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An integrated approach to the understanding of *Chlamydia trachomatis* infection

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ANIMAL MODELS FOR THE STUDY OF *CHLAMYDIA TRACHOMATIS* INFECTIONS IN THE FEMALE GENITAL INFECTION

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Summary

Despite intensive research on chlamydial pathogenesis and host immune responses, vaccine development has been hampered by incomplete understanding of the virulence factors and the critical factors governing protective immunity. It is unknown why certain *Chlamydia trachomatis* serovars cause asymptomatic or symptomatic infection or ascend to the upper genital tract. Former studies have used nonprimate monkey, mice or guinea-pig infection models. However, pigs are genetically and physiologically related to man and are susceptible to chlamydial infections. The main objective of the present study was the validation of the pig as an alternative animal model for *C. trachomatis* female genital tract infection.

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Introduction

Sustained genital tract infection with oculogenital serovars of *Chlamydia trachomatis* has been established in a number of animal

Table I: Animal models for the study of C. trachomatis infections in the female genital tract.

Animal model	First publication year (ref)	Origin	First author
Mouse	1981 (6)	Europe	M. Tuffrey
	1981 (7)	USA	A.L. Barron
Primates			
Baboon	1936 (1)	USA	P. Thygeson
Macaque	1983 (8)	USA	D.L. Patton
Pig	2005 (9)	Europe	D. Vanrompay

[a] versus [b]: $p < 0.05$ (bootstrap technique; 43); IgG: *C. trachomatis* IgG antibodies (micro)immunofluorescence test (Anilabsystems, Finland); lipopolysaccharide (LPS): *Chlamydia* LPS ELISA (Medac, Germany); IgA: *C. trachomatis* IgA enzyme immunoassay (Anilabsystems, Finland); cHSP60: cHSP60 IgG ELISA (Medac, Germany); high-sensitivity CRP (hs-CRP): CRP ELISA (DiaMed Eurogen, Belgium).

species beginning with the earliest effort to demonstrate Koch's principle of causality in cervicitis, which was performed in baboons in 1936 (1). In addition to a number of other nonhuman primates (2–4), seldom used models of both lower and upper female genital tract infection in rabbits, rats, and cats have been developed (5). Mice became and continue to be the small animal of choice since first shown to be susceptible to genital tract infection in 1981 with human isolates of *C. trachomatis* by Tuffrey and Taylor-Robinson (6) and with the mouse pneumonitis agent, MoPn, by Barron *et al.* (7).

This review will describe briefly the two major animal models to study *C. trachomatis* female genital tract infections, the primate model and the mouse model. Subsequently, a potential new model will be discussed, the pig model (Table I).

Mouse model

Following the simultaneous demonstration in 1981 of the susceptibility of the female mouse genital tract to infection with both the mouse pneumonitis agent (*Chlamydia muridarum*, MoPn) by Barron *et al.* (7) and human genital tract isolates of *C. trachomatis* by Tuffrey and Taylor-Robinson (6), the mouse became and remains the most common animal employed in experimental studies to identify the outcome influencing factors involved in the interplay between the host's complex immunopathic response to infection with *C. trachomatis*, and the equally complex, phenotypically variable, and environmentally responsive, biphasic intracellular developmental cycle of this unique pathogen. In addition to susceptibility to infection, there are many reasons for the selection of the mouse as a model host, including the existence of a large number of immunologically well-characterized inbred stains, the ability to modify disease-associated genes through crossbreeding and gene insertion and deletion techniques, as well as their size, ease of handling, and availability in sufficient numbers to assess the role of both major and minor components of this dynamic process.

