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This book discussed about the effect of certain parasitic infection on the heart, leading to serious health problems. Many people believe that parasites only happen to someone else and that they do not have them. But it has been estimated that more than 50% of all people in the Western world will have some sort of parasitic or semi-parasitic animal living in their bodies. It is also thought that parasites could be a co-factor in almost every illness that we now suffer from. We have seen parasites cause psoriasis, heart problems, constipation, diarrhea, muscle weakness, floaters in the eyes, junk food cravings, excessive appetite, allergies and depression. Many people who complete a parasite program report that they are happier and enjoy the company of others more. "In recent medical studies it has been estimated that 85% of the North American adult population has at least one form of parasite living in their bodies. Some authorities feel this figure may be as high as 95%."

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Cardio-parasitology overview

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Introduction

Many people believe that parasites only happen to someone else and that they do not have them. But it has been estimated that more than 50% of all people in the Western world will have some sort of parasitic or semi-parasitic animal living in their bodies. It is also thought that parasites could be a co-factor in almost every illness that we now suffer from.

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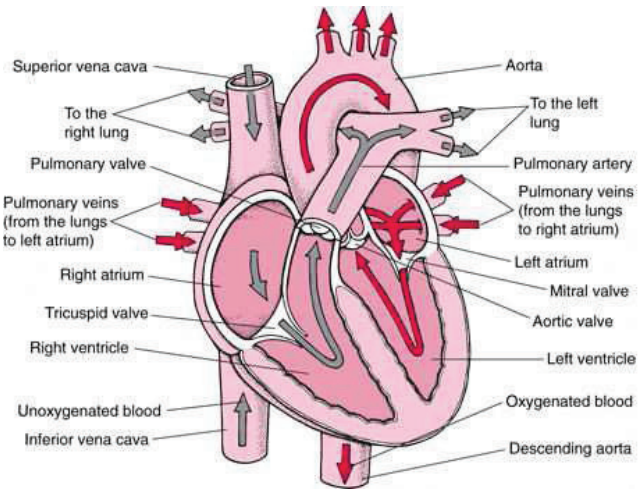

"In recent medical studies it has been estimated that 85% of the North American adult population has at least one form of parasite living in their bodies. Some authorities feel this figure may be as high as 95%."

There are two general groups of parasites. One consists of worms such as tape worms and roundworms. The second category is the protozoa, one-celled organisms. Parasites infect the intestines of both humans and animals and can cause a great deal of intestinal distress. We often associate parasites with Third World countries and poor sanitation, but parasites are a problem everywhere, even in North America. In countries where sanitation is a priority people mistakenly assume that no problem exists and they don't need to worry. Parasites are everywhere, waiting

for the opportunity to latch onto an unsuspecting host. Backpackers have long been aware of the danger of drinking water from streams and lakes. Open water even in the backcountry is often contaminated with parasites waiting for a host.

Some of the most serious parasitic infections are those involving the blood vascular and lymphatic system. These are the human heart parasites and parasites in the blood. Both protozoan and nematode parasites that need a blood sucking or biting arthropod intermediate host are included. There is also a group of very specialized trematodes that are blood parasites that live in the veins.

Nematodes like Trichinella spiralis, an intestinal parasite of carnivores like wolves, foxes, dogs and bears infects people. Its larval stages occur in the muscles of a wide variety of mammals including pigs, rats and man. Preferred locations are the diaphragm, heart, jaws and striated muscle generally.

Heart failure is a disorder in which the heart pumps blood inadequately, leading to reduced blood flow, back-up (congestion) of blood in the veins and lungs, and other changes that may further weaken the heart.

Heart failure may result from other disorders that cause the heart's walls to stiffen, such as infiltrations and infections. For example, in amyloidosis, amyloid, an unusual protein not normally present in the body, infiltrates many tissues in the body. If amyloid infiltrates the heart's walls, they stiffen, and heart failure results. Infiltration by certain parasites into heart muscle can cause heart failure.

Parasitic infections produce a wide spectrum of cardiac manifestations. They may involve various anatomic structures of the heart and are manifested clinically as myocarditis, cardiomyopathies, pericarditis, or pulmonary hypertension in many resource-constrained settings. However, many parasitic infections involving the heart may also be currently diagnosed in developed countries due to growing worldwide travel, blood transfusions, and increasing numbers of immunosuppression states such as organ transplantation, use of immunosuppressive agents, or HIV/AIDS. Clinicians anywhere in the globe need to be aware of the potential cardiac manifestations of parasitic diseases. [1]

American trypanosomiasis

Chagas' disease, also known as American trypanosomiasis, is a zoonotic tropical disease caused by the flagellate protozoan parasite Trypanosoma cruzi. Most infections occur through vector-borne transmission by triatomine insects in areas of endemicity but can also occur through blood transfusion or organ transplantation, vertically from mother to infant, and, more rarely, by ingestion of food or liquid contaminated with T. cruzi or accidents among laboratory personnel who work with live parasites. Vector-borne transmission involves the transmission of the infective form of the parasite (the metacyclic trypomastigotes) to humans by the excreta of the triatomine insect through mucous membranes or through breaks in the skin. Trypomastigotes then invade local host cells, where they differentiate into amastigotes and multiply within the cell. When the cell is swollen with amastigotes, they transform back into trypomastigotes by growing flagellate. The trypomastigotes lyse the cells, invade adjacent tissues, and spread via the lymphatics and bloodstream to distant sites. The cycle is completed when a reduviid bug becomes infected by ingesting blood from an infected host. A minority of patients will develop an acute syndrome of 4 to 8 weeks' duration, which invariably involves prolonged fever in addition to a variable constellation of symptoms, which include inflammation at the portal of entry, subcutaneous edema (localized or generalized), lymphadenopathy, hepatosplenomegaly, myocarditis, and, more rarely, meningoencephalitis. The manifestations of the acute phase resolve spontaneously for the vast majority of individuals even if the infection is not treated with an antiparasitic drug. About 60 to 70% of these patients will never develop clinically apparent diseases. They remain asymptomatic and infected life long, being recognized only if serological tests are performed (the so-called indeterminate form of chronic Chagas' disease). Roughly 30 to 40% of infected patients will subsequently develop the cardiac and/or digestive (megaesophagus and megacolon) form of chronic Chagas' disease, usually 10 to 30 years after the initial infection.

