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Comparison of serological procedures for diagnosis of infection with *Chlamydophila* sp. in bovines

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ABSTRACT

In order to diagnose chlamydiosis caused by bacteria *Chlamydophila* sp., the humoral immunity response in domestic bovines was examined. A possible difference in titre of specific antibodies between latently, acutely and chronically infected animals was also studied. At the same time we confirmed the applicability and reliability of complement-fixation tests (CFT) and enzyme–linked immunosorbent assay (ELISA) on the basis of their specificity, sensitivity and comparativeness. Of 276 bovines examined, originating from 10 herds on Croatian territory, CFT revealed *Chlamydophila* sp. specific antibodies in 14.2% of sera samples, ELISA in 35.8%, and indirect immunofluorescent test (IIF) in 27.1% sera samples. These results suggest that CFT, ELISA and IIF can be used as reliable serological methods for diagnostics of chlamydiosis, being in accordance with Office International des Epizooties (OIE) recommendations.

Key words: *Chlamydophila*, diagnostics, serology, bovine, infection, Croatia

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Introduction

Chlamydiae are obligate intracellular, gram-negative bacteria with a typical developmental life cycle (STEPHENS, 1999). During the last ten years four chlamydia species have been isolated from humans and from different domestic and wild animals: *Chlamydia psittaci* (PAGE, 1968), *Chlamydia pecorum* (FUKUSHI and HIRAI, 1993), *Chlamydia pneumoniae* (GRAYSTON et al., 1986) and *Chlamydia trachomatis* (WANG and GRAYSTON, 1991). During 1999, many changes and corrections of taxonomic classification of Chlamydia were published. The family *Chlamydiaceae* is divided into two genera, *Chlamydia* and *Chlamydophila*. *Chlamydophila* genus assimilates the current species, *Chlamydia pecorum*, *Chlamydia pneumoniae* and *Chlamydia psittaci*. Three new *Chlamydophila* species are derived from *Chlamydia psittaci*: *Chlamydophila abortus*, *Chlamydophila caviae* and *Chlamydophila felis* (EVERETT et al., 1999). In bovine, *Chlamydophila* (C.) *abortus*, *C. caviae* and *C. psittaci* strains (WC) induce abortion, genital infections, and can also cause enteritis, conjunctivitis, mastitis, pneumonia, polyarthritis, meningoencephalitis and seminal vesiculitis (IDTSE, 1984; PEREZ-MARTINEZ and STORZ, 1985; STING, 1997; EUZEBY, 1999). *C. pecorum* usually generates infections in ruminants which are not apparent, although in some cases it can develop enteritis, pneumonia, intestinal infections and polyarthritis, but without severe clinical signs (FUKUSHI and HIRAI, 1993). All these species present various common antigens, the most important of which is the lipopolysaccharide antigen (LPS), which can easily be demonstrated by complement-fixation tests (CFT) (BRADE et al., 1986). The agents may be transmitted by ingestion, inhalation, *in utero* transfer, natural or artificial insemination (PEREZ-MARTINEZ and STORZ, 1985). For the above mentioned reasons there are great risks of transmitting agents from infected herds to a healthy one. This is why the cultural and serological tests confirmed the existence of the disease in all countries with intensive and semi-intensive rearing of bovines (GERBERMANN, 1991).

An objective diagnosis should involve several laboratory methods. CFT is the most commonly used and widely accepted serological test for diagnosing chlamydiosis (ANONYMOUS, 2000a). Another test system for group-specific chlamydial antibodies, which promises to gain importance, is the enzyme–linked immunosorbent assay (ELISA). An indirect

immunofluorescent test (IIF) for chlamydia has been also developed and evaluated (MARKEY et al., 1993). CFT, or ELISA, are often the only methods available for a large number of laboratories. Isolation of chlamydophilae in embryonated hen eggs or cell culture is not routinely performed by veterinary laboratories because it is labour intensive and prone to contamination (RODOLAKIS et al., 1998).

