

USE OF ELISA FOR THE DIAGNOSTICS OF OVINE SARCOCYSTOSIS

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Abstract. A modification of micro ELISA was used for the detection of specific antibodies in the diagnostics of ovine sarcocystosis. The results of ELISA compared to those of indirect fluorescence reaction (IFR) were identical in 94.7%. The titres of specific antibodies were higher in 70.8% of samples if ELISA was used. The coincidence of the two tests is statistically highly significant ($P < 0.005$). ELISA can be recommended for both experimental and routine examinations for ovine sarcocystosis.

Ovine sarcocystosis is a frequent disease in Czechoslovakia. Černá and Merhautová (1981) found 82% of animals to be positive. Similarly Gut (1982) demonstrated sarcocysts in muscles of 92% of sheep. Svobodová and Nevole (1985) detected sarcocystosis in 64.9% of mixed muscle samples of ewes, rams and lambs. These papers indicate the wide occurrence of ovine sarcocystosis in Czechoslovakia.

Cases of sarcocystosis have not yet been recorded in this country. However, it is necessary to point out the difficulties in its diagnosis, since the clinical symptoms are nonspecific: fever, anaemia and loss of weight. According to Černá (1989), without the determination of antibody titre dynamics sarcocystosis may be mistaken for another nosological unit. In case of a latent course of the disease, the loss of weight causes economical losses in lambs (Munday 1986). The presence of macrocysts is important for the decision about the meat quality in adult animals (Pleva et al. 1987). The preventive measures consisting in the separation of intermediate hosts from the definitive ones can hardly be applied in sheep breeding (Levine 1985).

It is therefore necessary to improve and spread the diagnostic methods. The direct detection of cysts in the muscles has been one of the most commonly used methods. Indirect haemagglutination (Červa and Gut 1983) and indirect immunofluorescence were used for experimental diagnosis of sarcocystosis.

At present time, ELISA is increasingly applied for the diagnostics of various diseases including parasitary ones. Boch et al. (1979) and Reiter et al. (1981) used it for the detection of antibodies against sarcocystosis. The aim of this study was to prepare a modification of ELISA suitable for the diagnostics of ovine sarcocystosis in Czechoslovakia and by a comparison with IFR to estimate the possibility of its practical utilization.

MATERIALS AND METHODS

Since ELISA has been previously used for the diagnosis of sarcocystosis in Czechoslovakia, we used a set of 301 serum samples from sheep slaughtered at the abattoir in Tišnov and Strakonice and the samples were tested simultaneously by indirect immunofluorescence reaction (IFR). Cystozoites of *S. gigantea* were used as corpuscular antigen. Macrocysts were isolated from oesophagus muscles and washed in saline. After the macrocyst wall had been teared by operation needle, the zoites were released into PBS. The obtained suspension was dropped on slides and after drying stored at -18°C till further use. A lyophilized conjugate RASH/FITC (Bioveta, Ivanovice na Hané) was used. The sera were diluted from 1 : 10 to 10,240. The reliability of IFR was verified by the following controls:

- a) with negative serum, i.e. serum of lambs before drinking the colostrum,
- b) without serum,
- c) with positive serum, i.e. serum obtained by three times repeated examination of highly positive animals.

The preparations were evaluated using Jenalumar 30–600500 fluorescence microscope at 630 magnification.

The sandwich test for antibody titration was verified for the diagnosis of sarcocystosis by means of ELISA. Soluble antigen for the binding on a solid phase was prepared also from *S. gigantea* macrocysts. The released zoites were washed by centrifugation in PBS. The sediment diluted by sterile distilled water was disrupted by five fold freezing and slow thawing in a mixture of ice and salt and then centrifuged for 15 min at 300 g.

The supernatant was clarified by ultracentrifugation at 30,000 g 30 min. The obtained soluble antigen contained 740 μg protein in 1 ml. EIA RASH/Px glycerine conjugate with IgG concentration 1.3 mg/cm^3 and Px/IgG molar ratio 1.44 was used. The examined sera were diluted from 1 : 10 to 10,240. 5-aminosalicylic acid was used for the detection of peroxidase activity. The following control wells were on each plate:

- a) blank, i.e. without serum,
- b) with negative serum, i.e. serum of lambs before drinking the colostrum,
- c) with positive serum, i.e. serum exhibiting the highest titre in IFR.

To verify the specificity of the reaction, sera with positive antibody titre against toxoplasmosis (detected by Sabin-Feldman's reaction) and with negative titre against sarcocystosis were used and the titration curves were evaluated. Samples with values twice as high as those of negative controls were considered as positive.

Experimental design

1. Binding of 0.05 ml of antigen diluted in carbonate bicarbonate buffer, pH 9.5, in wells of microtitration plates. Incubation at 4°C for 18 h.
2. Washing by washing solution (PBS–0.05% Tween 20) ($3 \times$).
3. Application of 0.05 ml of tested serum and control sera diluted in dilution solution (PBS – 2% BSA) always into 2 wells in parallel. Incubation at 37°C for 90 min.
4. Washing by washing solution ($3 \times$).
5. Application of 0.05 ml conjugate diluted by dilution solution. Incubation at 37°C for 90 min.
6. Washing by washing solution ($3 \times$).
7. Visualization by prepared substrate of 5-aminosalicylic acid (0.1 ml). Incubation at 37°C for 30 min.
8. Reading of the result by Dynatech MI–700 photometer at 490 nm. The results obtained by ELISA were compared with those obtained by IFR and the relations were statistically evaluated (Hamilton and Rinaldi 1988).