Epidemiology.

Trypanosoma cruzi is endemic in South America, Central America, and parts of North America (Southern United States and Mexico). Historically, the disease disproportionately affected the poor because the transmission of T. cruzi infection occurred mainly in rural areas where humans live in poor-quality houses and in close contact with potential vectors. However, rural-to-urban and international migrations have changed the epidemiology of Chagas' disease, affecting periurban areas, urban areas, areas of endemicity, and areas of nonendemicity alike. As a result of these dynamic changes in the population and the coordinated efforts of countries where disease is endemic to interrupt vectorial and transfusional transmission, the prevalence and incidence of the disease are constantly changing. In the 1980s the overall prevalence of T. cruzi infection was estimated to reach 17 million cases in 18 countries where disease is endemic, with 100 million people at risk. Multinational vector control programs and compulsory blood bank screening achieved enormous success in the 1990s, decreasing the incidence of new infections

(700,000 cases per year in 1983) by 70% and the number of annual deaths by approximately 50% for the whole continent and eradicating the transmission of T. cruzi by the main domiciliary vector species, Triatoma infestans, from three of the countries where T. cruzi is endemic (Uruguay in 1997, Chile in 1999, and Brazil in 2006). According to the most recent estimates, there are currently 7.6 million people infected with T. cruzi in Latin America. Although precise figures documenting the total burden of cardiac involvement with T. cruzi are not available, it can be assumed that 20 to 30% of the 7.6 million infected individuals are or will potentially be developing chronic cardiac lesions. Chagas' cardiomyopathy, in turn, is thought to represent the principal cause of cardiac morbidity and mortality among young adults in countries where T. cruzi is endemic and has been estimated to result in at least 21,000 deaths each year. Additionally, because of the constant influx of immigrants from countries where the disease is endemic, Chagas' disease is becoming an important health issue in North America (United States and Canada) and many parts of Europe, where a growing number of individuals are suspected to be infected (e.g., 300,000 individuals in the United States and 48,000 to 67,000 individuals in Spain).

Pathology and pathophysiology of acute myocarditis and chronic cardiomyopathy.

Initially, the abundance of parasites associated with the acute phase of T. cruzi infection argued in favor of the parasite's direct implication in the tissue damage and myocarditis observed during this stage of the disease. Shortly afterwards, a demonstration of myocytolysis of nonparasitized cardiomyocytes led to the implication of parasite-directed cellular immune-mediated inflammatory damage. This immune-mediated damage, associated with large numbers of amastigotes in cardiac myocytes, translates into the hyaline degeneration of muscle fibers, the coagulation necrosis of myocytes and surrounding tissues, as well as the involvement of the epicardium and pericardium. Cellular and, possibly, humoral immune responses elicited by the parasite eventually control the acute infection but fail to completely eliminate the parasite. A variably long asymptomatic phase then ensues, where factors such as the parasite strain, the parasite load during the acute phase, the quality of the immune response, and the presence or absence of reinfection all might influence the course of chronic disease. Regarding the pathogenesis of the interstitial fibrosis, myocytolysis, and ongoing lymphocytic infiltration observed for the chronic phase of Chagas' disease, the paucity of parasites in cardiac tissue probably reflects the use of insensitive histological techniques in past decades. In recent years, more powerful and sensitive methods of parasite detection, such as immunohistochemistry and PCR, have demonstrated a higher frequency of T. cruzi antigens or parasite DNA in chronic lesions. The spectrum of outcomes for patients infected with T. cruzi is varied and probably stems from intrinsic genetic differences of both the parasite and the host and perhaps even the likelihood of ongoing infection in areas where the disease is highly endemic. Evidence exists for multiple hypotheses to explain the etiology of chronic cardiac lesions that implicate the parasite directly, the immune reaction to the parasite, and autoimmunity elicited either directly by the parasite (mimicry) or indirectly (bystander activation). The products of these lesions are various degrees of necrosis, neuronal damage, microvascular damage, and fibrosis. The contributing role of each mechanism to the pathogenesis of Chagas' cardiomyopathy is a whole other topic of debate.

There is evidence for both functional and anatomical parasympathetic neuronal damage in Chagasic patients. Patients with Chagas' disease lack the tonic inhibitory parasympathetic action on the sinus node and, thus, the chronotropic mechanism to respond to changes in blood pressure or venous return. Neuronal loss is thought to occur during the acute stage of the disease; the extent of neuronal damage, however, does not correlate with disease stage. Therefore, despite possible contributions of parasympathetic impairment to the impact of Chagas' heart disease (e.g., increasing vulnerability to malignant arrhythmias and sudden death or accentuating existing contraction abnormalities that can culminate in chamber dilatation), cardiac dysautonomia is unlikely to explain the main pathogenic mechanism underlying Chagas' cardiomyopathy.

