Abstract:

Besnoitia besnoiti, an economically important disease in cattle in many countries of Africa and Asia is re-emerging in Europe. Serological identification of infected cattle is important because introduction of these animals into naive herds seems to play a major role in the transmission of the parasite. We report new, simplified immunoblot-based serological tests for the detection of B. besnoiti specific antibodies. Antigens were used under non-reducing conditions in the immunoblots, because reduction of the antigen with β-mercaptoethanol diminished the antigenicity in both, tachyzoites and bradyzoites. Ten B. besnoiti tachyzoite and ten bradyzoite antigens of 15-45 kDa molecular weight were recognized by B. besnoiti infected cattle, but not or only weakly detected by cattle infected with related protozoan parasites, Neospora caninum, Toxoplasma gondii, Sarcocystis cruzi, Sarcocystis hominis, or Sarcocystis hirsuta. The sensitivity and specificity of B. besnoiti immunoblots were determined with sera from 62 German cattle with clinically confirmed besnoitiosis and 404 sera from unexposed German cattle including 214 sera from animals with a N. caninum-specific antibody response. Using a new scoring system, the highest specificity (100%) and sensitivity (90%) of the immunoblots were observed when reactivity to at least four of the ten selected tachyzoite or bradyzoite antigens was considered as positive. When a cut-off based on this scoring system was applied to both the tachyzoite- and the bradyzoite-based immunoblots, there was an almost perfect agreement with the indirect fluorescent antibody test with a titre of 200 as the positive cut-off. We identified and partially characterized 10 tachyzoite and 10 bradyzoite B. besnoiti antigens which may help to develop new specific and sensitive serological tests based on individual antigens and in the identification of possible vaccine candidates.