min of incubation. After 2 h a few ureaplasmas were adhered on the cells and a severe damage was observed.

Ureaplasma sp. has been isolated from cord blood and there have been numerous reports on their isolation from bloodstream of neonates and young infants, sometimes in associations with meningitis. It can be isolated from amnion liquid during early 20 weeks of gestation and without labor and intact membrane, indicating that *Ureaplasma* sp. could be an invasive microorganism (Brus and Van Warde, 1991; Cassell et al., 1991; Waites et al., 2005). Our scanning electron microscopy results showed ureaplasmas covered by cellular membrane (Figure 3D), suggesting that *U. parvum* serotype 3 could enter into the A549 cell.

A previous report showed *Mycoplasma genitalium* intracellular and Vero cells, with several vacuoles at 72 h after infection (Mernaugh et al., 1993; Jennsen et al., 1994; Readdy et al., 1996). *Ureaplasma* sp. that gets into A549 cells adherence was observed at less than 1 h, as *Mycoplasma gallisepticum* which has the capacity to invade host cell at 20 min after infection and to survive around 48 h into the cell (Jennsen et al., 1994; Winner et al., 2000); but by transmission electron microscopy, we observed several vacuoles in A549 cells infected with ureaplasmas. Immunoperoxidase staining assay showed that *U. urealyticum* T960 (serotype 8) attached to 3T6

cells and produced vacuolization of cytoplasm (Kotani and McGarrity, 1986).

We observed a structure like a “Tip” in some ureaplasmas by transmission electron microscopy. This structure has been reported in *M. pneumoniae* and other mycoplasmas (Razin et al., 1998; Razin, 1999; Krause et al., 1983; Krause et al., 1982; Razin and Jacobs, 1992).

Membranes of ureaplasmas dyed with ruthenium red highlight a structure like a “Tip” organelle was observed in *U. parvum* serotype 3. Adhesins have been found by immunoelectron microscopy to cluster at the surface of the “Tip” organelle. The high concentration of the adhesins at the “Tip” is apparently responsible for the remarkable strength of attachment of the mycoplasmas to erythrocytes through the attachment “Tip” (Kahane and Jacobs, 1995; Baseman et al., 1997; Baseman and Tully, 1997). “Tip” organelle plays an important role when entering the host cell (Razin et al., 1998; Razin, 1999; Razin and Jacobs, 1992). A report demonstrated that *Ureaplasma* sp. human has an extramembranous layer of polyanions and glucosyl-like residues that bound with red ruthenium, providing cytochemical evidences (Robertson and Smook, 1976). And this helps to identify ureaplasmas by transmission electron microscopy results.

We did not find different adherence between clinical strains of biovar 1 or biovar 2; also, there were no differences about adherence and damage among reference strains and clinical strains (biotypes 1 and 2).

Scanning and transmission electron microscopy data support the hypothesis of invasiveness, for ureaplasmas is likely not related to one or a few particular serotypes. Many serotypes have antigen variability and host factors could be important determinants for ureaplasma invasive infections (Brus and Van Warde, 1991; Cassell et al., 1991; Waites et al., 2005). The damage on A549 cell showed a plane form of the cell and loss of microvillus, similar as other pathogenic microorganisms as *E. coli*, *Campylobacter* sp., *Shigella* sp., and *Haemophilus ducreyi*, using other cellular lines (Donnenberg et al., 1997).

The host cell membrane is also vulnerable to toxic materials released by the adhering mycoplasmas. Although toxins have not been associated with mycoplasmas, the production of cytotoxic metabolites and the activity of cytolytic enzymes were well established (Rottem and Naot, 1998).

Damage observed could be due to urease activity; it has been suggested like a potential virulence factor of *Ureaplasma* sp. and the ammonium has a cytotoxic effect on the cell host (Waites et al., 2005). Previous reports have suggested that some different from urogenital tract producing urea (Jernigan, 1983). Urea production in tissues could explain why ureaplasmas can colonize tissues of the respiratory tract system. Small but essential amounts of urea growth factors suggest that ureaplasma present in these tissues is different from that in the urogenital system (Kotani and McGarrity, 1986).

