Comparison of indirect immunofluorescence and ID Screen® Toxoplasmosis indirect ELISA for the detection of antibodies against *Toxoplasma gondii* in cat and dog sera.

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(2) ID VET - Montpellier, Frankreich
Toxoplasmosis

- Toxoplasmosis is a zoonotic disease caused by a coccidian protozoan parasite, *Toxoplasma gondii*, that can infect warm-blooded animals, including man.

- Members of the cat family (Felidae) are the definitive hosts. They play an important role in toxoplasmosis spread because they excrete resistant *Toxoplasma gondii* oocysts into the environment.

- Many mammals and birds serve as intermediate hosts. Infection is contracted by ingesting either oocysts from the environment or meat containing live organisms.

- In dogs, infection may be asymptomatic. Abortion and central nervous system damage (in young dogs) may occur.
Lifecycle

Figure 1: Life cycle of Toxoplasma gondii (from Dubey JP, Toxoplasmosis. J Am Vet Med Assoc 189:166, 1986 to Dubey)

Fig. 2: Tissue cyst freed from mouse brain

Fig. 3: Electron microscopie photo of an intracellular tachyzoite

Fig. 4: Electron microscopie photo of a sporulated oocyst
Toxoplasmosis serology

- Toxoplasmosis serology is performed during human pregnancy, and thus is a question which has been studied extensively.
- ELISA, microagglutination and IFAT are the main tests used for Toxoplasmosis diagnosis.
- In the veterinary field, commercial ELISAs are currently available for ruminants, but not for other species.
Toxoplasmosis serology

- In Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana For dogs, microagglutination or IFAT is used. Agglutination, however, is difficult to interpret and therefore only IFAT is used.
- IFAT is difficult to implement for large sample numbers.

Comparison of indirect fluorescent antibody test and modified agglutination test for detecting *Toxoplasma gondii* immunoglobulin G antibodies in dog and cat

Gladia Macri · Marcello Sala · Alicia M. Linder · Nadia Pettirossi · Manuela Scarpulla
Aim

- ID VET has developed a multispecies kit that can be used on a large spectrum of mammals.
- This study compares IFAT and ID Screen ELISA
Method:
ID Screen® Toxoplasmosis indirect ELISA

+ Sample *

Wash

+ Conjugate

Wash

+ Substrate

P30 coated microplates

Multi-species-PO conjugate

* Sample = serum, plasma or meat juice
# Interpretation

- Read the optical densities at 450 nm
- Results are expressed as S/P%  

\[
S/P\% = 100 \times \frac{OD\ (Serum) - OD\ (NC)}{OD\ (PC) - OD\ (NC)}
\]

<table>
<thead>
<tr>
<th>Result</th>
<th>Status</th>
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</thead>
<tbody>
<tr>
<td>S/P ≤ 40%</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>40% &lt; S/P &lt; 50%</td>
<td>DOUBTFUL</td>
</tr>
<tr>
<td>50% ≤ S/P &lt; 200%</td>
<td>POSITIVE</td>
</tr>
<tr>
<td>S/P ≥ 200%</td>
<td>STRONG POSITIVE</td>
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</table>
The P30 antigen

- Major surface antigen in the external surface of the plasma membrane.
- Reference antigen in human diagnostics.

The Major Surface Antigen, P30, of *Toxoplasma gondii* Is Anchored by a Glycolipid*

(Received for publication, October 3, 1988)

Susana D. Nagel and John C. Boothroyd‡

From the Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, California 94305
Multi-species conjugate

- Proprietary conjugate recognizing a wide spectrum of mammalian antibodies
- Also used in other ID Screen kits (Toxoplasmosis, Neospororasis, Q Fever, Chlamydia, Trichinellosis)
SVILUPPO E VALUTAZIONE DELLE PERFORMANCE DI UNA ELISA HOME MADE DA UTILIZZARE PER LA DIAGNOSI INDIFFERENZIATA DI TOXOPLASMOSI OVINA

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2 : Istituto Zooprofilattico Sperimentale delle Sicilie
3 : ID-Vet - Innovative Diagnostics Montpellier France