In this study, infections caused with *Chlamydophila* sp. were determined serologically in the period between 1992 and 1999 in 10 bovine herds in Croatia. For detection of antibodies against *Chlamydophila* sp. in bovines, ELISA, CFT and IIF were used and compared with the direct immunofluorescent test (DIF) and chlamydia antigen detection kits "Clearview" (CW).

Also, the advantages and disadvantages of current methods most commonly used will be discussed.

Materials and methods

The prevalence of chlamydiosis was investigated during the period 1992-1999 in 10 bovine herds in Croatia. The number of the examined bovines and/or samples of their tissue was based on clinical test and symptoms specific for the disease of chlamydiosis.

Sera and pathological material. A total of 267 samples of bovine sera originating from different locations were tested (Table 1). Samples were taken from non-vaccinated bovines of different age groups from village homesteads or farm herds. Blood samples were collected by extracting blood clots by puncturing the jugular veins and were stored at a temperature of –20 ^{0}C. The tissue samples taken from brain, kidney, heart, spleen, and liver were fixed in formalin for pathohistological examination.

Differential diagnosis included culturing for *Salmonella* spp., *Escherichia* coli and *Campylobacter* spp. Sera were screened for Q-fiver by CFT. Brains were also examined for the presence of rabies and Aujeszky's disease.

Complement - fixation tests (CFT). The complement-fixing (CF) antibodies were detected by a Kolmer-type micromethod as described by

MEYER and EDDIE (1964) with a commercial group-specific chlamydia antigen (Department of Virology, Croatian Public Health Institute). A serum sample was considered positive when its end point was 1:32 (GERBERMMAN, 1991).

Table 1. Categories and numbers of serologically examined bovines for presence of antibodies and antigens to *Chlamydophila* sp.

Serologically tested bovines with methods						
Category	CFT	ELISA	IIF	DIF	CW	N^{o} of herds
Calves	14	0	0	2	0	1
Young bulls	189	110	50	15	15	7
Cows	24	24	20	0	0	1
Bulls	40	0	0	0	0	1
Total	276	134	70	17	15	10

Indirect immunofluorescent test (IIF). The IIF test was performed on slides (Chlamydia psittaci-Spot BIOMERIEUX, France). Serum samples were tested using a commercial fluorescent isothiocyanate conjugate (FITC Bovine IgG Sigma Chemical Company, U.S.A.) diluted in phosphate-buffered saline solution, and counterstained with Evans blue. End-point titers were recorded as the highest two-fold serum dilutions (1:40 to 1:640) with characteristic inclusions fluoresced (PEREZ-MARTINEZ et al., 1986).

Enzyme–linked immunosorbent assay (ELISA) CHEKIT - Chlamydia microtiter plates (producer: DR BOMELI AG) were supplied pre-coated with inactivated *C. psittaci* antigen. This test has been described by SCHMEER et al. (1987). Sera were tested in duplicate and diluted in CHEKIT-Washing&Dilution-Solution pH between 5.5 and 6.0. The sera and controls were added in a dilution of 1/400 (200µl/well), which were incubated at room temperature (+18 ^{0}C to +30 ^{0}C) for 90 minutes in a humid chamber. After a washing step, 100 µl volumes of monoclonal anti-ruminant IgG conjugate (diluted 1/200) were added and incubated at room temperature (+18 ^{0}C to +30 ^{0}C) for 90 minutes in a humid chamber. After a further

washing step, dilution CHEKIT-Chromogen pre-warmed to 25 ^{0}C was added (100 µl) to each well. After the colour reaction was developed, reaction was stopped by the addition of 50 µl per well freshly prepared CHEKIT- Stopping–Solution. The degree of colour (optical density measured at 405 nm) is directly proportional to the amount of antibody specific for *C. psittaci* present in the sera samples. The diagnostic relevance of the result was obtained by comparing the optical density (OD) of tested sera with OD of positive control. Optical density lower than 30% was classified as a negative result, the density between 30 and 40% was suspicious, while the density higher than 40% was considered a positive reaction.

