An inter-laboratory comparative study of serological tools employed in the diagnosis of *Besnoitia besnoiti* infection in bovines

P. García Lunar 1; L. M. Ortega-Mora 2; G. Schares 2; N. S. Gollnick 3; P. Jacquet 4; C. Grisez 4; F. Prevot 4; C. F. Frey 5; B. Gottstein 5; G. Álvarez-García 1

1SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid, Spain; 2Friedrich-Loeffler-Institut, Federal Research Centre for Virus Diseases of Animals, Institute of Epidemiology, Wusterhausen, Germany; 3Clinic for Ruminants with Ambulatory and Herd Health Services, Ludwig Maximilian University, Munich, Germany; 4Laboratoire de Parasitologie, Ecole Nationale Vétérinaire, Toulouse, France; 5Institute of Parasitology, University of Berne, Switzerland

**Background:** Bovine besnoitiosis is considered an emerging chronic and debilitating disease in Europe. The causative agent is the cyst-forming parasite *Besnoitia besnoiti*. Many infected animals remain subclinical and the only sign of disease is the presence of parasitic cysts in the sclera and conjunctiva. Serological tests are useful to detect asymptomatic/sub-clinical cattle for control purposes since there are no effective drugs or vaccines. In this sense diagnostic tools need to be further standardized. The aim of this study was to compare the serological tests available in Europe in a multi-centered study.

**Methods:** A coded panel of 401 well characterized sera from infected and non-infected bovines was provided by all the participants (SALUVET group - UCM; FLI, Wusterhausen; LP, ENV, Toulouse; IPB, Berne). The tests evaluated were: an in-house ELISA developed by SALUVET group, 2 commercial ELISAs (PrioCHECK Besnoitia Ab V2.0, INGENASA), IFAT and Western blots (tachyzoite and bradyzoite extracts under reducing and non-reducing conditions). Two different definitions of a gold standard were used: i) the decision of the majority of tests (Majority); ii) pre-test information based on clinical signs. WinEpiscope 2.0 was employed to estimate sensitivity (Se), specificity (Sp) and agreement, expressed as Kappa (κ)-values.

**Results:** Relative to the gold standard “Majority” almost 100% Se and Sp were obtained with 3 out of four bradyzoite based Western blots regardless the conditions and laboratory. A slight variability was observed when tachyzoite based Western blots were compared. However all Western blots showed almost perfect agreement. Regarding ELISAs, the in-house ELISA, PrioCHECK and INGENASA showed very good diagnostic characteristics (95-100% Se and Sp). IFAT FLI Wusterhausen performed better than IFAT SALUVET-UCM with 100% Se and 96% Sp. Relative to the gold standard “pre-test info”, Sp significantly decreased as expected due to the existence of subclinical infections.

**Conclusions:** In house, PrioCHECK Besnoitia Ab V2.0 and INGENASA ELISAs performed very well and are suitable tools to be used in epidemiological studies. However Western blot tests based on bradyzoite extracts performed better and could be employed as a *posteriori* test to re-test doubtful results in valuable samples for control purposes.