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CYSTICERCOSIS (A LOURDES SANCHEZ, SECTION EDITOR)

# The Parasitological, Immunological, and Molecular Diagnosis of Human Taeniasis with Special Emphasis on *Taenia solium* Taeniasis

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**Abstract** Human neurocysticercosis, caused by the larval stage of the tapeworm *Taenia solium*, is an important neurological disorder reported as a major cause of epilepsy. An important risk factor for neurocysticercosis is the presence of human *T. solium* carriers who, upon open defecation, disseminate tapeworm eggs, which are infective to both humans and pigs. In the latter, infection also results in cysticercosis, with associated health and economic consequences. Control of *T. solium* therefore depends greatly on accurate detection and treatment of carriers. However, the currently available direct diagnostic tests depend on detection, in feces, of either parasite stages or parasite antigens and genetic material. The former are low cost but lack adequate sensitivity and specificity; the latter are too expensive to be routinely utilized in endemic communities. Indirect tests based on antibody detection may only show exposure and not active infection. An ideal diagnostic test should be one that is low cost and is able to quickly and reliably detect tapeworm carriers so that appropriate treatment can be prescribed in order to eliminate the source of infection. Such a test remains elusive. Efforts should, therefore, be directed at formulation of a test that is not only sensitive and specific but also affordable for use in endemic countries.

**Keywords** *Taenia solium* diagnosis · Coproparasitology · Immunodiagnosis · Coproantigen ELISA · PCR

## Introduction

*Taenia solium*, *Taenia saginata*, and *Taenia asiatica* are important tapeworms causing taeniasis in humans, who, as the natural definitive host of these cestodes, harbor the adult worm in the small intestine. Cattle serve as intermediate hosts for *T. saginata*, while pigs fulfill this role for *T. solium* and *T. asiatica*. Upon ingestion of infective eggs, intermediate hosts develop metacestode larval stages (also called cysticerci), resulting in bovine and porcine cysticercosis, respectively. Unlike the other two species, *T. solium* can also cause cysticercosis in humans. This occurs after inadvertent ingestion of *T. solium* eggs when metacestodes develop in organs and tissues, giving raise to cysticercosis, one of the most important parasitic conditions in humans. People acquire taeniasis following ingestion of undercooked pork or beef meat or viscera containing viable cysticerci. These develop into adult intestinal tapeworms, which, when mature, release proglottids (worm segments) laden with infective eggs. Proglottids may be passed relatively intact in feces, but frequently they disintegrate within the intestine, and so free eggs can be found in feces. The excreted eggs are immediately infective to the intermediate hosts [1], thus making the tapeworm carrier a fundamental key player in the transmission of cysticercosis. Garcia-Garcia et al. [2] demonstrated that the presence of tapeworm carriers in households is the main risk factor attributed to human cysticercosis. In the absence of sanitary facilities and/or adequate personal hygiene, these carriers become

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a major risk for members of their household and also community members [3].

In non-endemic countries, taeniasis is most likely to be imported by immigrant tapeworm carriers or people travelling to endemic areas, where they may acquire the infection through consumption of infected pork. Similarly, returning travelers may import cysticercosis if they ingest infective eggs from the contaminated environment, from food, or directly from carriers [4••]. Additionally, migration of tapeworm carriers from rural to urban areas increases the risk of transmission of cysticercosis when there are poor environmental and social conditions [3].

While *T. saginata* has a more cosmopolitan distribution, *T. solium* is mostly reported in developing countries in Africa, Asia, and Latin America. *T. asiatica*, also known as Asian *Taenia*, is restricted to East Asian countries and has not been reported elsewhere in the world, including Africa [5]. *T. solium* endemicity in developing countries is associated with poverty, free-ranging pigs, and poor sanitary conditions, especially lack of latrines [1, 6, 7]. Many reports have documented *T. solium* infection in pigs in Africa, with prevalence rates as high as 64 % [8].

As mentioned, the lodging of the metacestodes of *T. solium* in the central nervous system (CNS) results in neurocysticercosis (NCC), one of the most important neurological parasitoses in humans, and the main preventable cause of acquired epilepsy in endemic areas [9•]. Unlike taeniasis, where symptoms are not of major clinical importance, the pathology caused by the establishment of *T. solium* metacestodes in the CNS may be responsible for a high disease burden and morbidity in endemic areas [1]. Unfortunately, the cysticercosis/taeniasis disease complex remains a neglected tropical disease, with very little information on its current global burden. As a consequence, and as for many other parasitic zoonoses, its true burden still needs to be determined [10, 11]. The current global burden of *T. solium* cysticercosis in terms of disability-adjusted life-years (DALYs) has been estimated at $2–5 \times 10^6$, an estimate comparable to those of other neglected parasitic zoonoses but less than those of the "big three" global infectious diseases—malaria, HIV, and tuberculosis [12•]. Also, NCC is reported to account for about 30 % of all reported cases of acquired epilepsy in endemic areas [13].

