Is species-specific diagnosis of porcine cysticercosis using loop-mediated isothermal amplification (LAMP) possible?

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BACKGROUND

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.The latter however have serious drawbacks, in particular regarding the specificity at the species level and above.

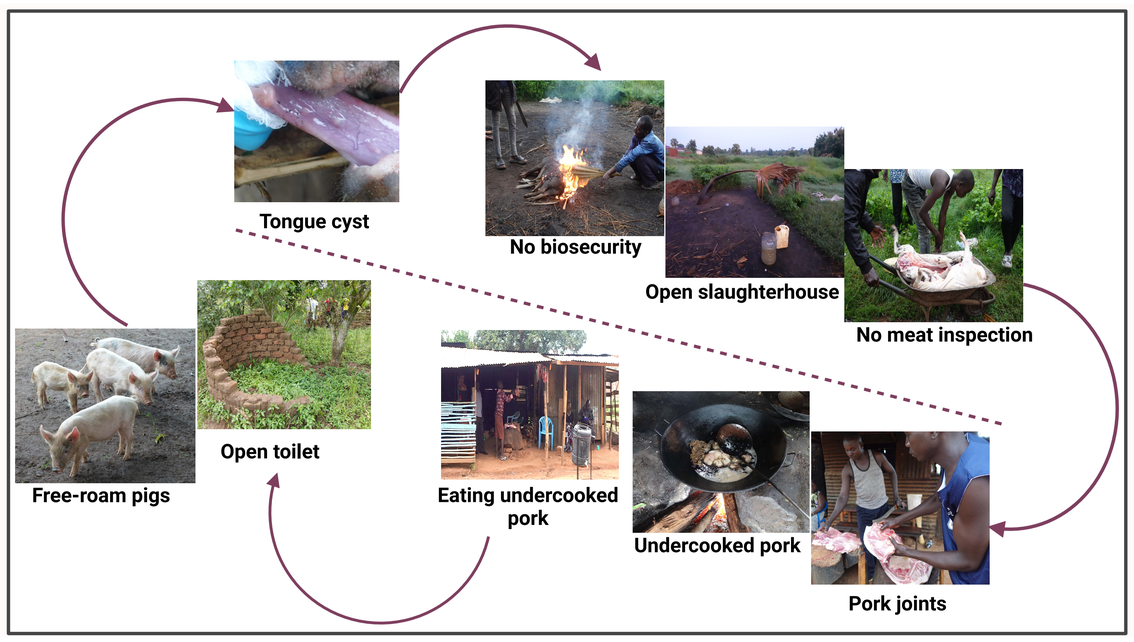

FIG.1 Disease cycle of *T. solium* in Uganda. Free-roaming pigs and eating undercooked pork by humans maintain the disease cycle in endemic areas.

⊕ **Objective**: Establish LAMP technology to distinguish porcine cysticercosis caused by *T. solium* from the related, co-endemic *T. hydatigena* (non-zoonotic).

METHODOLOGY

LAMP experiments were carried out in our laboratory in Giessen, with non-infected pig sera spiked with *T. solium* and *T. hydatigena* DNA. The saliva of humans was also used for spiking experiments. Once established the LAMP assay was used under field conditions in the ILRI/CDL lab in Uganda, using stored positive and negative pig sera (69) and saliva (10). Fresh serum and saliva samples were collected from 10 tongue cyst-positive pigs in Lamwo, Uganda, and tested with LAMP assay. The gold standard diagnostic, carcass dissection[1] was also performed for one tongue-cyst positive pig in Lamwo, Uganda.

FIG.2 Saliva and blood collection from cyst-positive pigs for LAMP-based analysis.

FIG.3 Lingual palpation[2] of a pig to identify cyst-positive pigs.

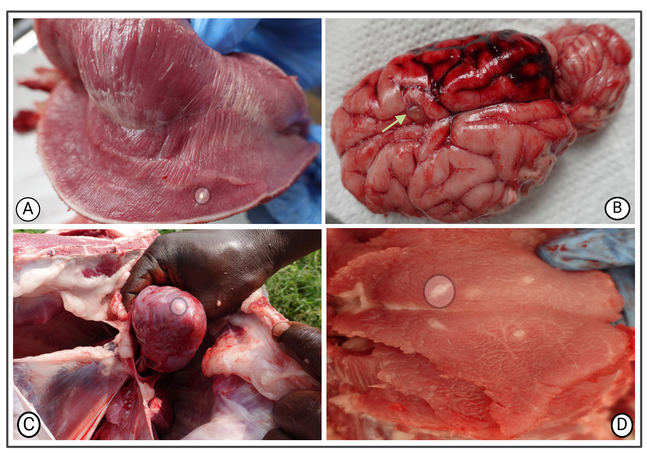

FIG.4 Carcass dissection of a tongue cyst-positive pig in Kitgum, Uganda. A) Calcified cyst in tongue, B-C) Viable cyst in brain and heart surface, D) Dead cyst in muscle fibre.

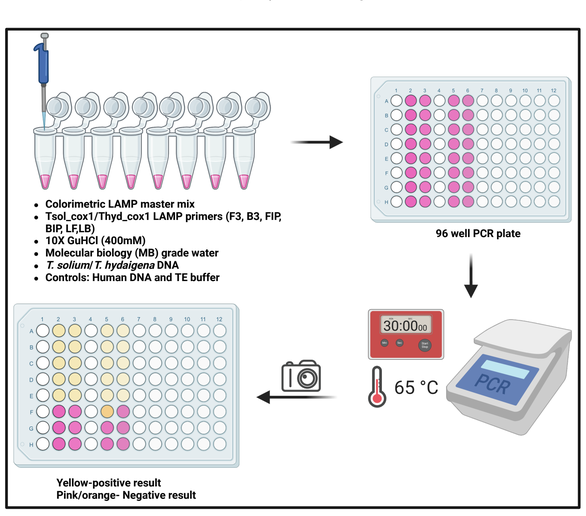

FIG.5 LAMP-based diagnostic protocols[3] for porcine cysticercosis. The LAMP reactions are incubated at 65°C for 30 minutes. Colour change from pink to yellow indicates the positive LAMP reactions.

