TAENIA SOLIUM
TAENIASIS/CYSTICERCOSIS DIAGNOSTIC TOOLS

REPORT OF A STAKEHOLDER MEETING
Geneva, 17–18 December 2015

World Health Organization

TDR For research on diseases of poverty
UNICEF • UNDP • World Bank • WHO

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CONTENTS

LIST OF ABBREVIATIONS

WHO: World Health Organization
TDR: Special Programme for Research and Training in Tropical Diseases
TPP: target product profile
MDA: mass drug administration
NTD: neglected tropical diseases
FAO: Food and Agriculture Organization of the United Nations
OIE: World Organisation for Animal Health
ILRI: International Livestock Research Institute, Nairobi, Kenya
UNICEF: United Nations Children's Fund
FERG: WHO Foodborne disease burden Epidemiology Reference Group
DALY: disability-adjusted life-years
Ab: antibody
Ag: antigen
ELISA: enzyme-linked immunosorbent assay
LAMP: loop-mediated-isothermal-amplification
PCR: polymerase chain reaction
CIETUS: University of Salamanca, Salamanca, Spain
CNM: Instituto de Salud Carlos III, Madrid, Spain
CDC: Centers for Disease Control and Prevention
EDCTP: European & Developing Countries Clinical Trials Partnership
ITM: Institute of Tropical Medicine, Antwerp, Belgium
TUM: Technical University Munich, Munich, Germany
HIV: human immunodeficiency virus
POC: point of care

EXECUTIVE SUMMARY

The World Health Organization (WHO) Special Programme for Research and Training in Tropical Diseases (TDR) and the Department of Control of Neglected Tropical Diseases convened a meeting at WHO headquarters in Geneva, Switzerland on 17–18 December 2015 to identify mechanisms to improve tools for diagnosis of *Taenia solium* taeniasis/cysticercosis for programme implementation in endemic low-resource settings. The two-day meeting was attended by participants from endemic countries and further experts (see *Annex 1*: List of participants). Country representatives from China, Madagascar, Mexico, Peru, Vietnam and Zambia presented situation analyses which informed the discussion on the process needed to acquire optimal tools for diagnosis in resource-limited settings, and an overview of tests used. Veterinary public health (pig/food safety) and mental health aspects and pathways from development to usage of diagnostic tools were also discussed (see Annex 2: Meeting agenda).

The participants identified the priorities for diagnostic tests and three working groups discussed the possible settings of use and drafted a target product profile (TPP) for each setting. Seven diagnostic test priorities were defined. A work plan was generated and the participants confirmed their support for continued cooperation in generating appropriate diagnostic tools for control of *T. solium* taeniasis/cysticercosis.

KEY MESSAGES

Common country needs for diagnostic tests

- Base the prioritization of test characteristics on setting (clinical versus research versus control).
- Design tools for evaluation of control programmes.
- Develop diagnostic tools for surveillance of taeniasis and asymptomatic neurocysticercosis for screening of patients before and after mass drug administration (MDA) with praziquantel.
- Devise rapid, easy-to-use diagnostic tests for 1) diagnosis of neurocysticercosis in epileptic patients in resource limited settings and 2) those able to differentiate neurocysticercosis from general cysticercosis.
- Implement new, validated diagnostic tests in national health and distribution systems in countries with full transmission including pigs and humans.
- Endorse political and international partner commitment for implementation.
- Define clinical implications of positive test results and provide decision trees for clinicians.

- Integrate control programmes with other neglected tropical disease programmes e.g. schistosomiasis control programmes (including development of tests with multiple targets coordination and harmonization of ethics).

Common needs expressed by diagnostic tool developers

- Focus on diagnostic tools for control and elimination programmes, on the short term first. Define tools/components/reagents already approved and available for potential further use.
- Evaluate and validate diagnostic tools based on the evidence and according to common protocol and standards.
- Elucidate factors causing false–positive and false–negative test results.
- Determine the geographical distribution of cross reacting parasitic infections.
- Conduct more profound studies on the specificity of existing tools (e.g. for cysticercosis tests in pigs confirmed by necropsy).
- Exchange test reagents and protocols, and share expertise between developers and industry.
- Define the role of point-of-care (POC) tests within the control toolbox.
- Conduct innovative research in order to develop new POC tests appropriate for field settings.

The development of an appropriate test requires that several steps to be followed. In order to overcome challenges with this process, it will be necessary to:

- define clear product standards and setting-specific target product profiles;
- share resources, such as targets and reagents;
- standardize and publish evaluation protocols;
- establish networks of evaluation sites pre-approved for standardized protocols; and
- accelerate policy development through modelling of health impact and cost-effectiveness.

DECLARATIONS OF INTEREST

All invited country representatives and experts completed the WHO declaration of interest form before the meeting. The forms were submitted to and reviewed by the WHO Secretariat. No conflicts of interest were identified.

1. BACKGROUND AND OBJECTIVES

Taenia solium taeniasis/cysticercosis infection is an important zoonosis of considerable (veterinary) public health concern that mainly affects poor communities. *T. solium* taeniasis/cysticercosis is also indicative of poor standards of sanitation and inappropriate pig husbandry practices *T. solium* is on the WHO list of neglected tropical diseases (NTD). On the roadmap defining strategic targets for elimination and control of the NTDs, *T. solium* taeniasis/cysticercosis has a goal of having validated strategies for control[1] and elimination[2] and scaled up interventions by 2020 (*1, 2*). In 2014, WHO in collaboration with the Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (OIE) and the International Livestock Research Institute (ILRI) convoked an informal consultation on intensified control of taeniasis and neurocysticercosis caused by *T. solium* infections. The consultation called for a WHO-led network to support countries in their efforts and to identify national gaps in control.

