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ELISA-assay for Pig *Taenia solium* Cysticercosis by using T18 recombinant antigen

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Abstract

Cysticercosis is caused by infection with the larval stage of tapeworm parasite *Taenia solium*. This zoonose is a major public health issue with high prevalence in Madagascar, around 21% and 7% to 20% respectively in swine and human. ELISA and Enzyme-linked Immuno-electro Transfer Blot (EITB) remained the main laboratory serological diagnostic tools available in the island. Four native glycoproteins are recognized by antibodies in a majority of sera from patients with cysticercosis and a similar pattern seems to be present in infected pigs. For a better management of this disease in Madagascar, the aim of this study is to set-up and validate the one majority glycoprotein antigen T18 (18KDa) for diagnosis test of pig cysticercosis, produced as recombinant protein.

Using a cDNA library prepared from *T. solium* cysticerci obtained from different regions of Madagascar, we first analyzed the polymorphism of the T18 gene. Then, gene was cloned into the pMAL-c2X plasmid for expression as an N-terminal maltose-binding protein (MBP) fusion protein and C-terminal hexa-His tagged protein. Product protein was then validated by ELISA test of pig sera.

Preliminary results show a strong conservation of T18 gene. And indeed larger amounts of soluble recombinant protein were obtained from these antigen. T18 recombinant-ELISA present 70% of sensitivity and 100% of specificity.

Keywords: Cysticercosis; ELISA-assay; Pig; Recombinant protein; T18

1. Introduction

Immunodiagnostic techniques are a classic tool in epidemiological studies to estimate the prevalence and to identify the risk factors associated with transmission of *Taenia solium* [1]. Surveying of people and pigs to detect cysticercosis-specific antibodies areas of disease transmission have an important role to play in control and elimination programs [2]. *Taenia solium* proteins used in antibody-detection assays especially for taeniosis, human cysticercosis and neurocysticercosis were reviewed by Deckers and Dorny [1]. The best characterized and the most specific immunodiagnostic for human cysticercosis diagnostic is the enzyme-linked immunoelectrotransfer blot using enriched and purified lentil-lectin chromatography glycoproteins (LLGP-EITB) extracted from a raw cysticercus [3]. With an initial reported sensibility of 98% and specificity of 100% when tested in human for detecting two or more cysts, reactivity with any of the seven glycoproteins fall into three families of antigens: GP50, T24/T42 and 8-kDa family (8, 13/14, 18, and 21kDa) was considered as positive test [4]. The LLGP-EITB technique was also tested in pigs [5]. However, the drawbacks of this assay lies in the preparation of the antigen complex which depends on infected pigs for

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supplying the source material and requires considerable technical expertise and sophisticated technology. Furthermore, batch differences can exist between different antigen preparations and a Western blot assay is not suitable and affordable for field studies [1, 6, 7]. To overcome these issues, characterization and production of the diagnostic LLGP as either synthetic proteins or recombinant forms have been realized and well documented in the literature specially for human cysticercosis [2, 4, 6, 8, 9]. The recombinant 24kDa antigen analyzed in EITB was the best candidate in detecting neurocysticercosis cases with two or more cysts [10]. Among the nine *Taenia solium* 8-kDa family antigens tested in synthetic forms and in FAST-ELISA: TsRS1 shown 100% of sensitivity and specificity, Ts18 var1 and Ts18 var3 (both at 18kDa) shown 100% and 97 to 100% of specificity and sensitivities respectively for human neurocysticercosis [6]. TSR1 was the best antigen for differentiating patients with multiple viable and racemose cysts against other parasitic diseases from countries not endemic for NCC and exhibited no problems of cross-reactivity when tested at higher concentrations [11]. TsRS1 was suggested to be an 8-kDa extracellular secreted protein that perhaps accumulates in the cyst fluid [6]. For porcine cysticercosis, the rT24H antigen tested in EITB , rT24H, rGP50 and the synthetic *Ts*18 var1 analyzed in Quick ELISA were also performed [12, 13]. Quick ELISA not requiring antibody-specific reagents for detection can be applied for testing human and pig cysticercosis. This completed automated ELISA was given a sensibility and specificity of 94.1% and 95.7% respectively for rGP50, of 97.1% and 88.9% respectively for rT24H, of 73.6% and 88.9% respectively for Ts18 var1 to detect porcine cysticercosis.

However, the rather heavy and complex preparation of these glycoproteins also led to the use of antigens from other sources. Cyst fluid of *Taenia solium* metacestodes was recognized as the most reliable and simple sources of diagnostic antigens, and present a more practical alternative for the serologic diagnosis of NCC [14, 7]. Characterization and cloning of *Ts* M 14 and 18kDa proteins forming the subunits of a 120 kDa protein complex from cyst fluid purified on single-step FPLC under native conditions was investigated [15]. Their native forms belonged to the *Ts*14 (14kDa) and *Ts*18 (18kDa) clades mostly found in the LLGP antigens [6]. As preliminary result, recombinant *Ts* M 14 and 18kDa antigens revealed high reliability for the differentiation of neurocysticercosis active and mixed stage with 90% and 85% of sensibility respectively and an overall specificity of 97.1% [15].

