Detection of IgG Antibodies against *Chlamydophila abortus* in Sheep with Reproductive Disorders

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Abstract

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Chlamydophila (Chl.) *abortus* is the most important causative agent of the enzootic abortion and other chlamydial infections of sheep. Complement fixation (CF) test is a very useful serological method as screening test, but the diagnosis can be complicated by non-specific, false positive reactions with some Gram-negative bacteria as well as with *Chl. pecorum*. Diagnosis can be improved by simultaneous use of indirect (i)ELISA. Moderate agreement (*Kappa* – 0.568) between CF test and iELISA was observed in this study. We observed 36.0 % (75/208) positive samples from clinically healthy sheep and 63.2 % (91/144) in the sheep with reproductive disorders by detecting anti-*Chl. abortus* IgG antibodies using iELISA. When the same sera samples were investigated by CF test, only 13.9 % seropositivity was observed in clinically health sheep whereas in the group with reproductive disorders, positivity observed was 45.8%. Sensitivity and specificity of iELISA used in this study was found to be higher than CF test.

Chlamydophila abortus, IgG antibodies, sheep, iELISA, CF test

Chlamydophila abortus, an etiological agent of enzootic abortion in sheep and goats, may also impair the overall reproductive performance (Papp and Shewen 1996). The organism has a zoonotic potential (Buxton 1986; Hyde and Bernischke 1997), and therefore must be dealt with great care with adequate microbiological precautions and laboratory containment. After revision of the family *Chlamydiacae,* Everett et al. (1999a) proposed to include this family into two genus, namely *Chlamydia* (Ch.) and *Chlamydophila* (Chl.). Genus nov. *Chlamydophila abortus* (previously *Chlamydia psittaci*, biotype 1) will substitute *Ch. psittaci,* the etiological agent for abortion of sheep, goats and cattle.

Complement fixation (CF) test can be used at a herd basis to detect the infective cause of abortion (Trávníček et al. 2001). IgG antibodies against *Chl. abortus* can be detected with CF test during active placental infection in the last months of gestation period and following the bacteraemia which often accompanies abortion. A significant level of IgG antibodies against *Chl. abortus* is possible to detect up to 8 weeks after abortion or parturition. A rise in antibody titre provides a basis for retrospective diagnosis. Antigenic cross-reactivity between *Chl. abortus* and *Chl. pecorum*, as well as with some Gram-negative bacteria like *Acinetobacter* can give rise to low false positive CF test results (Manual of Standards, 2000). False positivity can be avoided by using a more sensitive and specific method like ELISA. In our study we have not checked the cross reactivity of the CF test. Considering the cross reactivity of CF, it is possible to obtain more seropositivity by CF than ELISA, but the chances of getting more positivity are the least as cross reactive agents are not always present.

Indirect ELISA for detection of chlamydial antibodies in sheep was developed mostly for research purposes (Jones et al. 1997; Kaltenboeck et al. 1997; Salti-Montesanto et

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al. 1997; Gut-Zangger et al. 1999; Kennedy et al. 2001; Longbottom et al. 2001 and Buendia et al. 2001). Nowadays, iELISA kits are available commercially for detection of antibodies against *Chlamydophila abortus* in animals (Pospíšil et al. 1996; Věžník and Pospíšil 1997 and Buendia et al. 2001). iELISA test is more sensitive and specific as compared to CF test for detection of antibodies against chlamydias.

Our study aimed at monitoring of IgG antibodies against *Chl. abortus* in clinically healthy sheep as well as in sheep with reproductive disorders using iELISA and CF test and to compare both these tests.

Materials and Methods

A total of 208 serum samples were collected from clinically healthy sheep in 9 different farms with no evidence of chlamydial infection (i.e. abortion, stillbirths, pneumonia, conjunctivitis etc.), and 144 serum samples from seven farms of sheep with reproductive disorders. The animals with reproductive disorders were kept separately from the farms where healthy animals are bred. The reproductive disorders were abortion, stillbirths and infertility. The sera were examined by CF test as well as by iELISA. For CF test sera with titre 1:32 and higher were considered as positive (Manual of Standards, 2000). In CF test, we used species-specific antigen of *Chlamydophila abortus* (*Chlamydia psittaci*, biotype l) provided by Bioveta Ivanovice na Hané, Czech Republic.

We used commercially available iELISA kit by Cypress Diagnostics, Leuven, Belgium for detection of antibodies to *Chl. abortus* in ovine serum. The *Chl. abortus* specific antigen was coated in 96-well microtitre plate. Specific antibodies to *Chl. abortus* in diluted serum samples binds with coated antigen and remains attached even after washing with buffer. Anti-sheep antibodies conjugated with HRPO were added to bind with attached anti C*hl. abortus* antibodies. Substrate (OPD) was added after washing unbound conjugate for chromogenic reaction.

