# *Chlamydia trachomatis* infections and their impact in the adolescent population

*Infecciones por Chlamydia trachomatis y su repercusión en adolescentes*

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**ABSTRACT**

*Chlamydia trachomatis* is an obligate intracellular organism and can only replicate inside eukaryotic host cells. It has a unique developmental cycle, with metabolically inert, spore-like elementary bodies that infect host cells and develop into metabolically active, replicative or reticulate bodies (RBs) within a membrane-bound inclusion. RBs are divided once more into elementary bodies 24 to 48 hours after infection and they are eventually released through lysis of the host cell. The chlamydial infection, like the gonococcal infection, is the possibility of severe sequelae in both the eye and the genital tract. *Chlamydia trachomatis* infects epithelial cells in the eye and genital tract. The early stage of infection can present with a mucopurulent discharge, but infections are often asymptomatic at this stage. In most infected women, the infection has a resolution, but in women with persistent or repeated infections, it can spread upwards from the endocervix to the fallopian tubes, leading to infertility or ectopic pregnancy because of tubal occlusion by scar tissue. It is a common etiologic agent in acute salpingitis, mainly in the adolescent's population. With the exception of the lymphogranuloma *venereum* strains, which cause systemic illness and infect regional lymph nodes, *Chlamydia trachomatis* infection usually remains confined to mucosal surfaces, and it continues to produce enormous social and economic consequences despite advances in prevention, screening, and treatment.

**Keywords:** *Chlamydia trachomatis*; adolescent; biology; immunity.

**RESUMEN**

*Chlamydia trachomatis* es una bacteria intracelular obligada que solo se puede replicar en células eucarióticas. Tiene un ciclo de desarrollo que es único dentro de la microbiología. Consta de un cuerpo inicial o elemental metabólicamente inerte que ingresa en la célula eucariótica y allí se transforma en un cuerpo reticular o replicativo que es él que se divide. Luego, cada uno de estos cuerpos reticulares se transforma después de 24 a 48 horas en cuerpos elementales o iniciales que son los que se liberaran posteriormente a la lisis de la célula. La infección por *Chlamydia* y la infección gonocócica pueden dejar severas secuelas a nivel del tracto genital y ocular. En el estadio temprano de la infección, *Chlamydia trachomatis* puede presentar descarga mucopurulenta, pero a menudo es asintomática. En la mayoría de las mujeres, se resuelve favorablemente, pero en muchas de ellas, la infección puede ser persistente y/o recidivante. También puede diseminarse hacia el aparato genital superior (endometrio, trompas de Falopio), generando lesiones cuyas cicatrices dejan secuelas como esterilidad e infertilidad. Es la causa más común de la salpingitis aguda, principalmente en las adolescentes. Con excepción de las cepas responsables del linfogranuloma venéreo que pueden causar enfermedades sistémicas e infectar los ganglios regionales, *Chlamydia trachomatis* usualmente permanece confinada a las mucosas. Además, continúa produciendo consecuencias sociales y económicas con trascendencia a nivel mundial a pesar de los avances en su prevención, detección y tratamiento.

**Palabras clave:** *Chlamydia trachomatis*; adolescente; biología; inmunidad.

## INTRODUCTION

*Chlamydia trachomatis* (CT) is an obligate intracellular organism. This is an important fact, as CT is not developed in conventional culture media.

Similarly to *Neisseria gonorrhoeae*, its importance is based on its possible complications and sequelae both for women and for their contacts and descendants[1,2].

This text begins with a brief listing of the important points that will be developed in this article about CT, which will allow us to better understand the biology and pathogenesis of the infections produced by such elusive bacterium.

CT is a human pathogen, one of the leaders among the microorganisms that most produce sexually transmitted infections (STI) in the world, with more than 90 million new cases of genital infections every year. Around 70% of the infected women are asymptomatic, thus chronic infections may be established for months or even years. Although CT does not present symptoms, it can damage the reproductive organs and the conventional treatments, many times, are not able of completely eliminating it; therefore, it remains a persistent infection[3-5]. Recently, researchers from the Max Planck Institute for the Study of Biology Infections showed that CT infections might cause mutations in the DNA of the host, thus preventing the growth regulation mechanisms of damaged cells, which could be the path toward the development of tumors[6].

## EPIDEMIOLOGY

CT is an obligate intracellular Gram-negative microorganism, because it is unable of making metabolic energy and requires live cells for its development.

The prevalence of CT is estimated in 6.8% among sexually active women in the age range of 14 to 19 years[1,2].

The increase in reports about CT infection in the last years reflects the expansion of screening activities that were conducted in many communities, the use of more sensitive tests, the emphasis put in the detection of asymptomatic cases, and the use of statistical diffusion tools.

Study carried out in the *Cátedra de Microbiología y Parasitología*, School of Medicine, *Universidad del Salvador* – Buenos Aires, Argentina.
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When prevalence was compared between both the genders, the outcomes were higher in women, which was also seen in our experience[6].

It is worth mentioning that CT is part of a great group of microorganisms that currently form the *Chlamydiales* order. There are two important genera: *Chlamydia* y *Chlamydophila*[7]. While *Chlamydia* infects only mammals such as humans, rodents, and swine, the specificity of species for *Chlamydophila* is less strict and includes amphibians, reptiles, birds, and mammals. Both *Chlamydia* and *Chlamydophila* include some important species like human pathogens, as explained in **Table 1**[8,9].

CT causes several infections in humans and animals. It infects the epithelial cells and possesses 15 serovares, some of which are responsible for trachoma. Trachoma is the leading disease of blindness that can be preventable similarly to the STI. When the natural history of the disease is analyzed, it is found that there is limited knowledge regarding what happens in human infections without antibiotic treatment. There are two studies including females suggesting that it disappears after 1 year in 45 to 55% of them, reaching until 94%. In women, nontreated genital infections can have devastating consequences, such as inflammatory pelvic disease and its sequelae: ectopic pregnancy, infertility, and chronic pelvic pain[1,2,10].

## GENOMICS

The genome of a relative number of *chlamydiae* has been extensively studied since 1998[11,12].

As most of the microorganisms, CT has a chromosomic and plasma DNA. Its genome is small, and it only has 1.042.519 pb (58.7% of A-T). CT has a cryptic plasmid of 7.493 pb. The genomic analysis showed it codifies for around 875 proteins, which are not necessarily expressed and 70 of which are exclusively of CT. It is worth noting that in the area near the origin of the chromosome replication, there is a higher genetic diversity. This region includes genes that control tryptophan synthesis, and its use has been associated with the intervention of gamma interferon in the development of persistent infection. CT recovered from the human genital tract have a homologous gene of cytokines reported in the 0157 enterohemorrhagic *Escherichia coli* and in *Clostridium*[13], in such area.

Based on the genetic structure, different genotypes, which vary according to the areas and pathologies, are found. In a study carried out in Spain, the most frequent genotypes were E (45.3%), D (15.3%), G (10.2%), and F (9.6%). Other genotypes included B, H, I, J, K, and LGV II[14]. This study found that the genotype E was more prevalent among asymptomatic adolescents. This genotype is precisely associated with the asymptomatic infection[15,16].

*Chlamydophila abortus*, which has been considered a new species of *Chlamydophila psittaci*, is one of the causing agents of the real epizootic of abortion in the ovine cattle, resulting in economic damage. However, recently, the possible participation of this microorganism in an abortion condition has been described in a woman presenting abdominal pain and vaginal secretion, and culture or molecular studies were negative for *N. gonorrhoeae* and CT. The suspicion of a disease or of a pelvic inflammatory syndrome resulted in the determination of antibodies (Ac) study, which was consistently higher in the lipopolysaccharide (LPS) gender-specific antigen (Ag) of *C. psittaci*. A retrospective investigation found that the patient had Ac titers that were seen in the heat-shock protein 60 (HSP60), demonstrated in the ELISA test. The nested PCR reaction for the specific ompA of *Chlamydiaceae* spp. conducted in the liquid obtained by puncture of the Douglas pouch was positive.