In view of the advantages associated with the use of cyst fluid and of recombinant proteins, we want to test the possibility of exploring 18kDa (appointed T18 excreted and secreted antigen identified in the cyst fluid of *T. solium* metacestode for production in recombinant form. In the present study, we reported the evaluation of the *Ts M* 18kDa recombinant antigen expressed in *E. coli* as an immunodiagnostic protein for porcine cysticercosis in an IgG-ELISA assay and compared its performance with LLGP-EITB.

2. Methods

The polymorphism of the T18 gene was studied using total DNA extracted from pig carcasses cysticerci slaughtered at two slaughterhouses in Antananarivo and came from seven principal district suppliers of pigs (Ambanja, Ambilobe, Bealanana, Miarinarivo, Ambalavao, Ambohimahasoa, Marovoay). Extraction of the total DNAs was carried out with about 25 mg of cysticerci using the Nucleospin extract II Kit (Macherey-Nagel, ref: 740609.50) according to the manufacturer's recommendations.

The T18 gene (Genbank Accession number: AAX32918 Proteine) were obtained by PCR using the primers (Forward: AAACATATGCGTGTCTACATTGTGCTTC and reverse: AAAGGATCCTTAAGCAGTTTTGTTCTT) in 25 µL final reaction Mix containing 1 µl of each DNAs extracted from cysticerci. The presence of amplicon was verified by 2% agarose gel electrophoresis. Each sequence was verified by sequencing at Beckman Coulter Genomics (England) in both senses using the primer set above. The polymorphism of T18 sequence was analyzed by multiple alignment (Clustal2W).

The T18 DNA sequence was cloned into a pMalC2X expression vector introducing a His_6 tag in N-terminus and MBP tag in C-terminus. The recombinant *E. coli* Rosetta gami2 strain, specifically designed for proteins containing multiple cysteines, was used for the expression of T18 in soluble form. Cells were broken using Constant System Cell Disrupter (1 pass: 20KPSI). Supernatant was loaded onto a 3ml Resin talon CO^{2+} column (HisTrap H, GE Healthcare). Recombinants proteins were eluted using buffer at different concentration of imidazole (100mM - 200mM – 300mM). T18 recombinant was evaluated by ELISA in comparison with the commercial cysticercosis Antigen-ELISA Kit (ApDia Ltd., Turnhout, Belgium) based on the B158/B60 monoclonal antibodies and the EITB assay (EITB-LLGP) used as reference method adapted for pig according to the method described previously by Tsang et al., [1] for human. These two methods with the presence of cysticerci detectable by langueyage, carcass examination and/or scanner were applied to classify the biobank of serum used: true positive (tested positively with the three methods) and true negative (serums of pigs reared in non-endemic areas, Belgium and found negative in EITB-LLGP and B158/B60 Ag-ELISA Kit). The recombinant T18 was evaluated by ELISA adapted to the method described by Migliani et al. [16] previously tested in human.

3. Results

In this study, the genetic diversity between cysticerci isolated from principal district suppliers of pigs of Madagascar was observed by sequence alignment of *Taenia solium* T18 performed on ClustalX program. Analysis confirmed that no genetic variation occurs for T18 coding parts of cysticerci collected in different regions of Madagascar. This result is favorable for the continuation of study, in the production of T18 recombinante for pig cysticercosis diagnosis.

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T18        ----------------------------------------------------GAGAAAAAC   9
T18-7      AGCCTCATGCAAACCTACTACATGTTCCAATTCAAACGCTTTTTTGCCTTTAGGAAAAAC   116
T18-3      AGCCTCATGCAAACCTACTACATGTTCCAATTCAAACGCTTTTTTGCCTTTAGGAAAAAC   117
T18-4      AGCCTCATGCAAACCTACTACATGTTCCAATTCAAACGCTTTTTTGCCTTTAGGAAAAAC   120
T18-6      AGCCTCATGCAAACCTACTACATGTTCCAATTCAAACGCTTTTTTGCCTTTAGGAAAAAC   120
T18-5      AGCCTCATGCAAACCTACTACATGTTCCAATTCAAACGCTTTTTTGCCTTTAGGAAAAAC   117
T18-1      AGCCTCATGCAAACCTACTACATGTTCCAATTCAAACGCTTTTTTGCCTTTAGGAAAAAC   116
T18-2      AGCCTCATGCAAACCTACTACATGTTCCAATTCAAACGCTTTTTTGCCTTTAGGAAAAAC   116
                                                              *******

T18        AAACCGAAGTGTGATGGAAATAGTACTAAGAAAGAGATAGAATTTATCCACAATTGGTTC   69
T18-7      AAACCGAAGTGTGATGGAAATAGTACTAAGAAAGAGATAGAATTTATCCACAATTGGTTC   176
T18-3      AAACCGAAGTGTGATGGAAATAGTACTAAGAAAGAGATAGAATTTATCCACAATTGGTTC   177
T18-4      AAACCGAAGTGTGATGGAAATAGTACTAAGAAAGAGATAGAATTTATCCACAATTGGTTC   180
T18-6      AAACCGAAGTGTGATGGAAATAGTACTAAGAAAGAGATAGAATTTATCCACAATTGGTTC   180
T18-5      AAACCGAAGTGTGATGGAAATAGTACTAAGAAAGAGATAGAATTTATCCACAATTGGTTC   177
T18-1      AAACCGAAGTGTGATGGAAATAGTACTAAGAAAGAGATAGAATTTATCCACAATTGGTTC   176
T18-2      AAACCGAAGTGTGATGGAAATAGTACTAAGAAAGAGATAGAATTTATCCACAATTGGTTC   176
           ************************************************************