In addition to the neuronal damage, microcirculatory changes leading to ischemia have also been implicated in the pathogenesis of chronic Chagas' cardiomyopathy. A diffuse collapse of intramyocardial arterioles in the hearts of chronically infected patients has been observed. Occlusive platelet thrombi in small epicardial and intramural coronary arteries and increased production of cytokines and mediators that promote vasospasm and platelet aggregation have been demonstrated with experimental models of Chagas' disease. Clinically, despite consistently normal epicardial coronary arteries upon coronary angiography, reversible perfusion defects upon stress-induced myocardial perfusion scintigraphy that correlate with ischemia and abnormal coronary flow regulation have been shown for patients with chronic Chagas' cardiomyopathy.

As mentioned above, tissue damage caused directly by the parasite or, more likely, by the immune response elicited by it has been postulated to underlie and potentiate the aggression to cardiac myocytes and neurons. The inflammatory infiltrate in chronic Chagas' cardiomyopathy involves a predominance of macrophages, CD8+ and CD4+ lymphocytes (in a 2:1 ratio), and in some instances has been shown to correlate with more advanced stages of the disease. The persistence of parasites and antigens is thought to be involved in the recruitment of T. cruzi-specific CD8+ T lymphocytes, which predominate in the myocardial infiltrate in cases of chronic Chagas' myocarditis. The cytokine profile associated with this myocarditis is also shifted toward Th1 cytokines, so elevated gamma interferon (IFN-γ) levels and decreased interleukin-10 (IL-10) levels may potentially perpetuate an existent, ongoing inflammatory process. However, the exact mechanism responsible for the turning point from immunoprotection to immune-mediated aggression leading to irreversible tissue damage remains elusive: not only is parasite persistence true for both symptomatic and asymptomatic patients, but the presence of the parasite in heart tissue does not always correlate with inflammation.

Autoimmunity has also been postulated to be a plausible etiology for the chronic myocarditis observed for T. cruzi-infected patients. Several T. cruzi antigens that cross-react with cardiac and noncardiac host components have been identified, but only some have been shown to have functional activity. Among these, attention has focused on antibodies that cross-react with cardiac myosin and the immunodominant T. cruzi antigen B13 initially, because they were detected in 100% of patients with chronic Chagas' cardiomyopathy, in contrast to 14% of asymptomatic infected individuals, and later because T-cell clones derived from lesions of patients with chronic Chagas' cardiomyopathy were found to be simultaneously reactive to

cardiac myosin heavy chain and the B13 T. cruzi protein. Opponents of the molecular mimicry theory with specific relevance to anti-B13-cardiac myosin cross-reactive antibodies and derived cellular autoimmunity contend that these antibodies do not bind to intact myocytes, are not unique to T. cruzi infection, and are present in asymptomatic patients without heart lesions and that myosin autoimmunity is not essential for cardiac inflammation in experimental models. Arguing against autoimmunity developing as a result of parasite-specific immune responses, antigen exposure after tissue damage may also sensitize autoreactive T cells to self-antigens given a proinflammatory environment. The question, therefore, is not whether autoimmunity is present but whether it is a primary cause of or merely a contributing factor to the pathogenesis of chronic Chagas' myocarditis. As proposed by Kierszenbaum, this question remains to be answered by experiments that prove a clear association between the developments of similar cardiac lesions with the transfer of autoantibodies and/or autoreactive cells to susceptible hosts.

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Clinical manifestations of heart involvement:

(a) Myocarditis during the acute phase. Acute myocarditis as evidenced by autopsy studies probably happens in close to 100% of patients with acute Chagas' disease. However, there is an enormous discrepancy between autopsy findings and clinical data: first because acute infection is asymptomatic for approximately 90 to 95% of cases and second because even for symptomatic patients, acute Chagasic myocarditis is diagnosed for only 1 to 40% of them. Findings upon cardiac auscultation may include tachycardia (not always proportional to the degree of fever), cardiac murmurs, and muffled heart sounds. The principal electrocardiographic (ECG) alterations are first-degree

atrioventricular (AV) block, low Q-R-S (Q-R-S interval of the ECG) voltage, and primary T-wave changes. A chest radiograph may show variable degrees of cardiomegaly, and pericardial effusion is the most frequently reported echocardiographic abnormality. Death in the acute phase occurs occasionally (for 5 to 10% of symptomatic patients) as a result of congestive heart failure (due to severe myocarditis) and/or meningoencephalitis. After the acute phase most patients return to a normal or near-normal myocardial status, but some patients (30%) then chronically develop fibrosing myocarditis.

Among immunosuppressed hosts such as HIV-infected individuals and transplant recipients, reactivation of infection and de novo infection (including transmission with transplanted organs among transplant patients) have been reported. With reactivation in HIV-positive patients, myocarditis has been reported for up to 45% of cases. Reactivation of Chagas' disease among heart transplant patients has been estimated to occur in approximately 30% of cases. However, not all these cases are accompanied by florid symptoms or diagnosed by endomyocardial biopsy, and therefore, the true incidence of myocarditis with reactivation is difficult to estimate. Acute T. cruzi infection can also result as a consequence of donor-related transmission, which has been reported after kidney, heart, liver, and multiorgan transplants. Involvement in this setting can range from asymptomatic parasitemia that easily responds to treatment without further complications to severe, fulminant disease despite therapy (including death directly attributable to Chagasic myocarditis). In congenitally infected infants, the most common symptoms, which may be apparent at birth or develop within weeks after delivery, are hipotonicity, fever, hepatosplenomegaly, and anemia. Other findings include prematurity and low birth weight.

In utero infections are also associated with abortion and placentitis. Serious manifestations, including myocarditis, meningoencephalitis, and pneumonitis, are uncommon but carry a high risk of death.