**Table 1 –** Genera division of the *Chlamydiaceae* family and its division into two genera, *Chlamydia* y *Chlamydophila*.

| **Family I. Chlamydiaceae** | |
|---|---|
| | **Genus 2. Chlamydophila** |
| | *Chlamydophila psittaci.* |
| **Genus 1. Chlamydia** | *Chlamydophila abortus** |
| *Chlamydia trachomatis* | *Chlamydophila felis** |
| C/PK-2 Trachoma biovar | *Chlamydophila caviae** |
| LGV L2/434/BU Biovar | *Chlamydophila pecorum* |
| *Chlamydia muridarum** | *Chlamydophila pneumoniae* |
| *Chlamydia suis** | TWAR Biovar |
| | Equine Biovar |
| | Koala Biovar |
| **Families II, III, and IV** | |
| **Family II. Simkaniaceae fam.nov** | |
| *Simkania negevensis* sp.nov | |
| **Family III. Parachlamydiaceae fam.nov** | |
| **Genus 1** | **Genus 2** |
| *Parachlamydia* gen.nov | *Neochlamydia* gen.nov |
| *Parachlamydia acanthamoebae* sp.nov | *Neochlamydia hartmannellae* sp.nov |
| **Family IV. Waddliaceae fam.nov** | |
| *Waddlia chondrophila* sp.nov | |

## IMPORTANCE OF ENVIRONMENTAL CHLAMYDIAE

Recently, some intracellular microorganisms similar to that of the *Parachlamydiaceae* with new families were recently described. The use of techniques such as PCR together with phylogenetic techniques based on rARN allowed a substantial accumulation of genetic sequences associated with *Chlamydia.* Therefore, Chlamydia-like organisms are mentioned, in which some of them are much unknown and others have been associated with zoonosis. There is evidence that they might be the cause of several pathologies in the cattle that remained underdiagnosed.

Some species of *Parachlamydiaceae* replicate in different amoebae of free lives and can have a lithic action. Their DNA has been detected as antibodies in the materials of patients[17].

These bacteria might be currently considered as emerging pathogens, and thus they need to be investigated. *Simkania negevensis* (*Simkaniaceae* family)[9,17] and *Parachlamydia* (*Parachlamydiaceae* family)[18] could be essential human respiratory pathogens, whereas *Waddlia chondrophila* (*Waddliaceae* family) seems to be a new agent of abortions in ruminants[19].

## STRUCTURE

The elementary bodies (EB) of CT are microscopic rounded structures that are infectious, rigid, resistant to rupture because of disulfide bond of the proteins from the outer layer of the membrane, and they are released after lysis of the infected host cell. The size of EB

ranges from 200 to 400 nm. They are stained purple with Giemsa stain, and red with Macchiavello stain, which is different from the color stained by the cytoplasm of the host cell.

DNA and RNA are found in EB. The largest part of the DNA is found in the central nucleoid electron density and the largest part of the RNA, in the ribosomes. The EB show species-specific and serotype-specific Ag that lead to phagocytosis, they have no metabolic activity, cannot replicate, and are infective (**Figure 1**).

Reticulate bodies (RB) are the result of the EB differentiation after suffering phagocytosis, they have a bacillary morphology, do not have a dense nucleoid and their size ranges from 600 to 1,000 nm, but they are not infectious. They are stained blue with Giemsa stain, they can replicate, have metabolic activity and their DNA is disperse.

The membrane presents extracellular proteins rich in cysteine, including main protein of the outer membrane (MOMP) or 60-Kda protein, which is the biggest protein ; 12- to 15-Kda protein, which is present in the RB; and HSP, i.e., 60 and 70 HSP found in women with upper genital tract infections or pelvic inflammatory disease (PID).

In women with infertility and ectopic pregnancies, high Ac levels were found in the HSP 60 (anti-HSP60), in contrast with the Ac anti-HSP 70 that was reported in women with protective immunity[20-22].

## VIRULENCE FACTORS

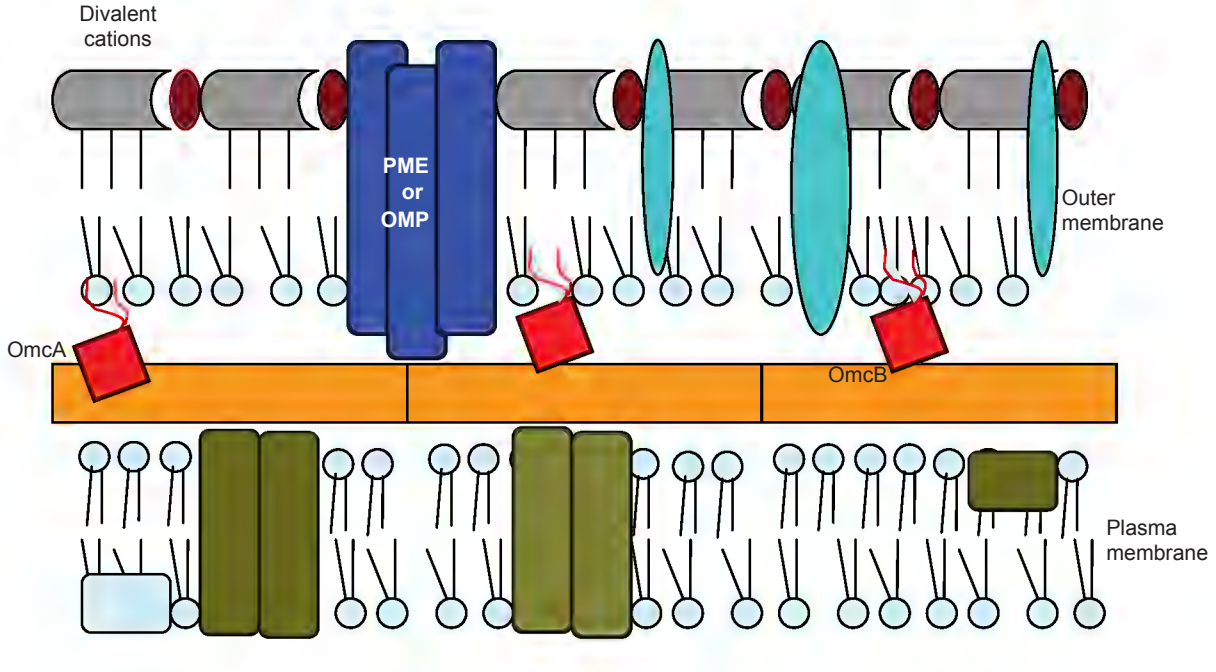
CT has been divided into 18 serotypes: A, B, Ba, C, D, E, F, G, H, I, Ia, J, K, L1, L2, L2a, L3, and L3a. This division is based on the analysis of the MOMP, which has four variable domains (VDs) that are flanked and interspaced through five constant domains. Three of four VD (VD1, VD2, and VD4) are found in the surface and contain antigenic epitopes, which are blank sites for serum typification. Both the LPS and the HSP60 have stimulated the innate response through the TLR4. However, the CT LPS has low endotoxic power[23,24], and the lack of significant expression of TLR4 in the cervix and in the upper genital tract suggests that the contribution of this molecule as a proinflammatory signal during the genital tract infections can be small[25]. Its union with the TLR2 seems more significant, and to be connected to the presence of a plasmid. When this plasmid is lost, the inflammatory response, the glycogen accumulation, and the infectivity are decreased[26-28].

The CT introduces proteins in the host cells through many mechanisms, including type III secretion[29], while some of them are translocated to the outer part upon the Sec-dependent pathway[30,31].

## INTERACTION OF *CHLAMYDIA* WITH THE HOST CELL

In order to understand the pathogenesis of any infection, attention should not only be toward the pathogen, but also to the interaction between the pathogen and the cells. This is even more notorious in the case of CT, because this is an obligate intracellular microorganism that depends on such interaction to replicate and survive.

CT uses the mononuclear cells for reproduction. The EB invades the cells through endocytosis and actively multiplies as a RB inside the phagosome. Then, each body is transformed to EB, and they are eliminated as such.




OmcA: former Omp2; OmcB: functional protein associated with the outer membrane complex (former Omp3).

**Figure 1 –** Cell wall of the elementary body.

When the EB infects the cells, it induces the centrosome to form an apparatus and damages the replication. The infection begins in the vaginal cells and climbs to the upper genital tract. The alterations in the cells require replicating cells. Thus, its preferential location is inside the metaplasic area of the lower genital tract between the ectocervix and the vagina, where the cells actively replicate themselves.[1,2]

Cervical dysplasia that originated in experimental injuries with rats were seen, which can be progressive. CT manipulates the cells in several ways:

- acknowledging the cell (adhesion);
- penetrating;
- constituting phagosome;
- transforming the EB to RB;
- dividing the RB;
- transforming the RB to EB;
- releasing the EB or persisting on it.

