

***Chlamydomphila pneumoniae* Antibodies in Swine**J. ČANDERLE¹, L. POSPÍŠIL¹, H. ŠTROBLOVÁ², Z. VĚŽNÍK¹¹Veterinary Research Institute, Brno, Czech Republic;²Department of Clinical Microbiology, Faculty Hospital Brno, Brno, Czech Republic

Received July 23, 2004

Accepted March 3, 2005

Abstract

Čanderle J., L. Pospíšil, H. Štroblová, Z. Věžník: *Chlamydomphila pneumoniae* Antibodies in Swine. Acta Vet. Brno 2005, 74: 81-86.

Sera from 80 boars of various breeds, all semen donors, kept in one insemination station in northern Moravia, were tested for the presence of *Chlamydomphila pneumoniae* antibodies. The employees of the insemination station suffering very often from respiratory diseases were repeatedly examined for a long-term, serologically demonstrated chlamydial infection. The sera of the animals were tested by enzyme-linked immunosorbent assay (ELISA) with a species-specific major outer membrane protein (cMOMP) *Chlamydomphila pneumoniae* and with a genus-specific chlamydial lipopolysaccharide (cLPS) antigen respectively. The antibodies against chlamydial heat shock protein (cHSP60) have been proved as well. In the ELISA IgG test with species-specific *Chlamydomphila pneumoniae* antigen (cMOMP) 18 sera (22.5%) were positive and 11 doubtful (13.7%) and with chlamydial genus specific antigen (cLPS) 26 sera (32.5%) were positive, 15 (18.7%) were doubtful, respectively. A positive result of ELISA with cHSP60 as antigen has been found in 26 sera (32.5%) and 7 doubtful (8.7%). Sera from the control group of four specific pathogen free pigs proved negative in all tests.

Boar, IgG antibodies, chlamydomphal antigens, chlamydial heat shock protein

Chlamydomphila (formerly *Chlamydia*) *pneumoniae* (*C. pneumoniae*) is a primary pathogen in humans. Everett et al. (1999) proposed a reclassification of the family Chlamydiaceae into four genera, based primarily on comparative sequence analysis of rRNA genes, and included *C. pneumoniae* into genus *Chlamydomphila*. Current research results suggest that of all *Chlamydomphila* species, *C. pneumoniae* plays the most important role in human pathology. It is the most frequent cause of respiratory infections and also probably one of the agents in pathological processes in other organs (e. g. joint inflammation, genital infection, etc.). It is also a very likely co-factor leading to atherosclerosis, broncho-pulmonary, cervical and ovarian carcinoma as well as some disorders of the central nervous system (Gran et al. 1993; Sriram et al. 1999; Wimmer et al. 1996; Wollenhaupt and Zeidler 1997; Wong et al. 1999; Paavonen 2000; Bernhard et al. 2001).

C. pneumoniae was isolated for the first time in 1965 from the conjunctiva of a Taiwanese child; the strain was marked TW-183. The second isolation from the throat of a student with a pharyngeal inflammation took place in 1983 and was marked AR-39. The original name of *C. pneumoniae* was TWAR agent (Kuo et al. 1986; Grayston et al. 1986). *C. pneumoniae* is the cause of a very cosmopolitan infection attacking most of the world-wide population more than once a lifetime.

Respiratory infections caused by *C. pneumoniae* are usually mild. Nevertheless, in a significant number of cases they can develop into a more serious disease (sinusitis, bronchitis, or complicated pneumonia) (Grayston et al. 1993). Another important discovery in recent years has shown that humans are not the only hosts in whom *C. pneumoniae* causes primary disease. Its presence in infections occurring in various animal species is being reported in an increasing number of cases.