RESULTS

Prior to the serum examination the box titration was performed to assess the optimal dilution of antigen and conjugate. The antigen and conjugate were tested in the dilutions 40, 80, 160 and 320, and 100, 200, 400, 500, and 1,000, respectively. The sera in which the highest antibody titre (10,240) was detected by IFR were used for the box titration. The optimal antigen dilution was 80, which means the protein concentration of 9.2 µg/l ml and optimal conjugate dilution was 200, i.e. 6.5 µg IgG in 1 ml.

Fig. 1 shows a comparison of the results obtained by ELISA with those obtained by IFR. The portion of middle and high antibody titres detected by ELISA is markedly higher. Of the 301 serum samples examined, identical results were obtained by the two methods in 285 samples (94.7%) and different results were obtained in 16 samples (5.3%). Titres of specific antibodies detected by ELISA were higher in 213 samples (70.8%), identical titres occurred in 72 samples (23.9%) and in 16 samples (5.3%), the antibody titres detected by ELISA were lower than those obtained by IFR. The titration curves are compared in Fig. 2. It is evident that no cross reaction with *Toxoplasma gondii* antigen occurred.

DISCUSSION

ELISA, due to its high sensitivity, is used for the diagnostics of various diseases including the parasitary ones. In Czechoslovakia it has been successfully applied particularly in human medicine, e.g. for the diagnostics of toxoplasmosis (Nemec and Čatár 1981) or larval toxocarosis (Uhlíková and Hübner 1983).

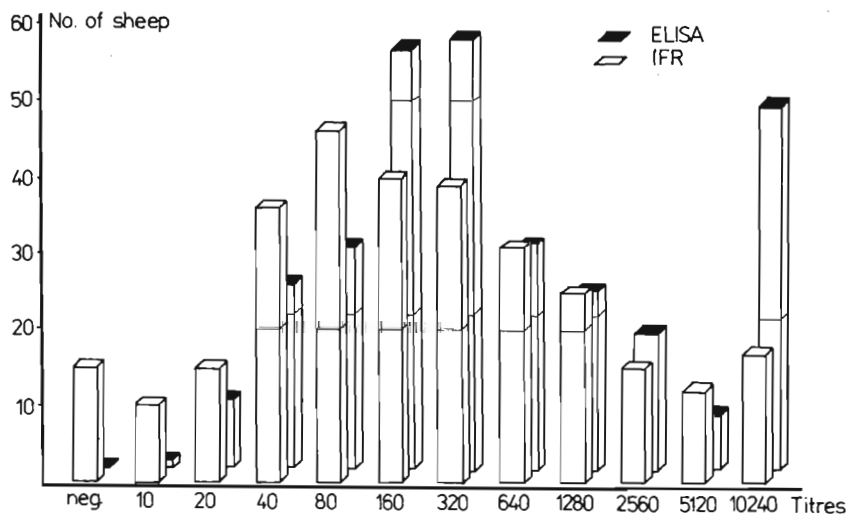


Fig. 1. Comparison of IFR and ELISA (frequency of antibody titres).

O'Donoghue and Weyreter (1984) used this method for the detection of antibodies against sarcocystosis. In our studies, ELISA was verified on a set of 301 serum samples previously tested by IFR.

Since there was no possibility to obtain positive control sera from experimental infection, the result was verified by triple repeat of IFR.

In agreement with Černá (1985) we consider IFR to be a reliable method and, moreover, the resulting titres were documented by a direct finding of the cysts in muscles. A significant correlation with the occurrence of muscle cysts was recorded at the titres higher than 40 (Svobodová 1989).

The coincidence of the two reactions was high and reached almost 95%. The methodical discrepancy was only 5.3% and was caused by the higher sensitivity of ELISA. In comparison with IFR, higher antibody titres were found in 70.8% of samples, identical titres in 23.9% of samples, and only in 16 samples the antibody titres detected by ELISA were lower. However, an erroneous reading of end point titres in IFR cannot be excluded in these cases. These results show that in general higher antibody titres were detected by ELISA. This is supported by the fact that the titres of 10–80 occurred less frequently, whereas the middle and high titres were

