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Diagnosis of porcine cysticercosis using rat basophilic leukemia (RBL) IgE reporters and loop-mediated isothermal amplification (LAMP) technology

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Abstract

Cysticercosis is a neglected zoonotic disease caused by larval stages of *Taenia solium*, with a huge impact on public health (neurocysticercosis in the brain) and the livelihoods of small-scale pig farmers in many low-income countries. Diagnosis of porcine cysticercosis is mostly based on tongue palpation and carcass inspection, complemented by IgG-based serological analyses. These diagnostic methods often show cross-reactivity or are not sensitive enough in pigs with low infestation. Here, we describe the development of a pig Immunoglobulin E (IgE) reporter system and selection, cloning, and recombinant expression of candidate diagnostic allergens of *T. solium*. In addition, we adopted a molecular approach using loop-mediated isothermal amplification (LAMP) technology to distinguish cysticercosis caused by *T. solium* from the related, co-endemic *T. hydatigena* (non-zoonotic). For developing the reporter system, Rat Basophilic Leukemia Neuropeptide Y-monomeric Red Fluorescent Protein expressing cells (RBL NPY-mRFP) were transiently transfected with a pig/rat high-affinity IgE receptor alpha chain (FcϵRIα) chimeric construct followed by antibiotic selection for stable transfectants. Putative IgE-binding *T. solium* allergens were selected by a combination of published work on transcriptomic and proteomic data and allergenicity predictions. Chosen allergens were cloned into expression vectors and transiently transfected into HEK-2936E cells in suspension. In LAMP method, we targeted cytochrome c oxidase 1 (cox1) gene of *T. solium* and *T. hydatigena*, incubated LAMP reactions at 65°C for 30 min and recorded the post-amplification colour (yellow-positive, pink-negative). Stable transfection of the chimeric pig-rat FcϵRIα was confirmed at the mRNA level by RT-PCR. Five candidate *T. solium* oncospheral diagnostic allergens (E5LBB8, K0A0S9, Q2XNL7, Q9NI46, W8P1J2) were identified through bioinformatic analysis and three HEK293-6E transfected allergens showed expected protein band size in Western blot analysis upon recombinant expression. The LAMP assay was able to detect 10 pg μL^{-1} of T. solium DNA in pig serum, without cross-reactivity with *T. hydatigena*. We are now focusing on the detection of *T. solium* DNA through LAMP assay in field studies. This study shows proof-of-principle for a serological reporter assay for a lab-based approach, while LAMP is expected to introduce a field-applicable point-of-care test for porcine cysticercosis.

Keywords: Allergen, cysticercosis, diagnosis, reporter system, *Taenia hydatigena*, *Taenia solium*

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