Address for correspondence:

MVDr. Jiří Čanderle, CSc.
Veterinary Research Institute
Hudcova 70
621 32 Brno, Czech Republic

Phone:+420 533 331 417
Fax:+420 541 211 229
E-mail:pospishil@vri.cz
<http://www.vfu.cz/acta-vet/actavet.htm>

Successively, the *C. pneumoniae* strain was isolated from horses (Storey et al. 1993), koala bears affected by ocular and genital infection (Glassick et al. 1996; Jackson et al. 1999; Bodetti et al. 2000), Australian and African frogs (Berger et al. 1999; Reed et al. 2000; Hotzel et al. 2001), from a Tanzanian chameleon, a green sea turtle from the Cayman Islands, an iguana, puff adders and a Burmese python (Bodetti et al. 2002). All of the *C. pneumoniae* positive animals suffered from some form of illness typical for humans affected by this Chlamydia species. All animal strains also showed a significant similarity with the human *C. pneumoniae* strain (up to 100%).

Sako et al. (2002) successfully demonstrated the presence of the *C. pneumoniae* antigen (immunohistochemically, electronoptically and by PCR) in atherosclerotic lesions of the aorta and coronary and splenic arteries of seven dogs suffering from atherosclerosis. Bodetti et al. (2002) describe six cases of disease or death in different species of animals as a consequence of *C. pneumoniae* infections, which indicates that the source of this microbe in the natural environment could be mammals, amphibians, reptiles or other animals.

It is surprising that we have not yet managed to find a published report on the presence of *C. pneumoniae* in pigs given the fact that *Chlamydia suis* and other species of *Chlamydia* or *Chlamydia* were identified in this animal species (*Chlamydia trachomatis*, *Chlamydia pecorum*, *Chlamydia psittaci* – Kaltenboeck et al. 1997; Schiller et al. 1997a, b; Busch et al. 2000). The pigs are a globally-distributed animal species and could be an important reservoir of certain microbial and viral pathogens. We began therefore to conduct serological research of antibodies to *C. pneumoniae* in boars from an insemination station in northern Moravia, the employees of which suffer for extensively from respiratory diseases probably caused by chlamydiae confirmed serologically (Bazala and Renda 1992; Pospíšil et al. 1997).

Materials and Methods

Origin of samples

Blood samples were collected from 80 boars kept at one insemination station in northern Moravia. Blood was taken from the *v. cava cranialis*, using the sterile collection set for swine (Hemos H-03, GAMA, Czech Republic). After coagulation and centrifugation the sera were stored at -70 °C. As negative control the sera from four pathogen-free three-month-old pigs (bred Large White from the institutional herd) were used. The sera were collected and stored in the same way.

Detection of chlamydial antibodies

For the quantitative detection of species-specific IgG antibodies the commercially enzyme immunoassay kit *Chlamydia pneumoniae*-IgG-sELISA medac (Medac, Germany), with the microtiter plates coated with *C. pneumoniae* major out membrane protein (a highly purified and species specific antigen - cMOMP) has been used. For the detection of chlamydial genus-specific IgG antibodies the commercially recombinant enzyme immunoassay kit (Chlamydia-IgG-rELISA medac) with chlamydial lipopolysaccharide (genus specific antigen - cLPS) has been applied.

Finally, the IgG antibodies against the chlamydial heat shock protein 60kD (anti-cHSP₆₀) by a recombinant enzyme immunoassay for the quantitative detection using genus specific the “cHSP60-IgG-ELISA medac” device were ascertained.

Preparation of the reagents and ELISA test procedure were performed in accordance with the recommendations of the manufacturer with the following exceptions: the rabbit anti-swine IgG with peroxidase (RASw/Px from Sevapharma, Czech Republic) as conjugate, and Ortho-Phenylene-diamine (OPD, BIO-RAD, France) as chromogenic substrate have been used. The swine serum for detection of the anti-cHSP60 antibodies was diluted to 1:300 by sample diluent (Medac).

The photometric reading of optical density (OD) at 490 nm (reference wavelength 630 nm) by a DYNEX MRX Reader was performed. For the evaluation of the ELISA test we calculated the OD cut-off (absorbance) in positive and negative control samples. The positive and negative (the sera from four specific pathogen-free pigs proved negative in all ELISA tests) samples were compared with OD of positive and negative control human serum (Medac), which were conjugated with goat anti-human IgG (Medac). The reaction was ascertained as negative for OD lower than the cut-off index (1.8), which corresponds to the summation of the average OD in negative control samples and a 10% increase on half the difference between positive control OD and negative control OD. An OD between 1.8 and 2.0 was marked as doubtful, higher than 2.0 as positive.

