

Field evaluation of an iELISA and CF test for detection of IgG antibodies against *Chlamydophila abortus* in goats, sheep and rams

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ABSTRACT: Blood sera samples from 99 clinically healthy goats, 230 sheep and 171 rams were investigated by CF test and indirect ELISA. In case of goats, 3.03% seroprevalence was detected, in sheep it was 3.04%, whereas, in case of rams seroprevalence was 0% by using complement fixation test. Using iELISA in the same groups the seroprevalences observed were, 24.24% in goats, 11.30% in sheep and 5.30% in rams. Indirect ELISA was found to be comparatively more sensitive than CF test in all three groups of animals for detection of IgG antibodies against *Chlamydophila abortus*. The iELISA used in this study can be used for screening at herd level like CF test, as there is moderate agreement ($Kappa = 0.426$) between these two tests.

Keywords: *Chlamydophila abortus*; CF test; iELISA; goats; sheep; rams

Chlamydophila (Ch.) abortus (previously *Chlamydia psittaci*, biotype 1) is the etiological agent of abortion in sheep, goats, and cattle. New genus *Chlamydophila* also includes species *Ch. psittaci* and *Ch. pecorum* which are etiological agents of other infectious and pathological processes like pneumonia, conjunctivitis, polyarthritis in sheep and goats (Everett *et al.*, 1999; O.I.E., 2000).

Complement fixation (CF) test can be applicable on a flock basis to detect evidence of chlamydial infection (Trávniček *et al.*, 2001). IgG antibodies against *Ch. abortus* can be detected with CF test during active placental infection in the last months of gestation period and also following the bacteraemia which often accompanies abortion. Significant levels of IgG antibodies against *Ch. abortus* can be detected up to 8 weeks after abortion or parturition. A rise in antibody titre provides a basis for retrospective diagnosis. Antigenic cross-reactivity between *Ch. abortus* and *Ch. pecorum*, as well as with some Gram-negative bacteria (e.g. *Acinetobacter*), can give rise to low false-positive CF test results (O.I.E., 2000).

Indirect ELISA for detection of chlamydial antibodies in ovines was developed mostly for research purposes (Gajdošová *et al.*, 1994; Kennedy *et al.*, 2001; Longbottom *et al.*, 2001; Buendia *et al.*, 2001). The application of monoclonal antibody technology in a competitive ELISA to discriminate between antibodies to *Ch. abortus* and *Ch. pecorum* is another advantage of iELISA (Salti-Montesanto *et al.*, 1997). ELISA test comparing with CF test is more sensitive and specific for detection of antibodies against chlamydias (Anderson *et al.*, 1990, 1995; Gajdošová *et al.*, 1994; Pospíšil *et al.*, 1996; Věžník and Pospíšil, 1997; Buendia *et al.*, 2001).

The study was aimed at monitoring of IgG antibodies against *Ch. abortus* in clinically healthy sheep, goats and rams using iELISA and CF test.

MATERIAL AND METHODS

Material was represented by 99 goat blood samples from 6 farms, 230 sheep blood samples taken from 8 different farms and 171 blood samples of

rams taken from 7 farms. The sheep farms were different from those with rams. All the animals were investigated preventively and were clinically healthy.

Sera samples were examined by micro complement fixation (CF) test for detection of IgG antibodies. Sera with titre 1 : 32 and higher were considered as positive (O.I.E., 2000). In CF, we used species-specific antigen of *Chlamydophila abortus* (*Chlamydia psittaci*, biotype 1) (Bioveta Ivanovice na Hané, Czech Republic.)

Simultaneously we used indirect iELISA test prepared by the Virological Institute of the Slovak Academy of Science. For iELISA, corpuscular antigen prepared from *Chlamydophila abortus* (previously *Chlamydia psittaci*, biotype 1) strain isolated in Slovakia from enzootic abortion of sheep was used (Sádecký *et al.*, 1978). Serum samples for iELISA were diluted for 1 : 1 000. Readings were taken at 492 nm according to the provided instructions. Standard negative and positive control sera were included in each iELISA assay. Average value of absorbance was 1.34 (Kováčová *et al.*, 1987).

Statistical analysis: CF test and iELISA were evaluated for their sensitivities and specificities and were compared by measuring agreement between tests (Kappa) according to Martin *et al.*, 1988.