With the exception of the infrequently used and unnatural direct upper genital tract injection models that demonstrated the ability to reproduce in the mouse a form of infertility associated with *C. trachomatis* infection (10, 11), most investigators currently employ slightly modified versions of the progesterone-enhancing model of Tuffrey and Taylor-Robinson (6). In this model, mice are pretreated with progesterone in order to induce a protracted and productive infection requiring diestrous/anestrous state within the genital tract (12), which allows for high incidence and prolonged infection following the noninvasive inoculation of the vagina with 10^2 to 10^7 inclusion-forming units (ifu) of *Chlamydia*. However, the number of infectious units necessary to establish infection and the course and outcome of infection varies significantly between MoPn and human isolates. Lower genital tract infection with MoPn, which is the most commonly used agent in this model, can be established with as few as 100 ifu and is a rapidly progressive process that causes severe upper genital tract pathology and infertility at high incidence following a single infection (13, 14), while infection with strains belonging to the human oculogenital biovar (serovars D–K) requires 10^4 ifu and infection is limited in its ability to ascend with any major pathological consequence from the initial site of infection within the lower genital tract of the mouse (14, 15).

The results obtained over the 20-year use of MoPn in the mouse model of *C. trachomatis* female genital tract were recently reviewed by Morrison and Caldwell (16) who emphasized the opinion that significant parallels exist in both the disease processes and immune responses that develop following infection of the mouse with MoPn and human infection with the oculogenital biovar. Common to murine and human infection are the development of protective immunity, the importance of Th1 CD4 T-cells in immunity, the induction but unknown significance of CD8 T-cells, a yet to be defined role of B-cells and antibody in protective immunity, and similar histopathology. Although perhaps true for the responses made during the most severe but infrequent upper genital tract sequelae associated with infection, the parallels with the asymptomatic and noninvasive course of infection that occurs in most women are less obvious and difficult to assess in such an invasive infection. This review excluded results obtained in studies using human isolates as being uninformative because of the noninvasive character of mouse genital tract infection with these strains.

Over the same period a much smaller number of studies have been conducted with strains belonging to the human biovar. Like MoPn, all commonly used mouse strains are susceptible to infection (14, 17, 18) and, although the dose required to establish infection with human isolates is significantly greater, variation in the level of shedding and duration of infection varies between serovars in an informative way among strains of the oculogenital biovar (15). With the exception of one inbred mouse strain, C3H, infection can spread in a retrograde fashion from the cervix and into the uterine horns where varying degrees of inflammation, as evidenced by hyperemia and distension, have been observed which varies in incidence and degree among mouse strains. Infection is self-limiting and without severe sequelae, and, where tested, appears to confer some level of Th1 dominant protection against reinfection. However, in C3H mice the course of infection with a strain of serovar E has been shown to run a more invasive course that results in disruption of the fallopian tube architecture and a loss of fertility in a low percentage of mice (14, 19). It was noted that the histological features of the inflammatory process were less severe and without accompanying hydrosalpinx, which is the hallmark of the upper tract disease associated with MoPn infection (19). The mechanisms that account for these differences is unknown but does hold promise that the more severe outcomes associated with human infection may have a closer parallel in this combination mouse strain and human isolate than is provided with MoPn.

The utility of an animal model of human disease is dependent upon its ability to mimic the disease process as it occurs in humans in order to identify characteristics of both the host and the agent that participate in the process, and in so doing to provide translationally valuable data on which to propose and test intervention strategies. Although compelling in its focus on the most severe outcomes of infection, the appropriateness of MoPn as a surrogate for the diverse collection of serovars that comprise

the human disease causing oculogenital biovar of *C. trachomatis* was recently questioned by Morré *et al.* (20). These investigators suggested that more translationally valuable information might be derived from a more systematic study of the human biovar in this model, based on an increasing number of *in vitro* and murine model studies (21–24) that describe significant differences in potential outcome altering phenotypes between these biovars (15, 16, 18), and the well-documented fact that infection of the mouse with human isolates mimics the more limited and less severe course of infection in most women with clinically confirmed *C. trachomatis* infection (25, 26). In a series of studies using an integrated approach described elsewhere in this supplement, Lyons *et al.* have demonstrated a correlation between differences in certain *in vitro* and murine model-derived phenotypic characteristics of strains belonging to the human oculogenital biovar and human epidemiological and clinical data (15, 24, 27). Expansion of this approach and a more systematic study of the human genital tract disease-causing biovar of *C. trachomatis* would likely provide a more productive path to follow in the future.

