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Immunodiagnosis of primary *Toxoplasma gondii* infection in sheep by the use of a P30 IgG avidity ELISA

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Abstract *Toxoplasma gondii* is a protozoan parasite with a worldwide distribution. In both sheep and humans, if the parasite is encountered during pregnancy, fetal infection and abortion can occur. Therefore, *Toxoplasma* infection in sheep has a major economic impact upon sheep farming. Clinically, there is a need to distinguish recent (acute) infections from longstanding (chronic) infections. However, current serological techniques, such as detection of anti-*T. gondii* IgG, cannot discriminate between acute and chronic infections. Increasing immunoglobulin avidity is a good determining factor of how recent an infection is. In this study, we describe the application and validation of a *T. gondii* IgG avidity ELISA, based on the use of an affinity-purified, native *T. gondii* P30 antigen. The assay was used to examine sera from eight sheep experimentally infected with *T. gondii* and found that all seroconverted within 21 days post-infection (p.i.), beginning with avidities that were initially low but that increased over time, with all sheep reaching high IgG avidity within 10 weeks p.i. In addition, sera from clinically healthy but *T. gondii*-seropositive lambs and ewes and seropositive ewes with a history of abortion were also subjected to a preliminary serological investigation. High IgG avidities were found in 80% of the seropositive lambs, in 90% of the clinically healthy ewes and in 97% of the ewes with abortion problems. These findings indicate that the animals had most likely contacted the parasite a longer time ago.

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Introduction

Toxoplasma gondii is a protozoan parasite that can infect many warm-blooded animals, including sheep and humans. The parasite has a worldwide distribution and in most cases infection does not cause severe illness. However, severe clinical disease can result when a primary infection is acquired during pregnancy or when the host immune response is compromised, such as with transplant patients undergoing immunosuppressive therapies or those infected with the human immunodeficiency virus (Joynson and Wreghitt 2001).

In most cases, immunocompetent individuals are protected by both cell-mediated and humoral immune mechanisms. The production of specific antibodies may help protect the host from subsequent re-infection.

In sheep, current serological assays are used to detect *T. gondii*-specific antibodies after abortion. However, they do not always indicate whether the infection is acute or chronic (Hill and Dubey 2002), although they do provide valuable epidemiological information (Seefeldt et al. 1989; Wastling et al. 1995; Werre et al. 2002). Recent work investigated the maturation of specific antibodies to determine when infection was acquired. Jenum and colleagues evaluated a *T. gondii*-specific IgG avidity enzyme immunoassay (EIA) and demonstrated its usefulness in obstetrics (Jenum et al. 1997); and an IgG avidity ELISA has been used in serological studies of neosporosis in cattle (Björkman et al. 1999; Sager et al. 2003). However, limited information is available concerning the serodiagnosis of acute toxoplasmosis in sheep. The *T. gondii* antigen used in the present study was P30 (also called SAG1), a dominant membrane protein of *T. gondii* (Lekutis et al. 2001), which has been used in the serodiagnosis of congenital toxoplasmosis in humans (Aubert et al. 2000; Villavedra et al. 2001), dogs (Silva et al. 2002) and camels (Abu-Zeid 2002). In the

present study, the development and first validation steps of a P30 IgG avidity ELISA for sheep is described.

Materials and methods

A commercially available native, affinity-purified P30 antigen (Z.I.SR2B, Avrille, France) was compared with a soluble somatic antigen ELISA (SA-ELISA) for its potential use in sheep. The *Toxoplasma gondii* SA was obtained from an in vitro-cultivated RH strain, as described for *Neospora caninum* by Sager et al. (2003). Additionally, a traditional endozoite antigen (TEA) and a recombinant H11 antigen were comparatively assessed in the same way. The preparation of the SA, the coating of the ELISA plates and all subsequent test steps were done as described by Gottstein et al. (1998) and Sager et al. (2003).

For comparing the conventional ELISAs with different antigens, 92 sera were taken from randomly selected healthy sheep at slaughter (Wyss et al. 2000). The measured absorption values were expressed as percentages of a positive control (antibody units; AU) and the cut-off was calculated from negative controls, as defined by the 3-fold standard deviation of a cluster of negative sera. Samples above the cut-off were considered as positive and those below as negative. The SA-ELISA was used as the "gold standard" (providing reference values) and the results obtained were compared with those of the three other antigens (P30, TEA, H11).