Direct immunofluorescent antibody technique (DIF). DIF using fluorescein-conjugated monoclonal antibodies (Chlamydia Direct IF, BIOMERIEUX, France) have been used to detect chlamydophila inclusions (VEZNIK et al., 1996) in impression smears of tissues. Positive and negative controls were run with each batch of the kits. The preparations were examined using an Axioskop-Opton microscope. Samples showing ten or more elementary bodies (diameter 0.3 µm uniform fluorescence) or reticular bodies (0.1 µm fluorescence in ring form) with bright, yellowish-green fluorescence against a red background were classified as positive. Chlamydophila-free samples were classified as negative.

Rapid immunoasay "Clearview Chlamydia" (CW). To apply the CW test (Unipath, Betford, UK) swabs taken from different organs and the meninges were placed in 600 µl of extraction buffer and heated at 80 ^{0}C for 10 minutes. The line forms due to binding of chlamydial antigen to the blue latex, and its immobilisation by a zone of antibody located beneath the "result window" remains clear. Formation of a blue line in a "control window" shows that the test has been performed correctly. Test performance was validated by appropriate positive and negative controls. This test has been described by WILSMORE and DAVIDSON (1991).

Statistical analysis. Standard procedures were used to calculate the sensitivity, specificity, and positive and negative predictive values for each test (ANONYMOUS, 2000b).

Results

In 104 (38.9%) of 276 sera tested, CF antibodies were detected, but only 38 (14.2%) sera had a significant antibody titre ≥1:32 for *Chlamydophila* sp. (Table 2).

Table 2. Number and percentage of sera with a positive and a significant (≥1:32) CF antibody titer for bacteria *Chlamydophila* sp. in bovines

Category	Herd	N° of tested sera	N° of sera with significant titer		CF antibody titres					
			n	%	Neg	1:8	1:16	1:32	1:64	1:128
Young bulls	herd 1	23	7	30.4	13	1	2	3	4	0
Young bulls	herd 2	18	2	11.1	13	3	0	1	1	0
Young bulls	herd 3	24	0	0	13	8	3	0	0	0
Young bulls	herd 4	19	0	0	12	6	1	0	0	0
Young bulls	herd 5	39	4	10.3	32	2	1	2	2	0
Young bulls	herd 6	51	13	25.5	18	12	8	11	2	0
Young bulls	herd 7	15	10	66.7	3	1	1	5	4	1
Calves	herd 8	14	1	7.1	12	0	1	0	0	1
Cows	herd 9	24	0	0	21	1	2	0	0	0
Bulls	herd 10	40	1	2.5	26	8	5	1	0	0
Total		267	38	14.2	163	42	24	23	13	2

Table 3. Chlamydophila-specific antibody response of bovines determined by ELISA

			Presence of specific IgG antibodies in bovine sera		
Category	Herd	N° of tested sera	N° of positive (%)	N° of ambiguous (%)	N° of negative (%)
Young bulls	herd 1	38	17 (44.7)	5 (13.2)	16 (42.1)
Young bulls	herd 3	23	9 (39.1)	5 (21.7)	9 (39.1)
Young bulls	herd 5	39	15 (38.5)	1 (2.6)	23 (59)
Young bulls	herd 7	10	7 (70.0)	0	3 (30)
Cows	herd 9	24	0	2 (8.3)	22 (91.7)
Total	5 herds	134	48 (35.8)	13 (9.7)	73 (54.5)

Table 4. Chlamydophila-specific antibody response of bovines determined by IIF

Category	Herd	N^o of tested sera	N^o of positive (%)
Young bulls	herd 5	39	11 (15.7)
	herd 9	20	0
	herd 7	11	8 (72.7)
Total	3 herds	70	19 (27.1)

Table 5. Antigen and antibodies detected in examined organs and sera by different tests