Ureaplasma sp. could produce damage by generation of nitric oxide radicals that stimulating release of cytokines or oxygen free radicals which are thought to induce oxidative stress in host cells, resulting in damage of the cell membrane (Waites et al., 2005). The intimate contact of the ureaplasmas with the host cell membrane might result from phospholipids changes or the potent membrane-bound to phospholipases A2 present in *Ureaplasma* sp. (Waites et al., 2005). *U. parvum* has two hemolysins in which hemolysis is mediated by H_2O_2 and may function as a virulence factor, which shows cytotoxic activity (Waites et al., 2005). Stimulation of proinflammatory cytokines (TNF - , IL-10, IL-6 and IL-8) could be related to damage on the host cell too (Ronald et al., 1997; Viscardi et al., 2002). There are several possible pathways to explain the damage produced by *Ureaplasma* sp. on the A549 cell. More studies are necessary to explain which mechanisms are involved in pathological changes of cell host.

In conclusion, these results are the first to show the binding of *Ureaplasma* sp. human to a cellular lineA549 cell. Our work suggests that A549 cellular line is a good model for the study of adherence of *Ureaplasma* sp. We used two ways to show adherence to host cell. The first step showed that the urease test (Bertholet assay) is a good indicator of colonization. Furthermore, the Bertholet data suggested that the ureaplasma’s adhesions have proteinaceous nature, and the adherence inhibition assays by neurominidase and glucose showed that residuals of sialic acid could be related with receptor. It is possible that the multi-banded antigen may act like an adhesin.

The electronic microscopy was the second step to show that *Ureaplasma* sp. binds to the cell, producing evident morphological changes, and can even develop localized micro colonies. Clinical isolated of *U. parvum* and *U. urealyticum* showed similar adherence, suggesting that there was no differential pathogenicity between biovar 1 and biovar 2 related with adherence, and invasive to the host cell.

ACKNOWLEDGEMENTS

We thank to Dra. Gail Cassell for providing monoclonal antibody 10C6.6 against multi banded antigen (MB 10C6.6) of *U. parvum*.

This work was supported in part by FUNSALUD- IMSS and PIFI-IPN. We thank to SGC Fellowship SNI, EDI, CoFAA-IPN. Project SIP- IPN 2007-20080521. ATM acknowledges fellowship by CONACyT (576ED96).

REFERENCES

Abele-Horn M, Wolff C, Dressel P, Pfaff F, Zimmerman A (1997). Association of *Ureaplasma urealyticum* biovars with clinical outcome for neonates, obstetric patients, and gynecological patients with pelvic inflammatory disease. J. Clin. Microbiol. 35: 1199-1202.

Alpha MJ, Embree JE, Degagne P, Olson N, Lertzman J, Mac Donalds KS, Half PF (1995). Transmission of *U. urealyticum* from mothers to full and preterm infants. Pediatr. Infect. Dis. J. 14: 341-345.

Baseman JB, Reddy SP, Dallo SF (1997). Interplay between Mycoplasma surface proteins, airway cells and the protean manifestations of mycoplasma-mediated human infection. J. Bacteriol. 179: 1317-1323.

Baseman JB, Tully JG (1997). *Mycoplasma*: Sophisticated, reemerging, and burdened by their notoriety. Emerg. Infect. Dis. 3: 21-32.

Basolo F, Zanchetta R, Bertoloni G (1984). Mycoplasmic localization patterns on spermatozoa from infertile men. Fertil. Steril. 42: 412-417.

Beehrens A, Poumarat F, Le Grand D, Heller M, Rosengarten R (1996). A newly identified immunodominant membrane protein (pMb67) involved in *Mycoplasma bovis* surface antigenic variation. Microbiology 142: 2463-2470.

Bérubé LR, Schur MK, Latta RK, Hirama T, Kenzie CR Jarrell HC (1999). Phosphatidyl choline-mediated inhibition of *Streptococcus pneumoniae* adherence to type II pneumocytes *in vitro*. Microbiol. Pathol. 26: 25-75.

Brus F, Van WWM (1991). Fatal ureaplasmal pneumonia and sepsis in newborn infant. Eur. J. Pediatr. 150: 782-783.

