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Diagnostic parasitology

A newly developed membrane-based assay for the simultaneous serologic screening and differentiation of echinococcosis

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Background: Cystic (CE) and alveolar echinococcosis (AE) are both zoonotic diseases caused by the tapeworms *Echinococcus granulosus* and *Echinococcus multilocularis*, respectively. Initial diagnosis is usually made by imaging techniques. Biopsies and ultrasound-guided punctures, if feasible, can be used for differential diagnosis upon PCR. Increasingly overlapping geographic distribution of the causative parasites raises the demand for non-invasive diagnosis and species differentiation. Although selected serological tests that are currently used demonstrate very useful serological performances, some limitations still exist concerning species differentiation and cross reactivity to other helminth infections.
We determined the suitability of the Anti-Echinococcus EUROLINE-WB (IgG) for the serological detection of Echinococcosis and its potential to simultaneously differentiate between *E. granulosus* und *E. multilocularis* as causative agent.
Material/methods: Anti-Echinococcus EUROLINE-WB (IgG) contains electrophoretically separated, axenically in vitro produced *Echinococcus multilocularis* metacestode vesicle fluid (EmVF) antigen, in addition to 3 membrane chips each coated with recombinant *E. granulosus* AgB8 and the *E. multilocularis* antigens Em18 and Em95. 324 sera were tested for the presence of anti-Echinococcus ssp. specific IgG. This panel included 55 CE and 49 AE sera from patients at different disease stages, 50 sera from blood donors, 50 sera from tumor patients, and 120 sera of patients infected with other putatively cross-reaction parasites. Presence and intensity of the bands were automatically evaluated using a commercial software (EUROLineScan).
Results: Investigation of pre-characterized sera from patients with clinically confirmed infections as well as healthy controls revealed a sensitivity and specificity of 93% and 100%, respectively. Application of an automated evaluation algorithm allowed assignment of the respective *Echinococcus ssp.* in 81% of the positive results. Investigation of a parasite panel comprising of patients infected with *Fasciola hepatica, Strongyloides stercoralis, Taenia solium, Trichinella spiralis, Schistosoma ssp., Plasmodium ssp., Toxocara canis, Anisakis, Entamoeba histolytica, Leishmania ssp*. and *Filarioidea* types indicated no cross reactivity. Cross-reactive positive results were only obtained in 4 out of 11 *Ascaris lumbricoides* and 1 out of 16 *Anisakis simplex* samples.
Conclusions: The combination of the EmVF Western Blot and immobilized recombinant proteins in a single line assay offers a unique diagnostic tool for the simultaneous serological detection and differentiation of Echinococcosis with excellent sensitivity and specificity. Remarkably we observed no

serological cross reactivity to diagnostically highly relevant larval *Taenia solium*, *Schistosoma ssp.* and *Entamoeba histolytica* infections.