T18        TTCCACGATGACCCGATTGGAAATCAAATTGCTCAACTCGCAAAGGATTGGAAGGTAGCA   129
T18-7      TTCCACGATGACCCGATTGGAAATCAAATTGCTCAACTCGCAAAGGATTGGAAGGTAGCA   236
T18-3      TTCCACGATGACCCGATTGGAAATCAAATTGCTCAACTCGCAAAGGATTGGAAGGTAGCA   237
T18-4      TTCCACGATGACCCGATTGGAAATCAAATTGCTCAACTCGCAAAGGATTGGAAGGTAGCA   240
T18-6      TTCCACGATGACCCGATTGGAAATCAAATTGCTCAACTCGCAAAGGATTGGAAGGTAGCA   240
T18-5      TTCCACGATGACCCGATTGGAAATCAAATTGCTCAACTCGCAAAGGATTGGAAGGTAGCA   237
T18-1      TTCCACGATGACCCGATTGGAAATCAAATTGCTCAACTCGCAAAGGATTGGAAGGTAGCA   236
T18-2      TTCCACGATGACCCGATTGGAAATCAAATTGCTCAACTCGCAAAGGATTGGAAGGTAGCA   236
           ************************************************************

T18        ATGCTGAAAGCCAAAGGCGAAATTCGGGCGTCACTGGCTGAGTACTGCAGAGGTCTGAAG   149
T18-7      ATGCTGAAAGCCAAAGGCGAAATTCGGGCGTCACTGGCTGAGTACTGCAGAGGTCTGAAG   296
T18-3      ATGCTGAAAGCCAAAGGCGAAATTCGGGCGTCACTGGCTGAGTACTGCAGAGGTCTGAAG   297
T18-4      ATGCTGAAAGCCAAAGGCGAAATTCGGGCGTCACTGGCTGAGTACTGCAGAGGTCTGAAG   300
T18-6      ATGCTGAAAGCCAAAGGCGAAATTCGGGCGTCACTGGCTGAGTACTGCAGAGGTCTGAAG   300
T18-5      ATGCTGAAAGCCAAAGGCGAAATTCGGGCGTCACTGGCTGAGTACTGCAGAGGTCTGAAG   297
T18-1      ATGCTGAAAGCCAAAGGCGAAATTCGGGCGTCACTGGCTGAGTACTGCAGAGGTCTGAAG   296
T18-2      ATGCTGAAAGCCAAAGGCGAAATTCGGGCGTCACTGGCTGAGTACTGCAGAGGTCTGAAG   296
           ************************************************************

T18        AACAAAACTGCTTAA--------------  164
T18-7      AACAAAACTGCTTAGGGGATCCTTAGA--  323
T18-3      AACAAAACTGCTTAGGGGATCCTTASA--  324
T18-4      AACAAAACTGCTTAGGGGATCCTTAGAA-  328
T18-6      AACAAAACTGCTTAGGGGATCCTTAGA--  327
T18-5      AACAAAACTGCTTAGGGGATCCTTAGAAC  326
T18-1      AACAAAACTGCTTAGGGGATCCTTAGAA-  324
T18-2      AACAAAACTGCTTAGGGGATCCTTAGAA-  324
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Figure 1 T18 gene cysticerci alignment

The induction temperature was decreased from 37 °C to 20 °C, to increase the solubility. When T18 was expressed at 20 °C, the protein was observed in soluble and purified fraction in SDS-PAGE Coomassie blue stained gel, which was confirmed by blot analysis.

The verification of the antigen quality on SDS-PAGE polyacrilamide gel revealed that the recombinant 18 kDa protein tends to dimerize in unreduced native condition. However, this dimerization is greatly reduced in the presence of a reducing agent (mercaptoethanol or DTT) and did not interfere with the antigenicity of the recombinant protein.

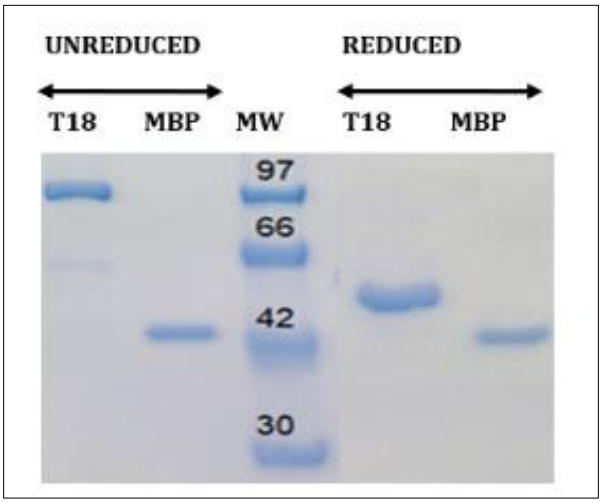

Figure 2 SDS-PAGE; MBP-fusion soluble recombinant protein T18 (51.7 kDa).
Molecular weight (MW) in KDa