Cassell GH (1993). *Ureaplasma urealyticum* intrauterine infections: role in prematury and disease in newborns. Clin. Microbiol. Rev. 6: 69-87.

Cassell GH, Waites KB, Crouse DT (1991). Perinatal mycoplasmal infections. Clin. Perinatol. 8: 241-262.

Chavez-Munguia B, Gonzalez-Robles A, Espinosa-Castellanos M, Cristóbal-Ramos AM, Martinez-Palomo A (1997). *Entamoeba dispar*: Ultrastructure and cytopathic effect. Arch. Med. Res. 28: 116-118.

Deguchi T, Yoshida T, Miyazawa T, Yasuda M, Tamaki M, Ishiko H, Maeda S (2004). Association of *Ureaplasma urealyticum* (biovar 2) with nongonococcal urethritis. Sex. Trans. Dis. 31: 192-195.

Díaz-García FJ, Herrera-Mendoza AP, Giono-Cerezo S, Guerra-Infante FM (2006). *Mycoplasma hominis* attaches to and locates intracellularly in human spermatozoa. Hum. Reprod. 21: 1591-1598.

Donnenberg MS, Kaper JB, Finlay B (1997). Interactions between enteropathogenic *Escherichia coli* and host epithelial cells. Trends Microbiol. 5: 109-114.

Duncan C, Krause DC, Taylor RD (1992). Mycoplasmas which infect humans. In: Maniloff JM, Elhaney CRN, Finch LR, Baseman JB (eds) Mycoplasmas molecular biology and pathogenesis. American Society for Microbiology, Washington D.C. pp.429-430.

Dykstra MJ (1993). Specimen preparation. In: A manual of applied techniquesor biological electron microscopy. Plenum Press, New York and London. pp. 1-9.

Faye-Petersen OM (2008). The placenta in preterm birth. J. Clin. Pathol. 61: 1261-1275.

Giron JA, Lange M, Baseman JB (1996). Adherence, Fibronectin binding, and induction of cytoskeleton reorganization in cultured human cells by *Mycoplasma penetrans*. Infect. Immun. 64: 197-208.

Harasawa R, Dybvig K, Watson HL, Cassell GH (1991). Two genomic clusters among 14 serovars of *Ureaplasma urealyticum*. Syst. Appl. Microbiol. 14: 393-396.

Jennsen KJ, Blomm J, Lind K (1994). Intracellular location of *Mycoplasma genitalium* in cultured Vero cells as demonstrated by electron mycroscopy. Int. J. Exp. Pathol. 75: 91-98.

Jernigan HM (1983). Urea formation in rat, bovine and human lens. Exp. Eye Res. 7: 551-558.

Kahane I, Jacobs E (1995). Mycoplasma adherence to host cells: methods of quantifying adherence. In: Razin S, Tully JG (eds) Molecular and diagnostic procedures in mycoplasmology. Academic Press. Inc., U.S.A. pp. 397-405.

Kang F, Ma Z, James G, Cordon S, Gilbert G (2000). Species identification and subtyping of *Ureaplasma parvum* using PCR-based assays. J. Clin. Microbiol. 38: 1175-1179.

Kotani H, McGarrity GJ (1986). *Ureaplasma* infection of cell cultures. Infect. Immun. 52: 437-444.

Krause DC, Leith DK, Baseman JB (1983). Reacquisition of specific proteins confers virulence in *Mycoplasma pneumoniae*. Infect. Immun. 39: 830-836.

Krause DC, Leith DK, Wilson RM, Baseman JB (1982). Identification of *Mycoplasma pneumoniae* proteins associated with hemadsorption and virulence. Infect. Immun. 35: 809-817.

Lee A, Ramanujam HT, Ware P (1991). Molecular diagnostic of *Ureaplasma urealyticum* septic arthritis in a patient with hipogammaglobulinemy. Arthritis Rheum. 4: 443-448.

Li Y, Chen HM, Brauner A, Zheng C, Jensen JS, Tullus K (2002). *Ureaplasma urealyticum* induce apoptosis in human lung epithelial cell and macrophages. Biol. Neonate 82: 166-173.