From an economic point of view, the presence of cysticerci in the specific intermediate hosts (i.e., cattle for *T. saginata*, and pigs for *T. solium* and *T. asiatica*), may be of great importance because of carcass condemnation in countries where meat inspection at the abattoir level is implemented [1, 11, 12•]. NCC is of great economic relevance, resulting from the cost of medical treatment and lost working days. A minimum estimate of the cost of admissions to hospital and wage loss for NCC in the USA (a non-endemic country) was US$8.8 million annually, whereas in endemic countries such as Mexico and Brazil, treatment costs have been estimated at US$89 million and US$85 million, respectively [13].

Overall, *T. solium* has a higher public health impact than *T. saginata*, which mainly has economic implications for the meat industry [14]. Adult tapeworm infections are largely asymptomatic, though some people may experience abdominal discomfort, nausea, diarrhea, and loss of appetite, and in the case of *T. saginata*, itchiness of the anal area due to the actively migrating proglottids [15].

Taeniasis infections are increasingly being diagnosed in endemic areas of the world [1]. At the same time, there is growing recognition of *T. solium* as a serious emerging public health threat [16]. The data are, however, still very limited because of the lack of adequate surveillance, monitoring, and reporting systems. Compared with other helminth parasites, *T. solium* taeniasis tends to have a low prevalence, typically less than 1 %, even in endemic communities [17]. In fact, a prevalence >1 % is considered hyperendemic [18]. This is because in communities with inadequate sanitary infrastructure, a few tapeworm carriers have the potential to disseminate the infection to a great number of people and free-roaming pigs. Regions of endemicity have been identified [6, 19], with studies reporting prevalences ranging from 0.3 % to 11.5 % on coproparasitologic examination [20–24, 25••, 26••] and from 0.5 % to 24.1 % on coproantigen (copro-Ag) enzyme-linked immunosorbent assays (ELISA) [1, 25••, 26••, 27•].

*T. solium* is considered a potentially eradicable parasite [28]. However, since the most affected areas are within developing countries, many ongoing challenges continue to hinder the implementation of control measures for this parasite. Obstacles that need to be overcome include lack of diagnostic facilities, inadequate or absent health infrastructure in rural areas, inaccessibility to health care and treatment with effective taeniacides, minimal cooperation between medical and veterinary services, and lack of knowledge about the parasite [1]. Several control options that target the various potential intervention points in the life cycle of the tapeworm have been described (Fig. 1). It is clear that control of taeniasis requires a multifaceted approach, as a single-intervention control program would not achieve the required results [29]. A control strategy that stands out is treatment of tapeworm carriers so as to remove the continued contamination of the environment in endemic areas. However, this strategy requires identification of such carriers, which has proven to be problematic because of the lack of low-cost and readily available diagnostic tools in resource-poor endemic areas.

This review looks at the currently available tools for taeniasis diagnosis and the strides made to date to improve them.

## Diagnosis of Taeniasis

Diagnosis of taeniasis is mainly based on the search for parasitic material in feces [30]. Several tests have been developed, and each has its own advantages and disadvantages