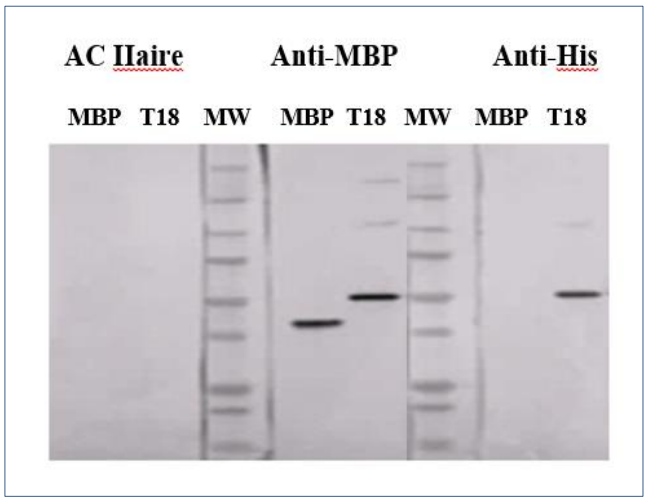

Figure 3 Blot analysis of reactvity of anti-Tag (Anti-MBP and Anti-His) for T18 recombinant protein

The pig sera forming the biobank used in this study were first characterized by the detection of antibodies (IgG) against *T. solium* antigens using the LLGP-EITB technique and by the detection of circulating antigens using the B158/B60 monoclonal antibody-based antigen ELISA. The twenty sera from pigs with cyst detected at slaughter and of 1 to 10 (low load: 7 pigs), 11 to 100 (mean load: 7 pigs) and over 100 (strong load: 6 pigs), classified as positive controls are all positive with both techniques used. They all showed in EITB bands of the 8kDa family of antigens (notably at 14 and 18kDa) with an antigen index greater than 30. The 40 sera of pigs from a country free from cysticercosis and 3 sera cysticerci free at slaughter, classified as negative controls were all tested negatively for antigen detection, whereas 39/42 and 50kDa bands were presented in LLGP-EITB on all these sera. These same bands were also found sera of pigs infected by Trichinellosis and Echinococcosis for cross-reaction tests. In addition, 3 out of 11 pigs with trichinellosis used in this study were tested positively for the circulating antigens detection with an antigen index between 2.4 and 5.8.

The positivity threshold for the T18 recombinant protein was calculated from 10 negative sera coming from a non-endemic zone to cysticercosis. Cut-off points between sera were set at 3 standard deviations from the mean of the negative sera giving a value to 0.16.

The LLGP-EITB serological reference test using native antigens was used to evaluate the ELISA tests developed on recombinant T18. The results obtained with the pig serum biobank are shown in the table. Thus the T18 recombinant protein had a sensitivity and specificity of 70% and 100% respectively.

Table 1 Serum samples tested by cysticercosis serologic diagnosis

samples	EITB-CS50 +	Apdia +	ELISA T18 +
Pig sera cysticerci+ (n=20)	20	20	14
Pig sera cysticerci- (n=33)	33	33	33
Pig sera Trichinellosis+ (n=11)	00	03	00
Pig sera Echinococcosis+ (n=3)	00	00	00

Although 3 out of 11 pigs sera trichinellosis were tested positively in B158/B60 Ag-ELISA Kit, none sera with other parasitic trichinellosis and echinococcosis showed cross-reactivity in ELISA-T18 recombinant test.

4. Discussion

There is only one report in the literature on immunotaxonomy of Taenia solium in which antigen differences between cysticerci collected from different pigs are reported [17]. Genetic variability has important epidemiological implications, such as the development of the parasite, its pathogenicity and drug susceptibility. Examining genetic heterogeneity of tapeworm infection at the family/household, community and eventually at geographical/regional levels will help determine transmission patterns in endemic regions [18].

The genetic differences between cysticerci allow the tracing of their spreading patterns and may be of relevance for the design of pig vaccination programs as well as for the clinical heterogeneity [19].

Although Rodrigo Vega et al., announced that the possibility of two drastically distinct disease syndromes differing in their preferred target tissue, neuro-tropic and extraneuro-tropic, affecting Madagascar could explain by in case of two forms of taenias (different cysticerci) [20]; in our survey, we didn't find any polymorphism T18 gene for cysticerci collected. We can say that cysticerci T18 gene is preserved to Madagascar.

Moreover, study in identification of new proteins in cysticercus fluid confirmed that no genetic variation occurs for these proteins for the cysticerci collected in different areas of Madagascar and development of a rapid test is in process [21].

The fidelity of T18 gene classified it among the key candidates in cysticercosis diagnosis. But, T18 is a low size protein, so it's necessary to fusion it with tag. Tag does not interfere with protein folding and did not affect the activity of recombinant protein.

Fusion tags have been extensively used for their ability to increase the yield, enhance the solubility and facilitate the detection and the purification of their partners [22]. In general, short tags have no effects on the solubility of their partners [23, 24]. Solubility tags, such as the maltose-binding protein (MBP) and the thioredoxin, have also been frequently used to increase the yield, anhance the solubility and the stability of the recombinant proteins [25].

