Transboundary and Emerging Diseases

ORIGINAL ARTICLE

An Inter-Laboratory Comparative Study of Serological Tools Employed in the Diagnosis of *Besnoitia besnoiti* Infection in Bovines

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The experimental design and the analysis of data were headed by SALUVET-Madrid. The serological tests evaluated in the present study were performed by the different partners (see Table 1), and all partners collaborated in the creation of the manuscript.

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Summary

Bovine besnoitiosis is considered an emerging chronic and debilitating disease in Europe. Many infections remain subclinical, and the only sign of disease is the presence of parasitic cysts in the sclera and conjunctiva. Serological tests are useful for detecting asymptomatic cattle/sub-clinical infections for control purposes, as there are no effective drugs or vaccines. For this purpose, diagnostic tools need to be further standardized. Thus, the aim of this study was to compare the serological tests available in Europe in a multi-centred study. A coded panel of 241 well-characterized sera from infected and non-infected bovines was provided by all participants (SALUVET-Madrid, FLI-Wusterhausen, ENV-Toulouse, IPB-Berne). The tests evaluated were as follows: an in-house ELISA, three commercial ELISAs (INGEZIM BES 12.BES.K1 INGENASA, PrioCHECK Besnoitia Ab V2.0, ID Screen Besnoitia indirect IDVET), two IFATs and seven Western blot tests (tachyzoite and bradyzoite extracts under reducing and non-reducing conditions). Two different definitions of a gold standard were used: (i) the result of the majority of tests ('Majority of tests') and (ii) the majority of test results plus pre-test information based on clinical signs ('Majority of tests plus pre-test info'). Relative to the gold standard 'Majority of tests', almost 100% sensitivity (Se) and specificity (Sp) were obtained with SALUVET-Madrid and FLI-Wusterhausen tachyzoite- and bradyzoite-based Western blot tests under non-reducing conditions. On the ELISAs, PrioCHECK Besnoitia Ab V2.0 showed 100% Se and 98.8% Sp, whereas ID Screen Besnoitia indirect IDVET showed 97.2% Se and 100% Sp. The in-house ELISA and INGEZIM BES 12.BES.K1 INGENASA showed 97.3% and 97.2% Se; and 94.6% and 93.0% Sp, respectively. IFAT FLI-Wusterhausen performed better than IFAT SALUVET-Madrid, with 100% Se and 95.4% Sp. Relative to the gold standard 'Majority of test plus pre-test info', Sp significantly decreased; this result was expected because of the existence of seronegative animals with clinical signs. All ELISAs performed very well and could be used in epidemiological studies; however, Western blot tests performed better and could be employed as *a posteriori* tests for control purposes in the case of uncertain results from valuable samples.