(b) Chronic Chagas' cardiomyopathy. Cardiac involvement is the most frequent and most severe manifestation of chronic Chagas' disease. Transition from the indeterminate form to the cardiac form of chronic Chagas' disease is usually manifested by the appearance of ECG changes such as incomplete or complete right bundle branch block (RBBB), left anterior fascicular block (LAFB), minimal ST-T (ST-to-T interval of the ECG) changes, and monomorphic premature ventricular contractions (PVCs), mostly in asymptomatic or oligosymptomatic patients. As the disease advances, associated intraventricular conduction defects (usually RBBB with LAFB), polymorphic PVCs, bradyarrhythmias, high-grade atrioventricular blocks, Q waves, nonsustained or sustained ventricular tachycardia, and, ultimately, atrial flutter or fibrillation may ensue. Symptoms like palpitations, atypical chest pain, presyncope, syncope, dyspnea upon exertion, and edema are usually observed throughout the course of chronic Chagas' cardiomyopathy (CCC). Findings upon physical examination vary according to the stage of the disease and the presence of conduction system abnormalities. These findings include cardiac rhythm irregularities, a displaced point of maximal impulse, gallop rhythms, a loud second heart sound implying pulmonary hypertension, mitral or tricuspid regurgitation murmurs, an

increase in the systemic venous pressure with liver enlargement and edema, and a borderline low systolic blood pressure with a reduced radial pulse implying systolic dysfunction. Upon echocardiogram, diastolic dysfunction usually precedes systolic dysfunction, potentially allowing the early detection of cardiac involvement in Chagas' disease. Characteristic echocardiographic findings include apical aneurysms, which are reported for 8.5 to 55% of cases (depending on the stage of the disease and the method of detection, i.e., if postmortem or at echocardiography or angiography); segmental left ventricular (LV) contractile abnormalities (more commonly at the posteroinferior wall); and reduced LV systolic function. Echocardiographic evidence of impaired LV function, as characterized by increased LV systolic dimension, reduced LV ejection fraction, or the presence of segmental or global LV wall motion abnormality and/or an LV aneurysm, is the most common and consistent independent predictor of death. Other important clinical and noninvasive adverse prognostic indicators, which not surprisingly reflect and parallel the degree of myocardial dysfunction, include advanced functional class (New York Heart Association [NYHA] classification III/IV), cardiomegaly, and nonsustained ventricular tachycardia upon 24-h ECG monitoring.

The clinical course of CCC is diverse and difficult to predict, with some patients remaining asymptomatic lifelong despite electrocardiographic and/or echocardiographic evidence of the disease, some presenting with signs, symptoms, and complications of progressive heart failure or advanced cardiac arrhythmias, and others dying unexpectedly without prior symptoms. Currently, several staging systems are available. These staging systems can help to identify patients at different degrees of risk, facilitate choices among treatment alternatives, and aid patient counseling. Most systems classify patients into four or five stages based on their functional capacity, ECG findings, and the presence or absence of heart enlargement and/or systolic dysfunction upon echocardiogram. The progression of disease to more advanced stages has been estimated to occur for 10% of patients over a follow-up period of 3 to 10 years. Another important aspect of staging is derived from its prognostic information: the rate of mortality for individuals at early stages of CCC is not significantly different from that of the general population. However, the life expectancy of patients with symptomatic and advanced CCC stages (involving systolic dysfunction and/or cardiomegaly) is less than 30% at 5 years, and their overall prognosis is worse than that of patients with dilated cardiomyopathies of other etiologies. Systemic and pulmonary embolisms arising from mural thrombi in the cardiac chambers are relatively frequent. Although the brain is by far the most common clinically recognized site of embolisms (followed by limbs and lungs), at necropsy, embolisms are found more frequently in the lungs, kidneys, and spleen. Chagas' disease is an independent risk factor for stroke in areas where the disease is endemic. Mortality in CCC is due to sudden cardiac arrest in 55 to 65% of patients, congestive heart failure in 25 to 30% of patients, and thromboembolic phenomena in 10 to 15% of patients.

Diagnosis:

Diagnosis of acute Chagas' myocarditis relies on the demonstration of the parasite and/or anti-T. Cruzi IgM in a patient with the correct epidemiological background and clinical picture. IgM

serology assays are not widely available in developing countries and not standardized, so diagnosis is usually performed by visualizing the trypomastigotes in fresh blood smears, thick drop preparations, or buffy coat smears. The level of parasitemia diminishes to almost undetectable levels by the 6th to 10th weeks of infection, making parasite identification in peripheral blood extremely difficult at this time. Diagnosis could also be attempted by hemoculture on specialized medium (which has improved sensitivity over direct examination) or xenodiagnosis (which involves detecting the parasite by infecting laboratory-reared triatomine bugs directly or indirectly with the patient's blood). However, even considering only congenital Chagas' disease, hemoculture is rarely performed for the diagnosis of acute infection since it requires a specialized laboratory and trained personnel and is usually not widely available. Indirect parasitological tests are of limited value in the diagnosis of acute Chagas' disease, as they can take more than 1 month to obtain results (retarding the beginning of trypanocidal therapy).

A diagnosis of CCC should be suspected for young or middle-aged patients who present with a segmental or dilated cardiomyopathy of unknown etiology if within areas of endemicity or within the right epidemiological context. It should be remembered that CCC presents years after the initial infection, and therefore, patients who migrated to areas where the disease is not endemic are still at risk of developing cardiac compromise. Given the low and probably intermittent parasitemia in the chronic phase, diagnosis relies on serological methods by detecting IgG that binds to T. cruzi antigens. Enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence (IIF), and indirect hemagglutination (IHA) methods are most commonly employed. Two positive tests using any of the three conventional techniques are recommended for a final diagnosis. Identification of the parasite by hemoculture or xenodiagnosis in the chronic phase of the disease is hampered by the low sensitivity of the methods, which is dependent directly on the level of parasitemia. However, these methods may have a role in confirming the diagnosis in rare cases of serologically doubtful results or in evaluating treatment failures at specialized centers.

