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To cite this version:
M. Pépin, Laurence Bailly, A. Souriau, Annie Rodolakis. AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE DETECTION OF CHLAMYDIAL ANTIBODIES IN CAPRINE SERA. Annales de Recherches Vétérinaires, INRA Editions, 1985, 16 (4), pp.393-398. <hal-00901600>

HAL Id: hal-00901600
https://hal.archives-ouvertes.fr/hal-00901600
Submitted on 1 Jan 1985

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AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE DETECTION OF CHLAMYDIAL ANTIBODIES IN CAPRINE SERA

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Résumé

ÉVALUATION D’UN TEST IMMUNOENZYMATIQUE (ELISA) POUR LA DÉTECTION DES ANTICORPS ANTI-CHLAMYDIA PSITTACI DANS LES SÉRUMS DE CHÈVRES. — Un test immunoenzymatique (ELISA) rapide et simple a été évalué pour le diagnostic sérologique de la Chlamydie abortive caprine. La souche de Chlamydia psittaci AB 3, multipliée sur membrane vitelline, a été utilisée comme antigène. La comparaison des titres obtenus avec la technique ELISA et la réaction de fixation du complément — méthode de référence — sur 204 sérum de chèvres montre qu’il existe une bonne corrélation entre les deux techniques (80 % de concordance). La cinétique des anticorps anti-Chlamydia après une infection expérimentale de 15 chèvres est tout à fait comparable, avec les deux techniques. La reproductibilité de la détermination des titres avec la technique ELISA est bonne.

Diagnosis of Chlamydia psittaci abortive infection of goats can be done by (i) isolation of the causative bacteria (ii) microscopic examination of colored smears of vaginal swabs or placenta and (iii) serological test (Rodolakis and Russo, 1984). Although several tests have been used (Storz, 1971), complement fixation (CF) test is the most widely used and considered as reference. This test has limitations: particularly, it requires good training, it cannot distinguish small serological reactions of intestinal chlamydia to those of systemic infection and it cannot be applied to serum exhibiting anticomplementary activity. These facts lead on to adopt a threshold value for positive response and to interpret results on a flock basis.

Enzyme-linked immunosorbent assays (ELISA) have been applied to determine specific antibody levels in many infectious diseases since their introduction by Engvall and Perlman (1971). Antibodies to Chlamydia trachomatis in human sera (Lewis et al., 1977; Jones et al., 1983; Levy et al., 1983; Duc-Golran et al., 1983) to Chlamydia psittaci in duck (Evans et al., 1983), to ewe (Milon et al., 1985) and caprine sera (Vitu et al., 1984) have been advantageously titrated by ELISA. It appears that this method is sensitive, reproducible and fast as a routine test. We used ELISA as a diagnostic test for chlamydiosis in goats compared with CF test, with an egg yolk propagated Chlamydia psittaci strain as antigen. Both methods gave similar results and ELISA may be proposed as an alternative technique to CF test.

Materials and Methods

Antigen

The abortive Chlamydia psittaci strain AB3 had been isolated from a vaginal swab of an aborted Solognote...
ewe. The third passage in chicken embryo stored at \(-70^\circ\text{C}\) was used as antigen. This suspension had a minimum infectivity titer of \(10^7\) plaque forming units per millilitre (PFU/ml) determined by plaque lysis on monolayer McCoy cells (Rodolakis and Chancerelle, 1977). The bacteria were killed before use by formaldehyde (0.1 %).

**Conjugate**

The conjugate used for ELISA was an anti-goat immunoglobulin G (IgG) horseradish peroxidase-labelled. This anti-IgG was prepared from rabbit and obtained from Cappel Laboratories, West Chester, PA 19380. The method of conjugating peroxidase (Boehringer Mannheim, 38240 Meylan, France) was performed as described by Nakane and Kawaoi (1974): the carbohydrate moiety of fluorodinitrobenzene - blocked peroxidase was oxidized with sodium periodate (Merck, 94130 Nogent-sur-Marne, France).

**Sera**

The caprine sera tested had two origins:

(i) 152 sera came from 47 goats raised at the Station de Pathologie de la Reproduction (INRA, Nouzilly, France) (group 1). Fifteen of these goats were inoculated with a suspension of *Chlamydia psittaci* strain AC1 propagated in yolk sac, by intradermal route (\(10^6\) PFU/animal) during the third month of gestation. Among these 15 goats, 12 had aborted. Blood samples were taken twice before the inoculation, each week during the four weeks after the inoculation and each month during the three months after the inoculation.

(ii) 52 sera came from two different flocks exterior to the Station de Pathologie de la Reproduction (22 and 30 goats respectively) (group 2). One flock had known an episode of enzootic abortion caused by *Chlamydia psittaci* a few years before this sample.

