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REVIEW ARTICLE
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Epidemiological tools for effective surveillance of porcine cysticercosis in Africa

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Abstract

Porcine cysticercosis is widely distributed in developing countries. Many tools were developed for effective control of the tapeworm in endemic countries. Tongue examination, meat inspection and Ag-ELISA were widely used in epidemiological survey. Both tongue examination and meat inspection are highly specific but less sensitive. To improve performance of Ag-ELISA, unambiguous test based on nanobodies was performed. Immunodiagnostic tests based on PCR, flows through assay (FTA), Surface Enhanced Laser Desorption–Ionization Time of Flight (SELDI-TOF) were also developed. Less data were reported using spatial statistical analysis hence multiples approaches were available for effective epidemiological survey of porcine cysticercosis.

Keywords: Ag-ELISA, cysticercosis, epidemiology, GIS, meat inspection.

Introduction

Taenia solium is a tapeworm responsible of serious public health problem in developing countries [1,2]. This tapeworm is transmitted to human by absorption of *T. solium* eggs or when eating undercooked pork. In humans, the larval stage of *T. solium* is responsible for conditions named cysticercosis or neurocysticercosis. Tongue examination and meat inspection are the main methods used for identifying infected pigs. The specificity of the tests is assessed to be close to 100%; however the sensitivity is variable, depending very much on the degree of infection in the pig [3]. Different techniques have been described to detect antibodies and antigens of *T. solium* infections in man and pigs: enzyme linked immunosorbent assay (ELISA), dipstick-ELISA, latex agglutination, immunoblot techniques, antigen detection based on sandwich enzyme-linked immunosorbent assay (Ag-ELISA) [3-9]. Antigens detection based on Ag-ELISA was therefore effectively used to detect porcine and human cysticercosis. However, Ag-ELISA is not highly specific and sensitive. Hence, other immunodiagnostics tests and spatial statistical analyses of porcine cysticercosis were developed in order to be used in epidemiological survey.

This paper aimed to review epidemiological tools developed for the control of porcine cysticercosis.

Macroscopic tools for porcine cysticercosis diagnostic

Porcine cysticercosis is widely distributed among different areas in developing countries. In Africa, the tapeworm was identified in Western Africa, Eastern Africa and Southern Africa. Meat inspection and lingual examination are frequently used for the diagnostic of the porcine cysticercosis in Africa.

Lingual examination: Lingual examination was widely used in all the diagnostic studies dealing with the assessment of *T. solium* cysticercosis infection in Africa [10]. To perform the test, the pig is firmly restrained in lateral recumbency and a hard wooden stick is used to open the mouth and keep it open. Using mutton cloth as an aid, the tongue is pulled out, examined and palpated along its entire ventral aspect to check for presence or absence of cysticerci. A pig found to have one or more cyst(s) on the tongue is considered positive for *T. solium* cysticercosis. If carried out correctly, the specificity of tongue palpation is close to 100% [3,10]. The sensitivity of the technique, is however variable, depending very much on the degree of infection in the pig. A Bayesian approach was used to estimate the performance of tongue inspection [3]. The performance indicators of this test were: 0.21 for sensitivity (se) and 1.00 for specificity (sp). None of the light infections and only about half of the heavy and moderate infections have been detected by tongue inspection. Nevertheless, despite of the low sensitivity of the lingual examination, this diagnostic test is useful in assessing of the spatial distribution of the tapeworm [11].