In response, control programmes are being established in several countries. Implementation programmes and clinical settings require easy-to-use and inexpensive diagnostics to establish a baseline, to measure the impact of interventions and to carry out regular surveillance, or to establish a diagnosis in clinical cases.

Diagnostic tools with sufficient performance and in formats that are cost-effective, easy to use and suitable for large-scale implementation in resource-limited settings are widely lacking. One aim of the working groups initiated at the 2014 consultation was thus to identify outstanding research needs and improvements in diagnostic tools. WHO has since published two landscape analyses on control and management of T. solium in humans and pigs, including current diagnostic approaches (*3, 4*).

As a next step towards addressing this gap in diagnostic tools, a stakeholder meeting was convened at WHO headquarters on 17–18 December 2015 to review current and future tools for diagnosis, with emphasis on resource-limited endemic countries (*Figure 1*).

MEETING OBJECTIVES

- to provide an update on the situation in selected endemic countries during implementation of diagnostic tools and the challenges of diagnostics in the field;
- to advance the agenda on mechanisms to identify and improve suitable programmatic tools for use in endemic low-resource settings;
- to specify needs for use of diagnostic tools in different settings adapted to local context and intended user as well as consideration of practical implementation aspects during test development;
- to generate a work plan for coordinated, harmonized searches for appropriate diagnostic tests.
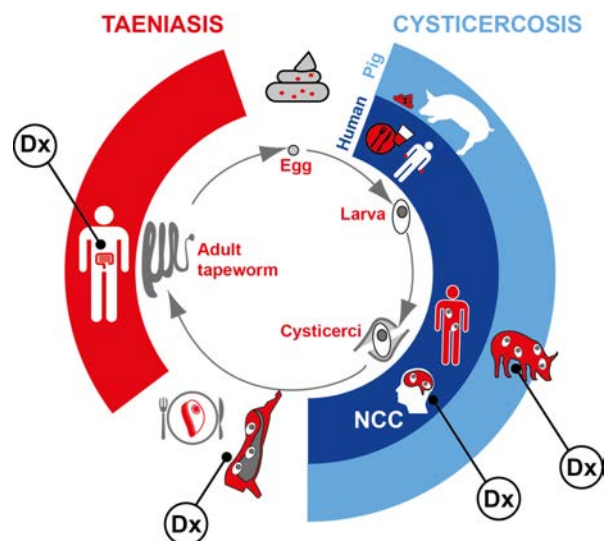
Figure 1. Targets for taeniasis/cysticercosis diagnostic tests (Dx): human: adult tapeworm (detection of taeniasis), larval cysts called cysticerci (detection of (neuro) cysticercosis); pigs: cysticerci in live pig or pork (cysticercosis)

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[1] Control to mean reduction of disease incidence, prevalence, morbidity, and/or mortality to a locally acceptable level as a result of deliberate efforts; continued intervention measures are required to maintain the reduction. Control may or may not be related to global targets set by WHO.

[2] Elimination as a public health problem is a term related to both infection and disease. It is defined by achievement of measurable global targets set by WHO in relation to a specific disease. When reached, continued actions are required to maintain the targets and/or to advance the interruption of transmission. The process of documenting elimination as a public health problem is called validation.

2. WHO UPDATES

2.1 DISTRIBUTION OF CYSTICERCOSIS

An updated map of the global distribution of *T. solium* infection and where intervention measures are needed was presented at the meeting (*Figure 2*). The status of endemicity was evaluated based on a compilation of data from different information sources (e.g. peer-reviewed publications and grey literature, Human Development Index, WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation, type of pig production). Distribution of *Taenia solium* infection, worldwide, 2015 target areas for surveillance programmes, and related diagnostic tools were identified.
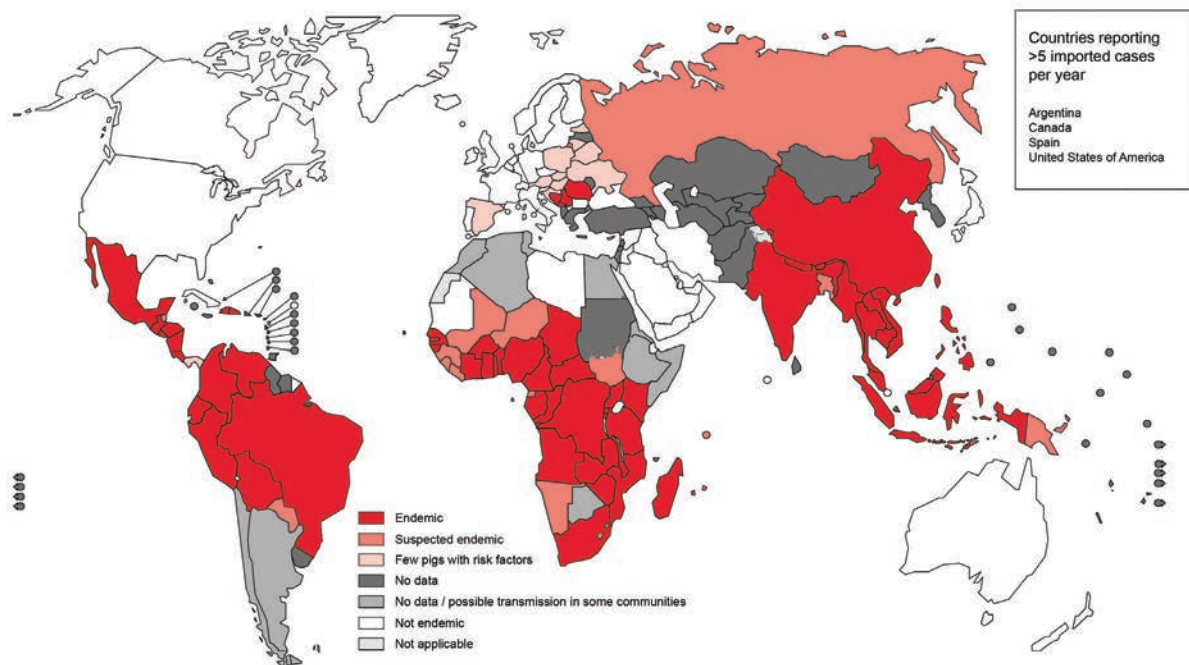
Figure 2. Distribution of Taenia solium infection, worldwide, 2015