For the IgG avidity ELISA, only the P30 antigen was used. For each serum, two 4-fold dilution series (starting at a dilution of 1:25) were loaded into the appropriate plate wells. Washing was performed with phosphate-buffered saline containing 0.3% Tween 20 (PBS/Tween) and with PBS/Tween containing 6 *M* urea for the two dilution series, respectively. The detailed avidity ELISA procedure was as described by Sager et al. (2003). The calculation of avidity (expressed as a percentage) was done according to Jenum et al. (1997). Four ewes were experimentally inoculated per os with 2,000 sporulated M_3 *T. gondii* oocysts; and serum samples were taken at weekly intervals (week 0, 1, 2, 3, 4, 6, 8, 12post-infection; p.i.) throughout pregnancy for the primary test validation. Another four ewes were tested serologically, either at 1, 4, 6, 8 and 12 weeks p.i. ($n=2$), or at 2, 4, 7, 10, 12 and 14 weeks p.i. ($n=2$). Additionally, sera from seropositive but healthy ewes ($n=41$) and lambs ($n=35$) at slaughter and *T. gondii*-positive sera from sheep originating from a herd with an abortion history ($n=114$) were tested in the P30 IgG avidity ELISA.

Results

Serological analysis of 92 healthy sheep at slaughter by different *Toxoplasma gondii* ELISAs revealed a high correlation of more than 95% between SA and P30 (Fig. 1) and between SA and TEA, respectively (data not shown). The soluble SA antigen was used as the "gold standard" for calculating sensitivity and specificity. Both P30 and TEA antigens exhibited a relative sensitivity of 96% and a relative specificity of 100%. In contrast, the corresponding sensitivity of the H11 ELISA was only 34% and the specificity 89% (Table 1).

With the anti-*T. gondii* P30 IgG avidity ELISA, all experimentally infected ewes seroconverted within 3 weeks p.i. IgG avidities were determined 2 weeks p.i. and a time-dependent increase was observed. At 10 weeks p.i., avidity values were above 35% in all animals (Fig. 2). Based on these results, avidities up to 25% were classified as low, those between 26% and 35% as intermediate and those above 35% as high.

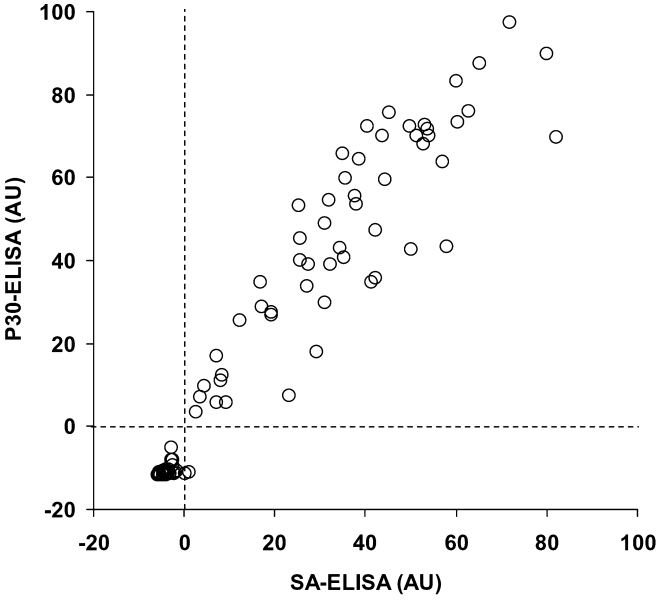

Fig. 1 Comparative dot-plot of values obtained with 92 sheep sera tested in the *Toxoplasma gondii* SA- and P30-ELISA (see Materials and methods). The values are expressed as antibody units (*AU*). *The dashed lines* indicate the negative/positive thresholds determined for each antigen