McGarrity GJ, Kotani H, Butter GH (1986). Ureaplasma eukaryotic cell interactions *in vitro*. Pediatr. Infect. Dis. 5: 316-318.

Mernaugh GR, Dallo SF, Holt SC, Baseman JB (1993). Properties of adhering and non adhering populations of *Mycoplasma genitalium*. Clin. Infect. Dis. 17: 69-78.

Monecke S, Helbig JH, Jacobs E (2003). Phase variation of the multiple banded proteins in *Ureaplasma urealyticum* and *Ureaplasma parvum*. Int. J. Med. Microbiol. 293: 203-211.

Naessens A, Foulon W, Breynaert J, Lauwers S (1998). Serotypes of *Ureaplasma urealyticum* isolated from normal pregnant women and patients with pregnancy complications. J. Clin. Microbiol. 26: 319-322.

Novy MJ, Duffy L, Axthelm MK, Sadowsky DW, Witkin SS, Gravett MG, Cassell GH, Waites KB (2009). *Ureaplasma parvum* or *Mycoplasma hominis* as sole pathogens cause chorioamnionitis, preterm delivery, and fetal pneumonia in rhesus macaques. Reprod. Sci. 16: 56-70.

Oue S, Hiroi M, Ogawa S, Hira S, Hasegawa M, Yamaoka S, Yasui M, Tamai H, Ogihara T (2009). Association of gastric fluid microbes at birth with severe bronchopulmonary dysplasia. Arch. Dis. Child. Fetal Neonatal Ed. 94: F17-F22.

Razin S (1999). Adherence of pathogenic mycoplasmas to host cells. Bios. Rep. 19: 367-372.

Razin S, Jacobs E (1992). *Mycoplasma* adhesion. J. Gen. Microbiol. 138: 407-422.

Razin S, Yogev, Naot Y (1998). Molecular biology and pathogenicity of mycoplasmas. Microbiol. Mol. Biol. Rev. 62: 1094-1156.

Readdy SP, Rasmussen WG, Baseman JB (1996). Molecular cloning and characterization of an adherence-related operon of *Mycoplasma genitalium*. J. Bacteriol. 177: 5943-5951.

Robertson J, Smook E (1976). Cytochemical evidence of extramembranous carbohydrates on *Ureaplasma urealyticum* (T-strain Mycoplasma). J. Bacteriol. 128: 658-660.

Robertson JA (1991). Hemadsorption by colonies of *Ureaplasma urealyticum*. Infect. Immun. 59: 2203-2206.

Robinson T, Davies HA, Sarathchandra P, Furr PM (1991). Intracellular localization of mycoplasmas in cultured cells demonstrated by immunocytochemistry and electron microscopy. Int. J. Exp. Pathol. 72: 705-714.

Ronald JB, Thomas EK (1997). *Mycoplasma hominis* and *Ureaplasma urealyticum* induce pro-inflammatory cytokines in an alveolar type II cell line (A549). Pediatr. Res. 41(4-2): 215.

Rosenshine I, Donnenberg MS, Kaper JB, Finlay BB (1992). Signal transduction between enteropathogenic *Escherichia coli* (EPEC) and epithelial cells: EPEC induces tyrosine phosphorylation of host cell proteins to initiate cytoskeletal rearrangement and bacterial uptake. EMBO J. 11: 3551-3560.

Rottem S, Naot Y (1998). Subversion and exploitation of host cells by mycoplasmas. Trends Microbiol. 6: 436-440.

Saada AB, Terespolski Y, Adoni A, Kahane I (1991). Adherence of *Ureaplasma urealyticum* to human erythrocytes. Infect. Immun. 59: 467-469.

Sachse K, Heilbig JH, Lysnyansky I, Grajetzki C, Müller W, Jacobs E, Yogev D (2000). Epitopes mapping of immunogenic and adhesive structures in repetitive domains of *Mycoplasma bovis* variable surface lipoproteins. Infect. Immun. 68: 680-687.

Sanchez PJ, Regan JA (1988). *Ureaplasma urealyticum* colonization and chronic lung disease in low birth weight infants. Pediatr. Infect. Dis. J. 7: 542-546.