Category	Detected antigens in organs		Detected antibodies in sera		
Young bulls (herd 7)	DIF	CW	CFT	ELISA	IIF
1	neg	neg	1:128	nd*	nd
2	neg	neg	1:32	pos	1:40
3	neg	neg	1:32	nd	nd
4	neg	neg	1:64	nd	nd
5	neg	neg	neg	neg	neg
6	neg	neg	1:16	pos	1:40
7	neg	neg	1:32	nd	neg
8	neg	neg	neg	neg	1:40
9	pos (lung, liver)	pos (lung, liver)	1:32	pos	1:40
10	neg	neg	1:8	pos	1:40
11	neg	neg	1:32	pos	1:40
12	neg	neg	neg	nd	nd
13	neg	neg	1:64	neg	neg
14	neg	neg	1:64	pos	1:40
15	neg	neg	1:64	pos	1:40
Calves (herd 8)					
1	neg	nd	1:16	nd	nd
2	pos (brain)	nd	1:128	nd	nd
Total	17	15	17	10	11

* nd = not done

Table 6. Sensitivity, specificity, and positive and negative values of CFT and ELISA

Method	CFT		Total (%)
ELISA	+	-	
+	15	33	48 (39.7)
-	1	72	73 (60.3)
Total (%)	16 (13.2)	105 (86.8)	121 (100)
Sensitivity =15/16×100 = 93% 33/105×100 = 31% falsely negative Specificity = 72/105×100 = 68% 1/16×100 = 6% falsely positive			

ELISA was performed on 134 sera samples, and 48 (35.8%) had positive titre on ELISA antibodies to Chlamydophila sp. (Table 3). The result of IIF showed that 19 (27.1%) sera of 70 tested were positive for antibodies against *Chlamydophila* sp. (Table 4).

Of 70 examined impression smears of tissues with DIF, and 60 swabs with CW test, the presence of *Chlamydophila* sp. antigen was detected in lungs and liver of one bull-calf and the brain sample of one calf (Table 5).

The sensitivity, specificity, and positive and negative predictive values of CFT and ELISA are shown in Table 6.

Discussion

Chlamydial CFT is a method which offers the advantage of being usable in several animal species. It is of low sensitivity in large ruminants (cattle) but less so in small ruminants (PEREZ-MARTINEZ et al., 1986; STING, 1997). It also has some disadvantages: the existence of sera with anticomplementary activity or haemolytic sera; difficulty in detecting specific antibodies below genus level (RODOLAKIS et al., 1998), and there are also serious problems of sensitivity when working with animals with low titers (MARKEY et al., 1993). With the CFT, 276 sera of bovines from different rearing categories were examined, the detected CF antibody titer ranging between 1:8 and 1:128. CFT confirmed a low titer ≤ 1:32 in 66 (24.7%) bovine sera while there

were 38 (14.2%) bovines with significant titer (≥1:32) (Table 2). According to the recommendation of GERBERMANN (1991) a low antibody titer was judged to be a non-specific or negative result. Significant titer (≥1:32) of antibodies indicates a possible infection with chlamydophilae, which causes abortions, chronic infections of respiratory organs, keratoconjunctivitis and nervous forms of the disease (GERBERMANN, 1991; ANONYMOUS, 2000a). Even though with CFT in 163 (87.8%) out of 276 sera examined titers were not detected, they cannot be considered as negative for chlamydophila infection and should be clarified using ELISA and IIF methods. The validity of the CFT has been questioned because sometimes, even though CF antibodies are present, and the result of CFT is yet negative, it is a fact that some classes of bovine immunoglobulin (IgG) cannot fix guinea pig complement (BASSAN and AYALON, 1971). The judgement of the falsely positive and falsely negative results in the CFT is held to be very demanding. Infection with *C. pecorum* causes major problems in serodiagnostic of chlamydiosis, because in addition to *C. pecorum*, several species (*C. psittaci*, *C. abortus* and *C. caviae*) have common LPS antigens (JONES et al., 1997; EUZEBY, 1999) and cross-reactions usually occur. CLARKSON and PHILIPS (1997) also warn about this and describe *C. pecorum* infection as ubiquitous on the territory of Great Britain. Many authors believe that the appearance of falsely positive results is a consequence of cross-reaction with gram-negative bacteria and occasional infections with *C. pecorum* which originates from birds (JONES et al., 1997). According to them, CFT is of no relevance for judgement of the results in single samples, or in the discovery of differences between vaccinated and non-vaccinated animals (ANONYMOUS, 2000a; DONN et al., 1997; JONES et al., 1997; RODOLAKIS et al., 1998). In order to avoid erroneous judgements of CFT results we believe that CFT can be a reliable method in the diagnostics of chlamydiosis in bovines only if we take into account clinical symptoms of the disease and significant antibody-titer rise (4-fold or greater) in paired sera, which is also recommended by GERBERMANN (1991).