Antigene polivalenti anti-specie - Sono stati utilizzati IgG policlonali anti-Toxoplasma (2) standard per la ricerca di anticorpi anti-Toxoplasma (2) in campioni di siero ovino positivi e negativi per la presenza di anticorpi anti-Toxoplasma e utilizzabili come controllo di reazioni della tecnica ELISA. Uno stock omogeneo di controllo positivo e negativo è stato conservato a -20° C ed utilizzato per la standardizzazione del metodo. Per la validazione del metodo ELISA è stata inizio costituita una banca di 416 campioni, scelti con criterio randomizzato tra quelli afferenti ai laboratori dell’IZSUM. Anche questi sono stati analizzati con il metodo IFAT.

Sviluppo test ELISA home made

Antigene - Transformatore grandi capsule RH si è coltivato su in vitro (chinese hamster ovary HBC5). Una volta raggiunta una concentrazione di tachiziti pari a 5x10⁶ c/c 1 ml di anticorpi anti-Toxoplasma- policlonale anti-spezie (ID-VET Innovative Diagnostics) per batteriario e il surnatante conservato a -20° C ed il surnatante conservato a -20° C fino al momento dell’uso.

SUMMARY

The performance characteristics of an ELISA test for toxoplasmosis were assessed using 416 samples collected from sheep kept in endemic areas of Italy. Indirect immunoenzymatic (ELISA) test was used as the reference method. The diagnostic accuracy of the home made method and that of a commercial kit was considered to be high, with values of sensitivity, specificity, positive and negative predictive values respectively of 96.7%, 95.9%, 96.7% and 95.9% and an area under the ROC curve of 0.96. The test showed the potential to be a useful tool in the serodiagnosis of Toxoplasma in the ovine species, allowing the development of a high-level diagnostic test.

MATERIALI E METODI

Campioni di siero - In assenza di esiti di sierotipico positivo, IFAT è stata utilizzata per individuare campioni di siero ovino positivi e negativi da utilizzare come controllo di reazione della tecnica ELISA. Uno stock omogeneo di controllo positivo e negativo è stato conservato a -20° C ed utilizzato per la standardizzazione del metodo. Per la validazione del metodo ELISA è stata iniziata una banca di 416 campioni, scelti con criterio randomizzato tra quelli afferenti ai laboratori dell’IZSUM. Anche questi sono stati analizzati con il metodo IFAT.

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Validazione del metodo - Lo standard di riferimento è stato individuato mediante l’utilizzo di un test ELISA home made utilizzato per la ricerca di anticorpi anti-Toxoplasma e utilizzabile come controllo di reazione della tecnica ELISA. Uno stock omogeneo di controllo positivo e negativo è stato conservato a -20° C ed utilizzato per la standardizzazione del metodo. Per la validazione del metodo ELISA è stata iniziata una banca di 416 campioni, scelti con criterio randomizzato tra quelli afferenti ai laboratori dell’IZSUM. Anche questi sono stati analizzati con il metodo IFAT.

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Case Study - A 6-month-old lamb was presented with episodes of dysentery. A high-level diagnostic test for toxoplasmosis was performed, allowing the diagnosis of toxoplasmosis in the ovine species. A high-level diagnostic test for toxoplasmosis was performed, allowing the diagnosis of toxoplasmosis in the ovine species. A high-level diagnostic test for toxoplasmosis was performed, allowing the diagnosis of toxoplasmosis in the ovine species.

Conclusions - The home made ELISA test for toxoplasmosis was shown to be highly accurate, allowing the diagnosis of toxoplasmosis in the ovine species. The home made ELISA test for toxoplasmosis was shown to be highly accurate, allowing the diagnosis of toxoplasmosis in the ovine species.

RESUMEN

La toxoplasmosis es una enfermedad de origen protozoario notoriamente diffusa en la especie ovina; la su difusión y localización de la enfermedad ha sido ampliamente estudiada en diferentes países. El presente estudio se ha realizado con el objetivo de determinar la prevalencia de la toxoplasmosis en diferentes zonas geográficas de Andalucía (España) y comparar los resultados obtenidos con los de otros estudios previos realizados en la misma región. Los resultados obtenidos muestran una prevalencia de la enfermedad similar a la obtenida en otros estudios realizados en la misma región, lo que sugiere una distribución geográfica similar. Los resultados obtenidos muestran una prevalencia de la enfermedad similar a la obtenida en otros estudios realizados en la misma región, lo que sugiere una distribución geográfica similar.
Detection of antibodies to *Toxoplasma gondii* in serum from experimentally infected pregnant ewes.

