Chapter

The Long Road to the Immunodiagnosis of Neurocysticercosis: Controversies and Confusions

Marcela Esquivel-Velázquez, Carlos Larralde, Pedro Ostoa-Saloma, Víctor Hugo Del Río Araiza and Jorge Morales-Montor

Abstract

To date, even widely studied, there is not a standard diagnostic method to detect neurocysticercotic patients. The later due to the complex nature of cysticercosis disease and the simplicity of common immunological assumptions involved in explaining the low scores and reproducibility of immunotests in the diagnosis of neurocysticercosis. To begin with, the few studies dealing with the immune response during neurocysticercosis are not conclusive, which of course it is crucial to develop an immunodiagnostic test. Their full recognition should clear confusion and reduce controversy as well as provide avenues of research and technological design. In here, logical arguments add that even under common immunological assumptions, serology of neurocysticercosis will always include false negative and positive results. Thus, serology is no strong support for medical diagnosis of neurocysticercosis (NC). In contrast, immunotests performed in the cerebrospinal fluid (CSF) of neurological patients should have fewer false positive and fewer false negatives than in serum. To conclude, it is argued that high scores in serology for NC will not yield to usual approaches and that success needs of a concerted world-wide effort. A more punctilious strategy based on the design of panels of confirmed positive and negative sera needs to be construed, shared and tested by all interested groups to obtain comparable results. The identification of a set of specific and representative antigens of *Taenia solium* (*T. solium*) and a thorough compilation of the many forms of antibody response of humans to the many forms of *T. solium* disease are also to be considered as one of the most importants factors to the disease.

Keywords: Cysticercosis, Neglected Diseases, Neurocysticercosis, Immunodiagnosis

1. Introduction

Neurocysticercosis (NC) is a disease caused by the larvae (or cysticerci) of the intestinal parasite *Taenia solium* (*T. solium*) when the cysticerci lodges in the central nervous system (CNS). It is considered one of the most important parasitic disease of the CNS [1–3]. Cysticerci may infect humans and may also locate elsewhere of CNS, in

skeletal muscles, heart, eyes, diaphragm, tongue and subcutaneous tissues, causing a condition simply referred to as cysticercosis. Cysticerci develop in humans and also in pigs from eggs produced by the adult tapeworm living in the intestine of humans and shed to the environment upon defecation, thus contaminating soil, waters and food.

The most serious condition of *T. solium* disease affecting human health is NC. An estimated 60% of NC cases are non-symptomatic [4], while the rest are symptomatic and exhibit a wide variety of neurological symptoms, being chronic epilepsy and headache the most noticeable [4–6]. Severe forms of NC develop meningitis, encephalitis, arteritis, areas of cerebral infarction and gliosis, as well as anatomical distortion and compression of intracranial structures causing blockade in the flow of cerebrospinal-fluid (CSF) [5–7], frequently leading to endo-cranial hypertension and requiring specialized medical attention and/or surgery to derive CSF and/or remove the parasite. The severe forms of NC seriously impair the patients´ health and may lead to death. Medical diagnosis of NC is impossible on clinical data alone as it presents a variety of nonspecific symptoms [8], while confirmatory diagnosis is established by biopsy, cranial CAT-scans and/or cranial NMR images showing nodular lesions of the brain usually suffice in most cases.

Immunodiagnosis of NC (IDxNC) has long been sought because of the disease's prolonged silent or ambiguous clinical pictures and also because of the low accessibility and impossibly high costs of CAT-scans and NMR-images in endemic countries [1, 9–11]. Not only an effective IDxNC would be a most practical way to facilitate medical diagnosis for millions of poor people in endemic countries, it would also supply sero-epidemiological studies with a low-cost indicator of prevalence of infection. In addition, a positive immune-test would rise the clinical suspicion of early non-symptomatic NC which, if confirmed, would allow to offer early treatment before the parasite does much irreversible CNS damage. Further, simplification of copro-parasitological studies in stools by an immune-test would help to identify carriers of live tapeworms and treat them in order to interrupt transmission in the explosive stage of massive egg production.