## ADHESINS AND ADHESION

We will further see more details regarding the analysis of the cell cycle, but, in each step, modifications and interactions of the microorganism with the compatible cell are being made.

CT is a Gram-negative microorganism, and thus it has an outer membrane with proteins, called MOMP, that work as adhesins. The characteristic of PME is that it promotes nonspecific interactions (electrostatical and hydrophobic) with the host cell. They are also known for having structures named VD I to IV, because they are separated by four symmetrical spaces whose sequence varies between the serum types. The importance of CT, on a clinical point of view, is that it is an in vivo determinant of the neutralizing antibody activity, because it works as a target[31].

In order to promote the adherence to the host cells, CT uses a trimolecular mechanism and requires the heparan sulfate similar to Glycosaminoglycan (GAG), that is, Heparan Sulfate Like Glycosaminoglycan (HSLG), in its surface. It seems CT elaborates a unique HSLG. However, an exogenous heparan sulfate, working as an adhesion analog, restores the suppressed CT infectivity through its early treatment with the heparan sulfate lyases.

## DEVELOPMENTAL CYCLE OF *CHLAMYDIA TRACHOMATIS*

All chlamydiae have a biphasic cycle. The infection begins when an infectious particle named EB invades the host cell. It is around the cytoplasmic membrane and constitutes the phagosome. This intracellular EB is differentiated in the RB, thus it is divided into two cycles: the early and the late cycle. A series of events is produced, and one of the most important ones is the modification of the RNA:DNA relation, which was 1:1 and becomes 3:1, thus indicating the synthesis of proteins (**Figure 2**).

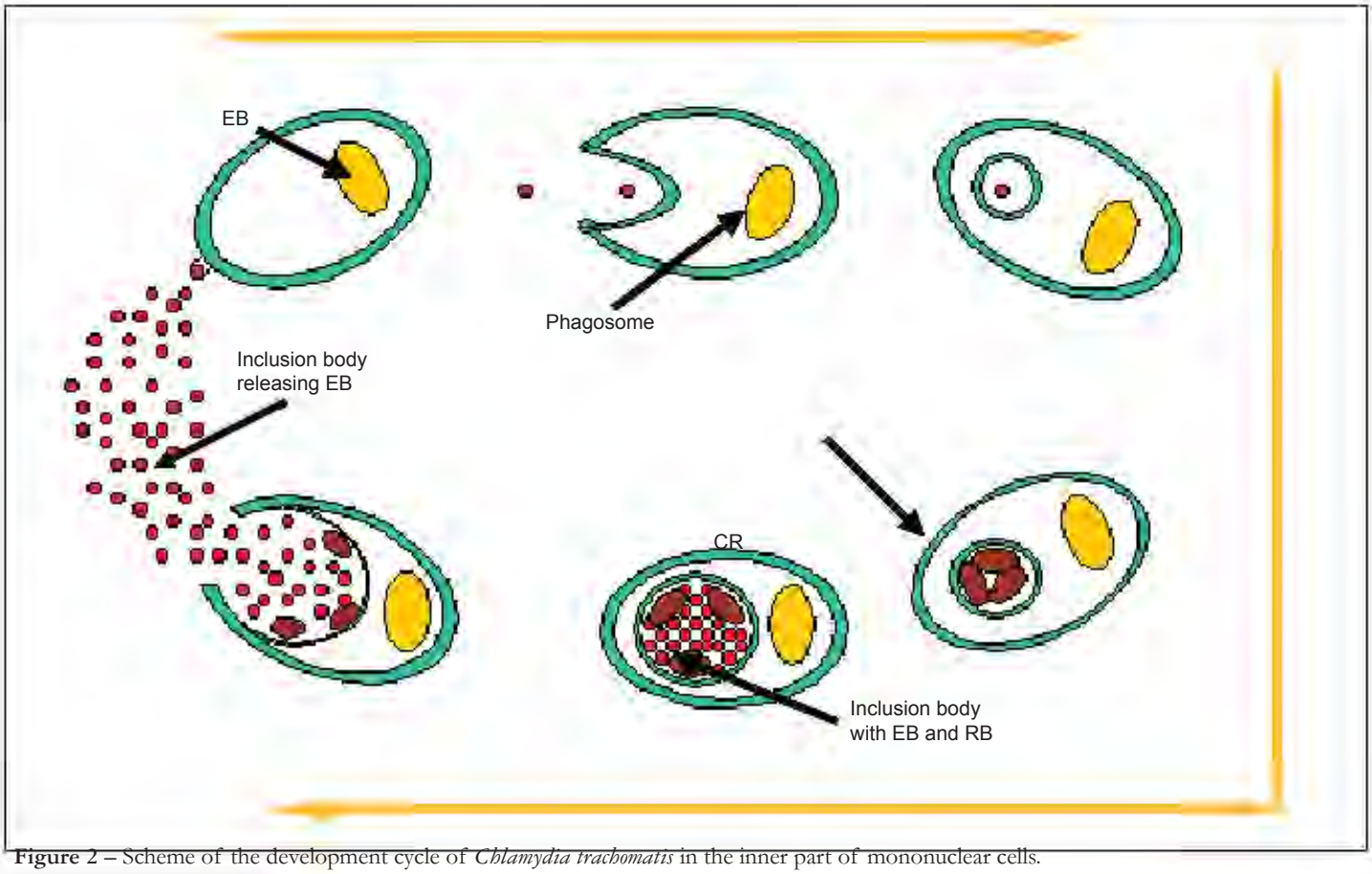



**Figure 2 –** Scheme of the development cycle of *Chlamydia trachomatis* in the inner part of mononuclear cells.

## PENETRATION AND CONSTITUTION OF THE PHAGOSOME OR PARASITOPHOROUS

In order to develop its intracellular life, CT needs to scheme its penetration and create an adequate niche, which is done through a non-flagellar type III secretion system that is an improved virulence factor commonly found in Gram-negative bacteria, but it is not located in the pathogenicity islands of CT because it is dispersed in the entire chromosome. The type III secretion collaborates to virulence, as already expressed, and consists in the introduction of proteins inside the cell, while the steps that allow the exportation from the host or the vacuole refer to translocation. These denominations have been standardized.

All chlamydiae have a biphasic cycle of development. It has been studied that the ingression is produced as an EB and, inside the vacuole, it is divided into RB, which is not infectious. This is carried out in the so-called early cycle, during which cellular modifications are done (**Figures 3 and 4**)[32,33]. After and during the medium and late cycles, the RB transforms to EB and leaves the cell.

## *CHLAMYDIA TRACHOMATIS* PRODUCES CELL ALTERATIONS

In cell cultures, the new inclusion migrates along the microtubules toward the perinuclear area that corresponds to the microtubule organizing center (MTOC) or centrosome. Many secretory organelles reside around the MTOC and possibly facilitate the interaction with lipids and with the compartments enriched by nutrients. Although CT cannot synthesize most of its requirements, it can, however, synthesize its own lipids, even though it is preferable to acquire them through the host cell. It was possible to show the presence of lipids in CT development areas in cellular lines (**Figures 5 and 6**) using cytochemical techniques[34,35]. It has been seen that the cell acquires neutral lipids through a nonvesicular pathway[36], interacting with lipid droplets and interfering with the energetic metabolism[37].

With regard to the alterations that CT produces in the cell[33], we can mention (**Figures 7 and 8**):

- fragmentation of Golgi apparatus;
- mitochondrial dysfunction;
- possible creation or facilitation of the supernumerary centrosome (abnormal mitosis)[38,39] appearance;
- exercise of an anti-immune strategy, thus suppressing cellular protection systems like apoptosis[40,41].

## ABERRANT BODIES

The formation of aberrant persistent bodies that prevent the CT cycle completion might occur.