2.2 RISK RANKING

In 2012, the Joint FAO/WHO Expert Meeting compiled a multi-criteria based ranking of food-borne parasites for public health concerns where *T. solium* in pork ranked first as food-borne parasite (5).

2.3 BURDEN ASSESSMENT

In 2015, the WHO Foodborne disease burden Epidemiology Reference Group (FERG) published estimates of the global burden of 31 bacteria, viruses, parasites, toxins and chemicals. *T. solium* was identified as a leading cause of deaths from food-borne diseases, resulting in a considerable total of 2.8 million disability-adjusted life-years (DALYs). The relative contribution of *T. solium* to the number of DALYs was especially high for many African, South American and some South-East Asian sub-regions (*Figure 3*). These data underscore the global importance of the *T. solium* disease complex (6).

2.4 RESOLUTION ON EPILEPSY AND NEUROCYSTICERCOSIS

Seizures, headaches and intracranial hypertension account for the high DALY burden. In endemic settings, approximately 30% of all epilepsy is due to neurocysticercosis. In May 2015, the World Health Assembly adopted resolution WHA68.20 on epilepsy, urging Member States to strengthen their ongoing efforts in providing care for people with epilepsy (7). Neurocysticercosis was indicated as a leading cause of preventable epilepsy.
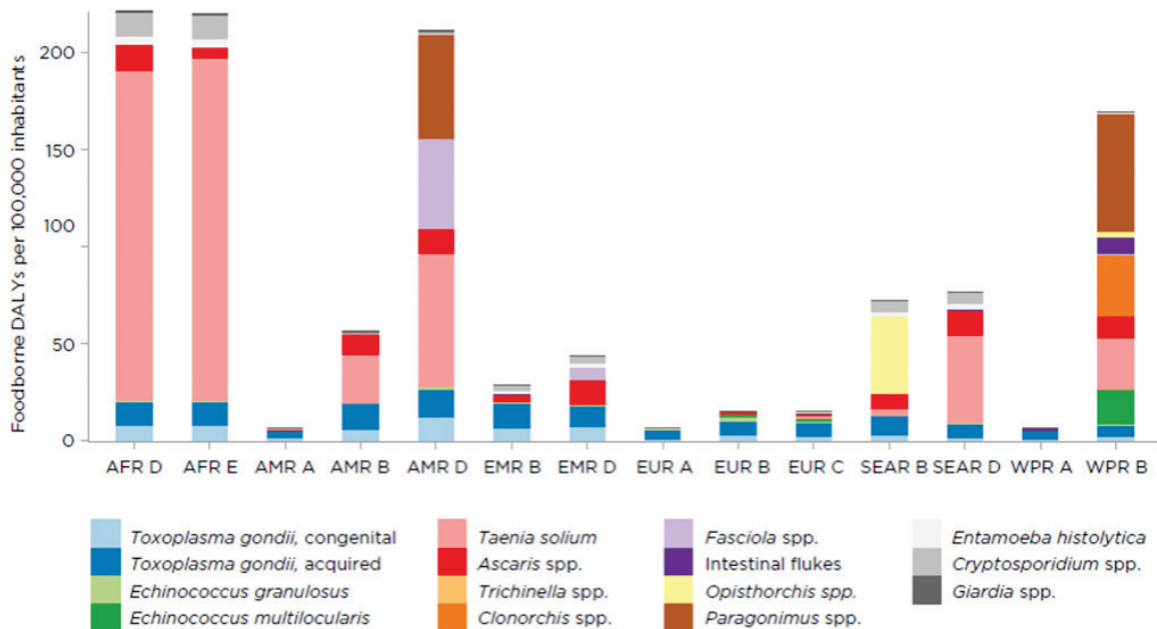
Subregions defined on the basis of child and adult mortality. Stratum A = very low child and adult mortality; Stratum B = low child mortality and very low adult mortality; Stratum C = low child mortality and high adult mortality; Stratum D = high child and adult mortality; and Stratum E = high child mortality and very high adult mortality. The subregions are not related to the six official WHO regions. For more detail see (4).

Figure 3. Relative contribution of causative agent to disability-adjusted life-years (DALYs), by incidence per sub-region

3. KEY ISSUES

3.1 COUNTRY NEEDS

Identification and discussion of specific diagnostic needs were based on presentations from representatives of six countries: China, Madagascar, Mexico, Peru, Vietnam and Zambia (see presentations: http://www.who.int/taeniasis/resources/diagnostic_tools_presentations/en/).