Primate model:
Pig-tailed macaque monkeys

In the early 1980s Patton *et al.* (28, 29) described a model of experimental acute salpingitis in pig-tailed macaque monkeys, *Macaca nemestrina*, by intratubal inoculation with *C. trachomatis* (serotypes E or F). The organisms were reisolated from both the endosalpinx and endocervix, and histopathological examination of the endosalpinx following infection showed epithelial degeneration and deciliation of ciliated cells. An antibody response to the infecting strain of *C. trachomatis* was demonstrated in monkey sera and in cervical secretions by the microimmunofluorescence test and cellular immune responses were demonstrated. These results indicated that the pig-tailed macaque could be a suitable model for further studies of the pathogenesis and pathophysiology of, immune responses to, and therapy for acute *C. trachomatis* salpingitis. A few years later it was shown that this model could also be used for kinetic studies of chlamydial infection and immunity as based on the results obtained from subcutaneously implanted fallopian tube autografts (30).

As in humans, it was shown that repeated homologous and heterologous infections with the serovars F, D, and J produced extensive tubal scarring, chronic salpingitis, and distal tubal obstruction, findings not apparent following a single infection (31). On the other hand, when pig-tailed macaques are inoculated in the cervix with *C. trachomatis* and after spontaneous cessation of cervical shedding of *C. trachomatis*, repeated inoculation either failed to produce infection, or resulted in infection of shorter duration with lower inclusion counts. Thus, cervical infection may lead to protective local immunity in pig-tailed macaques (32).

The role of delayed-type hypersensitivity (DTH) in the pathogenesis of *C. trachomatis* has been suggested in humans as the major cause of the development of late complications. DTH to chlamydial antigens was analyzed in the primate subcutaneous salpingeal autotransplant model by *C. trachomatis* serovar E by Patton *et al.* They showed that heat shock protein 60 was the only antigen to induce DTH from among the antigens tested, which included UV-inactivated organisms, recombinant major outer membrane protein, purified outer membrane proteins, and heat shock protein 10 (33). Interestingly, histological findings of the salpinx were consistent with the type of DTH reaction observed in ocular *C. trachomatis* infection, suggesting a similar pathogenesis for both salpingitis and trachoma (34).

The clear differences in the course of infections between women, symptomatic versus asymptomatic, and the development of late complications versus no complications, could be due to individual genetic differences. In the macaque model the development of chlamydial pelvic inflammatory disease (PID) varies between individuals in the conditions associated with the onset of intrapelvic adhesions. Some animals develop adhesions rapidly, within 2 weeks after a single tubal inoculation with *C. trachomatis*, while in others, adhesions are not observed until 2 weeks after a second tubal inoculation. Lichtenwalner *et al.* (35) tested whether this variability correlated with major histocompatibility complex (MHC) class I haplotype. They showed that as many as five different MHC class I alleles, or as few as two different MHC class I alleles could be correlated with risk of adhesion formation, concluding that in macaques, susceptibility or relative resistance to rapid formation of tubal adhesions is correlated with expression of MHC class I alleles, consistent with reports of MHC class I restriction of chlamydial immunopathology in humans.

Pig model

Pigs were studied as an alternative large animal model for studying human *C. trachomatis* infections. Pigs are physiologically and genetically more related to man than mice are, in addition to being practically and ethically more convenient to use compared to nonhuman primates. Moreover, Tuggle *et al.* (36) have produced cDNA libraries containing the majority of genes expressed in major porcine female reproductive tissues and subsequently confirmed the broad expression of many of the same genes ubiquitously expressed in human genital tissues. Pigs are susceptible hosts for *Chlamydiaceae*, which cause clinically inapparent infections as well as a wide variety of disease syndromes, including reproductive disorders. Pigs are naturally infected with *Chlamydophila abortus*, *Chlamydophila pecorum* and *Chlamydia suis*. The latter species being phylogenetically related to human *C. trachomatis* strains.