Many immunological methods have been tried to detect antibodies and/or antigens of *T. solium* in serum or CSF and feces, and even in urine and saliva [12–16], with variable levels of success in detecting NC cases and tapeworm carriers [17, 18]. The gallery includes *in vitro* tests using complement fixation, precipitation, agglutination, radioimmunoassay and enzyme-based detection systems (ELISA and Western Blots) [8, 19, 20]. Antigens used in diagnosis also vary from whole antigen extracts [14, 21, 22], secreted antigens [23–27], semi-purified fractions and purified natural proteins [6, 12, 16, 28] to recombinant proteins [4, 6, 8–11, 29, 30], and synthetic peptides [31–33], either from *T. solium* or from homologous parasites as *Taenia crassiceps* [2, 13, 19, 21, 32, 34], *Taenia saginata* [22, 35] or *Taenia taeniaeformis* [36]. Most reports initially claim very high specificity/sensitivity scores, sometimes even as high as 100/100%. Enthusiasm soon calms as the methods are applied by different laboratories, in larger numbers of cases and in various epidemiological scenarios of the disease [14, 28, 37, 38]. A sober statement about the state of the art at present times would claim a sensitivity that ranges from 50 to 85% (15–50% false positives) and a specificity of about 80–90% (10--20% false negatives), with large variations within and between tests and low reproducibility between laboratories [14, 28, 38].

2. Generalities of immune response to *Taenia solium* cysticercosis

In recent times it has been found that cysticercosis is importantly driven by the hosts neuroendocrine system function, particularly sex steroid hormones (Morales-Montor and Larralde c, 2005). *Taenia* parasites have developed elaborate

mechanisms of interacting with their intermediate hosts. The oncospheres which invade the intermediate host are susceptible to antibody and complement. However, by the time the host has generated an antibody response, the parasites have begun to transform to the more resistant metacestode. The metacestodes have elaborate means of evading complement-mediated destruction, including paramyosin which inhibits C1q, taeniaestatin which inhibits both classical and alternate pathways, and sulfated polysaccharides which activate complement away from the parasite. Similarly, antibody does not seem to be able to kill the mature metacestode. In fact, the parasites may even stimulate the host to produce antibody, which could be bound via Fc receptors and used as a source of protein. Finally, taeniaestatin and other poorly defined factors may interfere with lymphocyte proliferation and macrophage function, thus paralyzing the cellular immune response. Since the symptoms of NC are typically associated with a brisk inflammatory response, we hypothesize that disease is primarily caused by injured or dying parasites. This hypothesis raises important questions in assessing the role of chemotherapy in the management of NC, as well as in the evaluation of clinical trials, most of which were uncontrolled (Morales-Montor et al., 2006).

The generation of protective T cell responses to cysticercosis is a complex process in which cytokines and costimulatory molecules provide signals that direct the development of adaptative immunity (13). The characterization of T cell responses as belonging to either Th1-type responses (dominated by the production of IFN-γ and associated with cell-mediated immunity) or Th2-type responses (characterized by production of IL-4 and IL-5, and associated with humoral immunity) was important because it provided a basis for understanding how T cells contribute to resistance, or susceptibility to cysticercosis (14). Subsequent studies distinguished the role of IL-12 and IL-4 in the development of Th1 and Th2 responses, respectively, but there are other cytokines involved in this process (13). Succintly, it can be sustained that immune response to the worm (adult stage of *T. solium*) is limited to Th2-type mechanisms, while the line of defense against the cysticercus is a mixed