PCR-based methods using one of two target sequences (the variable region of the minicircle kinetoplast DNA and a 195-bp reiterated DNA sequence of the parasite) have achieved higher sensitivities than those of xenodiagnosis and hemoculture. The major problems with PCR-based techniques are the lack of standardization and commercial availability of assays for T. cruzi, the high level of complexity required, and the reliance of their sensitivity on the level of parasitemia (which is low, by definition, in chronic disease). PCR is therefore still considered among nonconventional methods and is recommended only as an adjunct in specialized centers to confirm parasitemia in congenital T. cruzi infection (since in this context, its sensitivity seems to be greater than that of microscopic examination) or for the evaluation of antiparasitic drug therapy. However, a single or even repeated negative post treatment PCR results do not necessarily mean parasitological cure. The negative results are indicative only of the absence of parasite DNA at those moments. The value of PCR lies mainly in the positive results that they yield, which usually reflect treatment failure. Whether assessments of parasite load by quantitative real-time PCR will correlate with the impact of trypanocidal treatment on

parasitological cure or resistance, as well as on disease evolution, should be a matter for further investigation.

The initial evaluation of the newly diagnosed patient with chronic T. cruzi infection includes a complete medical history and physical examination and a resting 12-lead electrocardiogram. Asymptomatic patients with a normal ECG have a favorable prognosis and should be followed up only annually or biannually. Patients with ECG changes consistent with CCC should undergo a routine cardiac evaluation, including ambulatory 24-h Holter monitoring (complemented with an exercise test whenever possible) to detect arrhythmias and assess functional capacity, chest radiography and two-dimensional (2D) echocardiography to assess ventricular function, and other cardiologic tests as indicated. Based on the results of these tests, it is possible to stratify individual patients by risk and implement appropriate therapy

Treatment and preventive measures:

(a) Etiological treatment. Only two drugs, benznidazole and nifurtimox, are recommended for the treatment of Chagas' disease. Of the two, benznidazole (a nitroimidazole derivative) has been more extensively investigated in clinical studies and is better tolerated overall. Adverse reactions such as generalized or sometimes localized allergic dermatitis occur in approximately 20 to 30% of patients and consist of pruritic and nonbullous polymorphous erythematous rashes, often followed by desquamation. Severe exfoliative dermatitis can occur and should lead to a prompt discontinuation of treatment. Another adverse effect that occurs in approximately 5 to 10% of patients, most commonly late in the treatment course, is a dose-dependent peripheral sensitive neuropathy affecting mainly the distal parts of the lower limbs; it also should prompt the cessation of treatment. Rare serious adverse events include leukopenia with granulocytopenia or agranulocytosis (sometimes followed by fever and tonsillitis) and thrombocytopenic purpura. Additional reported side effects include nausea, vomiting, anorexia, weight loss, insomnia, loss of taste, and onycholysis. Nifurtimox, a nitrofuran compound, has been associated with gastrointestinal side effects in 30 to 70% of patients as well as central and peripheral nervous system toxicity. Both compounds are better tolerated by children, allowing increased dosage regimens. Children should be treated with 10 mg/kg of body weight of benznidazole per day in two divided doses for 30 to 90 days or 15 to 20 mg/kg of nifurtimox per day in four divided doses for 90 to 120 days. Adults should be treated with 5 to 7 mg/kg of benznidazole per day in two divided doses for 30 to 60 days or 8 to 10 mg/kg of nifurtimox per day in three to four divided doses for 90 to 120 days.
Treatment has been recommended for all cases of acute and congenital infection, reactivated infection, and early chronic Chagas' disease (particularly children/adolescents ≤18 years of age) based on evidence of the shortening of the disease's clinical course, cure of infection, or reduction in numbers of parasites. For infected adults without advanced cardiomyopathy up to the age of 50 years, etiological treatment should generally also be offered. The rationale for these recommendations stems from evidence of a slowing of the progression of cardiomyopathy. In a recent observational trial, 566

chronically infected adults (30 to 50 years of age) without heart failure were assigned, in alternating sequences, to benznidazole or no treatment. After a median follow-up period of 9.8 years, fewer treated patients had a progression of disease or developed ECG abnormalities. Negative seroconversion was more frequent for treated patients. Another recently published controlled study, including 111 patients (17 to 46 years of age) with chronic Chagas' disease and a normal electrocardiogram, showed similar favorable results with benznidazole over a mean follow-up period of 21 years. For those patients over the age of 50 years, etiological treatment is considered optional because of the lack of any available data. A multicenter, randomized, placebo-controlled trial of benznidazole enrolling 3,000 patients with mild to moderate CCC who were 18 to 75 years of age is currently under way and should help clarify treatment decisions for this population.

In contrast, etiological treatment is contraindicated during pregnancy and for patients with severe renal or hepatic insufficiency, and it should generally not be offered to patients with advanced Chagasic cardiomyopathy or megaesophagus with significant impairment of swallowing. A more controversial issue is prophylactic treatment for transplant patients: some authors have recommended it for patients with Chagasic cardiomyopathy who undergo cardiac transplantation to prevent disease reactivation, while others recommend it for all infected donors pretransplant and for their respective recipients post-transplant. For chronic Chagas' disease, cure is documented when previously positive serological tests are negative, usually years or decades after treatment.