Of the 134 bovine sera samples, ELISA detected the presence of specific IgG antibodies to *Chlamydophila* sp. in 48 sera (35.8%) (Table 3). During research the ELISA method, compared to CFT, showed a higher sensitivity in proving antibodies to *Chlamydophila* sp. in the sera of infected

animals. Namely, we examined 121 bovine sera samples in parallel. The ELISA method confirmed 39.7%, and CFT 13.2%, positive animals (Table 6). The sensitivity of the ELISA method was 93%, specificity 68%, with respect to CFT (Table 6). Results of our investigation are in accordance with the results obtained by STING and MANDL (1993) who judged ELISA as a highly sensitive method compared to CFT, based and confirmed by extensive serological examinations of 4049 sera in a cow herd with a history of reproductive disorders, while ELISA showed the presence of *C. psittaci* antibodies in 79% and CFT in only 8% of examined sera. During our investigation, ELISA and IIF were methods easier to perform and easier to read. At the same time, the ELISA method is the most objective method for its automatised performance. The CW method proved to be an effective one for a rapid orientation method and for avoiding inadequate samples for further laboratory examinations. The method proved to be extremely valuable in routine diagnostics of field samples. CW method was used for a direct proof of LPS antigens in bovines suspected of infection with bacterium *Chlamydophila* sp. Of the 60 swabs taken from different organs, the presence of antigens of *Chlamydophila* sp. was detected in swabs from the lungs and liver of one bull-calf which, at the same time, had CF antibodies in titer 1:32 and IgG ELISA antibodies (Table 5). On the basis of clinical, pathoanatomic and pathohistologic examinations (not expressed in this study), as well as antibody titer and the antigen detection (DIF), it is obvious that pathoanatomic changes which indicate encephalomyelitis caused by chlamydophilae were present in calves whose CF antibody titer was 1:16 and 1:128. The general pathologic picture in the calf with the antibody titer 1:128 corresponded to the descriptions of the disease in the literature (IDTSE, 1984; JUBB et al., 1991). The presence of chlamydophilae was confirmed in the calf by DIF detecting elementary bodies, which we failed to detect in the calf with CF antibody titer 1:16. The fact that early antibiotic treatment of a large number of calves has an influence on the amount of antibodies in blood sera should not be neglected. We also noticed that the calf with antibody titer 1:16 had the most significant pathoanatomic changes in the brain and liver. The presence of antibodies to *Chlamydophila* sp. was not confirmed in other calves from the same herd. On the basis of these results, and data from other researchers (JUBB et al., 1991), we can observe that low

morbidity and high mortality in herds with encephalomielitis caused by chlamydophilae is an important clinical characteristic of infected calves.

Results of our research on the comparison of diagnostic methods for establishing infections of bovines with the bacteria *Chlamydophila* sp. showed that all the applied serological methods can be widely used as a routine practice in veterinary clinical laboratories. Also, together with serological methods and the methods of isolation, it is necessary to introduce molecular methods such as polymerase chain reaction (PCR) and restriction-fragment length polymorphism (RFLP) for the discovery of differences in DNA structure which are specific for individual isotypes of chlamydophila.