RESULTS

In the group of goats ($n = 99$) average 3.03% (0.0–6.66%) of seroprevalence was detected in 6 different farms; while, in case of sheep, ($n = 230$) from 8 different farms the average seroprevalence was 3.04% (0.0–8.57%), whereas in case of rams ($n = 171$) no positivity was obtained from 7 farms using CF test. Using iELISA in the same groups the average seroprevalences were 24.24% (11.76 to 40.0%), 11.30% (0.0–20.93%), and 5.26% (0.0–7.69%) in goat, sheep and rams, respectively (Table 1).

DISCUSSION

Complement fixation test is recommended by the O.I.E. (2000) for the use in the international trade of sheep and goats. Serological investigation can be combined by the ELISA or indirect micro immunofluorescence test. It is necessary to stress

that CF test and iELISA are the screening tests and the results obtained should be interpreted in every occasion in context with the clinical status of the animal or animals. The animals with bad physical and health conditions can give false positive results (O.I.E., 2000).

All sera samples positive in CF test were also found to be positive by iELISA. This finding is important to exclude the possibility of cross reactivity with other antigens and to correlate the specificity of iELISA with CF test. Lukáčová *et al.* (1999) discussed the cross reactivity among *Coxiella burnetti*, Phase II and chlamydiae. Therefore, it is important to take an account of possibility of latent infection of *Coxiella burnetti* in differential diagnosis and testing for chlamydial infection.

The sensitivity and specificity obtained by iELISA was higher as compared to CF test in this study, while both these tests were moderately in agreement (Kappa – 0.426). For iELISA homologous antigen *Ch. psittaci* (PK – 5082) was used. Whereas, in CF test heterologous antigen (Bioveta na Hané, Czech Republic) was used. Use of homologous antigen in iELISA explains the higher specificity than CF test.

According to Anderson *et al.* (1990) and Kováčová *et al.* (1994), results obtained by CF test were variable and with low sensitivity when compare to results obtained by iELISA. Because of higher sensitivity and specificity of iELISA, Kováčová *et al.* (1994), recommended it as a substitute test for CF test.

Markey *et al.* (1993), tested field sera samples, sera from experimentally aborted ewes, subclinically infected sheep and vaccinated sheep, by ELISA, CF test and immunofluorescence assay (IFA). The ELISA and IFA were found much more sensitive than CF test. Ovine abortion or ovine enteric isolates of *Ch. psittaci* possess sufficient antigenic difference for resolution of the status of borderline and complete positivity by iELISA or CF test for sera from flocks with no history of chlamydial abortion in ewes (Markey *et al.*, 1993).

All animals in the present study were clinically investigated and the history of their health status was checked. Every animal was clinically healthy and without previous history of chlamydial infections. Positive serological results obtained by CF test and iELISA may be a result of permanent presence of chlamydias in the digestive tract, which are able to stimulate the antibody production in animal.

Table 1. Seroprevalence of IgG antibodies in clinically healthy goats, sheep and rams detected by CF test and indirect ELISA

CF test	iELISA			
	Inv./posit.	%	Inv./posit.	%
Goats				
Farm No 1	19/0	0.0	19/2	10.52
No 2	17/0	0.0	17/2	11.76
No 3	16/1	6.25	16/4	25.00
No 4	16/0	0.0	16/5	31.25
No 5	15/1	6.66	15/6	40.00
No 6	16/1	6.25	16/5	31.25
Total	99/3	3.03	99/24	24.24
Sheep				
Farm No 1	33/0	0.0	33/2	6.06
No 2	38/1	2.63	38/3	7.89
No 3	24/0	0.0	24/2	8.33
No 4	30/0	0.0	30/2	6.66
No 5	5/0	0.0	5/0	0.00
No 6	35/3	8.57	35/7	20.00
No 7	22/0	0.0	22/1	4.54
No 8	43/3	6.97	43/9	20.93
Total	230/7	3.04	230/26	11.30
Rams				
Farm No 1	18/0	0.0	18/1	5.55
No 2	20/0	0.0	20/0	0.00
No 3	27/0	0.0	27/2	7.40
No 4	25/0	0.0	25/1	4.00
No 5	28/0	0.0	28/2	7.14
No 6	27/0	0.0	27/1	3.70
No 7	26/0	0.0	26/2	7.69
Total	171/0	0.0	171/9	5.26

CONCLUSION

Our result shows that iELISA is more sensitive and detects more sero-reagents when compared with CF test. Interpretation of serological results should be done in relation with clinical status in every case. The iELISA and CF are convenient methods for screening and control of vaccination in sheep against enzootic chlamydial abortion.

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