Meat inspection: *Post-mortem* inspection for diagnostic of *T. solium* cysticercosis was described [12, 13]. Meat inspection includes long and parallel incisions into the external masseter muscles on both sides of the face in an upward direction to severe completely the parotid

gland below the ear. The abdominal and diaphragmatic muscles, the root of the tongue and the tongue of all pigs should be incised and the blade of the tongue viewed and palpated. The heart of all pigs will be viewed, palpated and opened up. A deep incision will be made into the septum. Incisions will also be made in the triceps brachii muscle proximal to the elbow joint. Heavy infestation with *Cysticercus cellulosae* calls for carcass condemnation. In light or moderate infestation, the carcass may be conditionally approved pending heat or freezing treatment. Cysts that were encountered on incisional and intact surfaces were classified as either viable (translucent, fluid-filled with invaginated whitish protoscolices visible) or degenerated (black, sand-like or powdery contents) [14]. The specificity of meat inspection is estimated to be close to 100% [3, 15]. But it is admitted that, meat inspection is less sensitive [3]. This test is unable to exclude *T. solium* infected pork from the food chain since it failed to detect infections in 61.3% of infected animals [15]. Thus, the method is imperfect for use in epidemiological surveys and cannot be applied to monitor control programs of *T. solium*. Light infections go undetected in carcass inspection. Therefore, to control the parasite effectively in endemic areas, more sensitive and specific diagnostic methods have been used, such as immunodiagnostic tools [3].

ELISA for the detection of circulating *T. solium* antigens (Ag-ELISA)

Ag-ELISA (Antigens detection based on sandwich enzyme-linked immunosorbent assay) test for the detection of circulating *T. solium* antigens consists of collection of about 5 ml of blood from the jugular vein of large pigs or anterior vena cava of small pigs into plain tubes with clot activator. After centrifugation, the serum from each tube is transferred into two labelled cryovial tube aliquots for each pig and stored at -20 ^{0}C until tested. The samples are first treated with trichloroacetic acid (TCA) to break the antigen-antibody complexes and then tested at a final dilution of 1:4. Briefly the sandwich assay consisted of coating the plates with capturing monoclonal antibody (B158C11 A10), blocking, addition of TCA treated sera, after which the second biotin labeled antibody (B60H8A4), streptavidin labeled peroxidase and ortho phenylenediamine (OPD) substrate are added consecutively. Washings are carried out between the steps. The reaction is stopped using sulphuric acid and the plates are read in a spectrophotometer at a wavelength of 492 nm. The cutoff is calculated using a modified Student t-test programmed in MS Excel sheet, by comparing the optical density of each serum sample with a series of negative serum samples obtained from a pig without any history of cysticercosis in each study area at a probability level of $P < 0.001$. A serum sample is considered positive when the ratio (optical density of test sample/optical density cut-off) is $\geq$ 1.0. The specificity and sensitivity of Ag-ELISA test were found to be 86.7% and 94.7%, respectively [3]. For several authors, Ag-ELISA test is useful for identification of active infection [16,17]. In contrast, cross reactions were detected with *Taenia hydatigena* [3, 18].

To improve the performance of Ag-ELISA in the detection of porcine cysticercosis, nanobodies derived from camels was used to develop unambiguous specific tests [19]. Nanobodies owned remarkable characteristics such as being small, nonimmunogenic, very stable, highly soluble, and easy to produce in large quantities. Nanobodies technologies were developed and used in order to control disease such as cancer. [20]. Nanobodies are antibody-derived single domain proteins that contain the unique structural and functional properties of only heavy chain antibodies that naturally occur in camels [20]. Nanobodies are encoded by single genes and are efficiently produced in eukaryotic and prokaryotic hosts including bacteria and yeast [21]. Because of their unique three-dimensional structure, nanobodies have access to cavities or clefts on the surface of proteins [22, 23]. Nanobodies are ideal tools to selectively stabilize specific conformational states of membrane proteins [24,25]. Hence, nanobodies were cloned after immunization of dromedaries with *T. solium* cysticercosis and phage procedure [19]. The nanobodies that were obtained did not cross react with *Taenia hydatigena*, *Taenia saginata*, *Taenia crassiceps* or *Trichinella spiralis* and were categorized into four epitopebinding groups. The glycoprotein 14kDa (Ts14), that was found, reacted with antigen but the nanobodies also reacted with other proteins of the same family. When tested with a sandwich ELISA on cyst fluid, one particular nanobody detected its cognate serum antigens in a species-specific inhibition ELISA.