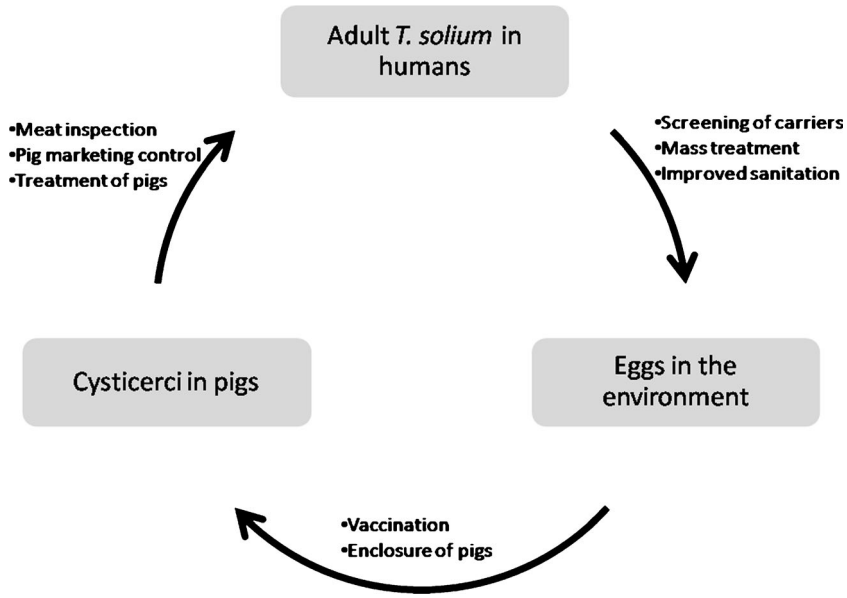


**Fig. 1** Potential intervention points for preventing transmission of *Taenia solium*

(Table 1). Importantly, diagnostic gold-standard and cost-effective tests are still lacking. The most widely used methods for taeniasis diagnosis are coproparasitological examination of feces to demonstrate the presence of *Taenia* spp. proglottids or eggs, and detection of specific coproantigens by an ELISA [31]. The possibility of detecting *T. solium*-specific antibodies in serum has also been demonstrated [32], and molecular methods have been reported.

## Self-Detection Tool for Tapeworm Carriers

A common symptom associated with taeniasis is the expulsion of proglottids [33••], and carriers may report the presence of these in their feces or feel them in their undergarments [34]. However, while for *T. saginata* and *T. asiatica*, the proglottids may be spontaneously expelled independently of defecation, the expulsion of *T. solium* proglottids is passive and they appear together with feces. The reliability of self-detection for taeniasis diagnosis has been evaluated [35–38], with sensitivity ranging from less than 50 % in Honduras to over 80 % in Sichuan, China [38, 39]. This wide variation has been explained by differences in the predominant *Taenia* species and the habits/customs of inhabitants in endemic areas [32]. Regardless, to be implemented as a reliable diagnostic tool, self-detection requires prior public health education campaigns [37].

**Table 1** Currently available taeniasis diagnostic tests, with their main advantages and disadvantages

| Test | Advantage | Disadvantage |
|---|---|---|
| Coproparasitologic | | |
| Self detection | Inexpensive | Unreliable |
| Microscopy | Highly specific | Low sensitivity |
| Immunological | | |
| Copro-Ag ELISA | Reasonably sensitive | Many false positives |
| Western blot | Highly specific | Many false positives |
| Molecular | | |
| PCR-based | Species differentiation | Very expensive |
| LAMP | Species differentiation | No field validation |

*Copro-Ag ELISA* coproantigen enzyme-linked immunosorbent assay; *LAMP* loop-mediated isothermal amplification; *PCR* polymerase chain reaction

## Coproscopic Examination of Feces

The microscopic examination of stool samples (coproscopy) has remained the routine method for the diagnosis and identification of *Taenia* spp. eggs or proglottids to date. Direct wet mounts or concentration methods such as Kato-Katz and the formol-ether concentration technique [40] are widely used for the detection of *Taenia* spp. eggs in feces. The diagnostic sensitivity of these techniques, however, is not optimal, with reports ranging from 38 to 69 % [41, 42]. Such low sensitivity is primarily due to the intermittent nature of egg release, which leads to an underestimation of the prevalence of taeniasis [43]. Allan et al. [44] reported that coproscopic studies from patients with active tapeworm infection are commonly negative because, firstly, eggs may not appear in feces every day, and secondly, eggs are not uniformly distributed in feces. For these reasons, the authors recommended collection of samples over a 3-day period. Further, if destrobilation (i.e., the breakage of gravid proglottids from the worm's body—the strobila) has led to a massive discharge of eggs, these may be absent from feces for up to several weeks thereafter, until more proglottids mature and become gravid [45]. In addition, the specificity of

coproscopic methods is limited at the genus level because the eggs of these tapeworms are identical under light microscopy [1]. This is particularly relevant given the risks associated with *T. solium* infection [30].

Parasitological identification of human adult intestinal taeniids to species level relies on the recovery of gravid proglottids or scolices. This recovery is difficult because of the disintegration of the proximal end of the worm when modern cestocidal drugs are used [45]. Jeri et al. [46] improved the treatment method to obtain a recognizable tapeworm by using pre-niclosamide and post-niclosamide electrolyte-polyethyleneglycol (PEG) salt purges to improve bowel cleaning and collection of the tapeworm scolex, making differentiation between *T. saginata* and *T. solium* easier. Nevertheless, since PEG has to be dissolved in 2 L of water, it might not be well accepted/perceived, especially in community studies.