Fifteen, 16-week old specific pathogen-free (SPF) outbred female pigs (gilts) (Intervet Akzo Nobel) were randomly assigned to three groups (A–C) of five, each reared in separate isolation units. Group A was intravaginally infected with 10^8 $TCID_{50}$ of *C. trachomatis* strain 486, a serovar E genital isolate from a female patient with a symptomatic clinical course in both the patient and her partner (37), while group B received the trachoma type E Bour strain (ATCC, VR-348B) (3). Group C was inoculated with 2-SP serving as a noninfected control.

Clinical signs were not observed except for a reddish vulva and fever, which has been described in women (38, 39). At autopsy, group A showed more severe gross lesions as compared to group B, although most prominent gross lesions for both groups could be observed in the cervix and the uterus of all gilts euthanized at 21 days postinfection. The observed inflammation and edematous thickened cervical and uterine tissue has been reported in women with acute *C. trachomatis* salpingitis (40).

Chlamydial excretion was observed in all gilts examined at 7, 14 and 21 days postinfection. Vaginal excretion was significantly higher ($p \leq 0.05$) for group A as compared to group B. A possible explanation could be the additional replication of strain 468 in the urethra, whereas Bour strain was undetectable in the urethra (Fig. 1). Bour strain was mainly present in epithelial cells of the lower genital tract with clear replication in the vagina and the cervix, while strain 468 was found more often in epithelial cells throughout the urogenital tract and even replicated in the oviducts. Thus, experimental intravaginal inoculation resulted in an ascending infection, although strain 468 ascended faster and probably more intensely than Bour strain. However, the latter remains to be proven as all animals were sacrificed at 21 days postinfection. The ascending character of chlamydial genital tract infection has also been demonstrated in guinea pigs, mice and humans (37, 41). There was no replication outside the genital tract, which is in accordance with the literature stating that only lymphogranuloma venereum strains invade the submucosa to infect macrophages and disseminate to regional lymph nodes (42).

Both serovar E strains caused acute inflammatory infiltrations in the uterus and oviducts. Uterine edema and cell vacuolization, as well as oviduct cell vacuolization and hyperplasia were observed. Lesions were similar to those observed by Ramsey *et al.*

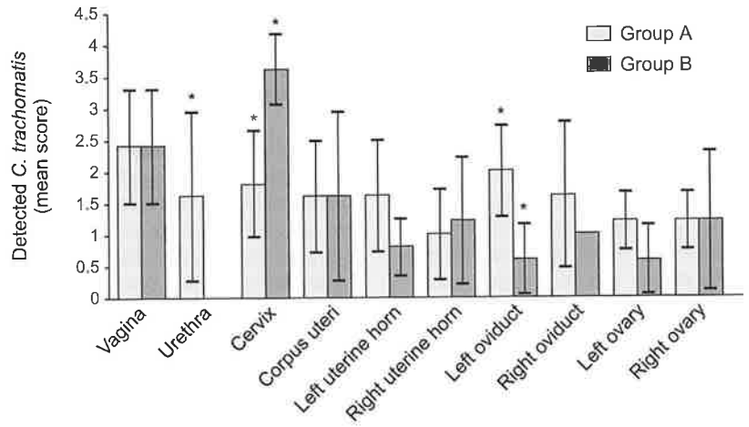

Fig. 1. Detection of *C. trachomatis* in urogenital tract tissues 21 days postinfection. *Statistically significant difference ($p<0.05$) between group A and group B.

(21) following a primary murine intravaginal infection with a strain of *C. trachomatis* serovar E in the highly susceptible C3H mouse strain.

The infection resulted in antibody titers in sera and genital secretions. At 14 days postinfection, mucosal IgA titers, likely synthesized locally in the genital tract, were significant higher than those observed in the sera. This is consistent with the observation made in women by Kozlowski *et al.* (43). Mucosal antibody titers for group A were significantly higher than for group B, probably due to more severe epithelial lesions and diffusion of serum antibodies and/or greater antigenic stimulation as a result of more intense replication.