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 clinically healthy sheep (n = 208), a of total 13.9 % seroprevalence was detected by using CF test. Seroprevalence in these clinically healthy sheep from different

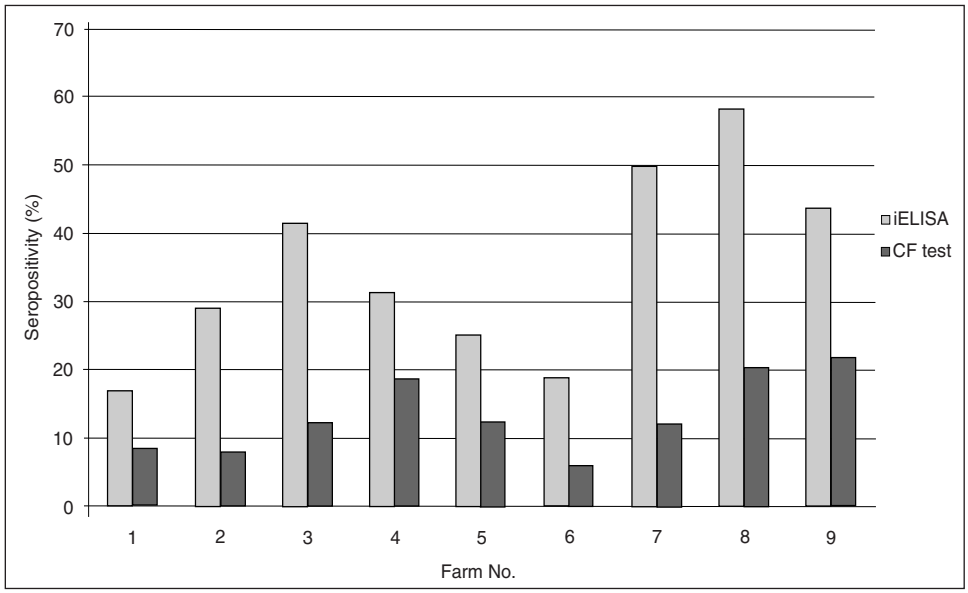

Fig. 1. Detection of IgG antibodies in clinically healthy sheep using iELISA and CF test

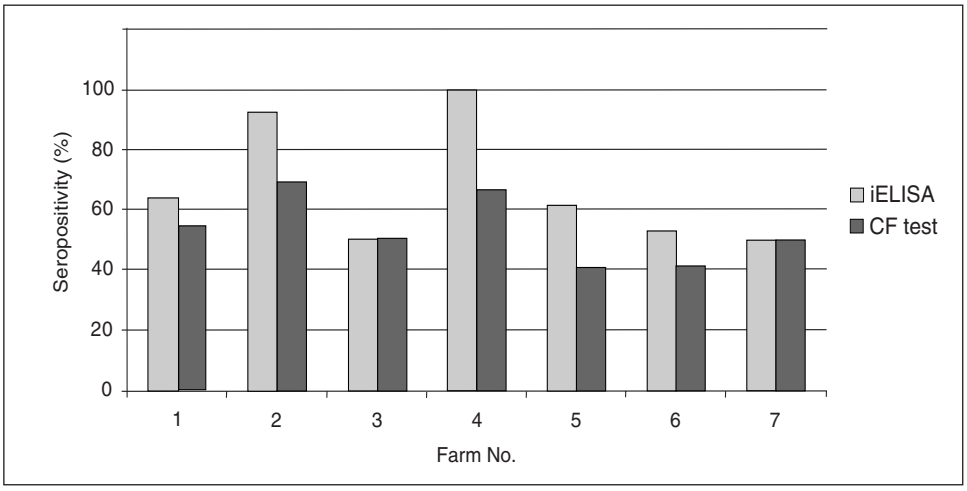

Fig. 2. Detection of IgG antibodies in sheep with reproductive disorders using iELISA and CF test

farms ranged between 6.2-21.9%. When the same group of sheep was screened by iELISA, a total 36.0% (16.6-58.3%) seroprevalence was detected (Fig. 1).

In the second group, that is sheep with reproductive disorders (n = 144), seroprevalence was 45.8% (40.9-69.2) by using CF test, whereas, the same was 63.2 % (50.0-100.0 %) by using iELISA (Fig. 2).