On the other hand and in perspective to improve Ag-ELISA response, the vesicular cyst antigen with 2-Dimensional IgG immunoblot was used to test the reactivity of cysticercotic sera of pigs [26]. The author reported diversity among the proteins and antigens of vesicular cyst and a diverse IgG antibodies response. Furthermore, using the random amplified polymorphic DNA markers (RAPDs) to assess the genetic variability among cysticerci infected pigs, authors found genetically difference in cysticerci infected pigs [27]. Therefore, a possible significant source of diversity in *Taenia solium* infectiveness and pathogenicity was suggested [26].

Surface enhanced laser desorption–ionization time of flight (SELDI-TOF)

The SELDI-TOF was used to identify biomarkers of *Taenia solium* cysticercosis for viable cyst (active disease) and degenerated cyst (inactive disease) [28]. SELDI-TOF is a proteomic high-throughput technique, which has been improved with the progression of protein-chip systems. Three major components constitute the instrumentation of SELDI-TOF MS technology: protein chip arrays, a mass analyzer, and data analysis software. The protein chip arrays are the

heart of the SELDI-TOF MS technology. Using chromatographic surfaces to retain proteins and peptides on ProteinChip arrays based on their physicochemical properties, the platform performs direct analysis via Time-Of-Flight-Mass Spectrography (TOF-MS). Peak patterns are formed via MS, which represent protein expression profiles. The location and intensity of every peak in the pattern reflect the mass over charge ratio (m/z) and abundance of the corresponding protein [29,30]. This high-throughput technique allows the screening of low molecular weight proteins compared with other traditional methods, such as 2D gel electrophoresis. Combined with bioinformatic approaches, SELDI-TOF is valuable in establishing protein expression profiles and in discovering new biomarkers with high sensitivity and specificity [29,31-33]. SELDI-TOF-based techniques with bioinformatics have also been successfully applied for early detection of several cancer types [34, 35], liver fibrosis, cirrhosis in patients with chronic hepatitis B virus (HBV) [36,-38] and chronic hepatitis C virus (HCV) infection [39,40]. Nevertheless, a major concern in study design was reported [41-43] and twenty sources of variation affecting SELDI-TOF process was found [44]. SELDI-TOF MS is a high throughput technique that allows hundreds of samples to be screened for disease biomarker identification in a relatively short time [45]. Otherwise, the system is manual, time consuming, and prone to human error. The cost of protein chip arrays seems to be high at $75/chip (8 spots). Using SELDI-TOF, 13 specific biomarkers were found for the viable phenotype, 9 specific for the degenerated phenotype and 8 specific for either viable or degenerated cysts [28]. Among biomarkers discovered in the experimental sample, five biomarkers (clusterin, lecithin-cholesterol acyltransferase, vitronectin, and apolipoprotein A-I) were identified to increase serum expression in pigs infected with viable cyst. Haptoglobin was identified as biomarker that increase serum expression in animal infected with degenerated cysts. However, when tested biomarkers with field samples, only three biomarkers were to specific to increase serum expression.

Development of immunodiagnostic test with polymerase chain reaction (PCR)