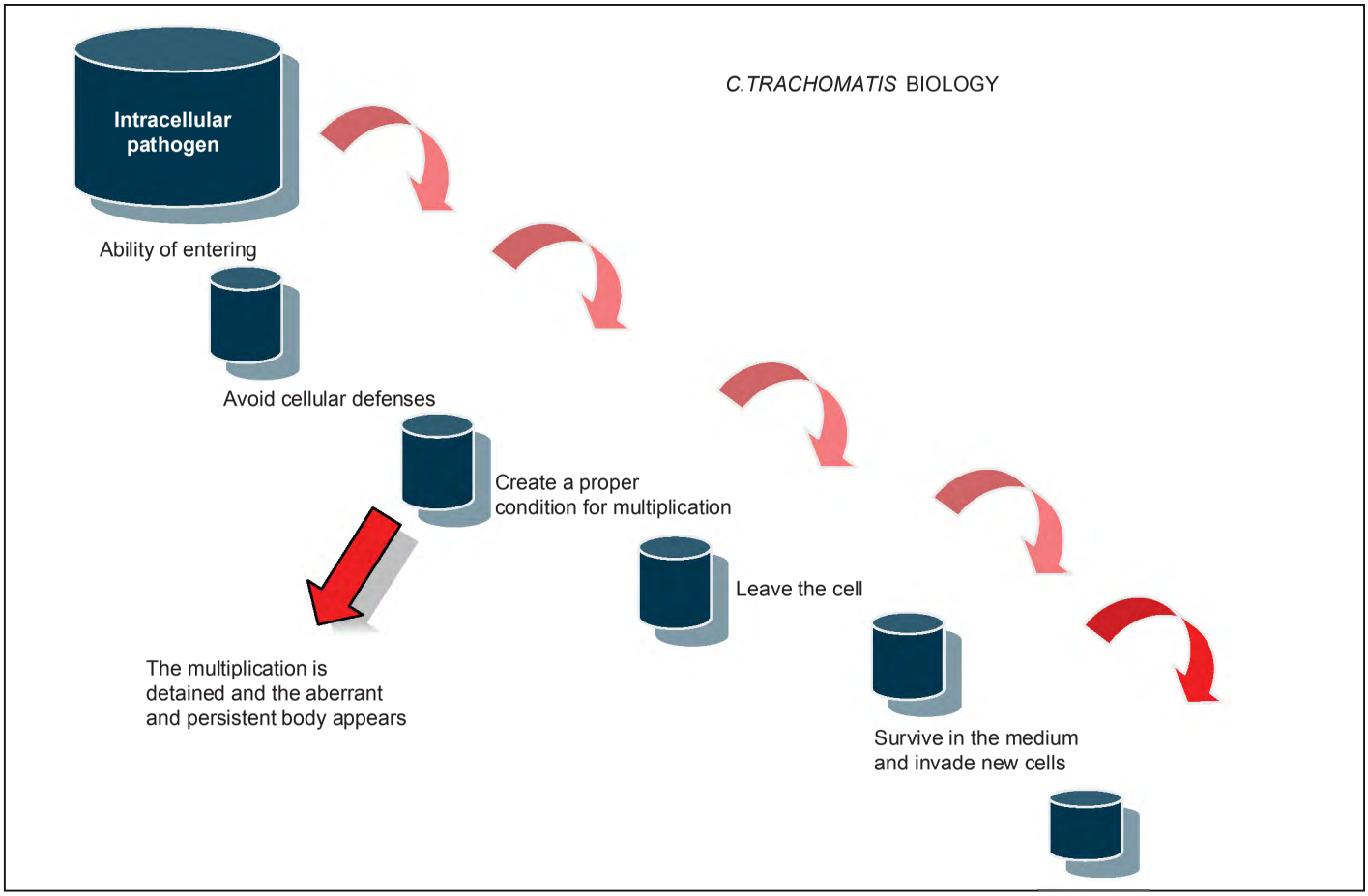



**Figure 3 –** Step followed by *Chlamydia trachomatis* to reach the intracellular inclusion body.

The in vitro persistence of large aberrant bodies follows several factors, among them the IFN-γ can remain in this condition for long period.[21] It is similar to what happens to other microorganisms such as *Toxoplasma gondii* and *Plasmodium*. The factors mentioned as facilitators of aberrant bodies and their persistence are fundamentally: penicillin use, depletion of tryptophan, and IFN-γ activity.

These aberrant bodies by remaining in the cells replicate with them and, based on a biological point of view, behave as bacterial plasmids; thus they remain inside the cells around 2 or 3 years. When they are not active, they are insensitive to the action of antimicrobials, even in the presence of those with the capacity of intracellular penetration.

