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A systematic review and meta-analysis on the validation of serological techniques for detection of anti-Toxoplasma gondii antibodies in humans and animals

HUERTAS LÓPEZ A. [1,2], CANTOS BARREDA A. [3,4], SÁNCHEZ SÁNCHEZ R. [2], MARTÍNEZ-CARRASCO C. [3], IBÁÑEZ LÓPEZ F. [5], MARTÍNEZ SUBIELA S. [1], CERÓN J. [1], ÁLVAREZ GARCÍA G. [2]

[1] Interdisciplinary Laboratory of Clinical Analysis, Interlab-UMU, University of Murcia - Regional Campus of International Excellence "Campus Mare Nostrum", 30100 Espinardo, Murcia, Spain; [2] SALUVET group, Animal Health Department, Complutense University of Madrid, Ciudad Universitaria s/n, 28040, Madrid, Spain; [3] Animal Health Department, University of Murcia - Regional Campus of International Excellence "Campus Mare Nostrum", 30100 Espinardo, Murcia, Spain; [4] Department of Biochemistry and Molecular Biology-A, University of Murcia - Regional Campus of International Excellence "Campus Mare Nostrum", 30100 Espinardo, Murcia, Spain; [5] Statistical Support Section (SAE), Scientific and Research Area (ACTI), University of Murcia - Regional Campus of International Excellence "Campus Mare Nostrum", 30100 Espinardo, Murcia, Spain

Introduction: *Toxoplasma gondii* is a paradigmatic parasite from One health perspective that causes toxoplasmosis, a zoonosis ranked as one of the most important foodborne parasitic diseases (1). A wide variety of serological techniques have been developed to detect the infection in humans and animals (2,3). Our aim was to describe and compare the main characteristics of these serological tests and the validation process followed, critically analysing whether such tests meet the standards required to ensure an accurate *T. gondii* serological diagnosis.
Methods: A systematic search of articles and a meta-analysis were performed (period: 2014 to 2022; databases: PubMed, Web of Science and Scopus; MeSH terms “Toxoplasm*” and “diagnosis” (4)). After applying the selection criteria and QUADAS 2 tool (4), 113 studies done in humans (n=65), animals (n=47) or both (n=1) were selected. A total of 42 variables regarding characteristics of the techniques and analytical and diagnostic validation parameters were studied. Data analyses were made by Pearson’s Chi-Square or Fisher exact tests. SPSS software was employed for statistical analysis and statistical significance was considered when $p < 0.05$.
Results: Significant differences between tests used for humans and animals were detected, as well as common relevant limitations. In humans, the most employed reference techniques were enzyme immunoassays (EIA) (49.2%; 32/65) with predominance of commercial techniques (67.7%; 44/65); moreover, different immunoglobulin isotypes were measured (95.4%, 62/65 IgG; 35.4%, 23/65 IgM; and 6.2%, 4/65 IgA) and tests discriminated between acute and chronic infections. In animals, the most used reference techniques were indirect fluorescent antibody test (IFAT) (21.3%; 10/47), EIA (19.1%; 9/47) and agglutination techniques (19.1%; 9/47) with predominance of in-house techniques (42.6%; 20/47), and most tests detected IgG (93.6%; 44/47). Common and relevant limitations identified in the studies were the absence of information about the negative (55.8%; 63/113) and positive (61.1%; 69/113) controls or duplicate samples (66.4%; 75/113) and, in addition, the lack of analytical validation (87.6%; 99/113), with no consideration of potential cross-reactivity with other pathogens (78.8%; 89/113).
Conclusions: Future tests developed for animals should add additional information regarding the kinetics and the phase of the infection of *T. gondii*. Nor commercial tests, widely employed in humans, nor in-house tests guarantee robust and comparable results in the absence of analytical validation and comparative studies including interlaboratory ring trials.

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