A key aspect of control is the separation of pigs from human faeces infected with tapeworm eggs. Improvements in sanitation and pig husbandry practices in developed countries reduced and/or eliminated T. solium. Similarly sharp drops in prevalence have more recently been noted in China and in some remaining foci in Europe (e.g . Portugal).

Although progress has been made in many developing countries its pace is slow. Optimal surveillance of human taeniasis and porcine cysticercosis and identification of neurocysticercosis patients are of paramount importance in these exposed populations.

Currently, diagnosis and case management of neurocysticercosis in endemic countries are problematic, especially in rural settings where health-care centres are small and personnel and laboratory equipment are limited. Imaging techniques (magnetic resonance imaging, computed tomography scanning) are required to confirm diagnosis, yet referral of suspected patients for confirmatory imaging is often impossible and patients consequently receive, if any, only symptomatic treatment (e.g. carbamazepine for epilepsy). Many patients do not seek treatment or have access to any form of health care.

Diagnostic tools are largely unavailable in developing countries (e.g. very low numbers of magnetic resonance imaging units per million population in Mexico, and only one diagnostic reference laboratory in Zambia used exclusively for research purposes to date) and misdiagnosis occurs as a result. The development of a practical, easy-to-use serological screening test to identify those individuals requiring subsequent imaging is thus crucial to facilitate and support diagnosis in endemic countries with limited resources. Such a test should meet the following criteria:

- available in rural areas where access to radiological infrastructure is lacking;
- at low cost for government or patient;
- of high sensitivity and specificity for diagnosis of neurocysticercosis.

Additionally, the detection of false–positive results due to viable parasites outside the central nervous system can impair optimal case management and lead to inappropriate treatment. To date no serological test is available to specifically test for neurocysticercosis in the

brain. The feasibility of adding some markers for cerebral inflammation, indicating central nervous system involvement, should thus be investigated. Finally, tools to differentiate viable from dead parasites are essential. Different diagnostic targets for screening are needed for

• human tapeworm carriers;
• infected pigs; and
• as a mixed approach tackling humans and pigs.

The ultimate indicator and target of elimination of transmission should be a decrease in the number of cases of symptomatic human neurocysticercosis. The lag time of several years between infection and symptoms requires lengthy follow up. The use of (neuro)cysticercosis monitoring is thus impractical in short- and long-term control programmes. Rather, indirect monitoring through passive surveillance in health centres in a given region would be more appropriate and provide evidence of the clinical efficacy of interventions. Moreover, a convenient, rapid screening test for human cysticercosis is currently not available.

Tapeworm carriers play a pivotal role in transmission as source of infection for pigs and cysticercosis in humans. However, monitoring tapeworm carriers is difficult due to their typically low prevalence in the population (mostly around 1–2%). Given their impressive biotic potential, few surviving tapeworms may be sufficient to reinstall in the population; therefore, very high screening coverage is required to find all carriers. Overall, an appropriate, user-friendly test for large-scale screening of taeniasis or fast patient testing is lacking but urgently needed in endemic countries.

As pigs with cysticercosis are the ultimate source of human taeniasis and thus a major factor in maintaining transmission, pig screening is equally important. The prevalence of porcine cysticercosis is typically much higher in endemic areas than either human taeniasis or cysticercosis and for this reason it can be used as a monitoring tool. However, the currently available methods for diagnosis of porcine cysticercosis are either impractical (necropsy), are known to be non-specific or have been inadequately validated in relation to specificity (serology). Overall, an appropriate, user-friendly test for large-scale screening of porcine cysticercosis is lacking but urgently needed in endemic countries.

Data presented at the meeting confirmed the lack of sufficient epidemiological information on the distribution of taeniasis/cysticercosis in developing countries and the largely unknown impact in many regions. In endemic settings, collection of data for surveillance purposes often faces several logistical challenges, notably:

poor infrastructure (e.g. only one central laboratory in Madagascar); limited availability of human resources; topography and climate; and limited financial resources. Furthermore, despite the availability of some diagnostic tests in some countries, if outside research activities, their availability is often restricted to a few central laboratories (e.g. Madagascar, Zambia) or tertiary referral centres (e.g. Latin America) that are rarely accessible for most of the population.

Reliable epidemiological data will be paramount as base for efficient surveillance and evaluation in these countries. Specifically, there is need for practical, affordable tests with appropriate performance in rural settings. The growing opportunities for integration with other NTD control programmes should be explored further (e.g. linkage in Madagascar with the schistosomiasis control programme, MDA initiatives) in order to share human resources and manage programmes cost effectively.

Presentations link: http://www.who.int/taeniasis/resources/diagnostic_tools_presentations/en/

COMMON COUNTRY NEEDS FOR DIAGNOSTICS INDICATED BY THE SITUATION ANALYSES FROM CHINA, MEXICO, PERU, VIETNAM AND ZAMBIA AND SUBSEQUENT DISCUSSIONS

• Setting-based (clinical versus research versus control) prioritization of diagnostic test characteristics.
• Evidence-based evaluation of diagnostic tests and guidelines for use in close collaboration with governments of affected countries.
• Diagnostic tools to evaluate control programmes.
• Diagnostic tools for surveillance of taeniasis and identification of asymptomatic neurocysticercosis patients (e.g important pre- and post-screening tool during MDA activities with praziquantel).
• Rapid easy-to handle diagnostic tests for neurocysticercosis pre-diagnosis in epileptic patients with limited financial resources living in remote areas; diagnostic tools to differentiate neurocysticercosis from other cysticercosis forms.
• Define clinical implications of positive test results and provide decision trees for clinicians;
• Integration of control programmes with other NTD programmes;
• Implementation of new diagnostic tests in national health systems and in national distributor systems;
• Endorsement of political and international partner commitment for implementation.