In summary, serovar E strains Bour and 468 could ascend in the genital tract of gilts. Both strains replicated in the superficial epithelial cervical and uterine layers, which are known to be target sites for a *C. trachomatis* genital infection. Inflammation and pathology occurred at the replication sites and the organisms triggered antibody responses. Our findings imply that the pig may be useful for studying the pathology, pathogenesis and immune response of *C. trachomatis* genital infection, and may function as either an intermediate animal model between mouse and nonhuman primates or as a substitute for the latter during the development of vaccines for use in human clinical trials.

Conclusions

None of the animal models currently used to study human female genital tract infection with *C. trachomatis* perfectly mimics the human reproductive system (anatomy, histology, endocrinology) or the pathogenesis and immune responses that occur during a human genital infection. Successful vaccine development will depend on research that meaningfully extends the findings of the murine model in a way that translates into possible candidates for testing in primates for ultimate use against human genital tract infection. The newly developed pig model could contribute to and expedite this process, as the anatomy, histology, endocrinology and immunology of the pig genital tract are quite comparable to those of the human genital tract. However, further research is needed to improve and standardize this new and potentially valuable model.

References

1. Thygeson, P., Mengert, W.F. *The virus of inclusion conjunctivitis.* Arch Ophthalmol 1936, 15: 377–410.
2. Ripa, K.T., Moller, B.R., Mardh, P.A., Freundt, E.A., Melsen, F. *Experimental acute salpingitis in grivet monkeys provoked by Chlamydia trachomatis.* Acta Pathol Microbiol *Scand* 1979, 87B: 65–70.
3. Johnson, A.P., Hetherington, C.M., Osborn, M.F., Thomas, B.J., Taylor-Robinson, D. *Experimental infection of the marmoset genital tract with Chlamydia trachomatis.* Br J Exp Pathol 1980, 61: 291–5.
4. Moller, B.R., Mardh, P.A. *Experimental salpingitis in grivet monkeys by Chlamydia trachomatis. Modes of spread of infection to the fallopian tubes.* Acta Pathol Microbiol Scand 1980, 888: 107–14.
5. Woodland, R.M., Johnson, A.P., Tuffrey, M. *Animal models of chlamydial infection.* Br Med Bull 1983, 39: 175–80.
6. Tuffrey, M., Taylor-Robinson, D. *Progesterone as a key factor in the development of a mouse model for genital-tract infection with Chlamydia trachomatis.* FEMS Microbiol Lett 1981, 12: 111–15.
7. Barron, A.L., White, H.J., Rank, R.G., Soloff, B.L., Moses, E.B. *A new animal model for the study of Chlamydia trachomatis genital infections: Infection of mice with the agent of mouse pneumonitis.* J Infect Dis 1981, 143: 63–6.
8. Patton, D.L., Halbert, S.A., Kuo, C.C., Wang, S.P., Holmes, K.K. *Host response to primary Chlamydia trachomatis infection of the fallopian tube in pig-tailed monkeys.* Fertil Steril 1983, 40(6): 829–40.
9. Vanrompay, D., Hoang, T.Q.T., De Vos, L. et al. *Specific pathogen free pigs as an additional animal model for studying Chlamydia trachomatis genital infection.* Infect Immun 2005 (in press).
10. Swenson, C.E., Schachter, J. *Infertility as a consequence of chlamydial infection of the upper genital tract in female mice.* Sex Transm Dis 1984, 11: 64–7.
11. Tuffrey, M., Falder, P., Gale, J., Quinn, R., Taylor-Robinson, D. *Infertility in mice infected genitally with a human strain of Chlamydia trachomatis.* J Reprod Fertil 1986, 78: 251–60.
12. Ito, J.I., Harrison, H.R., Alexander, E.R., Billings, L.J. *Establishment of genital tract infection in the CF-1 mouse by intravaginal inoculation of a human oculogenital isolate of Chlamydia trachomatis.* J Infect Dis 1984, 150: 577–82.
13. de la Maza, L.M., Pal, S., Khamesipour, A., Peterson, E.M. *Intravaginal inoculation of mice with the Chlamydia trachomatis mouse pneumonitis biovar results in infertility.* Infect Immun 1994, 62: 2094–7.
14. Darville, T., Andrews, C.W., Laffoon, K.K., Shymasani, W., Kishen, L.R., Rank, R.G. *Mouse strain-dependent variation in the course and outcome of chlamydial genital tract infection is associated with differences in host response.* Infect Immun 1997, 65: 3065–73.
15. Ito, J.I., Lyons, J.M., Airo-Brown, L.P. *Variation in virulence among oculogenital serovars of Chlamydia trachomatis in experimental genital tract infection.* Infect Immun 1990, 58: 2021–3.
16. Morrison, R.P., Caldwell, H.D. *Immunity to murine chlamydial genital infection.* Infect Immun 2002, 70: 2741–51.
17. Stagg, A.J., Tuffrey, M., Woods, C., Wunderink, E., Knight, S.C. *Protection against ascending infection of the genital tract by Chlamydia trachomatis is associated with recruitment of major histocompatibility complex class II antigen-presenting cells into uterine tissue.* Infect Immun 1998, 66: 3535–44.
18. Ito, J.I., Lyons, J.M. *Role of gamma interferon in controlling murine chlamydial genital tract infection.* Infect Immun 1999, 67: 5518–21.
19. Ramsey, K.H., DeWolfe, J.L., Salyer, R.D. *Disease outcome subsequent to primary and secondary urogenital infection with murine or human biovars of*