In this study, *Escherichia coli* Rosetta gami2 was transformed with pMalC2x-6xHis-T18-MBP to produce 6xHis-T18-MBP protein and it was purified with Co^{2+}affinity chromatography.

A high yield expression of soluble recombinant protein was achieved by examining the effects of the induction temperature, the inducer concentration and the host strains change on the expression of this recombinant protein. To improve the solubility of the recombinant protein, the temperature of growth was decreased from 37 °C to 25 °C. Result showed that the highest production of soluble protein was obtained when performing the culture of the Rossetagami2

at 20 °C in the presence of IPTG at 0.1mM. Indeed, the yield of soluble T18-MBP is from 8mg/L. Fig 2 shows that after IPTG induction (0.1mM), there is a protein around 51KDa in the soluble fraction.

At the present time, the routine diagnosis of porcine cysticercosis in pigs in Madagascar based for live animals on lingual palpation, and for carcasses on visual postmortem and incisional examination during veterinary inspection at abattoirs. Althought, these methods have high specificities (99-100%), there are few appreciable, especially in detecting cysticercosis in pig with of low levels of cysts. On the other hand, diagnosis of cysticercosis by immunological methods can be tested. Several methods are available for antigen or antibody detection.

The immunodiagnostic properties of potential new antigens for antibody detection of porcine cysticercosis were also evaluated in recombinant forms, including the enzyme families like the cysteine protease [26], and the α-enolase [27]. Recombinant TSOL45, and especially TSOL18 antigens cloned from mRNA obtained from hatched and activated oncospheres of *T. solium* were largely tested in pig vaccination [28, 29]. Combination of the rTSOL18 vaccine and medication based on oxfendazole treatment in pigs at 3-monthly intervals was predicted to lead to an interruption of *T. solium* transmission within a year of initiation of the program that was evaluated in the field [30, 31].

Immunization of pigs with the TSOL18 recombinant antigen induced complete, or near-complete (99,5%), protection against the development of cysticerci following experimental challenge infection. This is the highest level of protection that has been achivied by vaccination against *T. solium* infection in pigs with a defined antigen [32, 33, 34, 35].

There is compelling evidence for complement-fixing antibody playing a critical role in vaccine-induced immunity to taeniid cestode infection [36]. Solid evidence supporting the role of protective antibodies is available for numerous parasite species, including *Taenia ovis*, *Taenia pisiformis*, *Taenia saginata, Taenia taeniaeformis* and Echinococcus granulosus [37].

Apart the protective role observed in vaccination, antibody is likely to play an important role in anti-oncosphere immunity in infection detection with formation of the complex antigen-antibody, these complex is observed in serologicals methods.

Serodiagnosis developed in human cysticercosis infection (ELISA-CS50, EITB-CS50) are adapted for pig serodiagnosis in our study. EITB-CS50 and B158/B60 Ag-ELISA Kit tests are considered as gold standard associated to a postmortem inspection of carcasses in our case. We aimed to determine the diagnostic performance of ELISA-T18 recombinant in relation to the gold standard for porcine cysticercosis detection in Madagascar. T18 recombinant correspond to immunogenic GP18 glycoprotein, a major antigenic glycoprotein in CS50 antigen. ELISA-T18 test results showed high specificity 100% with sensitivity 70%, sensitivity is lower than native glycoprotein CS50. Different authors synthetised 10, 7-10 and 14KDa recombinant polypeptides used in immunoblot and ELISA reported the high specificity of antigens and the sensitivity is generally low enough [38, 39]. This decrease sensitivity is probably due to the absence of glycosylation of the recombinant protein produced in *Eschericia coli*. Eukaryotic cloning systems (HEK293, CHO, CO 7) or bug cloning systems (S2, Sf9) can be used to overcome this problem. Association of severals antigenic recombinant proteins could be consireded to improve sensitivity [40].

To part the sensitivity, the specificity is also a performance parameter of a diagnosis test. Serodiagnosis test results should be interpreted considering possible cross-reactions with other parasites. Gavidia at al. [41] and Jayashi et al. [42] found that pigs from andemic areas that were EITB positive had no cysts upon necropsy. Similar results were reported by Devleesschauwer et al. [43] using the B158B60 Ag-ELISA in sentinel pigs that tested Ag-ELISA positive, no *T. solium* cysts could be found in the carcass. It is well documented that infection with *T. hydatigena* causes falses positives in B158B60 Ag-ELISA [44]. In addition, when carcass inspection was used as reference standard, the EITB assay and the B158B60 Ag-ELISA were found to highly specific for detection of porcine cysticercosis, suggesting that for these assays cross-reactivity with other parasitesis no a major concern in this setting [45].

Fourteen serum samples from pigs with other parasitic infections were used in the study for cross-reactivities (11 sera with Trichinellosis and 3 sera with Echinococcosis). Even though, 3 out of 11 pigs with trichinellosis were tested positively for B158/B60 Ag-ELISA Kit tests, none of these serum pig samples with other parasitic reacted with antigen T18 recombinant. A similarity is observed for the study of Jayashi et al. [37], used TSOL18 as vaccine antigen; which cross-reactivity has not found between this antigen. It reinforces the fact that antibody responses were targeted specifically to antigen.