Proctor AF¹, O’Donovan J², Marques PX¹, Gutierrez J¹, Sammin D³, Brady C², Worrall S¹, Nally JE¹, Bassett H¹, and Markey BK¹

3. Regional Veterinary Lab, Department of Agriculture, Fisheries and Food, Kilkenny

Other external validations ongoing (swine, chicken, dogs etc..)
Methods:
Indirect Immunofluorescence (IFAT)

IFAT was performed with:
- Commercial slides (Toxoplasma gondii, Fuller-Laboratories Fullerton, CA USA, Ref TX-12)
- Anti-dog IgG conjugate (Anti-dog IgG FITC conjugate, SIGMA Ref. F4012) or an anti-cat IgG conjugate (Anti-cat IgG FITC conjugate, SIGMA Ref. F4262).
- Sera were tested at a screening dilution of 1:20 and positive sera diluted twofold and re-assayed to the end point.
Materials and Methods

- Samples tested:
  - 110 cat sera
  - 85 dog sera

- Tested by IFAT and the ID Screen® ELISA in the Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana (IZS LT), Rome, Italy
Results – cat sera

- 66/67 cat sera negative by IFAT were also negative by ELISA. One serum was IFAT-neg and ELISA-pos, and had a S/P near the cut-off (55%).

- 42/43 cat sera positive by IFAT were also positive by ELISA. The discordant serum had an IFAT titre of 20.

<table>
<thead>
<tr>
<th></th>
<th>ELISA Positive</th>
<th>ELISA Negative</th>
<th>Total</th>
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<tbody>
<tr>
<td>IFAT positive</td>
<td>42</td>
<td>1</td>
<td>43</td>
</tr>
<tr>
<td>IFAT negative</td>
<td>1</td>
<td>66</td>
<td>67</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>67</td>
<td>110</td>
</tr>
</tbody>
</table>
Results – dog sera

- 40/44 dog sera positive by IFAT were also positive by ELISA. The four discordant sera had mainly low IFAT titres and S/P values near the cut-off.

- 39/41 dog sera negative by IFAT were also negative by ELISA; among the two discordant sera, one serum was ELISA-doubtful and one ELISA-positive.

<table>
<thead>
<tr>
<th></th>
<th>ELISA Positive</th>
<th>ELISA Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFAT positive</td>
<td>40</td>
<td>4</td>
<td>44</td>
</tr>
<tr>
<td>IFAT negative</td>
<td>2</td>
<td>39</td>
<td>41</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>43</td>
<td>85</td>
</tr>
</tbody>
</table>
S/P distribution dog sera n=85

- Negative
- Positive Zone
- Doubtful zone

Number of sera tested:
- 0-10
- 11-20
- 21-30
- 31-40
- 41-50
- 51-60
- 61-70
- 71-80
- 81-90
- 91-100
- 101-110
- 111-120
- 121-130
- 131-140
- 141-150
- >150

Legend:
- IFAT-positive
- IFAT-negative
Discussion

- For cat sera, the agreement between ELISA and IFAT was high, with only 2/110 discordant results.
- For dog sera, the observed agreement between ELISA and IFAT was slightly lower, with 6/85 samples giving results around the ELISA or IFAT cut-off. The borderline titers could be the result of older infections.
- Such discrepancies are commonly observed when comparing serological techniques, especially for samples near the threshold. This phenomenon can be explained as small difference in analytical sensitivity of these tests.
Conclusion

- The ID Screen Toxoplasmosis Indirect ELISA, already validated for ruminants and swine, gives reliable results for the detection of anti-**Toxoplasma gondii** antibodies in cat and dog sera.

- It is an easy-to-use method for **T. gondii** antibody detection in multiple species.
Thank you for your attention!