3.2 DEVELOPERS' PERSPECTIVES

Updates on assays and tools under development were supplemented by experiences shared by the Foundation for Innovative Diagnostics and a background on point-of-care (POC) test development for resource-limited settings by the Chair of the meeting (see presentations: http://www.who.int/taeniasis/resources/diagnostic_tools_presentations/en/).

Research in Peru indicates that elimination of *T. solium* from an area is feasible. Elimination programmes generally follow several phases: mapping, monitoring, evaluation, and detection of reintroduction through post-programme surveillance. Appropriate diagnostic tools play a key role within elimination and control programmes and their characteristics should thus be adapted to each of the specific phases.

MAPPING AND MONITORING OF TAENIASIS AND CYSTICERCOSIS

START PHASE OF CONTROL PROGRAMMES

During the first phase of large-scale control programmes, a combination test could be used to integrate the mapping of several targets within diseases (e.g. taeniasis and cysticercosis; antigen (Ag) and antibody (Ab) and/or co-investigation with other diseases (e.g. schistosomiasis) to increase the cost-efficiency of control programmes). A quantitative or semi-quantitative test format could be considered, allowing the possibility of altering the sensitivity (changing the threshold) of the test.

For further monitoring purposes, POC copro-Ag techniques are preferred for rapid detection and treatment of taeniasis cases and a POC test for cysticercosis- Ab and Ag detection in sera in rural settings.

ADVANCED PHASES OF CONTROL PROGRAMMES

In advanced phases and during post-programme surveillance of large-scale control programmes (and screening for possible recrudescence of the parasite in the population), detection of cases becomes more challenging due to a very low prevalence. At this stage, the use of a more robust, field-friendly, sensitive non-POC test for taeniasis and cysticercosis Ab and Ag detection could be acceptable. To evaluate small-scale control programmes, such tools would be required for evaluation before and after the intervention.

In 2015, WHO published two landscape analyses on control and management, focussing on control of *T.*

solium (*3*), and on management of neurocysticercosis in low- and middle-income countries (*4*). Both analyses provide a non-exhaustive yet comprehensive overview of diagnostic tools currently available, their performance parameters, benefits and barriers.

TOOLS FOR DIAGNOSIS OF HUMAN CYSTICERCOSIS

The most commonly used tools for sero-diagnosis of human cysticercosis are summarized in *Table 1*. Further diagnostic tests for human cysticercosis are described in the literature (8, 9). Only a few assays (cysticercosis-immunoblot/EITB and Ag-ELISA) are commercially available; most of these tools are available only within research activities. However, all of the currently used techniques require laboratory and imaging capacity and are expensive. No POC Ag detection tool for mapping or any low-cost, easy-to-handle and appropriate test for monitoring is available. Moreover, some companies providing commercial cysticercosis-immunoblots have problems with distributing high numbers (because these tests are mainly based on crude Ag preparations which are limited). However, the use of Ab-detecting tools was discussed controversially during the meeting and will be restricted to specific needs (e.g. epidemiological screening of populations).

The need of **point-of-care (POC) tests**, especially rapid-diagnostic-tests, was highlighted by all developers. Nevertheless, only a few assays are currently under development:

A lateral flow format of the HP10 Ag-ELISA is being tested at the International Livestock Research Institute (Kenya) but it is restricted to pigs(see presentation E. Fevre). In Mexico, another HP10 lateral flow assay was developed and is currently tested in human patients (see presentation A. Fleury). Moreover, a new loop-mediated-isothermal-amplification (LAMP) PCR for detection of *T. solium*-DNA in human stool and tissue is under development at the University of Salamanca (CIETUS), in collaboration with Instituto de Salud Carlos III (CNM), Spain of Madrid, Spain (see presentation). First results of this LAMP-assay are promising, but restricted availability of required reagents and high costs will limit its use in developing countries. Furthermore, the United States Centers for Disease Control and Prevention (CDC) Atlanta – in collaboration with the Cysticercosis Working Group in Peru and CYS-TINET (COST Action TD1302)– is developing one qualitative and one quantitative lateral-flow assay to detect human taeniasis/cysticercosis Ab. The evaluation and validation of the qualitative assay will be conducted soon in an European & Developing Countries Clinical Trials Partnership (EDCTP) project coordinated by Belgium (ITM), in collaboration with African (Zambia, Tanzania) and European (Germany (TUM), Denmark) institutions.

TOOLS FOR DIAGNOSIS OF TAENIASIS

The most commonly used diagnostic tools for human taeniasis are summarized in *Table 2*. Further diagnostic tests for human taeniosis are described in the literature (*17*). Except for microscopy, these techniques require laboratory facilities and are costly. Currently, few co-pro-PCR techniques and non-commercial copro-Ag-ELISA assays are available. In contrast to the PCR assay, most copro-Ag-ELISA assays are genus, not species specific and thus cross-react with *T. saginata*. Therefore, these assays could not accurately identify *T. solium* carriers. However, a hybrid assay combining polyclonal Abs against Taenia adult tapeworm somatic extracts and an enzyme-conjugated rabbit IgG against *T. solium* adult excretory-secretory antigen demonstrated a species specificity of 100% (*18*). Further species specific monoclonal Abs – produced by ITM, Antwerp, - are on the way.