Three morphological characteristics to distinguish *T. solium* from *T. saginata* were proposed by Verster [47] in a taxonomic review of the genus *Taenia*. These characteristics are the presence of an armed rostellum on the scolex, a three-lobed ovary, and the absence of a vaginal sphincter. Additionally, the number of uterine branches in gravid proglottids is an indicative but not absolute difference between the two *Taenia* species [48]. Fixation and staining of proglottids with Semichon's acetocarmine allows for identification of these differences, as does injection of liquid black ink through the genital pore. In addition to the absence (*T. saginata*) or presence (*T. solium*) of rostellar hooks on the scolex, Morgan and Hawkins [49] described a differential method based on the number of uterine branches in gravid segments. They reported that *T. solium* had between 8 and 14 unilateral uterine branches, whereas *T. saginata* had 15–24 branches. However, several authors have reported overlapping numbers, thus questioning the specificity of this method [49, 50].

The differential diagnosis of the adult worm causing taeniasis is very important for control purposes, but, in light of the factors explained above, diagnosis using morphological characteristics from parasite material is plagued with challenges.

## Parasite Coproantigen Assays

Parasite coproantigens constitute specific products in the feces of the host that it is possible to detect using immunological tests. These products are associated with parasite metabolism, are independent of the presence of eggs or proglottids, and are reported to disappear from feces shortly after treatment [30, 51]. Coproantigens can also be detected as early as 2 weeks postinfection [52••].

Several assays detecting *Taenia* coproantigens have been developed in different formats but all in the form of antigen-capture ELISA, using polyclonal antibodies obtained from hyperimmunized rabbits with either adult worm somatic or excretory–secretory products [25••, 53–56]. These assays are reported to be genus specific and are independent of reproductive material (e.g., eggs). Furthermore, coproantigens are not detectable after treatment, and the antigens are stable in fecal samples [30], making the test very useful for early detection and evaluation of antiparasitic treatment efficacy in human *T. solium* taeniasis [51]. In epidemiological studies, the Copro-Ag ELISA is reported to detect around 2.5 times more cases of taeniasis than basic microscopy [42, 44].

The levels of sensitivity of these assays are dependent on the assay format (both microplate and dipstick formats have been used to date) and the quality of the rabbit sera used in their production (high-titer sera being better). Some studies have reported that these assays have specificity and sensitivity of 100 % and 98 %, respectively [31, 55]). Other studies in Guatemala and Peru have, however, recorded lower sensitivities [17, 56–58]. Using Bayesian analysis, a study by Praet et al. [59•] reported sensitivity and specificity of 85 % and 92 %, respectively. The tests are genus specific; as such, *T. saginata* and *T. solium* infections cannot be differentiated. No cross-reactions with other infections, including *Hymenolepis* spp., *Ascaris lumbricoises*, *Trichuris trichiura*, hookworm and parasitic protozoa, have been identified [25••, 30]. To achieve species specificity, Guezala et al. [60] combined both polyclonal antibodies against *T. solium* adult whole worm extract and *T. solium* adult excretory–secretory proteins (ESP) in a hybrid sandwich ELISA format. This assay was reported to perform with 100 % specificity and 95 % sensitivity in the detection of *T. solium* carriers [60].

Though Allan and colleagues [41] already pointed out the presence of false positive results with the copro-Ag test in a field study conducted in Guatemalan communities, cross-reactions with other parasites other than *Taenia* spp. have not been reported [30]. Nevertheless, potential nonspecific reactions of the polyclonal antibodies should be further investigated. In a study by Praet et al. [59], a sample that was *T. saginata* positive by copro-polymerase chain reaction (copro-PCR) was also copro-Ag positive, highlighting the nonspecificity of the copro-Ag test using polyclonal antibodies against adult *T. solium*. This calls for further improvements in the copro-Ag ELISA test, as the differential diagnosis of taeniasis has public health implications.

The copro-Ag ELISA is reported to detect immature tapeworm stages, and this could explain the higher number of copro-Ag ELISA-positive cases, compared with coproscopy (only detecting eggs and thus adult, gravid tapeworms) reported in studies that have used both tests. Further, studies that have used the copro-Ag ELISA test together with the molecular tests indicate that not all samples that are positive on copro-Ag ELISA are also positive on PCR [26, 58]. In contrast with the copro-Ag ELISA, which is able to detect immature tapeworms, molecular-based tests are dependent on reproductive material such as eggs. This highlights the

inadequacies of the latter to detect mature adult tapeworm carriers. Although based on one voluntarily self-infected subject, a study by Tembo and Craig [52••] reported that for *T. saginata*, coproantigens were detected 14 days postinfection, whereas proglottid patency occurred 86 days postinfection. If this is true for *T. solium*, then it could probably explain the higher number of copro-Ag ELISA positives compared with PCR reported in studies that have used both of these tests.