(b) Symptomatic treatment. Medical treatment of heart failure for patients with CCC should follow specific treatments targeted at each stage according to current guidelines for heart failure of other etiologies. An important exception is the use of beta blockers, which should be used with caution for CCC due to a higher incidence of atrioventricular conduction defects and associated bradyarrhythmias. Cardiac transplantation has been performed with good results for patients with advanced CCC but may not be available or accessible in all countries where this disease is endemic. Although not tested in randomized controlled clinical trials, amiodarone has been associated with a survival advantage in case-control studies among patients with ventricular tachycardia and has therefore been proposed for the management of patients with sustained and nonsustained ventricular tachycardia. However, treatment with amiodarone has been associated with pulmonary, cardiac, thyroid, liver, ocular, skin, central nervous system (CNS), and genitourinary toxicities, so treatment needs to be individualized. Pacemaker implantation is the recommended treatment for severe bradyarrhythmias and advanced conduction abnormalities. The roles of cardioverter-defibrillator implantation and cardiac resynchronization, however, are not well established for CCC. Finally, anticoagulation has been advocated for patients with atrial fibrillation, previous thromboembolic phenomena, or an LV aneurysm with thrombus.

(c) Preventive measures. Most T. cruzi infections can be prevented by decreasing vectorial transmission, improving blood product screening, and detecting and treating transplacental transmission. Multinational programs involving countries where the disease is endemic have achieved enormous success in decreasing both the prevalence

and incidence of Chagas' disease by following several operational stages. The tools for interrupting transmission are based on the implementation of vector control activities such as insecticide spraying, housing improvements, and education as well as the strengthening of the implementation of policy for use and screening of blood products for transfusion. Since the end of January 2007, the American Red Cross and other blood collection agencies in the United States began screening blood donations for antibodies to T. cruzi donations with the approved ELISA (referred to as universal testing). All initially reactive donations are retested in duplicate by using the same screening test; donations testing repeatedly reactive undergo confirmatory testing by the radioimmunoprecipitation assay (RIPA). Continued surveillance is also important to consolidate and maintain the success achieved.

For persons traveling to areas of endemicity, compliance with general food and water precautions is advised to prevent the extremely rare occurrence of food-borne Chagas' disease. More importantly, travelers should avoid sleeping in poorly constructed houses or else consider sleeping in insecticide-impregnated bed nets. However, the protective efficacy of insecticide-treated materials in reducing Chagas' disease transmission and eliminating the vector population has yet to be demonstrated. Finally, no vaccine for Chagas' disease is available. [(2)]

African trypanosomiasis

Human African trypanosomiasis is caused by microscopic parasites of the species Trypanosoma brucei. It is transmitted by the tsetse fly (genus Glossina). As the disease progresses, it causes meningoencephalitis and, eventually, coma and/or death, hence its common name, "sleeping sickness." It is estimated that 60–70 million people in 36 sub-Saharan African countries are at risk of infection.

Also known as sleeping sickness, African trypanosomiasis is caused by a microscopic species of parasites known as Trypanosoma brucei and is spread by the tsetse fly (Glossina). Very rarely, however, infections via mother to child, blood, and sexual activity are also observed.

The two types of African trypanosomiasis are named for the regions in Africa where they were found historically: East African trypanosomiasis (Trypanosoma brucei rhodesiense) and West African trypanosomiasis (Trypanosoma brucei gambiense). Incidentally, West African sleeping sickness mainly infects people, while East African sleeping sickness can infect wild animals and cattle.

Symptoms:

Symptoms in the early stage are relatively mild and may include fever, headache, and muscle and joint pain. However, mental impairment, seizures, and difficulty walking can also manifest in cases where the parasite crosses the patient's blood-brain barrier and into the central nervous system. If left untreated, the infection worsens, and death may occur in several months to several years.

Both East and West African sleeping sickness are accompanied by the following symptoms. After the first infection, people can be repeatedly re-infected.

West African Sleeping Sickness:

Ninety eight percent (98%) of sleeping sickness in Africa is West African sleeping sickness. Early symptoms include lymphadenoma, headaches, intermittent fever, and muscle swelling, none of which are specifically attributable to African sleeping sickness. However, distinct symptoms appear when the infection reaches the central nervous system, including personality change, mental impairment, and seizures. If untreated, it can kill within three years.

East African sleeping sickness:

East African sleeping sickness accounts for less than 2% of all cases of African sleeping sickness. Normally the disease develops rapidly, showing the first symptoms within a few weeks of infection. In some cases, symptoms may include a large sore (chancre) on the area bitten by the tsetse fly. Unless treated in the early stages of infection, the parasite will invade the central nervous system within a space of few weeks. Death usually occurs within several months. [(3)]

Diagnosis:

The diagnosis of African Trypanosomiasis is made through laboratory methods, because the clinical features of infection are not sufficiently specific. The diagnosis rests on finding the parasite in body fluid or tissue by microscopy. The parasite load in T. b. rhodesiense infection is substantially higher than the level in T. b. gambiense infection.

T. b. rhodesiense parasites can easily be found in blood. They can also be found in lymph node fluid or in fluid or biopsy of a chancre. Serologic testing is not widely available and is not used in the diagnosis, since microscopic detection of the parasite is straightforward.

The classic method for diagnosing T. b. gambiense infection is by microscopic examination of lymph node aspirate, usually from a posterior cervical node. It is often difficult to detect T. b. gambiense in blood. Concentration techniques and serial examinations are frequently needed. Serologic testing is available outside the U.S. for T. b. gambiense; however, it normally is used for screening purposes only and the definitive diagnosis rests on microscopic observation of the parasite.