Sanchez PJ, Regan JA (1990). Vertical transmission of *Ureaplasma urealyticum* from mothers to preterm infants. Pediatr. Infect. Dis. J. 9: 398-401.

Scharamm N, Wyrick PB (1995). Cytoskeletal requirements in *chlamydia trachomatis* infection of host cells. Infect. Immun. 63: 324-332.

Shepard MC (1983). Culture media for ureaplasmas. In: Razin S, Tully JG (eds) Methods in mycoplasmology, mycoplasma characterization. Academic Press New York. pp. 87-95.

Shimizu T, Kida Y, Kuwano K (2008). *Ureaplasma parvum* lipoproteins,

including MB antigen, activate NF-B through TLR1, TLR2 and TLR6. Microbiology 154: 1318-1325.

Smith DGE, Russell WC, Thirkell D (1994). Adherence of *Ureaplasma urealyticum* to human epithelial cells. Microbiology 140: 2893-2898.

Stancombe BB, Walsh WF, Derdak S, Dixon P, Hensley D (1993). Induction of human neonatal pulmonary fibroblast cytokines by hyperoxia and *Ureaplasma urealyticum*. Clin. Infect. Dis. 17: s154-s157.

Talbot VM, Paton AW, Paton JC (1996). Uptake of *Streptococcus pneumoniae* by respiratory epithelial cells. Infect. Immun. 64: 3772-3777.

Tarrant WP, Gonzalez-Berjon JM, Cernoch PL, Olsen RJ, Musser JM (2009). Spontaneous bacterial pericarditis with tamponade due to ureaplasma sp. J. Clin. Microbiol. 47: 1965-1968.

Thirkell D, Myles AD, Precious BL, Frost JS, Woodall JC, Burdon MG, Russell WC (1989). The urease of *U. urealyticum*. J. Gen. Microbiol. 135: 315-323.

Viscardi RM, Kaplan J, Lovchik JC, He JR, Hester L, Rao S, Hasday JD (2002). Characterization of a murine model of *Ureaplasma urealyticum* pneumonia. Infect. Immun. 70: 5721-5729.

Waites KB, Katz B, Schelonka RL (2005). Mycoplasmas and ureaplasmas as neonatal pathogens. Clin. Microbiol. Rev. 18: 759-789.

Waites KB, Talkington DF (2004). *Mycoplasma pneumoniae* and its role as a human pathogen. Clin. Microbiol. Rev. 17: 697-728.

Wang EE, Frayha H, Watts J, Hamberg O, Chernesky M, Mahoney JB, Cassell GH (1988). The role of *Ureaplasma urealyticum* and other pathogens in the development of chronic lung disease of prematury. Pediatr. Infect. Dis. J. 7: 547-551.

Watson HL, McDaniel LS, Blalock DK, Fallon MT, Cassell GH (1988). Heterogeneity among strains and a high rate of variation within strains of a major surface antigen of *Mycoplasma pulmonis*. Infect. Immun. 56: 1358-1363.

Winner F, Rosengarten R, Citti C (2000). *In vitro* cell invasion of *Mycoplasma gallisepticum*. Infect. Immun. 68: 4238-4244.

Yoder BA, Albertine KH (2008). Inflammation and lung disease in the neonatal period. NeoReviews 9: 447-457.

Young VB, Falkow S, Schoolnik GK (1992). The invasion protein of *Yersinia enterocolitica*: internalization of invasion-bearing bacteria by eukaryotic cells is associated with reorganization of cytoskeleton. J. Cell Biol. 116: 197-207.

Zheng X, Teng LJ, Watson HL, Glass JI, Blanchard A, Cassell GH (1995). Small repeating units within the *Ureaplasma urealyticum* MB antigen gene encode serovar specifity and are associated with antigen size variation. Infect. Immun. 63: 891-98.

Zheng X, Watson HL, Waites KB, Cassell GH (1992). Serotype diversity and antigen variation among invasive isolates of *Ureaplasma urealyticum* from neonates. Infect. Immun. 60: 3472-3474.