The results obtained were analyzed by means of correlation analysis using the Microsoft Excel program by Windows.

Results

The ELISA method detected, in the blood sera of 80 boars, IgG antibodies anti-species specific antigen of *C. pneumoniae* (cMOMP), with 18 positive cases (22.5%) and 11 doubtful cases (13.7%) see Table 1. The IgG antibodies anti-Chlamydophilal genus specific antigen (cLPS) proved by ELISA were detected, with 26 positive sera (32.5%) and 15 doubtful sera (18.7%). Out of the 18 anti cMOMP *C. pneumoniae* positive boar sera, 13 (72.2%) were also serologically positive for genus specific antigen (cLPS). The detection of the species specific antibodies anti- *C. pneumoniae* is in highly significant correlation with the detection of antibodies anti-chlamydial genus specific antigen ($r = 0.785$; $p < 0.001$). By the ELISA IgG antibodies anti- chlamydial specific heat shock protein (cHSP₆₀) 26 positive sera (32.5%) and 7 doubtful sera (8.7%) from the 80 boars were found. Out of positive and doubtful sera anti- cHSP₆₀ reacting sera a positive reaction with other chlamydophilal or chlamydial antigens was detected in 24 (74.7%) sera as well.

Table 1. Chlamydial antibody tests in the serum of boars ($n = 80$)

Test	ELISA anti- <i>C. pneumoniae</i>		ELISA anti- Chlamydia genus		ELISA anti- cHSP ₆₀	
		%		%		%
Positive	18	22.5	26	32.5	26	32.5
Doubtful	11	13.7	15	18.7	7	8.7
Negative	51	63.7	39	48.7	47	58.7

Discussion

The detection of the anti-chlamydial antibodies in pig sera is carried out rather sporadically and if so, then it is in the form of complement fixation test (CFT), with genus specific chlamydial antigen. The insemination station supplying boars for the examination described here was noted in the study using CFT about chlamydophilal infection in employees suffering from frequent respiratory diseases (Bazala and Renda 1992; Pospíšil et al. 1997). In another study performed in this insemination station the high prevalence of genus specific chlamydial antibodies detected by means of CFT in the sera of boars has been ascertained (Věžník et al. 1996). There are varying opinions on the evaluation of the CFT. The problem lies in the low sensitivity and specificity of the respective test (Henning et al. 2002). Nevertheless the test will be very often used by many authors as screening (e.g. Trávníček et al. 2001).

Other serological chlamydophilal reactions, commonly used in human medicine, such as indirect immunofluorescent reaction, ELISA, Western blot and others, are rarely used in veterinary medicine and the same applies to exceptional work with various chlamydial antigens (cMOMP, cLPS or heat shock protein – cHSP). Despite the fact that various chlamydial infections have been detected in pigs, namely *Chlamydia suis*, *Chlamydia trachomatis*, *Chlamydochila pecorum*, and *Chlamydochila psittaci* (Schiller et al. 1997ab; Busch et al. 2000), so far we have been unable to find in the professional literature evidence of spontaneous *C. pneumoniae* infection. Only Liuba et al. (2003) and Pislaru et al. (2003) describe the development and consequences of experimental *C. pneumoniae* infection in pigs.

The high prevalence of species-specific antibodies anti-MOMP *C. pneumoniae* (22.5%) in the sera from boars kept at the mentioned insemination station is surprising, though logical when juxtaposed to the frequent infectious diseases in the station's employees. The possibility of including non specific reactions into the