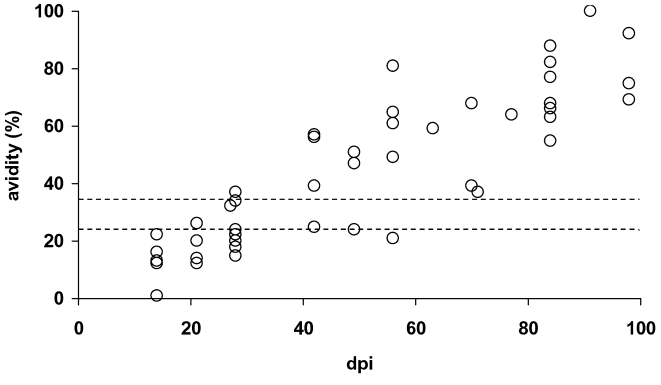

Fig. 2 Avidities obtained from eight sheep experimentally infected with *T. gondii*. *The dashed lines* mark the area borders of low (up to 25%), intermediate (26–35%) and high avidity (more than 35%). *dpi* Days post-infection

Table 1 Comparison of results from three different *Toxoplasma gondii* ELISAs (see Materials and methods) with sera from 92 clinically healthy sheep, sampled at slaughter

		Somatic antigen ELISA	
		Positive	Negative
P30-ELISA	Positive	54	0
	Negative	2	36
TEA-ELISA	Positive	54	0
	Negative	2	36
H11-ELISA	Positive	19	4
	Negative	37	32

Table 2 Anti-P30 avidities determined in sheep initially seropositive in a *T. gondii* somatic antigen ELISA

Avidity	"Normal" ewes	Lambs	Ewes with abortion history
Low	1	3	2
Intermediate	3	4	1
High	37	28	111
Total	41	35	114

When naturally infected lambs and sheep were investigated using the avidity ELISA, it was found that 90% of sera from adult sheep (without any abortion history) had high avidities, as did 80% of the sera from seropositive lambs (Table 2). The difference, however, was not significant, as determined by the χ^2 test ($P = 0.38$). Sheep from a farm with an abortion history showed a high avidity in 97.4% of animals. The χ^2 test did not reveal a significant difference, in comparison with the healthy ewes at slaughter ($P = 0.07$). Additionally, a Student's *t*-test was performed on the avidities of sheep with observed abortions ($n = 11$) and clinically healthy ewes ($n = 103$) from the flock with an abortion history. No significant difference could be found ($P = 0.49$).

Discussion

The affinity-purified, native *Toxoplasma gondii* P30 antigen proved to be suitable as a diagnostic assay for the presence of specific anti-*T. gondii* antibodies when compared with a reference SA-ELISA, as demonstrated by a high correlation of their respective results. Positive/negative discrimination appeared to be more distinct with the P30 ELISA. The P30 *T. gondii* IgG avidity ELISA was subsequently validated with sera from experimentally infected sheep. Within the first 4 weeks p.i., most of the sera remained below 35% avidity, whereas at 10 weeks p.i., all sera showed avidities > 35%. The cutoff line discriminating between higher and lower avidities was therefore positioned at this value.

The P30 IgG avidity ELISA can be used to discriminate between acute and chronic *T. gondii* infection in sheep. Thus, naturally infected healthy ewes at slaughter showed high avidities in 90% of cases. As 80% of the seropositive lambs had high IgG avidities, we may conclude that they were exposed to the parasite very early in life (Duncanson et al. 2001). The animals originating from a flock with an abortion problem showed a high seroprevalence (114 out of 117 were seropositive; i.e. 97%). Although this is indicative of a high exposure of the animals to *T. gondii* oocysts, no significant difference in the distribution of avidity could be observed when compared with randomly selected seropositive ewes. This may be due to the fact that primary exposure of the flock to the parasite occurred years before, as the blood samples used in this study came from a flock where the first abortions occurred 4 years previously (M. Hässig, personal communication). However, abortions persisted in this flock and continued after blood sampling. The fact that *T. gondii*-seroposive ewes with high avidity underwent subsequent abortions indicates that the parasite was most likely not the causative agent. Finally, sheep living persistently in high endemic areas should acquire the infection before their first pregnancy and should thus be immune at conception.

It is important to mention that the presented field data are only preliminary, but they give a first impression about the suitability of such a test system for routine diagnostics. There is evidence that the avidity ELISA is able to discriminate between acute and chronic infection. Once avidity has matured and reaches a high value (chronic stage), we can no longer obtain further information about the previous time-point of infection. In other words, the IgG avidity ELISA is very useful as a diagnostic tool over a relatively short time. We will therefore focus our further investigations on animals that have recently aborted, to further study antibody maturation at this important time, using either naturally or experimentally infected ewes.