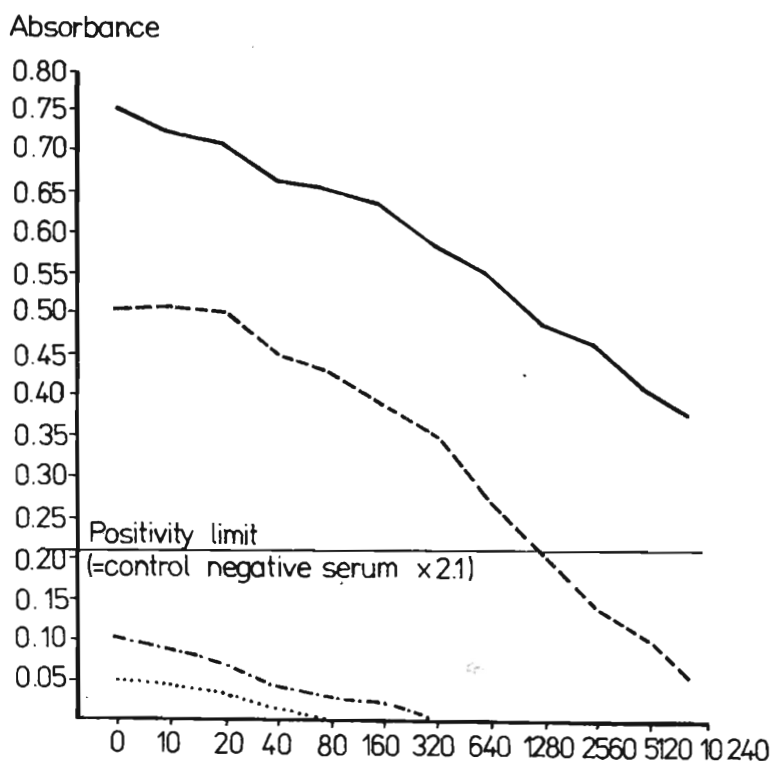


Fig. 2. Titration curves. — positive sera, - - - - control negative serum, control *T. gondii* positive serum.

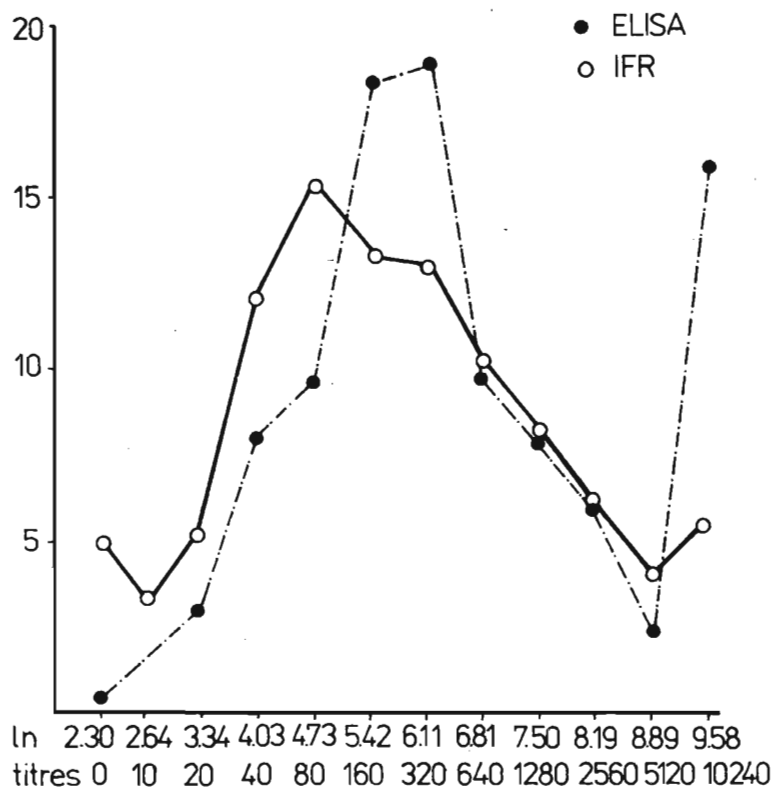


Fig. 3. Comparison of IFR and ELISA (statistical evaluation).
 x = logarithm of class centres in individual titres,
 y = percentage of frequency.

more frequent in ELISA. For example, the highest titre of 10,240 was shown $17 \times$ by IFR, but $45 \times$ by ELISA.

In our opinion, the decline from the normal distribution on the Gauss curve (see Fig. 3) was caused by a higher sensitivity of ELISA. Therefore a higher amount of antibodies was detected at a limit dilution (titre of 10,240).

On the other hand, the high sensitivity of ELISA brings also some problems and requires a precise performing. If the 5-aminosalicylic acid is used, the results of reaction can be read even visually. Consequently, every laboratory with a basic equipment can use ELISA for the diagnostics of sarcocystosis, whereas a special fluorescence microscope is needed for IFR. Of course, photometrical reading of ELISA results is more precise, especially if antibodies in highest dilutions are to be recorded. Our studies confirmed that the antigen obtained from *S. gigante* macrocysts did not cross-react with *Toxoplasma gondii* (see Fig. 2). The preparation of antigen from macrocysts is rather simple and the antigen is only generically specific. This antigen was used also by Boch et al. (1979) and Reiter et al. (1981).

The statistical evaluation of our results confirmed a correlation between the two methods at a high level of statistical significance ($P < 0.005$). It can be concluded that this first application of ELISA for the diagnostics of ovine sarcocystosis was found to be suitable and the method can be utilized for routine diagnostics.

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Received 16 February 1989

Accepted 5 March 1991