evaluation cannot be excluded, but it is quite unlikely when using species specific antigen in the ELISA test, and strictly set cut-off index for optical density for the estimation of positive reactions. According to the producer of the diagnostic sets, the probable cross-reactivity between different chlamydia species has been reduced by extracting a common component of all chlamydiae. ELISA is a suitable means of specific and exact diagnosis of Chlamydia infections nevertheless a dispute about the detection of anti- *C. pneumoniae* antibodies in the set of boar sera is possible. On the basis of serological examination only we are aware that we cannot rule out infection caused by *C. suis*, and/or by other members of Chlamydia family which were identified in pigs (*Chlamydia trachomatis*, *Chlamydia pecorum*, *Chlamydia psittaci* – Kaltenboeck et al. 1997; Schiller et al. 1997a,b; Busch et al. 2000). In accordance with the recent proposal for reclassification of the order *Chlamydiales* the former porcine serovar *Chlamydia trachomatis* will be classified in presence as *Chlamydia suis*. The pathogenic strain *Chlamydia suis* has been used by Sachse et al. (2004) for a challenge respiratory chlamydiosis in pigs. The antibody against *Chlamydia trachomatis* in 80 boars examined by us were ascertained only in 4 (5% – data not shown). *C. pneumoniae* infection in pigs based upon isolation of the pathogen, direct antigen detection or nucleic acid amplification tests (PCR or LRC) will be confirmed by us in near future as well. Isolation of the pathogen from cell culture normally needs 2 – 3 subsequent or different type passages. According to our experience direct immunofluorescence assays and antigen enzyme immunoassays seem to be of lower sensitivity and specificity. The findings of this study provide further support for the thesis of a wide-spread, cosmopolitan and interspecies *C. pneumoniae* infection, resulting in a broad spectrum of diseases (Shor 2000; Bodetti et al. 2002; Pospíšil and Čanderle 2004) and represents a health hazard in humans combined with an interesting epidemiological problem for both wild as well as domesticated animals, with a primary impact for people.

Protilátky proti *Chlamydia pneumoniae* u prasat

Séra 80 kanců různých plemen, dárců semene chovaných na jedné inseminační stanici, byla vyšetřena na přítomnost protilátek proti *Chlamydia pneumoniae*. U zaměstnanců stanice se dlouhodobě vyskytuje vysoká nemocnost s infekcí chlamydiemi potvrzenou sérologicky. Séra kanců byla vyšetřena enzymoimunoesejemi (ELISA) s třemi antigeny: rodově specifickým lipopolysacharidem chlamydií (cLPS), druhově specifickým proteinem vnější membrány (major outer membrane protein - cMOMP) *Chlamydia pneumoniae* a konečně s proteinem tepelného šoku chlamydií (cHSP₆₀). V ELISA testu IgG se specifickým antigenem *Chlamydia pneumoniae* (cMOMP) bylo pozitivních 18 sér (22.5 %) a 11 dubiálních (13.7 %). V ELISA testu IgG s rodově specifickým antigenem (cLPS) bylo pozitivních 26 sér (32.5 %) a 15 sér bylo dubiálních (18.7 %). Pozitivní výsledek ELISA s cHSP60 jako antigenem byl u 26 sér (32.5%) a u 7 dubiálních (8.7%). Kontrolní séra prasat (SPF) byla ve všech testech negativní.

Aknowledgements

The authors thank the management and the employees of the insemination station for their assistance with collection of blood samples and of other material. This paper was conducted with the support of the Grant Agency of the Ministry of Health of the Czech Republic, (IGA MZ CR No. NH/7026-3).

References

- BAZALA, E, RENDA, J 1992: Latent infection caused by chlamydias, which results in health problems for pig, cattle and sheep breeders. Berl Munch Tierärztl Wschr **105**: 145-149
 BERGER, L, VOLP, K, MATHEWS, S, SPEARE, R, TIMMS, P 1999: *Chlamydia pneumoniae* in a free-ranging giant barred frog (*Mixophyes iteratus*) from Australia. J Clin Microbiol **37**: 2378-2380

