SHORT COMMUNICATION

Comparison of a Commercial ELISA with the Modified Agglutination Test for the Detection of *Toxoplasma gondii* Infection in Naturally Exposed Sheep

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Summary

Surprisingly few commercial ELISAs are available for the detection of *Toxoplasma gondii* infection in animals, and none for use in sheep have been evaluated. We thus compared the Bommeli Diagnostics ELISA Toxotest for the detection of *T. gondii* antibodies in ruminants with the reference modified agglutination test (MAT) in a series of 180 sheep sera. ELISA results were analysed at two cut-off levels (30%, comprising both weakly positive and positive results, and 100%, comprising only positive results), and compared with MAT at three cut-off levels (titre of 1 : 25, 1 : 50 and 1 : 100). The results showed a moderate agreement of ELISA at both cut-offs ($\kappa = 0.46$ and 0.51) with MAT at a cut-off titre of 1 : 100. However, the specificity and positive predictive value were above 95% only at an ELISA cut-off of 100%, indicating its potential as a diagnostic test, particularly in areas with a high prevalence of infection. On the other hand, lower sensitivity and negative predictive value limit its value as a screening test. Thus, the ELISA Toxotest may be used for quick diagnosis of *T. gondii* infection in sheep in the field, i.e. for the differential diagnosis of ovine abortion storms.

Introduction

Toxoplasmosis is one of the most widespread zoonoses, with a significant public health and economic impact. In sheep, *Toxoplasma gondii* infection has long been known to cause significant reproductive and consequently, economic losses. Although there is a number of reports on *T. gondii* infection in sheep based on serology (Lunden et al., 1992; van der Puije et al., 2000; Pereira-Bueno et al., 2004), these studies have generally used in-house tests (based on fluorescence and ELISA techniques), the results of which are therefore difficult to compare. Field epizootiological studies as well as diagnosis in clinical situations, such as ovine abortion storms, require standardized ready-to-use serological assays. In Serbia, as in many other low-resource countries, however, testing for toxoplasmosis is not performed in veterinary laboratory services, mainly because of the lack of a quick, safe and cost-effective test easily performed in routine laboratories. Surprisingly few commercial ELISAs for the detection of *T. gondii* infection in animals in general are available to date. One such test has recently been evaluated for pigs (Gamble et al., 2005), and one for sheep and goats has been used (Natale et al., 2006) but only validated by its manufacturer (Institut Pourquier, Montpellier, France).

We have recently conducted a large seroepizootiological study of the prevalence and risk factors for *T. gondii* infection in major food animals in Serbia, which showed a seroprevalence of *T. gondii* in sheep as high as 85% (Klun et al., 2006). This study was based on the modified agglutination test (MAT) as the serological reference test for *T. gondii* in livestock (Dubey et al., 1985; Dubey and Beattie, 1988; Dubey et al., 1995). However, in addition to the length of time required to obtain the results and to being somewhat subjective to read, the MAT is not available to routine veterinary laboratories. It was thus considered important to examine if a commercially available ELISA may be used as an alternative to the MAT. The Bommeli Diagnostics

ELISA Toxotest, marketed since 2000, has been used in studies (Agerholm et al., 2006), but not evaluated. We here present the results of the evaluation of the Bommeli ELISA by comparison with the MAT in a series of sheep sera.

Materials and Methods

Sera

Of a sample of 511 sheep sera collected throughout Serbia between June 2002 and June 2003 as part of an epizootiological study and tested for *T. gondii*-specific antibodies by the MAT (Klun et al., 2006), a total of 180 sera were additionally analysed by ELISA. The sera to be tested by ELISA were selected to include all categories of MAT results, including negative and positive at low (1 : 25, 1 : 50 and 1 : 100), moderate (1 : 200, 1 : 400, 1 : 800), and high titres of specific antibody (≥1 : 1600); within each category, however, the sera were selected at random.

Serology

Modified agglutination test

The MAT for the detection of *T. gondii*-specific immunoglobulin (Ig)G antibodies was performed as described by Desmonts and Remington (1980). Formalin-fixed whole RH tachyzoites used as antigen for this test were prepared at the Institut de Puericulture de Paris (IPP). Sera were serially 2-fold diluted starting at 1 : 25.