5. Conclusion

In madagascar, cysticercosis had an impact directly on the public health sector, and the value of cysticercosis-infected meat is a major cause of income loss for poor farmers. Detection of cysticercosis in pigs is a key point to control the disease. The new challenge is to develop diagnostic tools usable in the dispensary for patients or directly at the farm gate for pigs. Recombinants antigens is one of the tools do not used crude antigens. Moreover, the gene coding for specific protein antigenic T18 of cysticercosis proves to be preserved to Madagascar. Only limited of tests is based on the lack of sensitivity (70%) wich may also be due to the near absence of glycosylation of the recombinant proteins produced in Escherichia coli and eukaryotic cloning systems can help to overcome this problem.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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References

[1] Jayashi, C.M.; Gonzalez, A.E.; Castillo Neyra, R.; Rodriguez, S.; Garcia, H.H.; Lightowlers, M.W.; 2014. Validity of the Enzymz-linked Immunoelectotransfer Blot (EITB) for naturally acquired porcine cysticercosis. *Vet. Parasitol*.; 199: 42 – 49.

[2] Hancock, K., Pattabhi, S., Whitfield, F.W., Yushak, M.L., Lane, W.S., Garcia, H.H., Gonzalez, A.E., Gilman, R.H., Tsang, V.C.W., 2006. Characterization and cloning of T24, a *Taenia solium* antigen diagnostic for cysticercosis. *Molecular & Biochemical Parasitology*. 147: 109 – 117.

[3] Noh, J.; Rodriguez, S.; Lee Y.M; Handali, S.; Gonzalez, E.A.; Gilman, R.H., Tsang, V.C.W.; Garcia, H.H.; Wilkins, P.P. 2014. Recombinant Protein and Synthetic Peptide-Based Immunoblot Test for Diagnosis of Neurocysticercosis. *Journal of Clinical Microbiology*. 52 (5): 1429-1434.

[4] Hancock, K., Pattabhi, S., Greene, R.M., et al.; 2004. Characterization and cloning of GP50, a *Taeni solium* antigen diagnostic for cysticercosis. *Molecular and Biochemical Parasitology*. 133: 115 -124.

[5] Arnau, J.; Lauritzen, C.; Petersen, G.E.; Pedersen, J.; 2006. Current strategies for the use of affinity tags and tag removal for the purification of recombinant proteins. *Protein Expr. Purif*. 48: 1 -3.

[6] Wang, Q.M.; Sun, S.H.; Hu, Z.L.; Wu, D.; and Wang, Z.C.; 2003. Immune response and protection elicited by DNA immunization against *Taenia* cysticercosis. *Vaccine*; 21: 1672 – 1680.

[7] Sako, Y.; Nakao, M.; Ikejima,T.; Piao,X.Z.; Nakaya,K.; Ito,A. 2000. Molecular characterization and diagnostic value of Taenia solium low-molecular-weight antigen genes. *Journal Clinical Microbiology*; 38(12):4439-44.

[8] Waugh, D.S; 2005. Making the most of affinity tags. *Trends Biotechnology*; 23: 316 – 320.

[9] Jayashi, C.M.; Gonzalez, A.E.; Neyra, R.C.; Kyngdon, C.T.; Gauci, C.G.; Lightowlers, M.W.; 2012. Characterisation of antibody responses in pigs induced by recombinant oncosphere antigens from *Taenia solium*. *Vaccine*; 30: 7475 – 7480.

[10] Dorny, P.; Phiri, I.K.; Vercruysse, J.; Gabriel, S.; Willingham lii, A.L.; Brandt, J.; Victor, B.; Speybroeck, N.; Berkvens, D.; 2004. A Bayesian approach for estimating values for prevalence and diagnostic test characteristics of porcine cysticercosis. *Int. J. Parasit.*; 34: 569 – 576.

[11] Park SK, Yun DH, Chung JY, Kong Y, Cho SY. 2000. The 10 kDa protein of Taenia solium metacestodes shows genus specific antigenicity. Korean Journal Parasitol ; 38(3):191-4.

[12] Malhotra, A.; 2009. Tagging for protein expression. *Methods Enzymol*; 463, 239 – 258.

[13] Migliani, R.; Rasolomaharo, M.; Rajaonarison, P.; Ravaoalimalala, V.E.; Rabarijaona, L.; Andriantsimahavandy, A.; 2000. La cysticercose dans le port de Mahajanga: plus fréquente qu'on ne l'imagine. *Arch Inst Pasteur Madagascar*; 66 (1&2): 39 – 42.

[14] Vega, R.; Pinero, D.; Ramanankandrasana, B.; Dumas, M.; Bouteille, B.; Fleury, A.; Sciutto, E.; Larralde, C.; Fragoso, G. 2003. Population genetic structure of *Taenia solium* from Madagascar and Mexico: implications for clinical profile diversity and immunological technology. *International Journal for Parasitology*; 33: 1479 – 1485.

[15] Deckers, N., Dorny, P., 2010. Immunodiagnosis of Taenia solium taeniosis/cysticercosis. *Trends Parasitol*. 26: 137 – 144.

[16] Esposito, D.; Chatterjee, D.K., 2006. Enhancement of soluble protein expression through the use of fusion tags. *Curr. Opin. Biotechnol*; 17: 353 – 358.