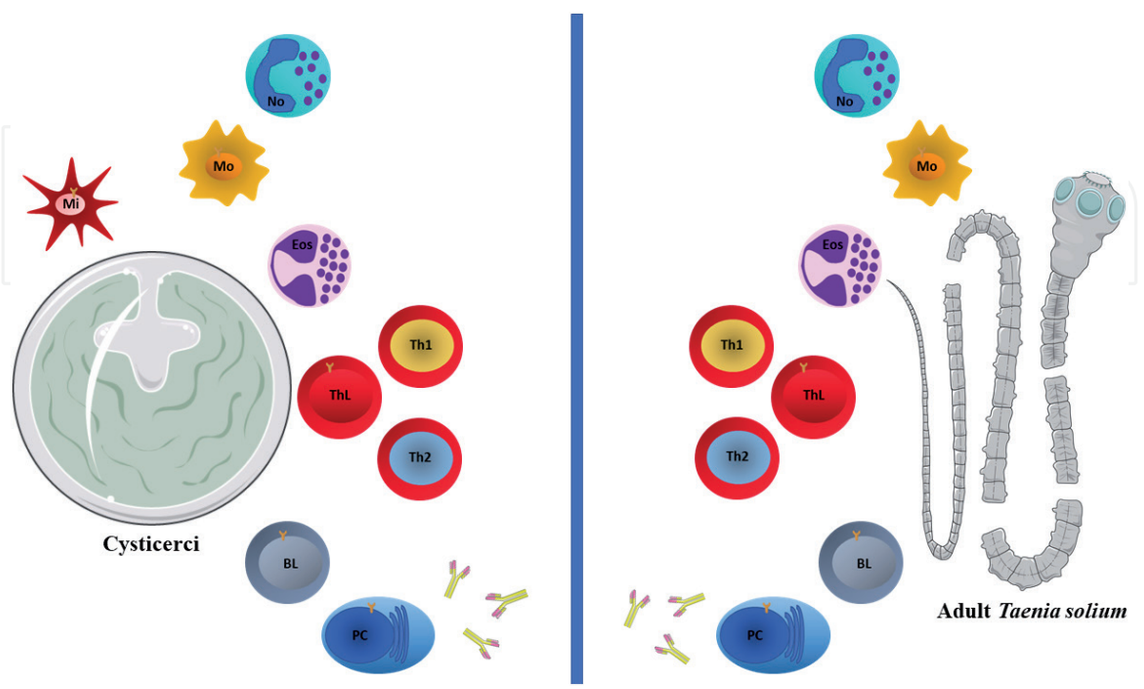

Figure 1.
Cysticerci or adult parasite and associated host immune cells. Mi, microglia/dendritic cell; Mo, macrophage; No, neutrophil; Eos, eosinophil; Th, T helper lymphocyte; Th1, T helper lymphocyte type 1; Th2, T helper lymphocyte type 2; BL, B lymphocyte; PC, plasmatic cell.

Th1-Th2-type immune response, with dominance of Th1-type immune response mechanisms involved in limiting parasite growth and expansion (**Figure 1**).

3. Sources and effects of controversy

Low sensitivity and specificity of IDxNC, as well as variability of results within each method and irregular reproducibility between different laboratories, are cause of discussion and confusion. More than 50 years of insufficiently planned and disaggregated individualistic research using different materials, reagents, techniques and conditions of endemial are involved. Policy of publication favoring alleged breakthroughs tells the luminous half of the stories, creating the false impression that similar results are to be expected by all. The surging of commercial kits and their accompanying propagandistic fanfare has fueled dispute and nurtured distrust because of suspected conflicts of interest without much improvement in diagnostic capacity. The serious problem caused by all this is that the jingle of controversy and confusion has reached medical practice and introduced doubts on the significance of serology in medical diagnosis and epidemiological study surveys. This has in turn retarded the recognition of *T. solium* disease as the great threat it is to human health and the high costs it incurs to public health in endemic countries. It might be of help to clarify the major causes behind the low performance of IDxNC as a preliminary step to reach a consensual agreement on the meaning of its results, its limitations and the ways for improvement.

Low performance and variability are usually thought to rise from the technical virtues or pitfalls of the different available immunological tools and reagents used. There is some reason for argument here but there is much more than that to fully explain the incoherent results and to incorporate in the design of a strategy with a chance of worldwide solid success. Rarely is it recognized faulty results may rise from over-simplified immunological assumptions about this particular host–parasite relationship, incomplete knowledge of the *T. solium* antigen repertoire and/or the immunological complexities derived from the many forms *T solium* has of affecting humans. Because the pleomorphism in *T. solium* disease sets the levels of difficulty for immunological discrimination and is the least recognized cause of controversial results, here we shall describe in somewhat fastidious detail its many different faces.

The exercise illustrates how hard it is the task of immunotests when put to effectively discriminate from the multiple faces of *T solium* disease the one and only of NC. It will also suggest ways of clustering the significant from the insignificant discriminations, for medical as distinguished from epidemiological purposes, as well as point to what is possible and what impossible. Inevitably, some of the major immunological assumptions behind the presence or absence of antibodies and/or antigen in an individual must be dealt-with to some extent since they interact with the disease polymorphism to increment the difficulties of immunodiagnosis for NC. The exercise also explains many of the discrepant findings and should clear some of the controversy as well as point to ways of improvement.

4. The many faces of *T. solium* disease

Any human population under consideration may be divided in two sets according to their having had come in contact with *T. solium* (*I*) or not (*0*) (**Figure 2**).