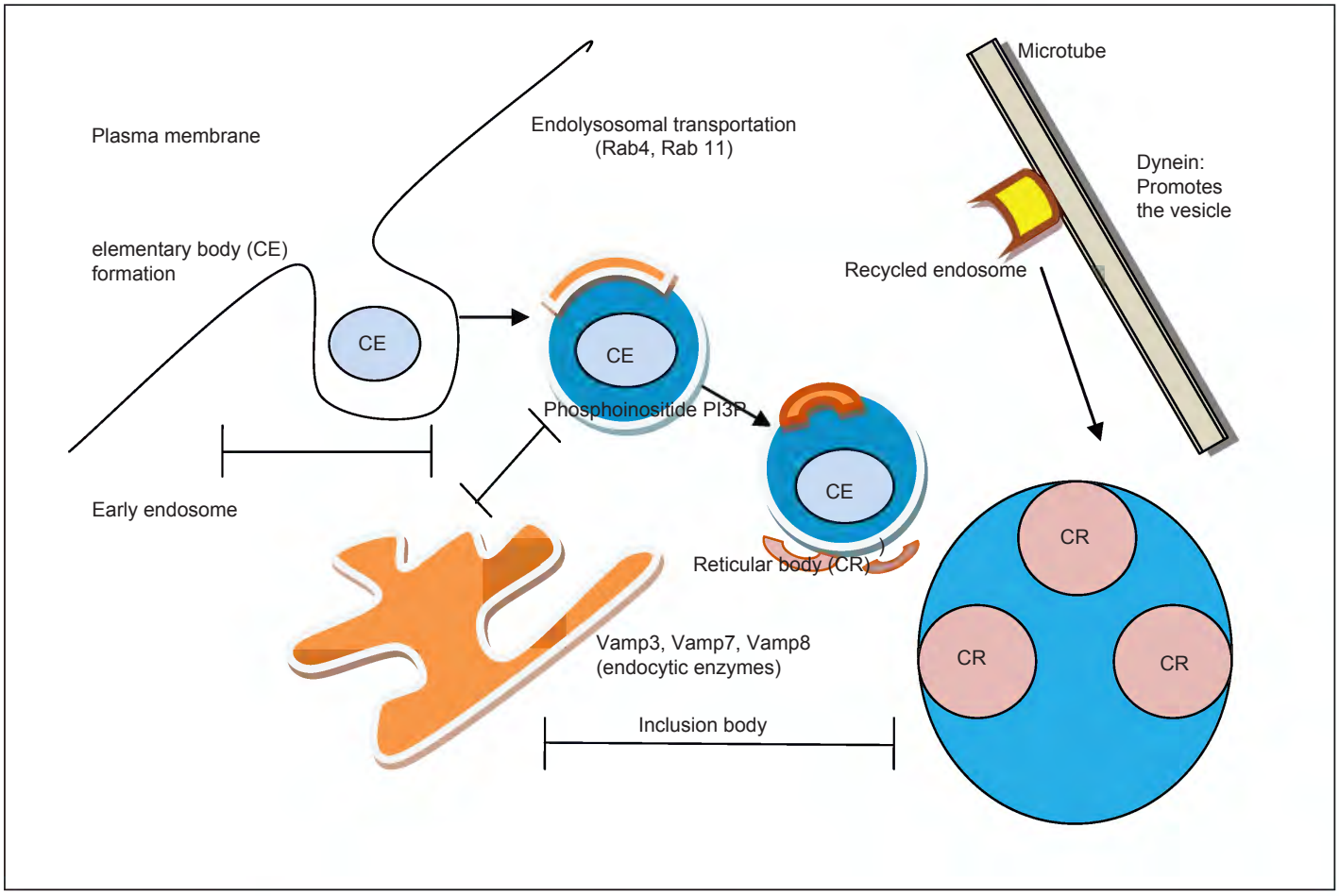



**Figure 4 –** Early intracellular cycle of *Chlamydia trachomatis*[33].

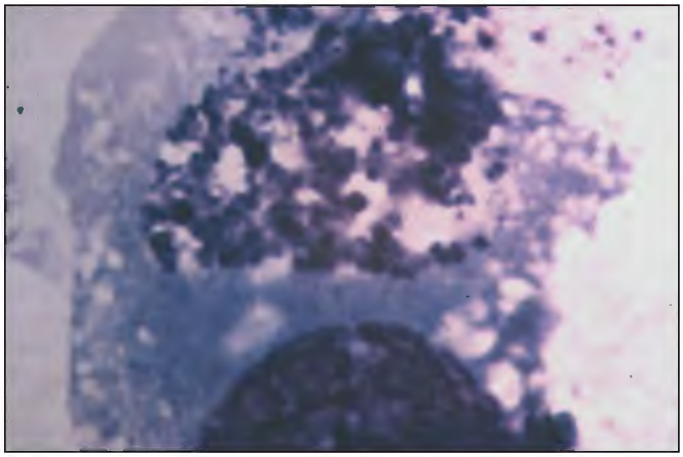


**Figure 5 –** Inclusion body releasing elementary body.

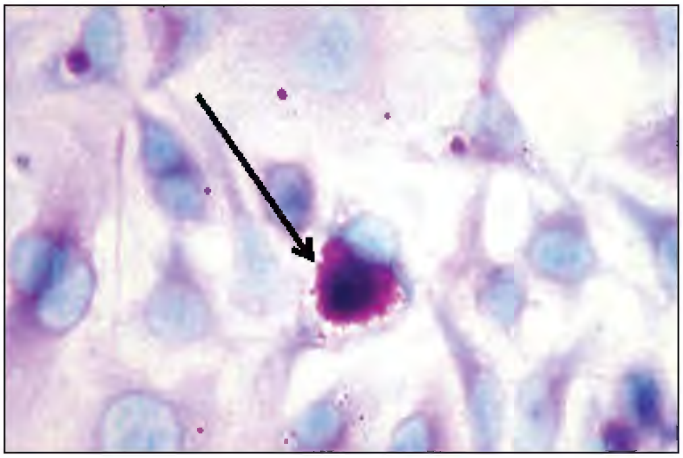


**Figure 6 –** Inclusion body in HeLa cells.

The detection of *Chlamydia* after a treatment with beta-lactam antibiotics suggests that its division is detained upon the incapacity of cell wall synthesis, and the presence of large aberrant bodies is seen. It is unknown how long it takes them to appear after exposure. The availability of biomarkers that might indicate this persistence is important[42].

Some points should be emphasized regarding the aberrant bodies of *Chlamydia* (suspended development):

- Cultures in cell lines that seem to be not infected, but the presence of *Chlamydia* with anomalous RB is found.
- The infection persists, because (1) the aberrant bodies can be transferred from a cell to another during cell division, (2) the aberrant bodies are capable of persisting during 2 or 3 years, (3) the infection can be induced through the immune response in the organism, (4) the aberrant bodies are relatively inert regarding the biochemical processes and less sensitive to the antimicrobials.

## IMMUNOPATHOGENESIS

The innate immunity is critical, since it is the early component of the host response. It was first identified by Rasmussen et al.[43] and Stephens[44], who demonstrated that the in vitro infection of cervical and colonic cells with CT induced the secretion of a great amount of chemoattractive cytokines and with proinflammatory activity. The CT internalization is not enough to promote a response, opposite to what happens with other microorganisms. The response is developed during the CT cycle. The endocervical cells release interleukin 1α (IL-1α) after the infection and the anti-IL-1α-specific antibodies inhibit the inflammatory cascade (Figure 9). Stephens' paradigm named "cell paradigm of clamidial pathogenesis" theorizes that "the inflammatory process of the pathogenesis is caused by infected cells and is necessary and enough for the development of a chronic inflammation and for the promotion of cell proliferation, tissue remodeling, and sequelae lesions"[45].

Observations using the electronic microscope reveal that the EB is associated with the epithelial cell 3 hours after the infection (HPI). After 12 hours of insertion, the transition to RB and the active division begins. However, the host response is early manifested at three HPI. There is also the appearance of 11 genes that codify receptors for CCR2 and CCR6 chemokines, the CCL3 chemokines (MIP-1 α), CCL20 (MIP-3 α), CCL24, CCL25 and CXCL15, the cytokines IL-1F8, IL-13, and the tumor necrosis factor alfa (TNF-α) in the cervical tissue. The CCL3 (MIP-1 α) and the CCL24 (eotaxin 2) are chemotactical for the immature dendritic cells and CXCL15 is chemotactical for polymorphonuclear. These are seen infiltrating the infected epithelium at 12 HPI, and the activity of Natural Killer (NK) cells has been early seen at 12 HPI, thus confirming that the chemokine gradient quickly develops after the infection[46]. The predictive value of the inflammatory response was seen in 1996[47], and then the migratory capacity of polymorphonuclear through endothelial cells infected with *C. pneumoniae* and stimulated with TNF-α[48,49].

## INFECTIONS

Recently, symptomatic and asymptomatic infections produced by CT in the area of gynecology and obstetrics were recognized: urethritis, cervicitis, PID, and perinatal infections. The asymptomatic condition and the latent infections constitute a real challenge for their research and prevention[50]. **Table 2** presents a summary of the infections and complications originated by CT.

### Cervicitis

Cervicitis can be asymptomatic or symptomatic. In general, it is seen as mucopurulent cervicitis (**Figure 10**). There may be hypertrophic, edematous, and bleeding ectropion. The presence of immature squamous metaplasia in the ectropion area has also been described

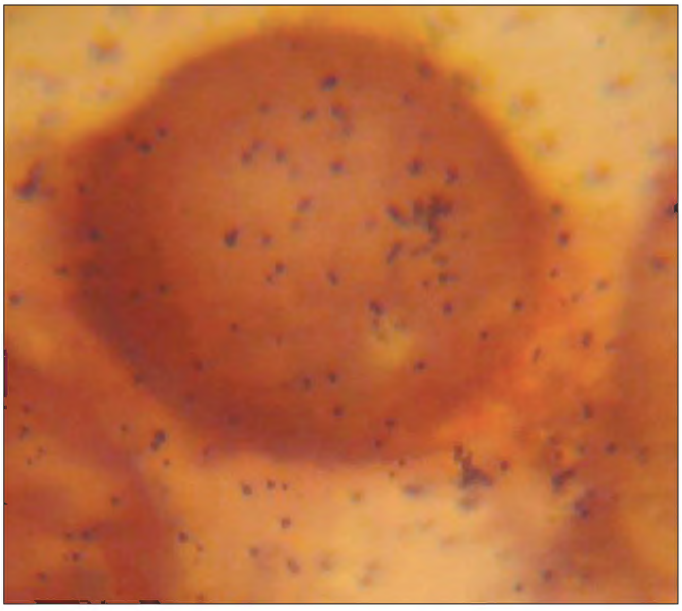


**Figure 7 –** *Chlamydia trachomatis* culture in HeLa. The black-colored intracellular lipids (LIC) are observed.

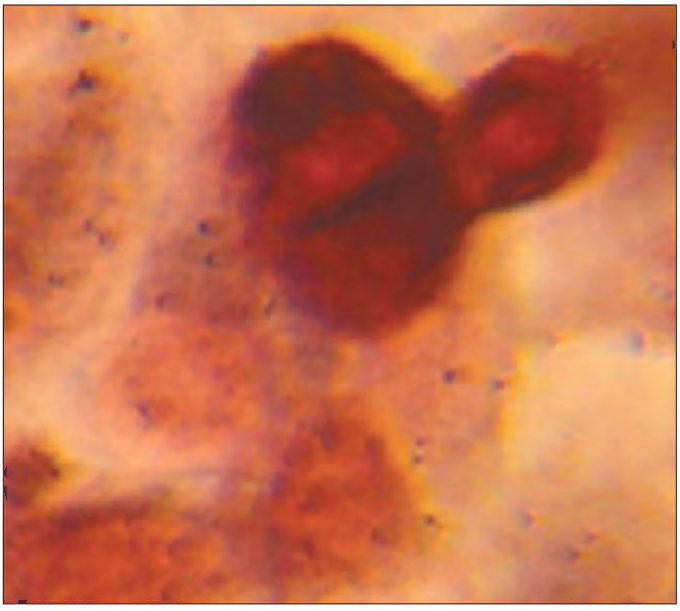


**Figure 8 –** Accumulation of lipid material.

by Paavonen and Eggert-Kruse[51] as an association with Chlamydia infection. Chlamydia infection can be related to the inflammatory response. The number of leucocytes can be a good index, although it is not exclusive.