All sera were stored at \(-20^\circ\text{C}\).

The reference positive serum was obtained from blood sample of an inoculated goat taken two months after the inoculation. The titer in CF test of this serum was 1/160. The reference negative serum was obtained from pool of blood samples of three goats; the titer in CF test was 1/10. These sera were diluted (1/2) in glycerol and stored at \(-20^\circ\text{C}\).

**CF test**

The complement-fixing antibodies were detected by Kolmer type micromethod (Lombard and De Saint-Hubert, 1976; Rodolakis *et al.*, 1977) with a yolk sac propagated chlamydial antigen (Rakeia, Roger Bellon, France). The highest serum dilution showing less than 50 per cent hemolysis was taken as the end point. A serum was considered positive when its end point was 1/80 or greater.

**Preparation of antigen-coated plates**

Polystyrene microplates (Greiner, 67240 Bischwiller, France) were coated with antigen (100 µl per well) by passive adsorption for 18 hours at 37 °C with gentle shaking (200 rotations per minute, rpm). The optimal dilution of the antigenic suspension was previously determined by titration against reference sera. This dilution was 1/1000 in 0.05 M carbonate-bicarbonate buffer (pH 9.5) giving a final concentration of approximately \(10^3\) PFU per well. Preliminary assays had also shown that sera weakly reacted only with antigens of yolk sac at low dilutions (1/10 to 1/40).

**Fig. 1.** — ELISA absorbance values for serial twofold dilutions of reference serum used to generate a positive control curve. This control was used to determinate the titers of tested caprine sera.
Coated plates were washed three times with desionated water and the wells were filled with 0.5 % bovine serum albumin in phosphate-buffered saline (PBS) (100 µl per well) and left at 37 °C for at least 4 hours to block remaining sites on the plastic. The plates were washed three times with desionated water and used immediately or dried and stored at 4 °C. Serial fivefold dilutions of caprine sera were performed in PBS containing 0.05 % (vol/vol) Tween 20 (PBST). The sera were tested at two dilutions: 1/125 and 1/625 (two wells per dilution; 100 µl per well); the choice of these two dilutions was previously determined and this procedure allowed (i) to estimate the titers of sera with a control curve (ii) to test 18 sera per plate. Serial twofold dilutions of reference sera were made and all dilutions were tested. After incubation for 2 hours at 37 °C, 200 rpm, the plates were washed three times in saline solution containing 0.05 % Tween 20 (ST) and the diluted conjugate (1/500) was added (100 µl). The plates were incubated for 2 hours at 37 °C, 200 rpm. After washing, 100 µl of 2,2'-azino-di-[3-ethyl-benzothiazolin-sulfonate] (ABTS) (Boehringer Mannhein, 38240 Meylan, France) (2.22 mg of ABTS in 10 ml of phosphate citrate buffer, pH 4.0 with 40 µl H2O2, 2 %) were added to each well. The plates were left for 30 minutes at 37 °C and the color reaction was stopped by adding 20 µl of sodium dodecyl sulfate 10 % in distilled water. Color was read without delay at 405 nm with Multiskan plate reading (Flow Laboratories, 92802 Puteaux, France). All readings were made against a blank row of wells which received all the above treatments, except that caprine serum was replaced by PBST.

Estimation of caprine sera titers

The titers of tested sera were determined from a control curve obtained with some titrations of reference positive serum. For each test, the reference sera were included for testing reproducibility and validity of assay. The estimation of titers was made with the following formula (Atanasiu and Perrin, 1979):

\[
\text{Titer (EU = equivalent units)} = \left( \frac{B \times C}{A} \right)
\]

with:

- \(C\): reciprocal of tested serum dilution
- \(B\): titer of reference positive serum (160 EU)
- \(A\): reciprocal of positive serum dilution corresponding to absorbance value of tested serum reading on the control curve (fig. 1).

This method of estimation of titers allowed to get rid of no-specific activity of sera.

Results

A positive threshold value for ELISA test was determined from titers of 62 sera of 47 healthy goats raised at the Station INRA; all these sera were sampled before experimental infection. The mean titer was 19.7 EU (standard deviation: 12.7). The positive threshold value was estimated with the following formula: mean titer + 2.57 standard deviation (2.57 is the value of deviation for a risk of 1%). The threshold value was 53 EU; this positive threshold value was chosen for all subsequent assays. The comparison of ELISA titers and CF for the 152 caprine sera of group 1 showed that there was a good agreement (fig. 2); the correlation coefficient for results with the two methods was 0.48 (P \( \leq 0.001\)). Analysis of discordant responses (+/- or -/+ ) did not show a significant difference between the two methods.
The most discordant responses were registered from samples taken either immediately after experimental infection or a long time after infection (tabl. 2). The kinetics of chlamydial antibodies was the same either with ELISA or with CF test (fig. 3 and tabl. 2). The comparison of ELISA and CF titers for the 52 caprine sera of group 2 showed that there was a difference of sensitivity or specificity between the two methods; ELISA gave more numerous positive responses than the CF test (tabl. 1): the difference between the discordant responses was significative ($P < 0.001$). The majority (12/14) of discordant responses came from sera of goats of the flock which had known an epidem of enzootic abortion.