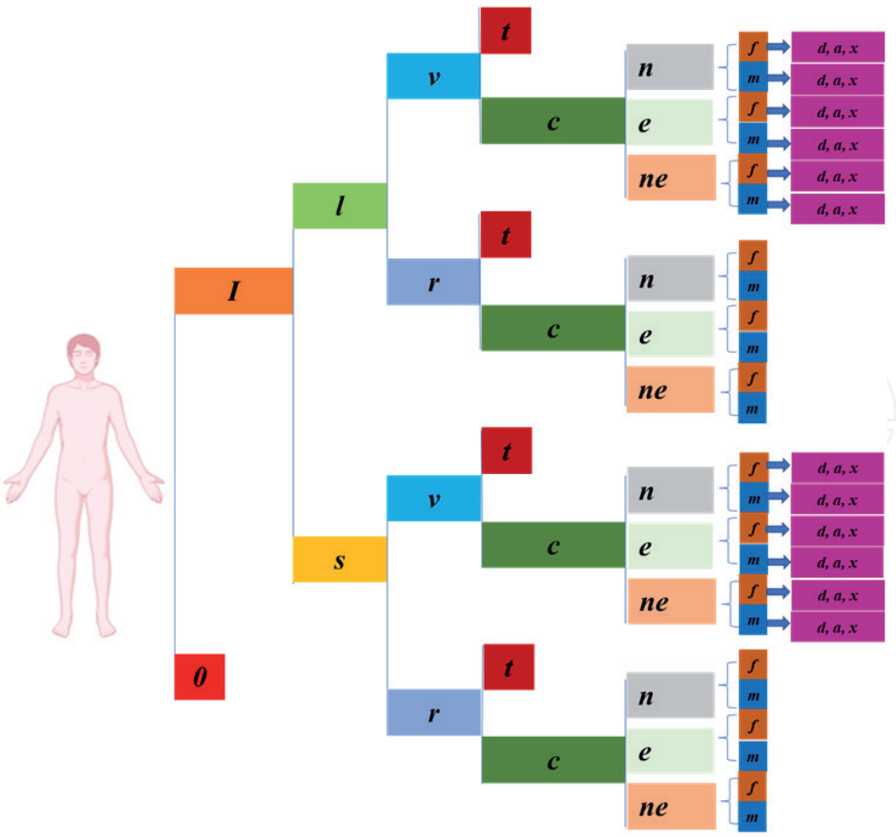

Figure 2.
Schematic representation of the different possible subsets of the contact and infection of human population with Taenia solium.

The set *I* includes at least 48 different subsets depending on whether the contact occurred a long (*l*) or short (*s*) time before sampling; the parasite was rejected (*r*) or it victoriously established in the host (*v*); the parasite is in the stage of a tapeworm (*t*) or as a cysticerci (*c*); if the cysticerci is located in the nervous system (*n*) or elsewhere (*e*) or in both (*ne*); if the cysticerci are few (*f*) or multiple (*m*); and if they are dead (*d*) or alive (*a*) or degenerating (*x*) (**Figure 2**).

The projection of positive serology upon the I set involve a number of immunological assumptions listed in **Table 1**.

Assuming the minimal, it may be concluded that:

1. Antibodies are to be found only in members of the *I* set in any of its subsets; and

2. Antigens are to be found only in members of the *I* subsets carrying live parasites at the time of sampling.

In consequence:

1. The finding of antibodies in an individual would be indicative of contact but would not establish the diagnosis of NC; and

2. The finding of antigen would establish the presence of a parasite in the form of cysticerci located either in the brain and/or elsewhere (i.e., skeletal muscle) or of an intestinal tapeworm.

If additional assumptions are added, then:

Minimal	1. Antigens used in the immunotest are specific of *T. solium* and present in all members of the species at all stages of development
	2. There are no natural antibodies to the parasite.
	3. There are no antiidiotipic antibodies simulating antigens.
	4. All humans produce antibodies to at least a small and the same subset of the *T. solium* antigens in the immunotest.
Additional	5. Antibodies and antigens tend to:
	a. disappear with time after an unsuccessful attempt of the parasite to establish in the host,
	b. increase with the number of parasites established and
	c. concentrate in the compartment where the parasite is located.