The prevalence of cervical infection seems to be higher in women with ectropion, which predisposes to the acquisition of *Chlamydia*, since several columnar cells are exposed to the receptors or adhesins of the microorganism. This would explain the high proportion of adolescents with Chlamydia, because cervical ectropion is present in 60 to 80% of the sexually active female adolescents.

Oral contraceptive pills also promote the presence of ectropion; therefore, they are also a risk factor.

Clinical diagnosis of the mucopurulent discharge through Chlamydia is not conclusive. The differentiation with gonococcal cervicitis, salpingitis, endometritis, intrauterine device-induced inflammation, among other causes, should also be investigated. Thus, the clinical diagnosis of Chlamydia by professionals with a few or no training has a small correlation with laboratory data.

Almost none of the women with cervical infection develop antibodies against Chlamydia, and the presence of local antibodies has been reported in only 20 to 50% of the cases. In nontreated women, the sequential cultural study showed that the Chlamydia infection can persist for many weeks or months without showing any symptoms, or it can be spontaneously solved.

The detection of CT cervicitis in women with high risk of STIs and its treatment have showed a decrease of the PID incidence, which was also seen for *N. gonorrhoeae*.

## Urethritis

About 50% of the women studied by cultures conducted in the cervix and urethra show positivity in both areas, and 25% in one or the other area. The causes of dysuria syndrome include frequency of infectious urinary sediment and negative urine common culture.

**Table 2 –** Summary of *Chlamydia trachomatis* infections.

| In men | In women |
|---|---|
| Urethritis | Cervicitis |
| Prostatitis | Urethritis |
| Epididymitis | Endometritis |
| Vesiculitis | Salpingitis |
| Orchitis | Oophoritis |
| Proctitis | Abortion |
| Reiter syndrome | Reiter syndrome (less frequent) |
| | Perihepatitis |
| Perinatal infections: conjunctivitis, pneumonitis, pneumonia. | |

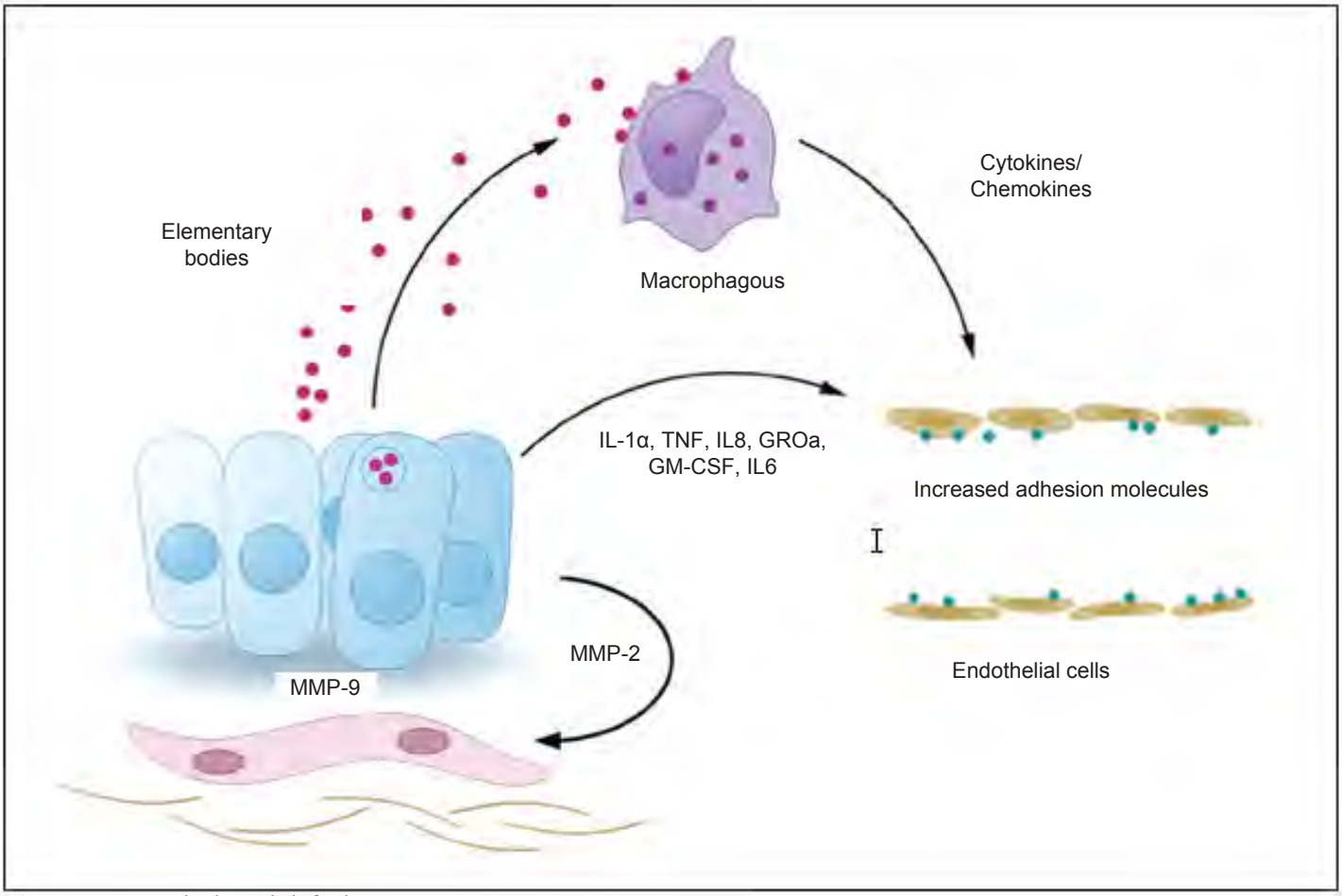



**Figure 9 –** Events in the early infection.

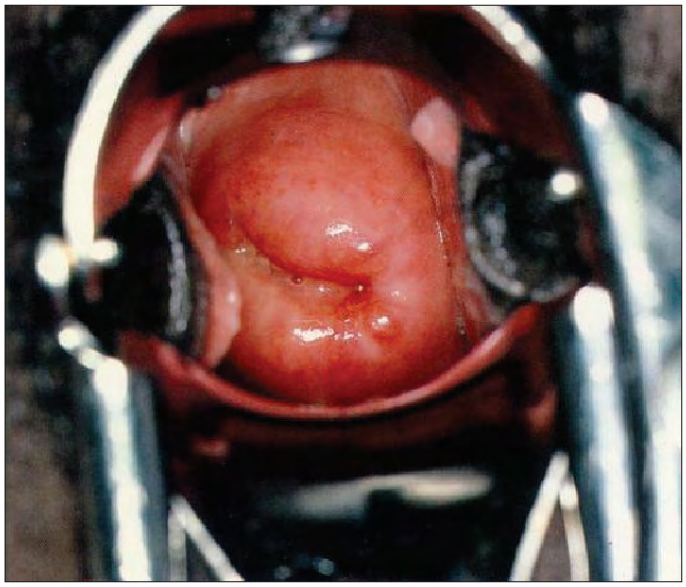


Figure 10. Cervicitis.

## Bartholinitis

The purulent infection of Bartholin's gland can be due to both *N. gonorrhoeae* and/or CT or other facultative or anaerobic microorganisms, which cannot be distinguished on a clinical point of view. The possibility of infection happens due to the existence of columnar epithelium in the glandular ducts. The concrete diagnosis is only achieved by means of a laboratory study.

## Endometritis

The disease is present in around half of the patients with mucopurulent cervicitis, and it is not frequent during pregnancy, although it can produce complications after such period.

It is characterized by the infiltration of the stroma with plasma cells and of the endometrial surface with neutrophil leukocytes.

## Lymphogranuloma Venereum

This is the only CT infection that leads to compromise and multisystemic manifestations. Many phases can be noticed: there is a transitory lesion followed by a secondary stage with suppurated regional lymphadenopathy. This is the period when the most important symptomatology is found, that is, the secretion, the painful adenopathy, and the fistulae. Sequelae associated with fibrotic changes and lymphatic drainage appear in the last or late phase.