ELISA

The 'CHEKIT-Toxotest' ELISA for ruminants (Dr Bommeli AG, Bern, Switzerland), was performed according to the manufacturer's instructions. In brief, after incubation of antigen-coated microplates with the test sera diluted 1 : 400, *T. gondii*-specific antibodies were detected by binding the antigen/antibody complex with a peroxidase-labelled anti-ruminant IgG monoclonal antibody conjugate for 90 min. Both the positive and negative controls provided in the kit were sheep sera. The optical density (OD) of the reaction was read on a Multiscan EX (Thermo Electron Corp., Vantaa, Finland) on a wavelength of 405 nm, while the reference wavelength was 492 nm. The results were calculated according to the control serum readings; i.e. as the per cent ratio between the ODs read for the sample and the positive control corrected for the OD of the negative control, and interpreted as recommended by the manufacturer, as follows: <20% negative, 20–30% ambiguous, 30–100% weakly positive and over 100% positive.

Statistical analysis

Test results (positive/negative) were classified into two by two contingency tables and the test descriptive parameters including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall (observed) agreement, were calculated. The concordance between the tests was determined by Pearson's correlation coefficient and in addition, by kappa statistics; kappa (κ) values of 0.01–0.20 indicated slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 substantial and 0.81–0.99 almost perfect agreement (Landis and Koch, 1977).

Results and Discussion

The results of a comparative serological investigation of a series of 180 sheep sera for *T. gondii* antibodies determined by MAT and ELISA are presented in Fig. 1. The

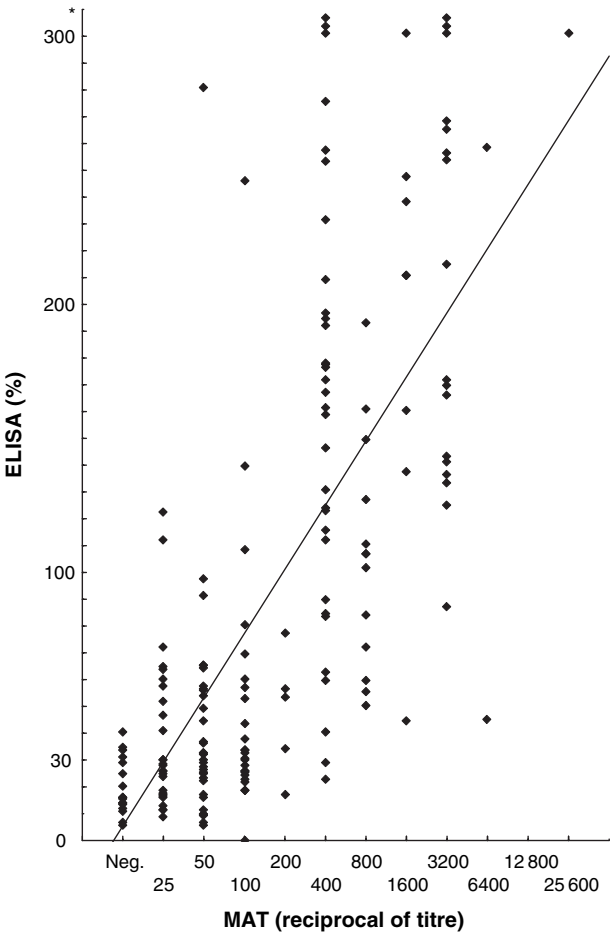

Fig. 1. Relationship between levels of *T. gondii*-specific antibody determined by MAT (titre) and ELISA (expressed as per cent ratio between the ODs read for the sample and the positive control corrected for the OD of the negative control) in a series of 180 sheep sera. Data shown for individual animals fit a linear regression line ($y = -27.47 + 27.072x$). Pearson's correlation coefficient $r = 0.667$, $P < 0.0001$. *ELISA results above 300% shown between 300% and 310% points; specifically, eight points with exact values of 317%, 346%, 357%, 376%, 391%, 497%, 520% and 603%.