For taeniasis-Ab detection only one in-house test – the rES33-immunoblot produced by CDC Atlanta – is currently available (*19*).

TOOLS FOR DIAGNOSIS OF PORCINE CYSTICERCOSIS

The objectives of diagnosis of porcine cysticercosis are to reveal areas of full *T. solium* transmission, to monitor the outcomes of *T. solium* interventions and to secure food safety. The most commonly used diagnostic tests and methods are described in *Table 3*. Tongue palpation and meat inspection are cheap methods which do not require any facilities, though are of very limited sensitivity, especially in light infections. Conversely, current serological tests are expensive and require a laboratory setting.

Porcine cysticercosis is definitively diagnosed by identifying cysticerci in pig tissues. However, local meat inspection regulations vary widely and tongue inspection provides only an indication of heavily infected pigs. For accurate diagnosis, the method of choice is thus nec-

ropsy. Although this method is time-consuming, a dissection restricted to heart, tongue and masticatory muscles might provide a more practical alternative, though entailing a loss in sensitivity.

Serology has been used for the diagnosis of porcine cysticercosis. In field studies comparing serology and full necropsy, all serological tests tend to have poor specificity. Test developers should elucidate the background of such low specificity, mainly with *T. hydatigena* (e.g. due to exposure to *T. solium* without cyst development, or cross-reactions). Overall, there is an important need for new or improved tests with good necropsy result concordance. A greater use of sera from field-reared pigs with known cyst burdens in the evaluation of serological tests would be ideal.

The meeting demonstrated that increased interaction among developers, industry, international organizations and national governments holds opportunities for the development of evidence-based tools appropriate for large-scale implementation in endemic countries.

COMMON NEEDS EXPRESSED BY DIAGNOSTIC TOOL DEVELOPERS

- Focus on diagnostic tools for control and elimination programmes.
- Define the role of POC tests within the control toolbox.
- Conduct innovative research in order to develop new POC tests appropriate for field settings.
- Initiate more profound studies on specificity of tools
- Consider cross-reacting of tests with other Taenia species and include in test evaluations.
- Exchange tests, reagents and protocols, and share expertise.

Table 1. Common laboratory diagnostic assays for human cysticercosis

Diagnostic assay	Technique	Reference
Ag-ELISA	HP10 (lateral flow format being tested)	Harrison et al. (*10*)
	B158/B60 (commercialized by ApDia)	Dorny et al. (*11*)
Ab-ELISA	oncospheral peptides	Ferrer et al. (*12*), and others
	crude Ag extract (commercialized by NovaTec)	Diaz et al. (*13*), and others
LLGP-EITB	glycoproteins (commercialized by several companies)	Tsang et al. (*14*)
EITB	rT24H (recombinant Ag) (lateral flow format being tested)	Hancock et al.(*15*), Nohet al. (*16*)

Ab-ELISA antibody enzyme-linked immunosorbent assay; Ag-ELISA antigen enzyme-linked immunosorbent assay; CC, cysticercosis; LLGP, lentil lectin purified glycoprotein-; EITB,enzyme-linked immunoblot

Table 2. Common diagnostic tests for human taeniasis

Diagnostic assay	Technique	Reference
Microscopy	Kato Katz, formol ether concentration techniques	Several
Copro-Ag-ELISA		Guezala et al. (18) and others
Copro - PCR	Nested PCR	Mayta et al.(2o), , and others
	Multiplex PCR	Yamasaki et al. (21) and others
	RT-PCR	Praet et al. (22) and others
EITB	rES33	Levine et al. (19)

EITB, enzyme-linked immunoblot; PCR, polymerase chain reaction

Table 3. Common diagnostic tests for porcine cysticercosis

Diagnostic assay	Technique	Reference
Meat inspection	local legislation Tongue check	Harrison et al. (*10*)
Ag-ELISA	HP10 (lateral flow format being tested)	Harrison et al. (10)
	B60/158 (commercialized by ApDia)	Dorny et al. (11)
Ab-ELISA		Assana et al. (23) and others
LLGP-EITB		Tsang et al. (14)

Ab-ELISA antibody enzyme-linked immunosorbent assay; Ag-ELISA antigen enzyme-linked immunosorbent assay; CC, cysticercosis; LLGP, lentil lectin purified glycoprotein-; EITB,enzyme-linked immunoblot

4. *T. SOLIUM* TEST DEVELOPMENT STRATEGIES FOR LOW- AND MIDDLE-INCOME COUNTRIES

Although a few commercial and several in-house assays for testing of *T. solium* taeniasis/cysticercosis in humans and pigs have been developed, none is accurate or available for large scale-implementation in low- and middle income countries. Reasons include insufficient test performance, high costs, limited accessibility, need of a laboratory, and/or required advanced training for use.

New evidence-based, setting-adopted and user-friendly products thus are urgently required. POC formats with a special emphasis on rapid diagnostic tests are prioritized.

Ideally, these new assays will optimally meet three criteria: affordability, accuracy and accessibility (*Figure 4*; see presentation Rosanna Peeling).