## Pelvic inflammatory disease

Although the etiology of salpingitis remains uncertain, after improving the use of laparoscopy as a diagnosis method that allows to directly approach the tube to obtain samples destined to the etiological investigation, in the last decade, it has been established that CT is probably the most common cause, together with *N. gonorrhoeae*, of acute salpingitis cases or PID. As in *N. gonorrhoeae*, neither PID nor CT is frequent during pregnancy.

## Perihepatitis

Perihepatitis is a common manifestation associated with salpingitis because its observation in pregnant women is less usual.

## Proctitis

Chlamydia proctitis represents a disease that is not commonly diagnosed in women with digestive or rectal manifestations. It is a cause of abdominal pain in adolescents. One should carry out lab tests to avoid subdiagnosis of these infections. It is important to remember that the intestinal tract might change to a reservoir for CT[52].

## Infections associated with arthritis

We must distinguish septic arthritis that has microorganisms in the joint and that is generally the product of systemic dissemination, as seen in the cases of disseminated gonococcal infection, from reactive arthritis, like in most of the cases, which are multifactorial. The most common form of arthritis associated with *Chlamydia* is the one developed after a urethritis condition that has been named: sexually acquired reactive arthritis (SAR).

## Participation in preterm birth

The CT infections in pregnant women vary from 2 to 30% based on the studied population. There is no reliable information regarding the influence of pregnancy in the physiopathology of Chlamydia infections, or about its role in perinatal prematurity and mortality. The association of premature birth with IgM antibody titer and CT-positive culture was confirmed by Sweet et al.[53]. This correlation is expected, but more prospective studies with a larger number of patients should be carried out.

The same happens regarding the relation between CT and spontaneous abortion. In animals, as explained, it has been seen that *C. psittaci* is an important cause of abortion. Some authors[54] have reported the relation of CT with spontaneous abortion. However, we believe it is still early to provide a definite role of this microorganism in the spontaneous abortion pathology. With regard to the post-partum or post-abortion infections, endometritis and salpingitis were the most significant ones. Some studies show these complications happen in a significantly higher level among women who were infected with Chlamydia during pregnancy. Several authors have found cervicitis due to Chlamydia in 6.7% of women during the 19th week or a lower stage of pregnancy. Women who are early infected have higher risks of giving birth to dead fetuses than women who are not early-infected (6 of 18.33% versus 8 of 23.34%, respectively). Some changes in the duration of pregnancy among positive women and 238 noninfected women ($p < 0.001$) were seen. However, in other studies including 9,000 patients, there was no significant association between cervical infection by Chlamydia and preterm birth. Techniques that are more sensitive might be needed to conduct studies in pregnant women, although all information should be

reviewed and the prevalence of the infection in the community and the risk factors for women to have STI should be considered as well.

## Neonatal and perinatal infections

A child can acquire Chlamydia through aspiration of the infected secretions that pass though the birth canal or if he/she is born via C-section, by infection of the membranes that suffered spontaneous early rupture before birth. The infections include conjunctivitis; nasopharyngitis; pneumonia or pneumonitis, and vaginitis.

## Other complications

CT can participate in oncogenic processes, thus increasing the risk of cervical cancer, causing the duplication of the host cell genome due to alteration and rupture of cell spindle and other alterations, as already seen [39,40].

It is worth mentioning that adolescents, mainly female ones, are under risk of acquiring this preventable infection, but most of the times, they do not know about the care that should be adopted. Therefore, if the infection is not seen early, it could cause severe damage to their lives.

# DIAGNOSIS

The diagnosis methods are based on:

- CT isolation in cell cultures;
- direct detection of different Ag in the clinical sample;
- molecular tests like PCR or LCR;
- serological studies that allow investigating antibodies, which are limited only to some pathologies, since they involve the antigenic persistence in the mucosa, during a relatively long period.

The used technique must be based on the characteristics of the studied population and such purpose (**Table 3**).

## Detection of antibodies

The techniques used are establishment of complement, microimmunofluorescence (MIF), immunofluorescence against the inclusion body, enzyme immunoassay, and detection of CT HSP. These techniques must be carefully evaluated and have limited value (**Table 4**).

We shall remember that for definite diagnosis, more or less complex methods are required, such as culture in cell lines. Currently, there are some molecular techniques available based fundamentally on the amplification of nucleic acids that provide great sensitivity and are very valuable for epidemiological studies, since they allow carrying out a study in the urine. The techniques that find Ag, ELISA, direct immunofluorescence (IFD), or chromatographic ones should not be used to evaluate the treatment in patients that had a positive clinical response, since Ag can continue for several months.

Several teams use different supports and methodologies to detect CT Ag and Ac. When a comparison evaluation is done on the same populations, notable differences are found and, based on it, one or the other method is used with different results. This can cause the adoption of erroneous measurements in the epidemiological research or in the establishment of therapeutic standards (**Tables 5 and 6**)[55-57].

**Table 4 –** Techniques for antibody detection.

| Test | Number of organisms/sample |
|---|---|
| DNA/RNA amplification | 1–10 |
| Culture | 10–102 |
| DFA | 10–103 |
| EIE | 103–105 |
| Probes | 103–104 |

DFA: direct immunofluorescence antibody; EIE: enzyme immunoassay.

**Table 3 –** Diagnostic guidelines of *Chlamydia trachomatis* in women.

| Diagnosis | Clinical criteria | Laboratory criteria | |
|---|---|---|---|
| | | Assumption | Certainty |
| Mucopurulent cervicitis | Mucopurulent cervical exudate, cervical ectropion, spontaneous, or induced easy bleeding. | Inflammatory response (>10 L/400´) in the absence of an acknowledged etiological agent (*T. vaginalis*, *C. albicans*, among others). | Positive culture or positive direct test in cervical smear test material. |
| Dysuria syndrome | Miccional difficulty with or without pain, including polyuria and frequent urination, in sexually active women without neither a structure change of the urinary apparatus nor immune deficiency, which is usually associated with a new partner. | Infectious sediment without conventional bacteriuria. | Positive culture or positive direct test in urethral and/or cervical swab. |
| Pelvic inflammatory disease | Pain around the lower abdomen, painful uterine or adnexal mobilization, palpable mass in the rectouterine pouch. Frequent presence of purulent cervical discharge. | It is similar to that of the mucopurulent cervicitis. Presence of purulent material in the puncture of the pouch of Douglas or in a direct tube laparoscopic sample. | Positive culture or positive direct test in endocervix. Tubal, endometrial, or rectouterine pouch material (rare in the latter). |
| Perihepatitis | Right upper quadrant pain, vomits, fever, sexually active female individual, evidence of recent or concurrent pelvic inflammatory disease. | It is similar to mucopurulent cervicitis or pelvic inflammatory disease. | IgM or IgG antibody titers against high *Chlamydia trachomatis*. |

CT screening in sexually active young population has low cost and great effectiveness when the population prevalence of the infection exceeds 3 to 6%. A nondiagnosed or treated STI can result in severe complications; therefore, we should pay attention to the asymptomatic characteristic that CT infection has in adolescents.

A very common error is to request the diagnosed and treated patients a new test to prove the treatment efficacy immediately. This is a problem since it could provide false-positive results that lead to unexplainable re-treatments. The Center for Diseases Control and Prevention (CDC) does not recommend the "healing" test in patients with CT continuously treated, but they recommend conducting a new monitoring after 3 or 4 months (**Chart 1**)[10]. The currently recommended treatment for cervical infection is the single dose of 1 g of azithromycin (the PID doses have not yet been reliably established).

Today, the use of azithromycin is recommended in other pathologies to avoid resistance to this microorganism.

For a successful treatment, these guidelines should be followed:

- complete the entire treatment, even if the symptoms disappeared before it is cured;
- sexual partners must be controlled and treated;
- no sexual intercourses until the infection is totally healed.

Today there are several efficient antibiotics to be used in short-term treatments, including single-dose antibiotics. Other antibiotics require a longer treatment, yet also effective, but they are usually abandoned after the symptoms disappear before the treatment conclusion or due to their side effects.