Chlamydia trachomatis. Infect Immun 2000, 68: 7186–9.
20. Morré, S.A., Lyons, J.M., Ito, J.I. Jr. *Murine models of Chlamydia trachomatis genital tract infection: Use of mouse pneumonitis strain versus human strains*. Infect Immun 2000, 68: 7209–11.
21. Suchland, R.J., Rockey, D.D., Bannantine, J.P., Stamm, W.E. *Isolates of Chlamydia trachomatis that occupy nonfusogenic inclusions lack IncA, a protein localized to the inclusion membrane*. Infect Immun 2000, 68: 360–7.
22. Morrison, R.P. *Differential sensitivities of Chlamydia trachomatis strains to inhibitory effects of gamma interferon*. Infect Immun 2000, 68: 6038–40.
23. Belland, R.J., Scidmore, M.A., Crane, D.D. et al. *Chlamydia trachomatis cytotoxicity associated with complete and partial cytotoxin genes*. Proc Natl Acad Sci USA 2001, 98: 13984–9.
24. Lyons, J.M., Ito, J.I., Peña, A.S., Morré, S.A. *Differences in the growth characteristics and elementary body associated cytotoxicity between Chlamydia trachomatis oculogenital serovars D and H and Chlamydia muridarum (MoPn)*. J Clin Path 2005 (in press).
25. Morré, S.A., van den Brule, A.J., Rozendaal, L. et al. *The natural course of asymptomatic Chlamydia trachomatis infections: 45% clearance and no development of clinical PID after one-year follow-up*. Int J STD AIDS 2002, 13 (Suppl. 2): 12–18.
26. van Valkengoed, I.G., Morré, S.A., van den Brule, A.J., Meijer, C.J., Bouter, L.M., Boeke, A.J. *Overestimation of complication rates in evaluations of Chlamydia trachomatis screening programmes – implications for cost-effectiveness analyses*. Int J Epidemiol 2004, 33: 416–25.
27. Lyons, J.M., Morré, S.A., Airo-Brown, L.P., Pena, A.S., Ito, J.I. *Acquired homotypic and heterotypic immunity against oculogenital Chlamydia trachomatis serovars following female genital tract infection* (submitted).
28. Patton, D.L., Halbert, S.A., Kuo, C.C., Wang, S.P., Holmes, K.K. *Host response to primary Chlamydia trachomatis infection of the fallopian tube in pig-tailed monkeys*. Fertil Steril 1983, 40(6): 829–40.
29. Patton, D.L. *Immunopathology and histopathology of experimental chlamydial salpingitis*. Rev Infect Dis 1985, 7(6): 746–53.
30. Patton, D.L., Kuo, C.C., Wang, S.P. et al. *Chlamydial infection of subcutaneous fimbrial transplants in cynomolgus and rhesus monkeys*. J Infect Dis 1987, 155(2): 229–35.
31. Patton, D.L., Kuo, C.C., Wang, S.P., Halbert, S.A. *Distal tubal obstruction induced by repeated Chlamydia trachomatis salpingeal infections in pig-tailed macaques*. J Infect Dis 1987, 155(6): 1292–9.