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References

Abu-Zeid YA (2002) Protein G ELISA for detection of antibodies against *Toxoplasma* SAG1 in dromedaries. J Egypt Soc Parasitol 32:247–257

Aubert D, Maine GT, Villena I, Hunt JC, Howard L, Sheu M, Brojanac S, Chovan LE, Nowlan SF, Pinon JM (2000) Recombinant antigens to detect *Toxoplasma gondii*-specific immunoglobulin G and immunoglobulin M in human sera by enzyme immunoassay . J Clin Microbiol 38:1144–1150

Björkman C, Naslund K, Stenlund S, Maley SW, Buxton D, Uggla A (1999) An IgG avidity ELISA to discriminate between recent and chronic *Neospora caninum* infection. J Vet Diagn Invest 11:41–44

Duncanson P, Terry RS, Smith JE, Hide G (2001) High levels of congenital transmission of *Toxoplasma gondii* in a commercial sheep flock. Int J Parasitol 31:1699–1703

Gottstein B, Hentrich B, Wyss R, Thür B, Busato A, Stärk KD, Müller N (1998) Molecular and immunodiagnostic investigations on bovine neosporosis in Switzerland. Int J Parasitol 28:679–691

Hill D, Dubey JP (2002) *Toxoplasma gondii*: transmission, diagnosis and prevention. Clin Microbiol Infect 8:634–640

Jenum PA, Stray-Pedersen B, Gundersen AG (1997) Improved diagnosis of primary *Toxoplasma gondii* infection in early pregnancy by determination of antitoxoplasma immunoglobulin G avidity. J Clin Microbiol 35:1972–1977

Joynson DHM, Wreghitt TG (2001) Toxoplasma infection in immunosuppressed (HIV-negative) patients. In: Joynson DHM, Wreghitt TG (eds) Toxoplasmosis: a comprehensive clinical guide. Cambridge University Press, Cambridge, pp 178–192

Lekutis C, Ferguson DJ, Grigg ME, Camps M, Boothroyd JC (2001) Surface antigens of *Toxoplasma gondii*: variations on a theme. Int J Parasitol 31:1285–1292

Sager H, Gloor M, Björkman C, Kritzner S, Gottstein B (2003) Assessment of antibody avidity in aborting cattle by a somatic *Neospora caninum*-tachyzoite antigen IgG avidity ELISA. Vet Parasitol 112:1–10

Seefeldt SL, Kirkbride CA, Dubey JP (1989) Comparison of enzyme-linked immunosorbent assay, indirect fluorescent antibody test, and direct agglutination test for detecting *Toxoplasma gondii* antibodies in naturally aborted ovine fetuses. J Vet Diagn Invest 1:124–127

Silva NM, Lourenço EV, Silva DAO, Mineo JR (2002) Optimisation of cut-off titres in *Toxoplasma gondii* specific ELISA and IFAT in dog sera using immunoreactivity to SAG-1 antigen as a molecular marker of infection. Vet J 163:94–98

Villavedra M, Battistoni JJ, Rossi S, Carol H, Nieto A (2001) Avidity analysis of the human immune response to a chitin binding protein of *Toxoplasma gondii*. Int J Parasitol 31:1087–1092

Wastling JM, Harkins D, Maley S, Innes E, Panton W, Thomson K, Buxton D (1995) Kinetics of the local and systemic antibody response to primary and secondary infection with S48 *Toxoplasma gondii* in sheep. J Comp Pathol 112:53–62

Werre SR, Jacobson RH, Bowman DD, Dubey JP, Mohammed HO (2002) Evaluation of kinetics and single-read enzyme-linked immunoassays for detection of *Toxoplasma gondii* antibodies in sheep. J Vet Diagn Invest 14:225–230

Wyss R, Sager H, Müller N, Inderbitzin F, König M, Audigé L, Gottstein B (2000) The occurrence of *Toxoplasma gondii* and *Neospora caninum* as regards meat hygiene. Schweiz Arch Tierheilkd 142:95–108