References

ANONIMOUS, (2000a): Enzootic abortion of ewes (ovine chlamydiosis). In: Manual of standars for diagnostic test and vaccines. Lists A and B diseases of mammals, birds and bess. Part 2, Paris. Office International des Epizooties. pp. 515-521.

ANONIMOUS, (2000b): Principles of validation of diagnostic assays for infectious diseases. In: Manual of standards for diagnostic tests and vaccines. Part 1, Paris. Office International des Epizooties. pp. 15-23.

BRADE, L., S. SCHRAMEK, U. SCHADE, H. BRADE (1986): Chemical, biological, and immunochemical properties of the *Chlamydia psittaci* lipopolysaccharide. Infect. Immun. 54, 568-574.

CLARKSON, M. J., H. L. PHILIPS (1997): Isolation of faecal *Chlamydia* from sheep in Britain and their caracterization by cultural properties. Vet. J. 153, 307-311.

BASSAN, Y., N. AYALON (1971): Abortion in dairy cows inoculated with epizootic bovine abortion agent (Chlamydia). Am. J. Vet. Res. 32, 703-710.

DOON, A., G. E. JONES, A. RUIU, M. LADU, J. MACHELL, A. STANCANELLI (1997): Serological diagnosis of chlamydial abortion in sheep and goats: comparison of the complement fixation test and an enzyme-linked immunosorbent assay employing solubilised proteins as antigen. Vet. Microbiol. 59, 27-36.

EUZEBY, J. P. (1999): Les taxons bacterriens d'interet veterinaire dectrits en 1999. Revue Med. Vet. 150, 923-928.

EVERETT, K. D., R. M. BUSH, A. A. ANDERSEN (1999): 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*, includind a new genus and five new species, and standards for the identification of organisms. Int. J. Syst. Bacteriol 49, 415-440.

FUKUSHI, H., K. HIRAI, (1993): *Chlamydia pecorum* the fourth species of genus Chlamydia. Microbiol. Immunol. 37, 516-522.

GERBERMANN, H. (1991): Chlamydiose beim Rind und ihre Bedeutung im Fruchtbarkeitsgeschehen. Wien. Tierärztl. Mschr. 78, 13-19.

GRAYSTON, J. T., C. C. KUO, S. P. WANG, J. ALTMAN (1986): A new *Chlamydia psittaci* strain, TWAR, isolated in acute respiratory tract infections. N. Engl. J. Med. 315, 161-168.

IDTSE, F. S. (1984): *Chlamydia* and *chlamydial* diseases of cattle: a review of the literature. Vet. Med. 79, 543-550.

JONES, G. E, J. C. LOW, J. MACHELL, K. ARMSTRONG (1997): Comparison of five tests for the detection of antibodies against chlamydial (enzootic) abortion of ewes. Vet. Rec. 141, 164-168.

JUBB, K.V. F., P. C. KENNEDY, N. PALMER (1991): Patology of Domestic Animals. 4th ed. Academic Press, Inc., Harcourt Brace Jovanovich, Publishers. San Diego, New York, Boston, London; Sydney, Tokyo, Toronto. Volume 1, pp. 171, 424, 471, Volume 2, pp. 253, 664, Volume 3, pp. 394, 415-417.

MARKEY, B. K., M. S. McNULTY, D. TODD (1993): Comparison of serological test for the diagnosis of *Chlamydia psittaci* infection of sheep. Vet. Microbiol. 36, 233-252.

MEYER, K. F., B. EDDIE (1964): Psittacosis-Lymphogranuloma venerum group (Bedsonia Infections). In: Diagnostic procedures for viral and rickettsial diseases (Lennete, E. H., N. J. Schmidt, Eds.) 3th ed. American Public Health Association, Inc., pp. 603-639.