Table 1.
Immunological assumptions involved upon positive serology within the I group (persons who has or had contact with Taenia solium*).*

3. Antibodies and antigens would be more likely (but not exclusively) to be found in all the *r* subsets of *I* that combine with *m* and *a* (that is, in all cysticercosis cases, acquired shortly or long before sampling, with many and live cysticerci located in the brain or elsewhere) and in tapeworm carriers. But more likely they would be found in the CSF in the neurocysticercosis (*n*) subsets combining with *m* and *a*, in the SERUM for the *e* (elsewhere cysticercosis) subsets also combining with *m* and *a*, and in the feces of *t* (carriers of live tapeworms). The precise magnitude of each likelihood is to be assessed in perhaps each endemic situation.

5. Clearing some discrepancy

From the above description of the variety in *T. solium* disease of humans and the usual and rather liberal assumptions about the quality of the immunological reactants and the nature of the immune response to this particular parasite many of the discrepancies in the performance of different immunotests in different trials may be explained. The most important being the variation in the composition of the set of control not-NC individuals (i.e., some containing more members of the *e* or *t* subsets of *I* would thwart specificity due to many false positive results) and/or in the control NC individuals (in which an undue number of the *d* subset would lower sensitivity). Likewise, the control NC individuals are frequently a mixed lot of NC patients, differing in time of evolution, number and location of cysticerci, form and time of medical and surgical treatment, general health and nutritional status, age, gender, race, etcetera, that can possibly affect their immune reactivity [39–46] (**Figure 3**).

The use of domestic and probably differently composed sets of presumed control *I* and *0* individuals and of NC and not-NC individuals accompanying each immunotest trial is widespread and thus suspect of being a major cause of incoherent results between trials.

Variation between different trials would also follow from differences in the probability distribution of immunologically positive and negative individuals in different situations of endemia (i.e., high and low endemia, urban and rural transmission) and in the simplification of the forms of disease by way of binomial variables (i.e., long or short time of exposure before sampling, single or multiple cysticerci,

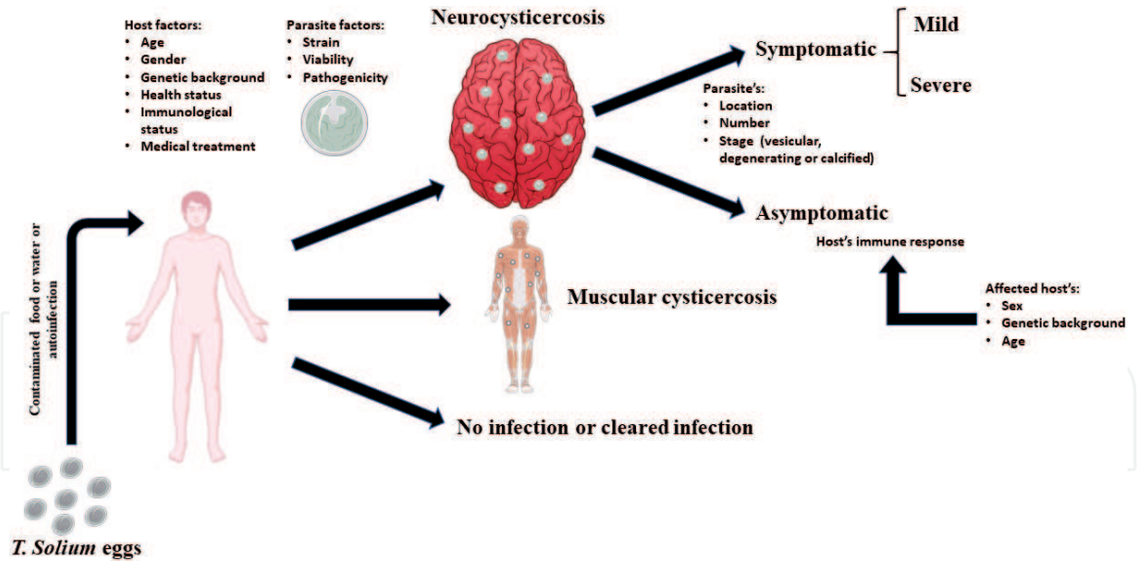

Figure 3.
Factors involved in neurocysticercosis. The development of neurocysticercosis depends on many factors from either the host or the parasite. The factors affecting the immune response of the host are particularly important for the immunodiagnosis of NC as they may affect the results between individuals.Differences in representativity of the whole of the parasite antigens and in cross-reactivity with other antigens in the geographic and endemic background, as well as differences in relative concentrations of reactants and conditions of reaction are additional suspect sources of variation.

dead or alive (not dying) when they are not really so (i.e., individual may be carrying 1, 2, ..., n cysticerci) and some are continuous (i.e., time of exposure before sampling) and even non-disjunctive (i.e. dead, degenerating and live cysticerci may coexist in an individual).