RESULTS

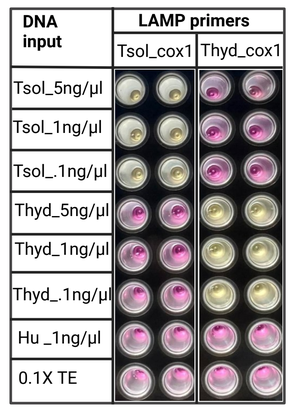

FIG.6 Spiking negative pig serum (collected from Veterinary clinic, University of Giessen) with genomic DNA of *T. solium* and *T. hydatigena* for LAMP assay with both respective and alternative LAMP primers. Human DNA and TE buffer were used for control.

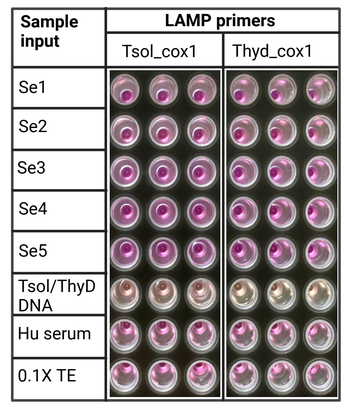

FIG.7 Pig serum collected by ILRI NZD team and archived at CDL, Makerere University. LAMP analysis was performed for apDia ELISA positive serum (Se1-Se3), and ELISA negative serum (Se4-Se5) with both *T. solium* and *T. hydatigena* cox1 primers. *T. solium* and *T. hydatigena* DNA used as positive control. Human serum and TE buffer were used as a negative control.

FIG.8 LAMP assay was performed using cyst fluid (1-6) collected and stored in ILRI/Uganda lab with both *T. solium* and *T. hydatigena* LAMP primers. Human serum and TE buffer (7,8) were used for control. Undiluted Cyst fluids (3 µl) induced colour change even before heating at 65°C.

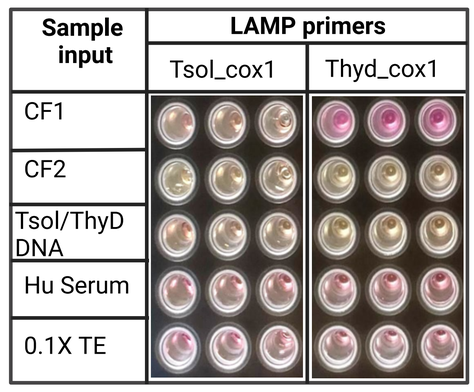

FIG.9 Cyst fluids (CF1-CF2) collected and stored in ILRI/Uganda lab tested with both *T. solium* and *T. hydatigena* LAMP primers for distinguishing types of cysticercosis in pigs. DNA of *T. solium* and *T. hydatigena* used as positive control. Human serum and TE buffer were used as a negative control.

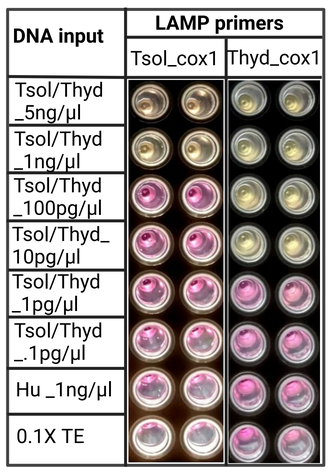

FIG.10 Spiking human saliva for LAMP assay in our laboratory in Giessen with both *T. solium* and *T. hydatigena* DNA with both respective and alternative LAMP primers. Human DNA and TE buffer were used for control.

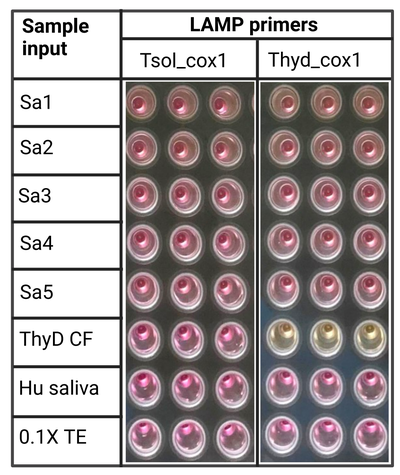

FIG.11 Saliva collected from tongue-cyst positive pigs in Lamwo, Uganda tested with LAMP for both *T. solium* and *T. hydatigena* primers. *T. hydatigena* cyst fluid used as a positive control. Human saliva and TE buffer were used as a negative control.

SUMMARY & CONCLUSION

⊕The cox1-based LAMP assay using cyst fluid can differentiate between *T. solium* and *T. hydatigena* cysticercosis infection in pigs.
⊖*T. solium* / *T. hydatigena* LAMP primers are unable to detect the presence of parasitic DNA in blood or saliva of pigs without prior DNA extraction.

✓**LAMP may not be a suitable technology for detection of parasitic DNA in serum or saliva of infected pigs without DNA extraction, but can be used for identification of *Taenia* species using cyst fluid.**

REFERENCES & ACKNOWLEDGMENTS

1. Thomas et al., 2015. World Health Organization. Geneva PP, 47.
2. Guyatt and Fèvre, 2016. Trop Med Int Health, 21(10): 1319–1323.
3. NEB Colorimetric LAMP assay. Link: https://www.neb.com.

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