- BERNHARD, J, LEHMANN, T, VILLIGER, PM 2001: *Chlamydia pneumoniae*: a cause of reactive arthritis? Schweiz Rundsch Med Prax **90**: 2060-2063
- BODETTI, TJ, TIMMS, P 2000: Detection of *Chlamydia pneumoniae* DNA and antigen in the circulating mononuclear cell fraction of humans and koalas. Infection and Immunity **68**: 2744-2747
- BODETTI, TJ, JACOBSON, E, WAN, C, HAFNER, L, POSPISCHIL, A, ROSE, K, TIMMS, P 2002: Molecular evidence to support the expansion of the host range of *Chlamydia pneumoniae* to include reptiles, as well as humans, horses, koalas and amphibians. Syst Appl Microbiol **26**: 146-152
- BUSCH, M, THOMA, R, SCHILLER, I, CORBOZ, L, POSPISCHIL, A 2000: Occurrence of *Chlamydiae* in genital tracts of sows at slaughter and their possible significance for reproductive failure. J Vet Med B **47**: 471-480
- EVERETT, KDE, BUSCH, RM, ANDERSEN AA 1990: Emended description of the order *Chlamydiales*, proposal of *ParaChlamydiaceae* fam. nov. and *Simkaniaceae* fam. nov., each containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species, and standards for the identification of organisms. Int J Syst Bacteriol **49**: 415-440
- GLASSICK, T, GIFFARD, P, TIMMS, P 1996: Outer membrane protein 2 gene sequences indicate that *Chlamydia pecorum* and *Chlamydia pneumoniae* cause infections in koalas. Syst Appl Microbiol **19**: 457-464
- GRAN, J, HJETLAND, R, ANDREASSEN, AH 1993: Pneumonia, myocarditis, and reactive arthritis due to *Chlamydia pneumoniae*. Scand J Rheumatol **22**: 43-44
- GRAYSTON, JT, KUO, CC, WANG, SP, ALTMAN, J 1986: A new *Chlamydia-psittaci* strain, TWAR, isolated in acute respiratory-tract infections. New Engl J Med **315**: 161-168
- GRAYSTON, JT, ALDOUS, MB, EATON, A, WANG, SP, KUO, CC, CAMPBELL, LA, ALTMAN, J 1993: Evidence that *Chlamydia pneumoniae* causes pneumonia and bronchitis. J Infect Dis **168**: 1231-1235
- HENNING, K, SACHSE, K, GROSSMANN, E 2002: Evaluierung eines Antikörper – ELISAs für Schweineseren. Dtsch tierärztl Wschr **109**: 449-450
- HOTZEL, H, GROSSMANN, E, MUTSCHMANN, F, SACHSE, K 2001: Genetic characterization of *Chlamydophila pneumoniae* isolate from an African frog and comparison to currently accepted biovars. Syst Appl Microbiol **24**: 63-66
- JACKSON, M, WHITE, N, GIFFARD, P 1999: Epizootology of *Chlamydia* infections in two free-range koala populations. Vet Microbiol **65**: 255-264
- KALTENBOECK, B, SCHMEER, N, SCHNEIDER, R 1997: Evidence for numerous omp1 alleles of porcine *Chlamydia trachomatis* and novel Chlamydophilal species obtained by PCR. J Clin Microbiol **35**: 1835-1841
- KUO, CC, CHEN, HH, WANG, SP, CAMPBELL, LA 1986: Identification of a new group of *Chlamydia-psittaci* strains called TWAR. J Clin Microbiol **24**: 1034-1037
- LIUBA, P, PESONEN, E, PAAKARI, I, BATRA, S, FORSLID, A, KOVANEN, P, PENTIKAINEN, M, PERSSON, K, SANDSTROM, S 2003: Acute *Chlamydia pneumoniae* infection causes coronary endothelial dysfunction in pigs. Atherosclerosis **167**: 215-222
- PAAVONEN, J 2000: *Chlamydia* and cancer. Proc. Fourth Meeting of the European Soc. for *Chlamydia* Res., Helsinki, 239-240
- PISLARU, SV, VAN RANST, M, PISLARU, C, SZELID, Z, THEILMEIER, G, OSSEWAARDE, JM, HOLVOET, P, JANSSENS, S, VERBEKEN, E, VAN DE WERF, FJ 2003: *Chlamydia pneumoniae* induces neointima formation in coronary arteries of normal pigs. Cardiovasc Res **57**: 834-842
- POSPÍŠIL, L, VĚŽNÍK, Z, DIBLÍKOVÁ, I, PEJČOCH, M 1997: The occurrence of the antibodies against the chlamydial antigen in human population of the Czech Republic. Epidemiol Mikrobiol Imunol **46**: 13-17
- POSPÍŠIL, L, ČANDERLE, J 2004: *Chlamydia (Chlamydophila) pneumoniae* in animals: A review. Vet. Med. Czech **49**: 129-134
- REED, KD, RUTH, GR, MEYER, JA, SHUKLA, SK 2000: *Chlamydophila pneumoniae* in a breeding colony of African clawed frogs (*Xenopus tropicalis*). Emerg Infect Dis **6**: 196-199
- SAIKKU, P, LAITINEN, K, LEINONEN, M 1998: Animal models for *Chlamydia pneumoniae* infection. Atherosclerosis **140**: Suppl. 1, 17-19
- SACHS, K, GROSSMANN, E, BERNDT, A, SCHÜTT, C, HENNING, K, THEEGARTEN, D, ANHENN, O, REINHOLD, P 2004: Respiratory chlamydial infection based on experimental aerosol challenge of pigs with *Chlamydia suis*. Comp Immunol Microbiol Infect Dis **27**: 7-23
- SAKO, T, TAKAHASHI, T, TAKEHANA, K, UCHIDA, E, NAKADE, T, UMEMURA, T, TANIYAMA, H 2002: Chlamydial infection in canine atherosclerotic lesions. Atherosclerosis **162**: 253-259
- SCHILLER, I, KOESTERS, R, WEILENMANN, R, KALTENBOECK, B, POSPISCHIL, A 1997a: Polymerase chain reaction (PCR) detection of porcine *Chlamydia trachomatis* and ruminant *C. psittaci* serovar 1 DNA in formalin fixed intestinal specimens from swine. J Vet Med B **44**: 185-191
- SCHILLER, I, KOESTERS, R, WEILENMANN, R, THOMA, R, KALTENBOECK, B, HEITZ, P, POSPISCHIL, A 1997b: Mixed infections with porcine *Chlamydia trachomatis* and infections with ruminant *Chlamydia psittaci* serovar 1 associated with abortion in swine. Vet Microbiol **58**: 251-260
- SHOR, A 2000: The pathology of *Chlamydia pneumoniae* lesions in humans and animal models. Trends Microbiol **8**: 541-542