The selection of the *T. solium* antigens to be used as reactants in the immunotests also vary widely among the different immunotests and also within the same immunotest applied to different endemic conditions and geographic locations.

6. Immunological assumptions

Of all the immunological assumptions necessary to interpret the results of immuno tests in diagnosis of NC, the less tenable are those implying there are no cross-reactions with other parasites endemic in the area, that all humans react equally to infection and that the set of antigens selected for the immunotest are shared by all individual cysticerci and tapeworm in the species (**Figure 4**).

The question of antigen cross-reactivity is usually dealt-with by selection of the set of *T. solium* antigens most reactive with positive control samples (confirmed NC cases) and less reactive with negative control samples (presumably without NC), all gathered from donors residing in the endemic areas [47, 48]: a sensible procedure in principle but usually lacking in proof of the statistical representation of the population affected by the other pathogens and in the certainty of negative control samples with respect to clinically silent NC and cysticercosis located elsewhere. Failure to control antigen cross-reactivity results in false positive tests. That not all humans react equally to infection is an additional source of false negative immunotests. Heterogeneous immune response of humans to pathogens is well known in a number of infections, possibly all, and although not thoroughly explored in *T. solium* disease it follows from differences in levels of antibodies of control and problem samples as well as differences in the published images of WB [47–49]. Besides, NC cases donating samples to use as positive controls usually differ in some

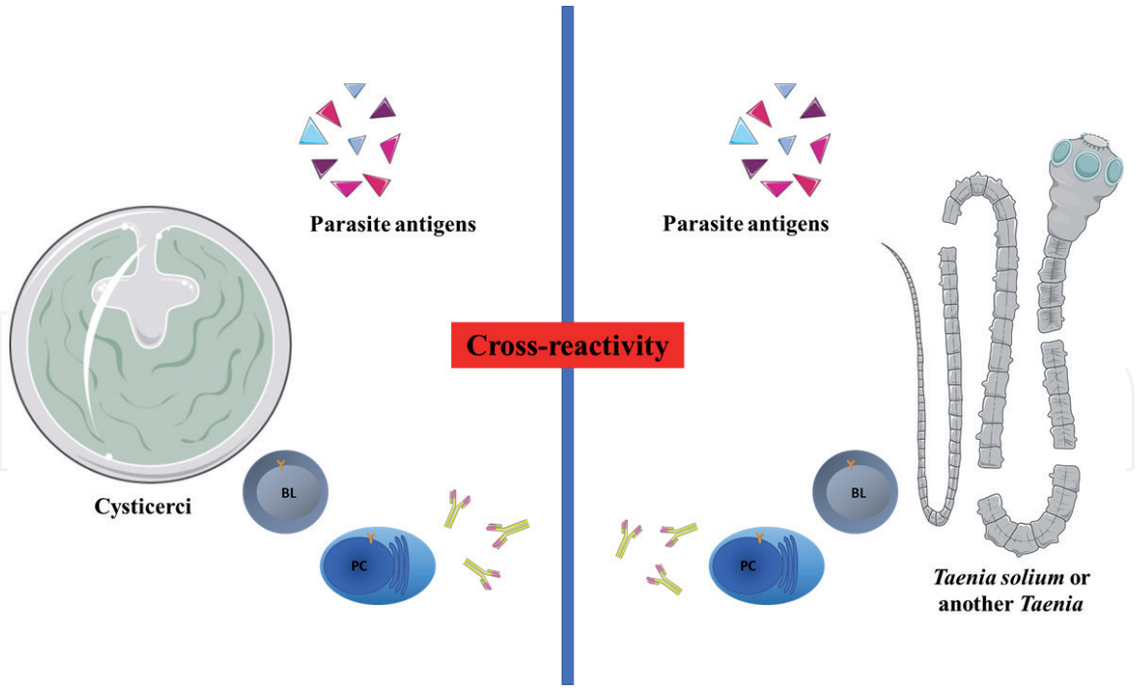

Figure 4.
Failure in immunodiagnosis. Cross-reactivity occurs due to some, but not all, of the secretion and excretion antigens of Taenia solium that are shared, not only during the different stages of its biological cycle, but also with some other endemic parasites (eg: Taenia saginata).

or various characteristics of the disease likely to be of immunological consequence (i.e., form and duration of treatment, natural history of the disease, site of residence, age [39], gender [40, 43–45, 50], race [28, 51–54]).