Some common aspects in the prevalence of CT infection include:

- it could be conducted without a speculum;
- screening is important to control PID;
- it can be conducted using enzyme immunoassay (EIE) if the prevalence is around 5 to 7%;
- universal screening if the prevalence corresponds from 10 to 12%, which is selective if below 5 to 6%;
- screening should be conducted every 6 months;
- diagnosis and treatment of CT infections seemed to significantly reduce the ectopic pregnancy.

**Table 5 –** Related limits of detection with different technologies[56].

| | Culture (%) | EIE (%) | DFA (%) | NAA (%) |
|---|---|---|---|---|
| S | 40–60 | 65–75 | 70–75 | 90–98 |
| E | 100 | 90–95 | 90–97 | 98–99 |

S: sensitivity; E: specificity; EIE: enzyme immunoassay; DFA: direct immunofluorescence antibody; NAA: nucleic acid amplification.

**Table 6 –** Techniques for the Ag detection of *Chlamydia trachomatis*[56].

| Techniques | Prevalence (%) |
|---|---|
| Immunofluorescence | 14.3 |
| Ag: MOMP | 14.3 |
| IgG detection with EIA | 11.9 |
| Ag: HSP 60 (cHSP60) (Medac) with EIA | 23.2 |
| Immunoassay PID-fluorescence (InoDiag) | 26.2 |

MOMP: Major Outer Membrane Protein; EIA: Enzyme Immuno Assay; HSP: Heat Shock Protein; PID: Pelvic Inflammatory Disease.

## PERSISTENT INFECTIONS DUE TO *CHLAMYDIA TRACHOMATIS*

Bear in mind what was explained about aberrant bodies that create persistent infections. It is very hard to establish an adequate therapy for the habitual behavior of these CT forms. It is possible that, despite the correct treatments, the infection persists. When these aberrant bodies persist on the cell, they multiply with them and remain inside them for 2 or 3 years.[55]

## VACCINES: PROGRESSES AND CHALLENGES

Still no vaccines are available for CT or *C. pneumonia*, which can be used in humans. However, the existence of some successful vaccines in the veterinary field might hold some expectations. They include mitigated or EB established as immunogens, they produce short-term immunity and, therefore, they require reinforcement doses to maintain a proper protection[58].

The Ag that develop protective immunity have been studied around three decades. Therefore, based on the conventional techniques, the findings were limited. When the CT genome was available, it was possible to find some advances. Around 900 proteins are codified, and each one can be cloned for the analysis of specific Ag based on the response that is manifested during the chlamydial infection. The potentially useful Ag for vaccinations are presented in **Figure 11**.

**Chart 1 –** Guide of general therapeutics[10].

| |
|---|
| There are several therapeutic proposals regarding antimicrobial doses and time of administration.<br>Based on our experience, we are in favor of a 15-day average for most of the medications used nowadays, with the exception of azithromycin.<br>In each scheme, we will mention the useful drugs that have been commercialized in our environment: |
| Noncomplicated male urethritis, cervicitis, or proctitis<br>Minocycline or doxycycline: 100 mg in 12 hours.<br>Tetracycline: 500 mg, four times a day.<br>Erythromycin: 500 mg, four times a day.<br>Roxithromycin: 150 mg every 12 hours.<br>Ofloxacin: 400 mg in 12 hours*.<br>Ciprofloxacin: 500 mg in 12 hours*.<br>Levofloxacin: 500 mg daily orally, for 7 days*.<br>If the clinical evolution has a favorable scenario, the recovery evaluation using a laboratorial diagnosis test will not be necessary. |
| Chlamydia trachomatis infections in pregnancy<br>Erythromycin: 500 mg, four times a day.<br>Roxithromycin: 150 mg in 12 hours.<br>The administration of erythromycin estolate during pregnancy period is not recommended because of the hepatotoxicity it might cause (thus, the azithromycin use is established). |
| Pelvic Inflammatory Disease<br>The treatment implies the association of an antimicrobial on activity over aerobic, anaerobic, and facultative bacteria, which are associated with an anti-chlamydia antimicrobial. |
| Dysuria syndrome: with macrolides or azalides. |

It should not be administered to subjects younger than 14 years old.

Efforts should be given to correctly identify the protective Ag causing vigorous response of T CD8+ cells.

Stary et al.[59] commented that CT infection induces a protective immunity that depends on interferon and on the T CD4 cells. We know, in general, that subcutaneous or intramuscular vaccination could create a systemic and cutaneous effective immunity to several pathogens, but the vaccination through these nonmucosae routes usually do not induce, or induce very little, the protection in mucosae surfaces like the one that CT requires. In very interesting experiments done by the investigators, the inactivated CT mucosa exposure through ultraviolet light (UV-CT), with charge-switching synthetic adjuvant particles (cSAP), creates an extended protection in rats. The UV-CT-cSAP vaccine induces a memory in T cells, regardless of the route that is employed, but only the mucosa route induces effective T cells that will be needed together with the T cells of resident memories (TRM) to produce the clearance or elimination of CT.

## MAIN POINTS

1. CT is a very common STI, more frequently reported in the United States, estimated in 3 million cases every year.
2. The cost estimated by the CDC of CT consequences is around two million or more dollars and that of the screening and treatment programs is around 175 million dollars.
3. Each dollar invested in the control saves 12 million in the complications of the nontreated cases.
4. It is the most common STI among female sexually active adolescents and young adults: 1 in every 10 subjects will be infected at some time. The most frequent age group is between 15 and 19 years. This group represents 46% of all infections.
5. It can be asymptomatic and its beginning indolent, and causes cervicitis, urethritis, and endometritis. Up to 40% of nontreated women develop the upper genital tract infection or PID, which can be silent, especially among adolescents.
6. The nontreated PID can cause sterility, chronic pelvic pain, and ectopic pregnancy.
7. Fifty percent of the children exposed to the birth canal develop conjunctivitis and from 10 to 16% develop pneumonia.
8. Diagnosis can be directly made in clinical samples by means of several immunological and molecular techniques.
9. These techniques should not be used for controlling the therapy and should never be used immediately after the treatment.
10. Culture in cell lines should be considered for conflicting cases.
11. Do not forget about the possibility of persistent cases, which do not respond to therapy.

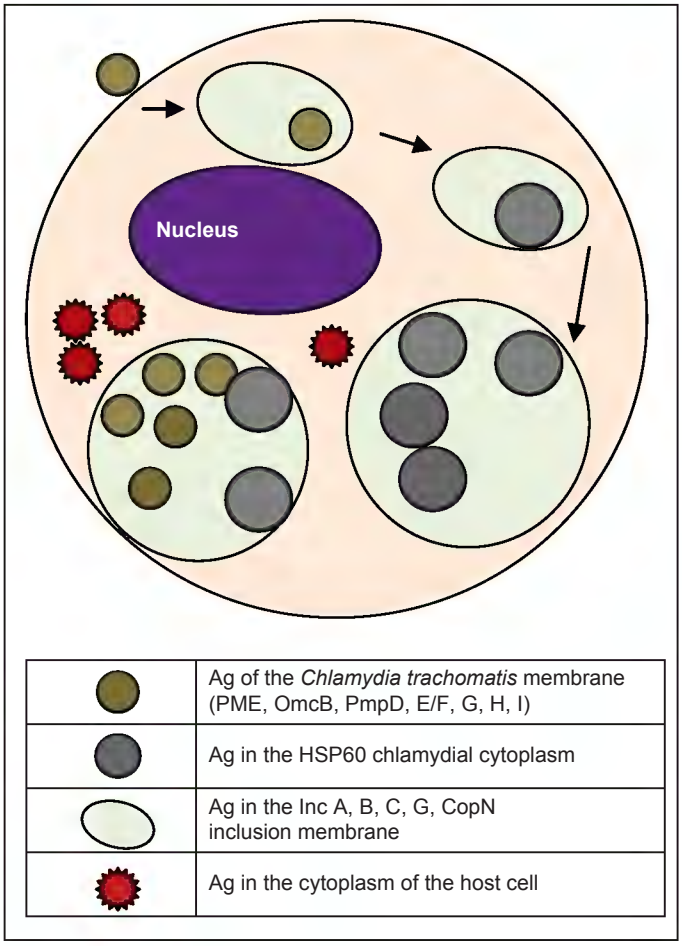



**Figure 11 –** Useful antigens for vaccines.

### Conflict of interests

The authors report no conflict of interests.

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