A PCR protocol targeting mitochondrial cytochrome oxidase (COI) gene in sera samples was used for the diagnosis of porcine cysticercosis [46]. According to these authors, the use of PCR in serum for the diagnosis of porcine cysticercosis has a high specificity and the assay did not showed cross-reactivity with toxoplasmosis and trichinellosis. However, this technique is also specific when used to test the other pig taeniids, mainly *Taenia hydatigena, Taenia asiatica* [47]. The same authors showed that bioinformatic analysis by means of NCBI/BLAST and ClustalW programmes has shown high homologies in mitochondrial cytochrome oxidase gene among the different *Taenia* species. Since, the diagnosis of porcine cysticercosis based on PCR protocol targeting mitochondrial cytochrome oxidase (COI) gene as reported [46] is useful to prove existence of the larval stages belonging to the *Taenia* genus [47]. In contrast, PCR was used for the diagnostic of porcine cysticercosis after inspection of the carcass [48]. Nevertheless to perform this PCR protocol, genomic DNA was extracted from *T. solium* cysticerci dissected from naturally infected cysticercosis positive pigs. The oligonucleotide primers used were TBR-3 (5'-GGC TTG TTT GAATGG TTT GAC G-3' from AB020395; positions 34–55) and TBR-6 (5'-GCT ACT ACA CCT AAA TTC TAA CC-3'; positions 319–297) and the primers Cox1 I (5'-TTG TTATAA ATT TTT GAT TAC TAA C-3' from AB066490 to AB066492; positions 165–189) and Cox1 II (5'-GAC ATA ACA TAA TGA AAA TG-3'; positions 1148-1129). The TBR primers and the Cox1 primers yielded products of 286 and 984 bp, respectively, in cysticercosis positive cases with the PCR test. Both sets of primers were found to be highly specific, since they did not yield any PCR product in negative controls.

Development of immunodiagnostic test with flow through assay (FTA) method

Flow-through assay (FTA) is a membrane based immunoassay. This assay is based on a principle of direct competitive ELISA [49,50]. Antibody of the tested molecule is coated on a membrane surface followed by an addition of tested molecule enzyme conjugate. The molecule and its conjugate compete for the limited antibody binding sites. After dried step in an incubator and a washing step, the enzyme substrate is added and reacts with the tested molecule-coupled enzyme and color develops. A FTA for the serodiagnostic of porcine cysticercosis using cyst fluid antigen (CFA) and the whole cyst fluid antigen (WCA) has been developed [51]. The test consisted of coating the *T. solium* metacestode antigen on cellulose acetate membranes in order to increase its capturing by the antibodies in the serum sample. The bound antibodies are visualized by the addition of protein A colloidal gold conjugate, which served as antigen-antibody detecting reagent imparting pink color to the membrane as a dot. Performing the techinique, cyst fluid antigen was prepared using Chung [52] method with modification. The proteins within the two antigens were estimated by using method of Lowry with bovine plasma globulins as a standard [53]. The performance observed for FTA were better with CFA (96.0% sensitivity; 96.0% specificity) compared to WCA (92.0% sensitivity; 96.0% specificity) [51]. When comparing result with the enzyme-linked immunosorbent assay, the sensitivity for both the antigens were 96 % while the specificities with CFA and WCA were 96 and 92 %, respectively. The authors found that the cross-reaction was observed in one out of eight hydatidosis positive pigs (12.5%) with CFA by both the assays. The highest diagnostic accuracy (96%) was obtained with CFA-FTA and CFA-ELISA. Those latest

antigens: CFA, Cyst wall antigen (CWA) and crude lysate antigen (CLA) were used to identify immune response in sera collected from neurocysticercosis patients [54]. The authors found the highest positive cellular response with CFA. This cellular response was followed by crude lyase antigen and finally by crude wall antigen. When fractioned the CFA, the fractions F1 (10.51±4.83) and F4 (9.91±3.91) revealed maximum response. Nevertheless, the significantly level of cytokine was revealed with the fraction F1 that induced TNF-α (115.18 ± 91.2) secretion, and F2 that induced IL-4 (15.69 ± 5.79) and IL-10 (117.81 ± 41.89) secretion. On the other hand, Excretory secretory antigen showed the highest sensitivity and Crude soluble extract antigen showed the highest specificity when comparing the performance of Crude soluble extract antigen, Excretory secretory antigen and Lower molecular mass antigen fraction (10–30 kDa) on the diagnostic performances of neurocysticercosis [55]. In contrast comparing vesicular fluid (VF) and a glycoprotein fraction (LLa-Gp fraction), purified from a whole parasite extracted by lentil lectin affinity chromatography, the vesicular fluid ELISA showed 100% sensitivity and specificity whereas the sensitivity and specificity was found lower with the LLa-Gp ELISA [56]. Despite the variation on the sensitivity and specificity registered using CFA and the WCA in Flow-through assay, this method of diagnostic is rapid, easy-to-use [57]. The method does not require any equipment and more importantly, any individual can perform this assay. However, since the method is semi-quantitative, interpretation of results may be difficult when the tested molecule concentration of the test sample is close to the method cut-off level.