Thus, IDxNC is placed between the wall of false positives and the sword of false negatives and forced to negotiate selecting the antigen(s) most frequently found to react with control NC samples in order to decrease false positives but conceding some false negatives with the consequent loss in both sensitivity and specificity scores.

The antigen repertoire of *T. solium* is known to be numerous and varied [47, 48, 55, 56] but the distribution of the antigens in the members of the species, in the different developmental stages of the parasite and in different geographic locations is perhaps the most neglected possibly crucial need of information for the design of successful immunodiagnosis of *T. solium* disease.

7. Proposals for improvement

1. *T solium* disease is too serious a human problem to make of it an arena of scientific and technological individualistic rivalry. Cooperation is necessary to concert a worldwide effort to design an exacting research plan concordant with the complexities of *T. solium* disease and to develop and test in the short term a minimal number of options from which to select the most proficient IDxNC possible to be put to immediate production and general use while further research for improvement continues.

2. There is no hope for immunodiagnosis of *T. solium* disease without clearing the problem of antigen cross-reactivity and species representation. Purification of antigen(s) or epitopes critically certified to be exclusive of *T. solium* and present in all members of a representative sample of parasite specimens of an endemic site is mandatory. Although some likely candidates have been

proposed [55, 57, 58] they are lacking in satisfactorily meeting with either one or both of these conditions. A way of avoiding the high costs and demanding technical skills involved in the purification of natural antigens is the use of those present in phage display peptide libraries [59–61]. Antigens present in only *T. solium* but not in all specimens of the species would constitute the candidate antigen preparation (CAP).

3. It is also necessary to study and characterize the presumed wide spectrum of humans' antibody production in *T solium* disease in order to calibrate the candidate antigen preparation that would include all infected individuals. WBs using CAP in reaction with representative samples of all subsets of I, if possible, or of *n* and *e* at least, would provide the images necessary to construct all immunological profiles of the infected individuals. Computer assisted image analysis of WB and cluster analysis could address this problem. The set of CAP that reacts with all or most infected individuals would constitute the definitive antigen preparation (DAP).

4. Rather than attempting to develop ways to distinguish each of the different subsets of *T. solium* disease, efforts in immunodiagnosis could focus in improving diagnosis of NC (to include all subsets with *n* and *ne* to the exclusion of all other subsets of *I* as *t* and *e*), whilst for prevalence of *T. solium* disease, in whatever its form, it should only clearly distinguish members of the *I* set from those of *0*.

   Three are the classes of *T. solium* disease that matter the most and perhaps require different strategies: the contact case (members of the *I* set), the NC case (all *n* and *ne* subsets) and the tapeworm carrier (*t* subset). For this purpose, it is indispensable to construct representative and certified negative and positive control panels of the samples CSF, serum and feces from each geographic area upon their reaction with DAP. Certification of the members of the *e* subset and *0* set is complicated by its need of whole-body scans in search of cysticerci located elsewhere of CNS. Additional negative control samples from a culturally and historically certified community or geographic area to be rid of *T. solium* disease and low in infectious disease in general would be useful to estimate blank readings of immunotesting with DAP.

5. Once the problem of antigen specificity and representation is solved there should be no major problem to IDxNC in the CSF of a symptomatic neurological patient nor of an intestinal tapeworm in the feces, preferably by antigen detection (this, to distinguish cysticercosis located elsewhere and live from dead cysticerci in the CNS because antibodies could persist after death of the parasite for unknown periods of time).

6. But there would remain serious problems to tackle for serology, the most accessible sample useful for detection of early nonsymptomatic NC cases in the general population and for epidemiological studies of *T. solium* disease prevalence. The major problem for serology in unambiguously detecting asymptomatic NC cases is the potential location elsewhere of the parasite (all *e* subsets and the *t* subset) that produces false positive results, and the low reactivity of patients with few or calcified cysticerci (*f* and *d* subsets) that produces false negative results. Adding to positive serology a marker of CNS damage [62, 63] as a sign of CNS involvement could help in discriminating NC from other forms of *T. solium* disease.

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Conflict of interest

The authors declare no conflict of interest.

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