Table 1. Comparison of ELISA and MAT (descriptive parameters and measures of agreement) at different levels of *T. gondii*-specific antibody taken as cut-off ($n = 180$ sera)

	Cut-off level					
	ELISA 30%			ELISA 100%		
	MAT 1 : 25	MAT 1 : 50	MAT 1 : 100	MAT 1 : 25	MAT 1 : 50	MAT 1 : 100
Sensitivity (%)	73.0	78.7	87.4	38.7	44.9	58.3
Specificity (%)	76.5	63.6	57.1	100.0	95.5	96.1
Overall agreement (%)	73.3	75	74.4	44.4	57.2	74.4
Positive predictive value (%)	96.7	87.0	73.2	100.0	96.8	95.2
Negative predictive value (%)	22.8	49.1	77.2	14.5	35.9	63.3
Kappa (κ) value	0.24	0.38	0.46	0.11	0.26	0.51
P for kappa	<0.001	<0.001	<0.001	0.001	<0.001	<0.001

specific antibody levels obtained by ELISA showed a good correlation with those determined by MAT ($n = 180$, $r = 0.667$, $P < 0.0001$).

The major point in further analysis of the agreement between ELISA and MAT was setting the cut-off. As the ELISA manufacturer does not provide data on the concentration of antibody corresponding to a particular test result, the ELISA results were analysed at two cut-off levels, one including all results positive at above 30% (comprising both those weakly positive and positive), and the second considering only results positive at above 100%, and compared with MAT results at titres of 1 : 25, 1 : 50 and 1 : 100 as cut-off levels. As there is no international reference serum for sheep (containing a specified amount of specific antibody), any taken cut-off is arbitrary. The MAT is not species-specific and has initially been introduced for humans (Desmonts and Remington, 1980), where a titre of 1 : 25 corresponds to 1.25 IU/ml (according to a WHO human reference serum). Therefore, any of these MAT cut-offs may be considered to indicate very low amounts of *T. gondii*-specific antibody. The sensitivity, specificity and positive and negative predictive values of the ELISA in comparison with MAT were thus analysed separately at all these levels (Table 1).

At the ELISA cut-off of 30%, despite a similar overall agreement at all three MAT cut-offs, κ statistics showed moderate agreement between the tests only at the MAT cut-off of 1 : 100. At the ELISA cut-off of 100%, the specificity and PPV were above 95% at all MAT cut-offs; however, at MAT cut-offs of 1 : 25 and 1 : 50 the sensitivity and NPV were below 50%, resulting in poor agreement. Setting the MAT cut-off at 1 : 100 resulted in an increase in the sensitivity and NPV, and consequently, in moderate between-test agreement.

At the MAT cut-off of 1 : 100, the tests had a similar agreement (as shown by an identical overall agreement and similar κ values) at both ELISA cut-offs, for, however, different underlying reasons. At the 30% cut-off, ELISA had a reasonable sensitivity (although lower than MAT) but a low specificity and moderate PPV and NPV. This indicates a higher proportion of false-positive results, particularly in a population with a high prevalence of infection, such as the one examined. Conversely, when only 100% positive ELISA results were considered, the specificity and PPV were very high but at the expense of a decrease in sensitivity and NPV. This indicates that some positive sera have been missed.

While the sensitivity of the Bommeli ELISA at the 30% cut-off may be satisfactory for screening purposes, its low specificity at this cut-off limits its value as a screening test. On the other hand, high specificity and PPV at the 100% cut-off indicate its potential for diagnostic purposes, particularly in field conditions in case of, for instance, abortion storms. However, due to decreased sensitivity and NPV at this cut-off, the results may be an underestimate. Naturally, in the absence of testing for specific IgM antibodies, serological diagnosis of *T. gondii*-induced abortions requires testing of paired serum samples (3–4 weeks apart).

One limitation of this study is the lack of parasitological verification of serological data obtained in either test. Regrettably, the MAT has not been validated by parasite recovery data (such as obtained by cat bioassay) in sheep. However, its extensive evaluation in swine (Dubey et al., 1995; Dubey, 1997) and chicken (Dubey et al., 2005b) showed excellent correlation with parasitological findings. Thus, in this evaluation of ELISA versus the MAT as the reference test, we could only extrapolate from the validation of the MAT in these species, as has been carried out in studies of the ruminants musk ox and caribou (Kutz et al., 2000, 2001), or the bottlenose dolphin (Dubey et al., 2005a).

Notwithstanding all above-mentioned limitations, this first report on the performance of the Bommeli ELISA in naturally exposed sheep shows that this assay may be a helpful diagnostic tool in local veterinary laboratory services.

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