

PREVALENCE OF ANTIBODIES AGAINST *CHLAMYDOPHILA PSITTACI* AND *CHLAMYDOPHILA ABORTUS* IN CATTLE IN POLAND. A PRELIMINARY REPORT.

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Abstract

In Poland bovine chlamydia monitoring programmes have not been carried out during the last decade, therefore epidemiological status of the disease remains unrecognized. Animal movements observed recently, including international trade and import of livestock into Polish territory, support the necessity of serological surveillance of chlamydia in a representative population of cattle in Poland. The aim of the study was to evaluate *Chlamydia psittaci* and *Chlamydia abortus* seroprevalence in a selected cattle population and comparative evaluation of serological techniques used in chlamydia diagnostics (CFT, ELISA).

Key words: cattle, chlamydia, serological diagnosis, statistical comparison.

Up till now, no wide scale monitoring of the epidemiologic status of chlamydia (former name chlamydiosis) has been conducted in Poland. Taking into account the movement of animals, especially those imported to Poland in last years and lack of such examinations of home cattle, serological monitoring that shows the presence of antibodies against *Chlamydia psittaci* and *Chlamydia abortus* (*Cp. psittaci*, *Cp. abortus*) in cattle seems highly reasonable. All the more that according to the literature, permanent outbreaks of the disease in cattle and sheep are occurring in our neighbours, for example in Germany and the Czech Moravia (20). Moreover, high titres (256 or 516) of antibodies against *Cp. abortus* in sheep and rams exported from Poland and the presence of antibodies against *Cp. psittaci* and *Cp. abortus* were found when examinations towards chlamydiosis were performed in the National Reference Laboratory and in the frame of multiyear program in the National Veterinary Research Institute in Pulawy.

Data considering the taxonomy of the microorganisms belonging to *Chlamydiaceae* along with

information on their pathogenicity towards people and animals were reported recently (7, 9, 15, 18, 20).

It seems also reasonable to mention that chlamydia as a zoonosis presents a serious hazard to people (Fig. 1).

The aim of the presented studies was to estimate the occurrence of antibodies against *Cp. psittaci* and *Cp. abortus* in home cattle population and compare serological qualitative method (complement fixation test) and quantitative one (ELISA) used in the diagnosis of chlamydia in ruminants.

Material and Methods

Serological examinations were done using the complement fixation test (CFT), a diagnostic technique which is recommended for such investigations by the World Organisation for Animal Health (OIE). This technique was validated under the laboratory conditions and accredited by the Polish Centre for Accreditation (according to the PN-EN ISO/IEC 17025:2001/Apl:2003). For the CFT Virion (Swiss Company) and Sera and Vaccines Manufactory (Biomed-Krakow) reagents were used. Before each examination, an intralaboratory evaluation including antigen titration against a positive control serum and checking the activity of the other reagents used in the reaction were carried out to find the actual titre versus activity ratio in relation to that declared by the manufacturers. The specific reaction of the CFT, its consecutive steps and results interpretation were performed according to the Manual of Standards for Diagnostic Tests and Vaccines (1) and the Instruction of the Chief Veterinary Inspector (13). In addition, each sample was examined using two ELISAs, the first for the detection of species specific antibodies against *Cp. psittaci* (Test A, producer Dr Bommeli) and the second for the detection of antibodies against immunogenic protein at a molecular weight of 80 to 90 kDa, specific

to *Cp. abortus* (Test B, producer Institute of Pourquier). A total of 1 333 sera from cows during their last months of pregnancy, both home bred and imported to Poland. The examinations involved 57 counties.

Parts of the placenta of cows that were positive serologically were used to prepare imprint preparations stained according to Stamp's method with the use of 10% carbolic fuchsine.

The χ^2 (chi-squared) test was used to evaluate the reliability of results obtained from the two methods that were compared including the ELISAs' results relevant to the data found by the CFT method. In

addition, a significant value of Pearson and Cramer factors correlation of $P=0.00$ were computed.

Results

Of the 1 333 sera used in our examinations, 257 (19.3%) were positive in both the CFT and ELISA. The statistical analysis embraced the sera collected from 200 animals which were either positively or negatively diagnosed. The analysis of the results obtained from these two tests was shown in Table 1 whereas Table 2 quoted selected data as an example.

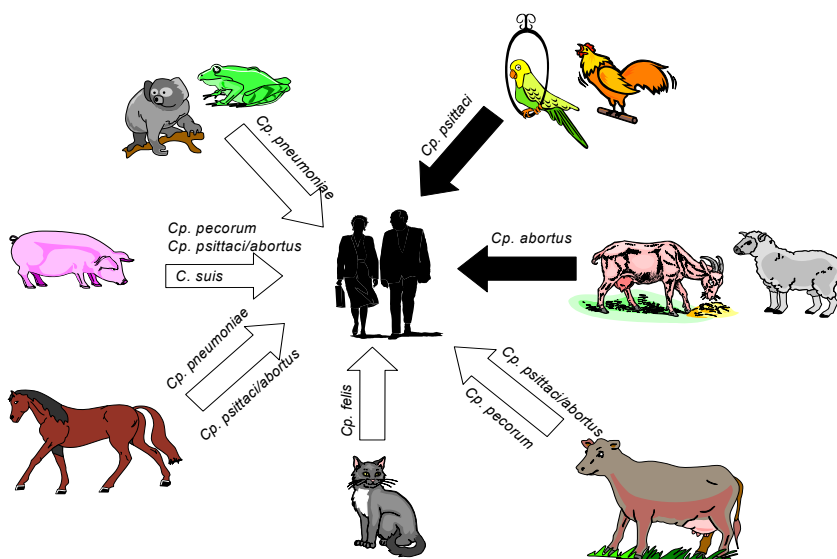


Fig. 1. The zoonotic potential of microorganisms belonging to *Chlamydiaceae*.