Figure 5. Target criteria for urban, semiurban and rural settings
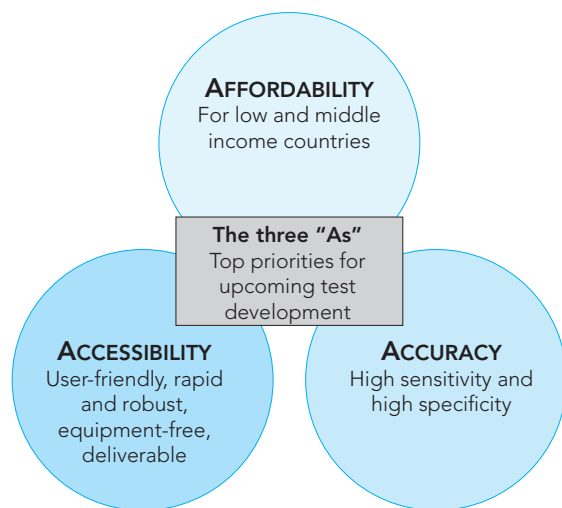
Figure 4. The three "As": optimal criteria for test development

However, test developers must strive for optimal balance between these criteria and develop tools adapted to specific purposes (e.g. surveillance versus case management, see above) and settings (urban, semiurban versus rural areas). Each of these purposes and settings will require specific priorities in test development (*Figure 5*; see presentation).

The full development and implementation pathway of envisaged new diagnostic tools has to follow several steps:

1. Specification of setting-orientated target product profiles.
2. Setting of diagnostic targets and definition of a technology platform.
3. Development of product prototypes.
4. Generation of proof of principle.
5. Laboratory and field evaluation.
6. Formulation of policy and guidelines for use.
7. Adoption and implementation in endemic countries.

According to experience in other fields, the estimated cost of the procedure for one ready-for-use test will be US$ 10– 100 million. Policy and full country adoption may take between 5–7 years depending on the country conditions. In order to reduce costs and time, sharing platforms, reagents etc. with existing tests and networks (e.g. surveillance programmes for Schistosoma spp.) is recommended.

PRIORITIES FOR TESTS

To specify the main groups of tests most urgently required, the participants were polled and priorities for diagnostic test development listed. Three groups of tests were identified (*Table 4*).

Table 4. *Taenia solium* taeniasis/cysticercosis diagnostic tools to be considered as top priorities

Species	Human		Pig
Disease	TS	CC	CC
Assay	Copro-Ag POC test	Ab/Ag POC test	Ag POC test
Additional suggestions	High Sp	Ag + Ab format;	high Sp
		Lateral-flow format;	
		inflammatory marker for NCC/CC differentiation	

CC, cysticercosis; POC, point of care; Sp, specificity; TS, taeniasis

Within the three main test groups seven separate required tools were identified, which will have to meet specific evidence-based criteria.

1. Human copro – Ag test group

   a. tool for screening and monitoring taeniasis with in control programmes;
   b. tool assisting surveillance interventions in the population (e.g. "track & treat").

2. Human cysticercosis – Ag/Ab test group

   a. tool for screening populations for (neuro)cysticercosis duing control programmes (e.g. before and after MDA);
   b. tool for diagnosis of (neuro)cysticercosis patients in clinical settings;
   c. tool for the specific diagnosis of extraparenchymal neurocysticercosis.

3. Porcine cysticercosis – Ag test group

   a. tool for monitoring of pig populations during control programmes;
   b. tool to aid ante-mortem diagnosis of porcine cysticercosis (decision test for farmers, pig pro ducers, meat inspectors etc.).

In addition, the importance of loop-mediate PCR assays for the detection of taeniasis carriers outside clinical settings was highlighted by members of the working groups. Overall, there is need for public–private partnerships to develop innovative new tests, as both sectors have different and complementary objectives and strengths. Full characterization of targets (e.g. definition of epitopes, assessment of affinities and genetic variability) and assessment of feasibility of production will accelerate test development.

Multicentre independent evaluations are important and opportunistic development is advised (e.g. multiplexing, combined tests for pig and human; one test for different levels of the health system).

Evaluation of the developed tools in different settings and cost-effectiveness studies will be required.

5. TARGET PRODUCT PROFILES

During the meeting the seven most needed test tools were identified. The next step, to specify the setting-oriented target product profiles, was initiated as a group work exercise. These profiles describe the ideal and minimal requirements for each priority diagnostic test and eventually contain more than 30 well-defined criteria. (*24*) Successfully implemented profiles of diagnostic tests for other infectious diseases in the same settings (e.g. HIV POC tests) can serve as guidance. Close collaboration between developers and producers is envisaged during development.

Draft target product profiles for the top three diagnostic test groups (*Table 4*) were generated (T*able 5, Table 6 and Table 7*). These drafts provide a working foundation for further development with the aim for the refined profiles to serve as an incentive for industry to generate setting-oriented, WHO-endorsed diagnostic tools for *T. solium* taeniasis/cysticercosis.