[17] Devleesschauwer, B.; Aryal, A.; Tharmalingam, J.; Joshi, D.D.; Rijal, S.; Speybroeck, N.; Gabriël, S.; Victor, B.; Dorny, P.; 2013. Complexities in using sentinel pigs to study Taenia solium transmission dynamics under field conditions. *Veterinary Parasitology*; 193: 172 -178.

[18] Bobes, R.J.; Fragoso, G.; Reyes-Montes, M.d.R.; Duarte-Escalante, E.; Vega, R.; Aluja, A.S.; Zuniga, G.; Morales, J.; Larralde, C.; Sciutto, E. 2010. Genetic diversity of *Taenia solium* cysticerci from naturally infected pigs of central Mexico. *Veterinary Parasitology*; 168: 130 – 135.

[19] Campbell, G; Garcia, H.H.; Nakao, M.; Ito, A.; Craig, P.S. 2006. Genetic variation in *Taenia solium*. *Parasitology international*; 55: 121 – 126.

[20] Gauci, C.G., Lightowlers, M.W. 1995. Developmental regulation of Taenia ovis 45W gene expression. *Molecular and Biochemical Parasitology*;73(1-2):263-6.

[21] Ponce R., León-Janampa N., Gilman R.H., Liendo R., Roncal E., Luis S., Quiñones-Garcia S., Silverstein Z., García H.H., Gonzales A., Sheen P., Zimic M., Pajuelo M.J.; Cysticercosis Working Group in Peru. 2018. A novel enolase from *Taenia solium* metacestodes and its evaluation as an immunodiagnostic antigen for porcine cysticercosis. *Experimental Parasitology*. 191:44-54.

[22] Rasamoelina-Andriamanivo, H.; Porphyre, V.; Jambou, R.; 2013. Control of cysticercosis in Madagascar: beware of the pitfalls. *Trends Parasitol*ogy; 29 (11): 538 -547.

[23] Villota, G.E., Gomez, D.I., Volcy, M., Franco, A.F., Cardona, E.A., Isaza, R., Sanzon, F., Teale, J.M., Restrepo, B.I., 2003. Similar diagnostic performance for neurocysticercosis of three glycoprotein preparations from *Taenia solium* metacestodes. *Am. J. trop. Med. Hyg.* 68: 276 – 280.

[24] Gonzalez, A.E.; Cama, V.; Gilman, R.H.; Tsang, V.C.W.; Pilcher, J.B.; Chavera, A.; Castro, M.; Montenegro, T.; Verastegui, M.; Miranda, E.; Bazalar, H; 1990. Prevalence and comparaison of serologic assays, necropsy, and tongue examination for the diagnosis of porcine cysticercosis in peru. *American Journal of Tropical Medecine and Hygiene*; 43 (2): 194 -199.

[25] Lightowlers, M.W.; 2006. Cestode vaccines: origins, current satus and future prospects. *Parasitology*; 133: 27 – 42.

[26] Poudel, I.; Sah, k.; Subedi, S.; Singh,D.K.; Kushwaha, P.; Colston, A. ; Gauci,C.G.; Donadeu,M.; Lightowlers, M.W. 2019. Implementation of a practical and effective pilot intervention against transmission of *Taenia solium* by pigs in the Banke district of Nepal. PLoS Neglected Tropical Diseases ; 25 :13(2).

[27] Nativel, P.; Rahantamalala, A.; Ramiandrisoa, S.; Rasoamampianina, V.; Duchateau, M.; Chamot-Rooke, J.; Guebey, R.; Rasamoelina-Andriamanivo, H.; Jambou R. 2016. Bio-guided identification of proteins for the diagnosis of cysticercosis in swine. *Veterinary Parasitology*; 220: 23 – 27.

[28] León-Janampa N.; Liendo R., Gilman R.H. , Padilla C., García H.H., Gonzales A., Sheen P., Pajuelo M.J., Zimic M. 2019. Characterization of a novel cathepsin L-like protease from Taenia solium metacestodes for the immunodiagnosis of porcine cysticercosis. *Veterinary Parasitology*; 267: 9-16

[29] Greene, R.M.; Hancock, K.; Wilkins, P.P.; Tsang, V.C. 2000. *Taenia solium*: molecular cloning and serologic evaluation of 14- and 18-kDa related, diagnostic antigens. *Journal Parasitology;* 86:1001-1007.

[30] Huerta, M.; De Aluja, A.S.; Fragoso, G.; Toledo, A.; Villalobos, N.; Hernandez, M.; Gevorkian, G.; Acero, G.; Diaz, A.; Alvarez, I.; Avila, R.; Beltran, C.; Garcia, G.; Martinez, J.J.; Larralde, C.; and Scuitto, E.; 2001. Synthetic peptide

vaccine against *Taenia solium* pig cysticercosis: successful vaccination in a controlled field trial in rural Mexico. *Vaccine*; 20: 262 – 266.

[31] Gauci, C.G.P.; Flisser, A.; and Lightowers, W.; 1998. Research note a *Taenia solium* oncosphere protein homologous to host-protective *Taenia ovis* and *Taenia saginata* 18kDa antigens. *International Journal for Parasitology*; 28(5): 757 – 760.