Discussion

Kennedy et al. (2001) developed two serological tests viz. indirect immunofluorescence test and ELISA for the detection of fetal antibody to *Chl. abortus* for diagnosis of ovine abortion. An indirect ELISA (R OMP 91B iELISA) based on recombinant protein fragment of the polymorphic outer membrane protein, POMP91B of *Chlamydophila abortus* was developed and used by Longbottom et al. (2001). Indirect ELISA proved more sensitive (84.2 %) and specific (98.5%) than CF test in experimentally as well as naturally infected sheep. Furthermore, iELISA was found better at differentiating *Chlamydophila abortus* from *Chlamydophila pecorum* in infected animals (Longbottom et al. 2001). Our results obtained in sheep with reproductive disorders using iELISA showed 63.2 % positivity, which is significantly higher then positivity obtained with CF test (45.8%). The sensitivity and specificity obtained for iELISA as compared to CF test was higher in this study, while both these tests were moderately in agreement (Kappa – 0.568).

A new commercially available ELISA (ELISAr-Chlamydia) for detecting antibodies against *Chlamydophila abortus* has been evaluated in sheep (Buendia et al. 2001). The ELISA is based on a recombinant antigen, which expresses part of a protein from the 80-90kDa, which is specific to *Chlamydophila abortus*. A total of 105 sera from six flocks with confirmed ovine chlamydial abortion (OEA), as well as 258 sera from 18 flocks, with out OAE were tested by ELISA with recombinant antigen. The ELISAr-Chlamydia was compared with the CF test and with ELISA using purified *Chlamydophila abortus* elementary bodies (ELISA-EB). The results showed that the sensitivity of ELISAr-Chlamydia was 90.9% with a specificity of 85.9%. As compare to above parameters the sensitivity of CF test was 71.0% with a specificity of 83.6%, whereas, the sensitivity and specificity of ELISA-EB were 95.2% and 54.2% respectively. The study demonstrated most

Table 1
Number of animals tested and farm wise positivity obtained for IgG antibodies in clinically healthy sheep as well as in sheep with reproductive disorders using iELISA and CF test

Farm No.	No. of animals	No. of positive animals	
clinically healthy sheep	tested	iELISA	CF test
1	24	4	2
2	24	7	2
3	24	10	3
4	16	5	3
5	24	6	3
6	16	3	1
7	24	12	3
8	24	14	5
9	32	14	7
Total	208	75	26
	sheep with reproductive disorders		
1	11	7	6
2	13	12	9
3	2	1	1
4	3	3	2
5	88	54	36
6	17	9	7
7	10	5	5
Total	144	91	66

balanced results between sensitivity and specificity for ELISAr-Chlamydia (Buendia et al. 2001). The sensitivity of CF test in our study in the group of sheep with reproductive disorders was 45.8% that is lower than iELISA but, comparing with the results obtained by Buendia et al. (2001), sensitivity of iELISA used by us was lower. According to Everret et al. (1999) and Everret and Andersen (1999), introducing Polymerase Chain Reaction (PCR) into practice can make possible an exact distinction of invasive and pathogenic species of chlamydiae.

Salti-Montesanto et al. (1997) compared three serological methods viz. competitive (c) ELISA, A-ELISA (containing solubilized outer membrane complexes) and CF test, using 125 field ovine sera. They detected 45.9% sensitivity for cELISA, 48.8% for A-ELISA and only 19.2% for CF test. The prevalences obtained by CF and iELISA in group of clinically healthy sheep and in sheep with reproductive disorders are significantly different (13.9%-45.8% by CF and 36.0%-63.2% by iELISA).

Indirect ELISA found to be more sensitive and specific than CF test. It is concluded that an indirect ELISA is suitable as a routine test for chlamydial diagnosis and seroepidemiological studies in sheep.

Detekcia IgG protilátok proti *Chlamydophila abortus* u oviec s poruchami reprodukcie

Chlamydophila (Chl.) abortus je najdôležitejším pôvodcom enzootického abortu a iných chlamýdiových infekcií oviec. Komplement fixačný test (KFT) je veľmi užitočným sérologickým testom používaným tiež ako screeningový test, výsledky ním dosiahnuté však môžu byť komplikované nešpecifickými, falošne pozitívnymi reakciami s antigénmi niektorých Gram negatívnych baktérií a tiež s *Chl. pecorum.* Sérodiagnostika môže byť

upresnená simultánnym použitím nepriamej ELISA metódy. Zistili sme rozdielnosť (*Kappa* – 0.568) medzi KFT a nepriamou ELISA metódou. Pri použití nepriamej ELISA sme zistili u klinicky zdravých oviec pozitívne hladiny IgG protilátok u 36.0% zvierat (75/208) a u oviec s poruchami reprodukcie séropozitivita bola 63.2% (91/144). Pri použití KFT u klinicky zdravých oviec séropozitivita dosiahla 13.9% a v skupine oviec s reprodukčnými poruchami bola 45.8%. Citlivosť a špecificita nepriamej ELISA metódy použitej v tejto štúdii bola vyššia ako u KFT testu.

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