Table 5. Draft target product profile for a human copro Ag-taeniasis test

Purpose/setting	Screening	Track and treat
Specimen	Stool (fresh)	Serum, stool
Test time	3 h	30 min
No. of operator steps	–	1–3
Performance	Se (95–99%) Sp (for species differentiation: 90%), golden standard: parasitological proof	Se (95-99%) Sp (for tapeworm detection: 90%)
Cost (US$)	1–5	0.5–1

Se, sensitivity; Sp, specificity

Table 6. Draft target product profile for a combined human Ag/Ab-cysticercosis test

Purpose/setting	Screening	Diagnosis	Extraparenchymal NCC
Specimen	Full blood; Ag: urine	Full blood; Ag: urine	CSF
Test time	< 30 min (max. 1 h)	< 30 min (max. 1 h)	< 30 min (max. 1 h)
No. of operator steps	Max. 4	Max. 4	Max. 4
Performance	Se: 80–90%	Sp: 90–98%	Sp: 90–98%
Cost (US$)	Ideally 1–3	Ideally 1–3	Ideally 1–3

CSF, cerebrospinal fluid; NCC, neurocysticercosis; Se, sensitivity; Sp, specificity

Table 7. Draft target product profile for a porcine Ag-cysticercosis test

Purpose/setting	Screening	Food safety
Specimen	Blood (serum, spots)	Blood
Test time	–	30 min
No. of operator steps	–	1–3
Performance	Species: Se (for < 50 cysts,90%) Sp (98%) Gold standard: necropsy	Se (for < 50 cysts, 95–99%) Sp (90%)

Se, sensitivity; Sp, specificity

Fully developed profiles out of the draft TPPs are envisaged as a follow up to this meeting through broad expert consultation.

6. SUMMARY OF GAPS

The following needs for diagnostic tool development were identified during the meeting:

- Landscape analysis/systematic review on existing diagnostic tests including reported performances
- Critical re-evaluation of current test performance and required plan for re-evaluation process
- Epitope mapping of parasite antigens recognised by monoclonal Abs and other reagents used in existing test/assays
- Multiple clear product standards and setting-specific target product profiles
- Review and approval of target product profiles by governments of endemic countries
- Clear agenda for upcoming development processes and priorities
- Close collaboration among research groups (and cysticercosis networks), commercial developers and governments of affected countries during diagnostic test development process
- Assessment of prices for diagnostic tests that governments in resource-poor settings would be willing to pay
- Sharing of information and resources (e.g. targets, reagents: e.g. biobank, targets, reagents) between research groups and developers
- Evidence-based, setting-adapted and user-friendly POC assays for diagnosis of porcine cysticercosis and human taeniasis/(neuro)cysticercosis in low- and middle-income countries (urgent need)
- Standardized and published evaluation protocols for clinical and field settings, including definition of a gold standard for serological testing, required sample sizes for evaluation, etc.
- Acceleration of policy development through modelling health impact and cost-effectiveness
- Formation of network for standardized evaluation and development hubs
- Diagnostic decision tree for neurocysticercosis and clinical case management guidelines for resource-limited rural settings
- Raise interest in and advertise the results of this meeting including target product profiles by informing industry
- Multidisciplinary approach to be reflected by the WHO Secretariat: concerned departments to work together on the way forward.

7. NEXT STEPS

The stakeholder meeting is the first step in the development pathway. The next steps are to:

- Advance landscape **of the available diagnostic tools and the performance of current tests** to elucidate their value, and thus to improve product profiles as well as guidelines
- Conduct a WHO-led **systematic review** to inform the development of guidelines on clinical case management of neurocysticercosis. To assist the review process, PICO-questions (population-intervention-comparison-outcome) will be sent to experts. Overall, the systematic review should be finished by the end of the 2016 summer.
- Generate **full target product profiles**, guided by the drafts developed by **the meeting's working groups**, in broad consultation with different stakeholders
- Disseminate the meeting results through different networks and publications
- Explore establishment of **virtual biobank of available resources** through established networks (e.g. CYSTINET Europe (COST Action TD 1302)) and extend to other cysticercosis working groups worldwide.

Overall, the stakeholders confirmed their support for continued cooperation in generating appropriate diagnostic tools to tackle *T. solium* taeniasis/cysticercosis.

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ANNEXES

ANNEX 1. LIST OF PARTICIPANTS

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ANNEX 2. MEETING AGENDA

DAY 1		
09h30–09h45	Welcome address	Director TDR, John Reeder
09h45–10h30	Setting the scene: Introduction to meeting context and vision Election of chair and rapporteurs Around the table: introduction of participants and expectations from meeting	Piero Olliaro Bernadette Abela-Ridder
11h00–12h00	Country presentations (10 min/ country)	China, Madagascar Mexico and Latin America Peru Vietnam Zambia
12h00–13h00	Discussion on country needs	
14h00–14h15	Epilepsy and neurocysticercosis	Tarun Dua
14h15–14h30	Diagnosis of porcine cysticercosis Identification of endemic countries using maps	Marshall Lightowlers
14h30–16h00	Developers/pipeline perspective Roundtable format. • 5 slides per developer on diagnostics being developed and marketed: target product profile; current use including strengths and weaknesses; challenges, limitations and possible solutions • Moderated discussion using pre-defined discussion questions	Sukwan Handali
16h30–18h00	Identification of subjects/thematic clusters and formation of working groups for day 2 based on needs, issues and questions identified in previous discussions Goal of group work: To develop a forward vision agenda and identification of concrete next steps	

DAY 2		
09h00–09h15	Summary of meeting day 1	Director TDR, John Reeder
09h15–09h35	Perspectives for innovative new diagnostics	Isra Cruz, Foundation for Innovative New Diagnostics
09h35–11h00	Group work Based on needs and issues identified in discussions of day 1	2 working groups
11h20–12h00	Presentation of group work results	
12h00–13h15	Discussion of group work results and next steps Discuss concrete next steps as consequence of results and suggestions from working groups: what/who/how/when; revisit the expectations formulated in the morning session of meeting day 1; make sure these are met or amended accordingly	Tarun Dua
13h15–13h30	Final remarks and closure	Bernadette Abela-Ridder Piero Olliaro