The rate at which tapeworms establish in the intestine following ingestion of cysticerci is not well known. It is generally assumed that only one tapeworm develops in a host (solitary worm). Competition between tapeworms of the same or different species, influencing their establishment, has been suggested by Conlan et al. [61]. Since people may consume pork meat infected with many cysts, potentially many of these can develop into adult worms within one host. However, an important proportion of infected individuals can harbor multiple tapeworms, as demonstrated in studies by Bustos et al. (8.2 %) [51] and Jeri et al. (20 %) [46]. It is also possible that some juvenile tapeworms are expelled before they reach maturity. Although cross-reactions have been demonstrated not to occur with the copro-Ag ELISA, additional studies to improve the test are required, and use of monoclonal antibodies to detect antigens in stool is suggested.

## Serological Diagnostic Assays

Wilkins et al. [32] described *T. solium*-specific antigens to detect antibodies against adult *T. solium* in serum by Western blot analysis, with sensitivity and specificity rates of 95 % and 100 %, respectively. Even though no cross-reactions were found in serum from individuals infected with *T. saginata* and other cestodes, including *T. solium* cysticercosis, one sample from a patient suffering from NCC but not harboring the intestinal worm tested positive [30]. The serological diagnosis of taeniasis has obvious advantages over the fecal-based methods (e.g., species specificity, avoidance of potential biohazard associated with collection and handling of fecal samples, and also the possibility of combining the test with other immunological assays in the diagnosis of cysticercosis). However, in treated individuals, antibodies remain detectable for a long time (period not yet established) and cause false positives [30, 62]. Further, as highlighted above, it is possible that after successful infection and initial establishment in the intestine, some tapeworms fail to progress into mature and gravid worms, consequently dying and being expelled from the body. In these situations, it is possible that individuals will remain positive for antibodies even when an actual infection cannot be demonstrated.

While these assays have been applied successfully as part of field research programs in endemic countries, issues such as cost and accessibility remain to be addressed if these tests are to be used routinely in these areas of the world [30]. The assays are also yet to be evaluated in large-scale field studies in endemic areas. For this reason, these tests are not yet commercially available for diagnosis but are available only for research purposes.

Handali et al. [63], described a rapid test method using recombinant proteins for immunodetection of taeniasis, which could be affordable, reliable, rapid, and easy to perform. Though feasible, the test still requires field evaluation and improvements of its sensitivity for taeniasis detection in endemic areas.

## Molecular Methods

Molecular techniques have also been developed that allow species-specific tapeworm detection in feces and differentiation of collected parasite material [64–68, 69•]. Differentiation of human *Taenia* spp. by molecular assays is normally done on proglottids expelled from carriers after treatment [50, 70, 71]. In recent years, PCR tests for species-specific confirmation of *Taenia* spp. have been developed, based on the detection of parasite DNA in fecal samples (copro-DNA) [65], cysticerci [65, 72], or eggs present in the feces and on proglottids [65]. Several methods and loci have been used for differentiating *Taenia* spp. Gonzalez et al. [71] designated primers that have been used in multiplex PCR, giving differential detection between *T. saginata* and *T. solium*.

Mayta et al. [48] used PCR–restriction fragment length polymorphism (PCR–RFLP) to differentiate *T. solium* and *T. saginata*. They amplified the 3′ region of the 18S and the 5′ region of the 28S ribosomal gene (spacing the 5.8S ribosomal gene) and used three restriction enzymes (AluI, D*de*I or *Mbo*I) for analysis of the PCR amplicons. Each enzyme gave a unique pattern for each species. In this assay, the primers amplified DNA from all cestodes, not only from *Taenia* spp.

Rodriguez-Hidalgo et al. [50] also differentiated *Taenia* spp. by PCR–RFLP using the 12S rDNA but developed new primers to reduce the nonspecific amplification found when using field samples. They, however, also used D*de*I as the restriction enzyme.