All patients diagnosed with African trypanosomiasis must have their cerebrospinal fluid examined to determine whether there is involvement of the central nervous system, since the choice of treatment drug(s) will depend on the disease stage. The World Health Organization criteria for central nervous system involvement include increased protein in cerebrospinal fluid and a white cell count of more than 5. Trypanosomes can often be observed in cerebrospinal fluid in persons with second stage infection. [(4)]

The diagnosis of T. b. gambiense HAT follows a three-step pathway: screening, diagnostic confirmation, and staging. The majority of control programs rely on active case detection through mass population screening. Screening tools therefore need to be sensitive, practical, quick, and cheap. For that purpose, the Card Agglutination Test for Trypanosomiasis (CATT/T. b. gambiense), currently used in most areas of endemic infection, is a more efficient screening method than the cervical lymph node (CLN) palpation and puncture. Diagnostic confirmation then relies on the finding of trypanosomes in the blood, lymph nodes, or cerebrospinal fluid (CSF). Unfortunately, it is estimated that 20 to 30% of patients are missed by the standard parasitological techniques. Staging of the disease is a key step that allows classification of the patient into the first (hemolymphatic) or second (meningoencephalitic) stage of the disease. In the absence of reliable blood tests able to detect CNS invasion by the parasite, HAT staging relies on the CSF examination.

It must be stated that the efficiency associated with implementing accurate diagnostic tools in HAT control programs based on active case finding can be offset by other crucial determinants such as a low attendance rate of the population or an insufficient proportion of patients completing treatment with subsequent cure.

Biological Parameters:

Biological blood parameters such as increased sedimentation rate and low hematocrit reflect the systemic chronic inflammation present in HAT patients and are therefore nonspecific.

Thrombocytopenia is generally mild or absent, and features of disseminated intravascular coagulopathy are not found. Liver transaminase levels, bilirubin, and renal function tests are usually within normal limits or slightly elevated. Protein measurements usually show decreased albumin and increased immunoglobulin concentrations, especially of IgM. Low serum C3 levels and split C3 products can be found, reflecting complement activation. These findings are of little use in most field settings, where only the erythrocyte sedimentation rate and the hematocrit can be measured.

Antibody Detection:

Indirect evidence for trypanosome infection can be obtained by demonstrating specific antibodies in the blood, plasma, or serum of infected hosts. Trypanosomes have a complex antigenic structure and elicit the production of a large spectrum of antibodies. T. b. gambiense specific IgG and IgM antibodies are present in high concentrations and are directed mainly against the immunodominant surface glycoprotein antigens of the parasite. The type of antigen(s) used greatly determines the sensitivity and specificity of the test. Current serological tests detect antibodies after 3 to 4 weeks of infection. Seropositivity must be interpreted with caution in previously treated patients since antibodies can persist for up to 3 years after cure.

CATT/T. b. gambiense:

Although not registered by any regulatory agency, the introduction of the CATT/T. b. gambiense (CATT) for mass population screening has been a major breakthrough in the diagnosis of T. b. gambiense HAT, limiting the number of parasitological examination to patients found with a positive serology. Developed in the late 1970s, the CATT is a fast and simple agglutination assay for detection of T. b. gambiense-specific antibodies in the blood, plasma, or serum of HAT patients. The antigen consists of lyophilized bloodstream forms of T. b. gambiense variable antigen type LiTat 1.3. Antigen production is a fastidious process based on the extraction of trypanosomes from infected rat blood. The trypanosomes are then fixed, stained with Coomassie blue, and freeze-dried. The reagent, which is produced under full quality control, is currently made only at the Institute of Tropical Medicine in Antwerp, Belgium, and field kits containing the reagent, control sera, and a 12/220-V card rotator are available. One drop of reagent is mixed with one drop of blood and shaken for 5 min on the rotator, and the result is visible to the naked eye. Up to 10 patients can be tested at the same time, and hundreds of individuals can be screened daily. The reported sensitivity of the CATT on undiluted whole blood (CATT-wb) varies from 87 to 98%, and the negative predictive value is excellent during mass population screening. Nevertheless, false-negative CATT results can occur, as suspected in patients infected with strains of trypanosomes that lack or do not express the LiTat 1.3 gene. This could explain the lower sensitivity of the CATT in some areas of endemic infection such as the Ethiope focus in Nigeria, where an alternative serological test should be used. Furthermore, when the CATT is performed on undiluted blood or serum with low dilution (<1:4), the agglutination can be inhibited, a phenomenon called prozone. To overcome this problem, which is caused by complement factors and affects the sensitivity of the test, addition of EDTA to the dilution buffer has been proposed, substantially increasing the sensitivity with only a minor loss in specificity (. The CATT buffer supplemented with EDTA can remain stable for at least 2 years at 45°C.

Despite a reported specificity of around 95%, the positive predictive value of the CATT-wb remains limited because the test is used for mass screening in populations where the prevalence of HAT is usually below 5%. False-positive results can occur in patients with malaria and other parasitic diseases such as transient infection by nonhuman trypanosomes. The specificity of the CATT is further improved when performed on serum diluted to 1:4. This remains insufficient for diagnostic confirmation but allows a significant gain of time and financial resources by decreasing the number of parasitological investigations.

The CATT can be performed with blood-impregnated filter paper (FP). This method is particularly useful for screening individuals who cannot be reached by full mobile teams during active case finding. The micro-CATT, a protocol using small quantities of both antigen (ca. one-fifth of the standard amount) and FP eluate (sample), showed promising results in Côte d'Ivoire. The major constraint for widespread use of the micro-CATT is the rapid decrease in sensitivity when FP are stored for more than 1 day at ambient temperature. Moreover, due to the minute volumes of antigen and test sample used, reading and interpretation of the agglutination patterns can be difficult with the micro-CATT. A recently modified method, the macro-CATT, was developed for testing blood-impregnated FP by using a standard amount of antigen and a higher volume of FP eluate. The macro-CATT was evaluated in southern Sudan and showed a sensitivity of 91% and excellent stability when FP were stored for up to 2 weeks at ambient temperature (25 to 34°C).