Table 1
Repeatability of the two test results

Compared assays	Number and percentage of animals reacting similarly in the two tests compared
CFT and ELISA - test A	159 (79.5%)
CFT and ELISA - test B	126 (63%)
ELISA-test A and ELISA - test B	117 (58.5%)
CFT, ELISA - test A and ELISA -test B	103 (51.5%)

Table 2

Demonstration of the titre distribution of antibodies against *Chlamydomphila* sp. obtained by the CFT and values of optical densities of the sera examined by ELISA

No.	Control serum	CFT					Results	Test ELISA (A)		Test ELISA (B)		Comments			
		1/4	1/8	1/16	1/32	1/64		OD value	Results	OD value	Results	* _ repeatabi lity of CFT results test A	** _ repeatabi lity of CFT results test B	*** _ repeatabi lity of CFT results- test A	**** _ all the same results
1.	-	-	-	-	-	-	neg	29.8	neg	-15.3	neg				****
2.	-	+	+	+	+	+	neg	12.2	neg	-14.5	neg				****
3.	-	-	-	+	+	+	neg	29.1	neg	12	neg				****
4.	-	3+	3+	2+	2+	+	pos	77.7	pos	13.3	neg	*			
5.	-	2+	2+	2+	2+	+	pos	60.6	pos	24.9	neg	*			
6.	-	-	-	+	+	+	neg	11.0	neg	45	neg				****
7.	-	-	+	+	+	+	neg	7.98	neg	5.4	neg				****
8.	-	4+	4+	3+	3+	2+	pos	185	pos	38.9	neg	*			
9.	-	-	-	+	+	+	neg	58.7	pos	304	pos			***	
10.	-	-	-	+	+	+	neg	87.2	pos	83.9	pos			***	
11.	-	3+	+	-	-	-	neg	47.1	pos	1.9	neg		**		
12.	-	3+	2+	-	-	-	neg	86.9	pos	-110	neg		**		
13.	-	4+	3+	2+	2+	-	pos	48.5	pos	99.4	pos				****

Discussion

Lately, in spite of an increasing interest in animal chlamydial infections and their zoonotic potential, there is a general lack of the data considering statistical comparisons of the serological methods applied to the disease diagnosis, especially those relevant to the official investigations including the laboratory techniques recommended by the OIE. Thus, it should be stressed that the recent investigations are, at least in part, pioneering. Numerous authors (2, 3, 6, 10, 19) have compared the serological results restricted only to percentage relations among their results.

It should be stressed that the highest repeatability (79.5%) of the results was evidenced when the CFT and ELISA – test A were used to detect specific antibodies only against *Cp. psittaci*. The CFT method included the group-specific antigen which detected specific antibodies both against *Cp. psittaci* and *Cp. abortus*. It may be assumed that in such cases carrier state or general infection is of concern. These cows did not miscarry and no elementary bodies of *Chlamydomphila* were found by bacterioscopic examinations of the placenta. Similar relationships were evidenced by Hoelzle *et al.* (8) and Stepanek *et al.* (20) with regard to the ELISA concerning the *Cp. psittaci* antigen. To eliminate false negative data related to genital chlamydia in cattle, the authors suggested the need for the use of the kits comprising a mixed antigen, *i.e.* which is able to detect antigens specific for *Cp. psittaci* and *Cp. abortus*.

The statistical analysis (the χ^2 test, at $P=0.00$) revealed a high conformity of the results of the tests compared. The 36.82 value permitted stating that the methods compared are interrelated and give significantly unanimous results. Moreover, the Pearson linear correlation coefficient (0.6570) was evaluated; its values ranged from 0 to 0.707 for a 2 x 2 chart and determined the dependency rate of the methods compared. It was found that the closer the value to zero the weaker the relationship was and in contrast, the closer the value to 0.707 the stronger the dependence was. A similar relationship was found in the case of the Cramer coefficient the values of which were also 0.650 in the range from 0 to 1.

Then, the relationship between the CFT and ELISA-test B detecting specific antibodies against the immunogenic protein occurring only in *Cp. abortus* was examined. This relationship was determined by the repeatability of the results with regard to 126 (63%) sera. The use of ELISA permitting the detection of antibodies against *Cp. abortus* is also recommended by other authors (2, 6, 16) and it is suggested that this method may be a valuable supplement to the CFT. According to the literature data (9, 16, 19), chlamydia of the genital tract causes marked economic losses and imposes a serious threat for people (abortion). The statistical analysis demonstrated a lower chi-squared test value as compared to that obtained when the CFT and ELISA-test B were used; in this case the value was 9.125 and the Cramer and Pearson coefficients was 0.581. On the basis of the statistical

values mentioned above, it may be stated that the two methods are interrelated and give statistically agreeable results.

The results of both ELISA tests (A and B), showing repeatability in 117 examined sera were not confirmed by the statistical analysis. The *chi*-squared value was only 0.13 at the Cramer coefficient attaining 0.0543 and negative values of the Pearson coefficient (0.0543).

Of a total of 200 animals 103 (51.5%) reacted positively in the two tests applied. Abortion was reported in 40 out of 200 tested animals (20%). All the placenta were positive in the Stamp method.

To establish statistical relationship between qualitative and quantitative serological methods undoubtedly further more detailed studies are needed. Thus the relationship found in the present studies should be regarded as the preliminary one. In the further studies the confirming methods such as PCR and cell culturing are intended to be used.

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