The major problem with PCR for DNA detection in stool samples has been that of sensitivity, owing to the presence of PCR inhibitors in stools [64, 73]. Mayta et al. [67] reported a nested-PCR assay targeting the Tso31 gene, which was developed for specific diagnosis of taeniasis due to *T. solium*. The specificity and sensitivity of the assay on archived samples were 97 % (31/32) and 100 % (123/123), respectively. Under field conditions, and using microscopy and/or Copro-Ag ELISA testing as the gold standards, the assay was 100 % sensitive and specific.

Praet et al. [59•] reported a novel real-time PCR using *T. solium*-specific primers, TsolITS_145F and TsolITS_230R (Biolegio, Nijmegen, The Netherlands) and the Tsol_ITS_169Tq_FAM double-labeled probe (Biolegio) to

detect *T. solium*-specific amplification. *T. saginata*-specific PCR primers and a detection probe were also chosen within the ITS1 sequence to amplify and detect *T. saginata* specifically. Using Bayesian analysis, this real-time PCR had sensitivity and specificity of 83 % and 99 %, respectively. This study highlighted the importance of using Bayesian analysis in the estimation of diagnostic tests in light of the absence of a diagnostic gold standard for taeniasis.

The high sensitivity of species-specific detection of *Taenia* spp. is a major advantage of the copro-PCR test for the diagnosis of taeniasis [59•, 64, 65]. However, molecular tools remain very expensive and unavailable in endemic areas. The current DNA extraction methods are too expensive to be used as a routine test, and many developing countries lack well-equipped laboratories needed for molecular tests [1], and this renders their use under field conditions unfeasible.

A report by Nkouawa et al. [68] described the development and evaluation of a loop-mediated isothermal amplification (LAMP) assay for differential diagnosis of infections with *Taenia* species. They demonstrated that the LAMP method was able to differentially detect *Taenia* species and had high sensitivity and specificity. The LAMP test is simple and highly cost effective compared with PCR, requiring simple inexpensive materials and equipment. The test was piloted on a limited number of clinical specimens, and therefore it requires field validation before it can be made available for routine differential diagnosis of taeniasis. If validated, the LAMP test has the potential to be used as an alternative and cost-effective tool for the detection of *T. solium* carriers globally.

## Conclusions

The presence of *T. solium* tapeworm carriers in a community where open defecation is frequent leads to high human and porcine cysticercosis prevalences. A contaminated environment exposes individuals to repeated contact with the parasite. This has been demonstrated by incidence studies reporting high antibody seroconversion rates [25••, 74•]. Many of those individuals may end up with NCC and, as a result, may suffer from epilepsy for life. It is therefore the authors' view that the detection and treatment of carriers would be a great leap toward the control and elimination of taeniasis and cysticercosis in endemic communities. However, diagnostic deficiencies in the detection of adult-stage intestinal tapeworm carriers hamper control strategies that are based on detection and treatment of carriers [75]. Detection of eggs in feces is insensitive and nonspecific, while immunological and molecular tests still require refinement before they are made available to endemic communities at a relatively cheaper price than currently prevails. As highlighted in this review, taeniasis diagnosis is hindered by the lack of a diagnostic gold-standard test for *T. solium* detection.

From the public health point of view, it might be argued that taeniasis control could be approached in the same manner as soil-transmitted helminths: with mass drug administration (MDA), utilizing drugs such as niclosamide, which is reported to be both safe and efficacious. Regrettably, niclosamide is not readily available in many endemic countries, or it is not accessible to poor communities where the infection is prevalent. Further, since the taeniasis/cysticercosis disease complex remains a neglected problem, few resources are devoted to its control, if any at all.

The use of mass treatment has resulted in decreases in taeniasis and porcine cysticercosis prevalences in endemic areas [18, 29]. However, since its effects last for only up to 2 years [76], MDA should be implemented for a number of years or should be combined with other control programs such as community education [77], vaccination of pigs [78–80], and improved veterinary control of pig slaughter [1]. As stated by Lightowlers [81], the future control of *T. solium* infections lies in an integrated approach, because a single control measure is unlikely to achieve effective and long-lasting control. Nevertheless, the reduction of environmental contamination with *T. solium* eggs by detection and treatment of carriers would be an important entry point. In resource-constrained settings, tapeworm carrier detection can be more cost-effective than MDA. Hence, low-cost, effective, quick, and easy to perform tests are urgently needed to detect these tapeworm carriers who are the cornerstone of taeniasis/cysticercosis transmission.

**Compliance with Ethics Guidelines**

**Conflict of Interest** Kabemba E. Mwape and Sarah Gabriël declare that they have no conflict of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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