Spatial analysis tools for porcine cysticercosis survey

Disease analysis at geographical level would be beneficial for a better understanding of cause of diseases and actions to be promoted in the field of epidemiological control. One of the techniques used to reveal spatial distribution of diseases is clusters detection. In epidemiology, a cluster is a number of health events located close together in space and/or time [58]. Identifying spatial and spatio-temporal clusters of cases could help: (i) to generate new information for further etiologic studies; (ii) to identify risk areas where to focus the surveillance and allocate the resources (antibiotics, rapid diagnostic tests…); (iii) to develop cost-efficient vaccination strategies [59]. Spatial analysis of diseases can be performed using approaches based on Bayesian geo-statistical, K-functions, Generalized Additive Models (GAM), Cuzick–Edwards test, Besag -Newell test [60,61] or SaTScan (Kulldorff M. and Information Management Services, http://www.satscan.org/). These aforementioned statistical spatial analysis approaches were used to detect clusters of the following conditions: meningococcal meningitis, *Campylobacter spp*. in humans and in broiler flocks, schistosomiasis, Rhodesian Human African Trypanosomiasis, Helminth Co-Infection and Co-Intensity, Hookworm, Dengue Virus, tuberculosis [62-69]. Both global and local clusters were assessed by using varying statistical spatial methods. To perform a global cluster in a study areas, the most frequently used tests are K-function, Diggle and Chetwynd's bivariate K-function [70-73] and the Potthoff-Whittinghill method (PW) [74-78]. Moran's I statistic, Cuzick and Edwards's nearest neighbors and Tango's maximized excess events tests (MEET) are less used [79-81]. Tango's MEET generally has the best statistical power, adjusts for multiple testing and has the added value of being able to evaluate spatial autocorrelation and spatial heterogeneity [82]. Local clusters of diseases are performed by using Anselin's local indicator of spatial association (LISA) and the Besag-Newell test [61,83-85]. Nevertheless, Kulldorff's spatial scan statistic is most frequently used for detection of local cluster because the test can adjust for multiple testing and heterogeneous background population densities, along with other confounding variables. Kulldorff's spatial scan statistic is also applicable to both point and aggregated data, and has been adapted to detect noncircular clusters [86,87,88]. Diggle's test and Stone's conditional test is used to evaluate focal clustering [89,90] and to determine whether risk declines from pre-specified point sources [76,85]. When performed power evaluation of diseases clustering tests, spatial scan statistic has good power in detecting local clusters [82]. Tango's MEET is useful for detection of urban clusters and Besag-Newell's test for detecting mixed clusters with a right choice of parameter. The Cuzick-Edwards' k-NN test was found to have a good power in detecting hot spot cluster but the power of the test depends upon the right choice of the parameter. With regard to the detection of global clusters, Tango's MEET has the highest power. Nevertheless, the spatial scan statistic performs diseases clusters well, but not as well as Tango's MEET [82]. However, Cuzick-Edwards' k-NN test is useful to perform clusters with very small distance if a right choice of parameter has been done. Statistical spatial analysis approaches were seldom used to control porcine cysticercosis [11,91]. By using K functions to assess general cluster of porcine cysticercosis and SatScan to detect local cluster, authors found that the two approaches match well [11]. In the district of Matapalo (Peru), distance to nearest neighbor was used to detect hotspots surrounding *taenia solium* tapeworm carriers [92]. The risk-adjusted nearest neighbour hierarchical spatial clustering (Rnnh) was used to detect clusters of porcine cysticercosis in a Rural Area of Eastern Zambia-A Community [93]. SaTScan was used to determine clusters of different indices of *Taenia solium* infection in Vellore district of Tamil Nadu State in India [94]. In western Kenya, the k-1 nearest neighbours local convex hull method was used to determine the spatial ecology of free-ranging domestic pigs [95].