[32] Ferrer, E.; Sanchez, J.; Milano, A.; Alvarez, S.; La Rosa, R., Lares, M.; Ganzalez, L.M.; Cortez, M.M.; Davila, I.; Harrison, L.J.S.; Parkhouse, R.M.E.; Garate, T.; 2012. Diagnostic epitope variability within *Taenia solium* 8kda antigen family: Implications for cysticecosis immunodetection. *Experimental Parasitology*; 130: 78 – 85.

[33] Gavidia, C.M.; Verastegui, M.R.; Garcia, H.H.; Lopez- Urbina, T.; Tsang, V.C.W.; Pan, W.; Gilman, R.H., Gonzalez, A.E.; The Cysticercosis Working Group in P.; 2013. Relationship between serum antibodies and *Taenia solium* larvae burden in pigs raised in field conditions. *PloS Negl. Trop. Dis.*; 7: 2192.

[34] Lightowlers, M.W.; Donadeu, M. 2017. Designing a Minimal Intervention Strategy to Control Taenia solium. *Trends Parasitology;* 33(6):426-434.

[35] Yakoleff-Greenhouse, V.; Flisser, A.; Sierra, A.; Larralde, C. 1982. Analysis of antigen variation in cystcerci of *Taenia solium*. *Journal Parasitology*. 68, 39 – 47.

[36] Ng-Nguyen, D.; Stevenson M. A.; Phan T. V.; Nguyen V. T.; Vo T. V.; Traub R. J.2018. The epidemiology of Taenia spp. Infection and Taenia solium cysticerci exposure in humans in the central Highlands of Vietnam. *Infectious Disease*; 18: 527.

[37] Tsang, V.C.W., Brand, J.A., Boyer, A.E., 1989. An enzyme_linked immunoelectrotransfert blot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). *J. Infect. Dis.* 159: 50 – 59.

[38] Scheel, C.M.; Khan, A.; Hancock, K.; Garcia, H.H.; Gonzalez, A.E.; Gilman, R.H., Tsang, V.C.W., and The Cysticercosis Working Group in Peru. 2005. Serodiagnosis of neurocysticercosis using synthetic 8-KD proteins: comparison of assay formats. *American Journal of Tropical Medecine and Hygiene.* 73 (4): 771 – 776.

[39] Flisser, A.; Gauci, C.G.; Zoli, A.; Ocana, J.M.; Rodriguez, A.G., Alpizar, J.L.D.; Maravilla, P.; Canul, R.R.; Avila, G.; Vega, L.A.; Kyngdon, C.; Geerts, S.; Lightowlers, M.W.; 2004. Induction of protection against Porcine Cysticercosis by vaccination with Recombinant oncosphere Antigens. Infection and Immunity; 72(9): 5292 – 5297.

[40] Cai, X.; Chai, Z.; Jing, Z.; Wang, P.; Luo, X.; Chen, J.; Duo, Y.; Feng, S.; Su, C.; and Jin, J.; 2001. Studieson the development of DNA vaccine against cysticercus cellulosae infection and its efficacy; *Southeast Asian J. Trop. Med. Pblic Health*; 32(2): 105 – 110.

[41] Handali, S.; Klarman, M.; Gaspard, A.N.; Noh, J.; Lee, Y.M.; Rodriguez, S.; Gonzalez, A.E.; Garcia, H.H.; Gilman, R.H.; Tsang, V.C.W.; Wilkins, P.P.; 2010. Multiantigen Print Immunoassay for Comparison of Diagnostic Antigens for *Taenia solium* Cysticercosis and taeniasis. *Clinical and Vaccine Immunology*; 17(1): 68 - 72.

[42] Dorny , P.; Brandt, J.; Zoli, A.; Geerts, S. 2003. Immunodiagnostic tools for human and porcine cysticercosis. *Acta* Tropica; 87(1):79-86.

[43] Porphyre, V.; Betson, M.; Rabezanahary, H.; Mboussou, Y.; Zafindraibe, N.J.; Rasamoelina-Andriamanivo, H.; Costard, S.; Pfeiffer, D.U.; Michault, A.; 2016. *Taenia solium* porcine cysticercosis in Madagascar: comparison of immune-diagnostic techniques and estimation of the prevalence in pork carcasses traded in Antananarivo city. *Veterinary Parasitol*ogy; 219: 77 – 83.

[44] Lee E.G. , Bae Y.A., Jeong Y.T., Chung J.Y., Je E.Y., Kim S.H., Na B.K., Ju J.W., Kim T.S., Ma L., Cho S.Y., Kong Y. ; 2005. Proteomic analysis of a 120 kDa protein complex in cyst fluid of Taenia solium metacestode and preliminary evaluation of its value for the serodiagnosis of neurocysticercosis. *Parasitology*; 131(6): 867-79.

[45] Hancock, K., Khan, A., Williams, F.B., Yushak, M.L., Pattabhi, S., Noh, J., Tsang, V.C.W., 2003. Characterization of the 8-Kilodalton Antigens of *Taenia solium* Metacestodes and Evaluation of Their Use in an Enzyme-Linked Immunosorbet Assay for Serodiagnosis. *Journal of Clinical Microbiology*. 41 (6): 2577 – 2586.