32. Wolner-Hanssen, P., Patton, D.L., Holmes, K.K. *Protective immunity in pig-tailed macaques after cervical infection with Chlamydia trachomatis*. Sex Transm Dis 1991, 18(1): 21–5.
33. Patton, D.L., Sweeney, Y.T., Kuo, C.C. *Demonstration of delayed hypersensitivity in Chlamydia trachomatis salpingitis in monkeys: A pathogenic mechanism of tubal damage*. J Infect Dis 1994, 169(3): 680–3.
34. Lichtenwalner, A.B., Patton, D.L., Van Voorhis, W.C., Sweeney, Y.T., Kuo, C.C. *Heat shock protein 60 is the major antigen which stimulates delayed-type hypersensitivity reaction in the macaque model of Chlamydia trachomatis salpingitis*. Infect Immun 2004, 72(2): 1159–61.
35. Lichtenwalner, A.B., Patton, D.L., Cosgrove Sweeney, Y.T., Gaur, L.K., Stamm, W.E. *Evidence of genetic susceptibility to Chlamydia trachomatis-induced pelvic inflammatory disease in the pig-tailed macaque*. Infect Immun 1997, 65(6): 2250–3.
36 Tuggle, C.K., Green, J.A., Fitzsimmons, C. et al. *EST-based gene discovery in pig: Virtual expression patterns and comparative mapping to human*. Mamm Genome 2003, 14(8): 565–79.
37. Lyons, J.M., Ito, J.I., Morré, S.A. *Chlamydia trachomatis serovar E isolates from patients with different clinical manifestations have similar courses of infection in a murine model: Host factors as major determinants of C. trachomatis mediated pathogenesis*. J Clin Pathol 2004, 57(6): 657–9.
38. Eckert, L.O., Hawes, S.E., Wolner-Hanssen, P.K. *Endometritis: The clinical-pathologic syndrome*. Am J Obstet Gynecol 2002, 186(4), 690–5.
39. Faro, S. *Chlamydia trachomatis*. In: Faro, S., Soper, D.E. (Eds.). Infectious Diseases in Women. W.B. Saunders Company: Philadelphia 2001, 451–67.
40. Møller, B.R., Weström, L., Ahrons, S. et al. *Chlamydia trachomatis infection of the fallopian tubes. Histological findings in two patients*. Br J Vener Dis 1979, 55: 422–8.
41. Rank, R.G., Sanders, M.M. *Pathogenesis of endometritis and salpingitis in a guinea pig model of chlamydial genital infection*. Am J Pathol 1992, 140: 927–36.
42. Igietseme, J.U., Black, C.M., Caldwell, H.D. *Chlamydia vaccines – Strategies and status*. Bio Drugs 2002, 16: 19–35.
43. Kozlowski, P.A., Neutra, M.R., Flanigan, T.P., Cu-Uvin, S. *Induction of antibodies in women after vaginal immunization during the mid-proliferative or mid-secretory phase of the menstrual cycle*. Faseb J 2000, 14(6) (Suppl. S): A1248.