- SRIRAM, S, STRATTON, CW, YAO, SY, THARP, A, DING, LM, BANNAN, JD, MITCHELL, W 1999: *Chlamydia pneumoniae* infection in the central nervous system in multiple sclerosis. *Ann Neurol* **46**: 6-14
- STOREY, C, LUSHER, M, YATES, P, RICHMOND, S 1993: Evidence for *Chlamydia pneumoniae* of nonhuman origin. *J Gen Microbiol* **139**: 2621-2626
- TRÁVNÍČEK, M, KOVÁČOVÁ, D, ZUBRICKÝ, P, ČISLÁKOVÁ, L 2001: Serosurvey of sheep and goats to *Chlamydia psittaci* in Slovakia during the years 1996-2000. *Vet Med – Czech* **46**: 281-285
- VĚŽNÍK, Z, POSPÍŠIL, L, DIBLÍKOVÁ, I, ŠVECOVÁ, D 1996: The frequency of antibodies against *Chlamydiae* in animals in the Czech Republic. *Veterinářství* **46**, 335-338 (in Czech)
- WIMMER, MLJ, SANDMANN-STRUPP, R, SAIKKU, P, HABERL, RL 1996: Association of chlamydial infection with cerebrovascular disease. *Stroke* **27**: 2207-2210
- WOLLENHAUPT, J, ZEIDLER, H 1997: Klinik, Diagnostik und Therapie der Chlamydien-induzierten Arthritis. *Akt Rheumatol* **22**: 176-182
- WONG, YK, GALLAGHER, P.J, WARD, ME 1999: *Chlamydia pneumoniae* and atherosclerosis. *Heart* **81**: 232-238