The reproducibility of results obtained from ELISA was good; 24 sera were titrated three times, only three discordant responses were registered: 3 sera identified as positive in two assays were filed negative in the third assay. The comparison of mean titer (in EU) obtained in these assays did not show difference. Day-to-day variations in the ELISA results was examined by evaluating the titers of reference sera. There was little day-to-day variation for negative serum, the mean titer was 22.6 EU (standard deviation: 3.6; standard error: 0.79) and for positive serum, the mean titer was 157 EU (standard deviation: 30; standard error: 6.4).

**Discussion**

We have developed an ELISA in which whole killed bacteria were used as antigen for the detection of chlamydial antibodies in caprine sera. For the detection of IgG class antibodies, the ELISA proved to be comparable to the CF test widely used in practice. Comparison of ELISA titers and CF test titers for the 152 sera of goats of group 1 shows a good agreement (fig. 2 and tabl. 2). For the 52 sera of goats of group 2, ELISA gave more numerous positive responses than CF test: this discrepancy which may be caused by a greater sensitivity or by a lack of specificity which may be due to use whole bacteria as antigen. These antigens can detect antibodies elicited by others microorganisms which possess cross reactivity with *Chlamydia psittaci* as *Acinetobacter calcoaceticus* (Brade and Brunner, 1979). However, it seems for our study that the discrepancy may be due to a greater sensitivity of ELISA: most of the discordant responses are registered with sera of the flock which had known an epide of enzootic abortion. This hypothesis is supported by the results obtained in other studies with a purified antigen either with *Chlamydia trachomatis* (Finn et al., 1983) or with *Chlamydia psittaci* (Vitu et al., 1984): it appears that the ELISA is more sensitive.

![Fig. 2](image_url) - Scatter diagram of serum chlamydia antibody titers obtained by ELISA and CF test. Correlation between the two techniques was determined by the least-squares method.

... positive threshold values: 80 for CF test, 53 for ELISA.
than CF test. The difference may be also due to the fact that the ELISA does not detect the same class of immunoglobulins as the CF test: IgG for ELISA, IgG1 and IgM for CF test. However, it seems that the levels of IgM do not increase the rate of complement-fixing antibodies in sheep (Fuensalida-Draper and Rodolakis, 1978): the level of IgG may only lead to this disagreement. ELISA seems to be more appropriate than the CF test for early detection of chlamydial antibodies in caprine sera after an experimental infection (fig. 3): we have also found this result with ovine sera (unpublished results) just as Vitu et al. (1984).

Our estimation of antibody titers in sera allows a great number of samples to be tested in an assay. This method, used in other studies (Levy et al., 1983) is more advantageous (rapidity and cheapness) than the method used by Finn et al. (1983): the titer of a tested serum is determined by the reciprocal of the last dilution placed just before a negative threshold value. ELISA has a good reproducibility: it is necessary, to ensure reproducible results, to respect rigidly the technical procedure of ELISA, especially during the washing steps. With this condition, ELISA appears more attractive for antibody detection than CF test: ELISA may be performed with minimal technical training, uses only small quantities of reagents and all reagents including antigen coated plates are stable for long periods of time. Furthermore, using a purified antigen, ELISA will be able to allow a distinction between serological reaction to intestinal chlamydia to those of abortive infection and practice of individual diagnosis.

Received, 15th February, 1985.
Accepted, 30th March, 1985.

Acknowledgements

We are grateful to Dr S. Bernard and Isabelle Lantier for providing the enzyme-conjugated immunoglobulin used and to Dr M. Plommet for his advice.

Summary

Chlamydia psittaci is one of the major cause of abortion in goats. The complement-fixation (CF) test is widely used for detection of chlamydial antibodies in caprine sera. A simple, rapid enzyme-linked immunosorbent assay (ELISA) has been developed for the measurement of immunoglobulins G (IgG). The
Chlamydia psittaci strain AB3 grown in yolk sac was used as antigen. ELISA proved satisfactory for the detection of serum IgG; a total of 204 sera were tested and the results correlated well with those obtained by CF test (80 per cent of agreement). The results obtained with ELISA were reproducible.

References