Table-1. Characteristics of porcine cysticercosis detection tests

Type of tests	Method of detection of porcine cysticercosis	Materials used for detection	Sensitivity	Specificity	type of output	Fields of application	References
Tongue examination	Visual exam	-	0.161-0.708	1.000	Qualitative	*Ante mortem* inspection Epidemiological survey	[3, 10, 11]
Meat inspection	Incision and visual exam	knife	0.221-0.387	1.000	Qualitative	*Post mortem* inspection Epidemiological survey	[3, 112]
Ag-ELISA	Serology base on antigen	ELISA reader Protein chip arrays	0.645-0.867	0.841-0.947	Quantitative	Epidemiological survey	[3, 105]
SELDI-TOF	Proteomic high-throughput	Mass analyzer, Data analysis software	-	-	Quantitative	Test not performed	[28]
PCR	Genomic DNA technique	PCR equipment	-	-	Quantitative	Test not performed	[46]
FTA	Direct competitive ELISA technique	Flow through module	-	-	Semi-quantitative	Test not performed	[51]

Table-2. Characteristics of statistical tools use for diseases clusters detection

Type of tests	Type of clusters	Method of detection of clusters	Software that implement the function	Categories of input data	Input data	Fields of application	References
Spatial analysis tools	Global	K-function,	CrimeStat, R, ArcGIS, MATLAB, ClusterSeer	Case event Case-control	P&D	Epidemiological survey	[113,114, 115, 116, 117]
	Global	Diggle and Chetwynd's bivariate K-function,	CrimeStat,	Case-control	P&D	Epidemiological survey	
	Global	Potthoff-Whittinghill method (PW).	R (Dcluster package)	Case event	P&D	Epidemiological survey	
	Global	Moran's I statistic*	CrimeStat, R, ClusterSeer, GeoDa	Continuous	P, Ag, D&C	Epidemiological survey	
	Global	Cuzick and Edwards's nearest neighbors*	Space Time Intelligence System (STIS), ClusterSeer, R	Case event Case-control	P&D Adaptive for Continuous	Epidemiological survey	
	Global	Tango's maximized excess events tests (MEET)*	R	Case-control	P, Ag & D	Epidemiological survey	
	Local	Anselin's local indicator of spatial association (LISA)	CrimeStat, R, GeoDa, ArcGIS, SpaceStat	Continuous	P & C	Epidemiological survey	
	Local	Besag-Newell test	R (Dcluster package) ClusterSeer.	Case event Case-control	P, Ag & D	Epidemiological survey	
	Local	Kulldorff's spatial scan statistic	SaTScan R, ClusterSeer	Case-control Case-event	P, Ag, D &C	Epidemiological survey	

*less used, P: Point, D: dichotomous variable, Ag: Aggregated data, C: continuous data

Indeed the use of frequentist approaches above describe, Bayesian approaches are also used to detect diseases clusters. A Bayesian approach might be more suited if study was conducted in order to estimate a priori probabilities of event [96]. Then, one of the biggest challenges in the Bayesian statistic is the choice of priors. Indeed, experience and a good understanding of the influence of prior distributions and convergence assessment of Markov chains are crucial in Bayesian analyses [97]. Nevertheless, Bayesian methods for the analyses are more flexible and suitable tool for inference in data sets with many missing values, or when accounting for detection probabilities [98-100]. Bayesian approaches were used to estimate prevalence of porcine cysticercosis [3] and in the test characteristics of coprology, coproantigen ELISA and a novel real-time PCR for the diagnosis of taeniasis [101].

Spatial statistic was therefore less used in identification of regions with cysticercosis in order to allow its sanitary control. Furthermore, the use of this new approach could increase the detection of most active sites for porcine cysticercosis transmission in endemic areas. Therefore, the detection of clusters become useful if authors assessed risks factors associated to diseases distribution in order to have better comprehension of cysticercosis distribution.