PAGE, L. A. (1968): Proposal for the recognition of two species in the genus *Chlamydia* Jones, Rake and Sterns, 1945. Int. J. Syst. Bacteriol. 18. 51-66.

PEREZ-MARTINEZ, J. A., N. SCHMEER, J. STORZ (1986): Bovine chlamydial abortion: Serodiagnosis by modified complement-fixation and indirect inclusion fluorescence tests and enzyme - linked immunosorbent assay. Am. J. Vet. Res. 47, 1501- 1506.

PEREZ-MARTINEZ, J. A., J. STORZ (1985): Chlamydial infections in cattle-Part 1 Modern Vet. Practice 66, 517-522.

RODOLAKIS, A., J. SALINAS, J. PAPP (1998): Recent advances on ovine chlamydial abortion. Vet. Res. 29, 275-288.

SCHMEER, N., K. L. SCHNORR, J. A. PEREZ-MARTINEZ, J. STORZ (1987): Dominance of *Chlamydia psittaci*-specific IgG2 subclass in the humoral immune responses of naturally and experimentally infected cattle. Vet. Imunol. Immunopathol. 15, 311-322.

SCHNORR, K. L. (1989): Chlamydial vaccines. J. Am. Vet. Med. Ass. 95, 1548-1560.

STEPHENS, R. S. (1999): Chlamydia: Intracellular biology, pathogenesis, and immunity. American Society for Microbiology, Washington. pp. 11-23.

STING, R. (1997): *Chlamydia-psittaci*-Infektionen bei Kühen und weiblichen Schafen im nördlichen Baden - Württemberg. Tierärztl. Umschau 52, 332-339.

STING, R., J. MANDL (1993): Detection of bovine antibodies to *Chlamydia-psittaci* by an ELISA.Tierarztl. Umschau 48, 512.

VEZNIK, Z., L. POSPIŠIL, D. ŠVECOVA, V. KUMMER, J. CANDERLE, I. DIBLIKOVA, J. MAŠKOVA (1996): Chlamydiae in reproductive disorders and pathology of reproductive organs in man and animals. Reprod. Dom. Anim. 31, 595-600.

WANG, S. P., T. GRAYSTON (1991): Three new serovars for *Chlamydia trachomatis*: Da, Ia and L2. J. Infect. Dis. 163, 403-405.

WILSMORE, A. J., I. DAVIDSON (1991): "Clearview" rapid test compared with other methods to diagnose chlamidyal infection. Vet. Rec. 128, 503-504.

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VLAHOVIĆ, K., A. DOVČ, Ž. ŽUPANČIĆ, M. PAVLAK, J. JERČIĆ: Usporedba seroloških postupaka za dokazivanje zaraze bakterijom *Chlamydophila* sp. u goveda. Vet. arhiv 71, 367-379, 2001.

SAŽETAK

Radi postavljanja objektivne dijagnoze klamidioze, uzrokovane bakterijom *Chlamydophila* sp., istraživan je humoralni imunosni odgovor u goveda. Istražena je i moguća razlika u razini titra specifičnih protutijela između latentno, akutno i kronično inficiranih životinja. Istovremeno, utvrđena je primjenjivost i pouzdanost seroloških postupaka imunoenzimnog testa (ELISA) i reakcije vezanja komplementa (RVK) na temelju specifičnosti, osjetljivosti i reproducibilnosti. U 276 goveda, koja su potjecala iz 10 različitih uzgoja s područja Hrvatske, nalaz protutijela za bakterije *Chlamydophila* sp. utvrđen je u 14,2% uzoraka seruma postupkom RVK, u 35,8% postupkom ELISA, a u 27,1% uzoraka postupkom neizravne imunofluorescencije (IIF). Ove bi spoznaje mogle poslužiti kao smjernice za uvođenje najpouzdanijeg od uporabljenih seroloških postupaka u dijagnostici klamidioze u Hrvatskoj.

Ključne riječi: *Chlamydophila*, dijagnostika, serologija, govedo, infekcija, Hrvatska

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