Contribution of tools in porcine cysticercosis control in developing countries

Ante-mortem tongue palpation and *post mortem* meat inspection are public health measures used to prevent porcine cysticercosis transmission to human. These available diagnostic techniques, widely used in developing countries, are easy to use, rapid and cheap. Nevertheless *ante mortem* tongue palpation and *post-mortem* meat inspection are reported to be specific and less sensitive when applied to pigs with low pigs' burdens [102,103]. Characteristics of different tests above describe were summarize in Table-1 and 2. Current available diagnostic techniques such as Ag-ELISA and EITB are found sensitive and specific. But neither of ELISA tests (B158/B60 Ag-ELISA and HP10 Ag-ELISA) used for the diagnosis of cysticercosis is perfect [3,104,105]. Hence, the epidemiological data collected on *T. solium* cysticercosis in Africa cannot reflect the real situation of the diseases [106]. However, Ag-ELISA has the advantage to detect the presence of active infection and the level of the infective burden [107]. Nevertheless, to date the same sensitivity was not observed with the current available antigen capture

assays and the reported specificities are not yet close to levels required for mass screening of pigs in endemic areas [107,108]. Performance of sandwich ELISA could be improved with nanobodies if good epitope could be found to bind with *T. solium* proteins [19]. Nevertheless, both Ag-ELISA and EITB is useful for epidemiological survey because require access to laboratory with proper instrument and training staff. The other immunodiagnostic techniques developed and present in this review need to be performed and require access to laboratory with proper instrument (Table-1) and well-trained staff. Hence, all those diagnostic techniques will be useful for epidemiological survey. Nevertheless, data collection in field on porcine cysticercosis will be limited by lack of laboratory instrument and fees to perform the tests. According to previous reports, pigs inspected are predominantly reared by unressources smallholder [2,109]. When considering the available resource and the social level of pig's owners and butchers, the process of testing pig sera in laboratory before purchase or before the marketing of meat is limited by fees and the delay of results [110]. Hence, processing like that could involve butchers in clandestinely. Then, to prevent transmission of porcine cysticercosis in developing countries, highest sensitive and specific diagnostic techniques, cheap, rapid and easy to use by veterinary inspector in field are needed. It is therefore important to couple any diagnostic technique with spatial statistic technique in order to identify porcine cysticercosis cluster, which allow any direct intervention or any epidemiological survey. Tools and software frequently used to detect diseases clusters are summarize in Table-2. Few applications using spatial statistic are done in cysticercosis field. Since some authors have described areas of spread of porcine cysticercosis and neurocysticercosis base on estimation [111], to date, there is lack of information on specific areas of spraying of porcine cysticercosis and neurocysticercosis. There also lack of information on spatial movement of pigs with porcine cysticercosis and on risk for urban areas to contribute of spraying the burden. Then, immunodiagnostics and spatial analysis tools are able to be developed and combined for a better control of porcine cysticercosis as well as for control of neurocysticercosis.

Conclusion and recommendations

Ante mortem tongue palpation and *post mortem* meat inspection are widely used in Africa for diagnosis of porcine cysticercosis. Nevertheless, both methods are limited on sensitivity. Current immunodiagnostic tests such as Ag-ELISA and EITB are found sensitive and specific. But Ag-ELISA is not perfect and both tests require access to laboratory with proper instrument and training staff.

All others methods described above were not well performed. Spatial statistic tools are available, but are less used in identification of geographical areas of distribution of porcine cysticercosis. Hence, for better control of porcine cysticercosis in zoonotic areas, it is interesting to develop diagnostic techniques with high sensitivity and specificity, handy to use, rapid and cheap. It is recommended to combine immunodiagnostic test with spatial statistical tool for better control of the tapeworm.

Authors' contributions

All authors were contributed in discussion, draft and revision of manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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