Other serological tests. The LATEX/T. b. gambiense has been developed as a field alternative to the CATT. The test is based on the combination of three purified variable surface antigens, LiTat 1.3, 1.5, and 1.6, coupled with suspended latex particles. The test procedure is similar to the CATT, including the use of a similar rotator. Compared to the CATT, the LATEX/T. b. gambiense showed a higher specificity (96 to 99%) but a lower or similar sensitivity (71 to 100%) in recent field studies conducted in several Western and Central African countries. Further evaluations are needed before it can be recommended for routine field use.

Immunofluorescence assays have been used with success for HAT control in Equatorial Guinea, Gabon, and the Republic of Congo, where they were shown to be highly sensitive and specific. The availability of standardized antigen at low cost has greatly improved the reliability of the test. It can be used with serum or FP eluates, but the test sensitivity has been reported to be as low as 75% when used with impregnated FP. Enzyme-linked immunosorbent assay (ELISA) methods can be performed with serum, FP eluates, and CSF with strict standardization and quantification. Interestingly, ELISA could also detect specific antibodies in the saliva from a group of 23 patients with confirmed HAT. Antibody levels were about 250-fold lower than in the serum and could not be detected by the CATT or the LATEX/T.b.gambiense in the vast majority of these patients. The sophisticated equipment required for IFA and ELISA methods limits their use to reference laboratories for remote testing of samples collected in the field during surveys.

Trypanosome Detection:

Parasitological diagnosis is made by microscopic examination of lymph node aspirate, blood, or CSF. It provides direct evidence for trypanosome infection and thus allows a definite diagnosis.

Unfortunately, parasite numbers in T. b. gambiense infection can vary between more than 10,000 trypanosomes/ml, being easily detectable, and less than 100 trypanosomes/ml, being below the detection limit of the most sensitive methods in use. Moreover, parasite detection can be rather labor-intensive. Failure to demonstrate parasites therefore does not necessarily exclude infection. Serial examination of blood on consecutive days can increase the test sensitivity but is rarely performed in practice. When possible, it is recommended to use methods that test a larger quantity of blood and/or that facilitate trypanosome visualization to improve the sensitivity of parasite detection. It is also essential to keep the time between sampling and examination as short as possible to avoid immobilization and subsequent lysis of trypanosomes in the sample. Trypanosomes are rapidly killed by direct sunlight but can survive for a few hours when the sample is kept in a cool and dark place. We review here the main parasite detection methods that are currently available for field use. A detailed description of most of these tests, including figures, can be found in the WHO Trypanosomiasis Control Manual.

Chancre aspirate:

Trypanosomes can be detected in the chancre a few days earlier than in the blood. The chancre is punctured, and the fluid obtained is microscopically examined as a fresh or fixed and Giemsa-stained preparation. This method is very seldom applied in the field because most infections are detected much later, when the chancre has already disappeared.

Lymph node aspirate:

CLN palpation should be done systematically in all patients with a positive CATT result. When enlarged CLN are present, they are punctured, the fresh aspirate is expelled onto a slide, and a coverslip is applied to spread the sample and facilitate the reading. The wet preparation is then quickly examined by microscope (magnification, ×400) for the presence of motile trypanosomes. The technique is simple and cheap. The sensitivity varies between 40 and 80% depending on the parasite strain, the stage of the disease (sensitivity is higher during the first stage), and the prevalence of other diseases causing lymphadenopathy.

The yield of CLN palpation and puncture in patients with a negative CATT is very low; between 1999 and 2001, trypanosomes were observed in only 316 (0.18%) of 174,295 lymph node aspirates from CATT-negative individuals in Democratic Republic of Congo. The authors calculated that a mean of 138 h of work per new case diagnosed would be necessary. Furthermore, all positive lymph node aspirates found in 1,000 individuals from two endemic foci located in Angola and Central African Republic were associated with positive CATT. Systematic CLN palpation and puncture is therefore not cost-effective for use with CATT-negative individuals unless indicative clinical signs are present.

Wet and thick blood films:

In wet blood films, 5 to 10 µl of finger prick blood is placed on a slide and examined microscopically (magnification, ×400) under a coverslip. Trypanosomes can be seen moving between the erythrocytes (the movement of the surrounding erythrocytes often attracts attention). Despite its very low sensitivity, with a detection limit as high as 10,000 trypanosomes/ml,

corresponding to 1 parasite/200 microscope fields, this method is still used in some centers because of its low cost and simplicity. Giemsa- or Field's-stained thin blood films have a similarly low sensitivity. Examination of 20 µl of stained blood in a thick blood film slightly improves sensitivity, with a detection threshold of around 5,000 trypanosomes/ml. It is the technique of choice for blood examination only when no centrifuge is available, although it is quite time-consuming (10 to 20 min per slide) and requires expertise to recognize the parasite, which is frequently deformed in this preparation. Apart from trypanosomes, other parasites such as microfilaria and Plasmodium can be detected.

Microhematocrit centrifugation technique:

The blood concentration technique of microhematocrit centrifugation (mHCT), sometimes referred to as the capillary tube centrifugation technique or as the Woo test, was developed more than 30 years ago and is still in use in many HAT control programs. In brief, capillary tubes containing anticoagulant are filled three-quarters full with finger prick blood. The dry end is sealed with plasticine. By high-speed centrifugation in a hematocrit centrifuge for 6 to 8 min, trypanosomes are concentrated at the level of the white blood cells, between the plasma and the erythrocytes. The capillary tubes, mounted in a special holder, can be directly examined at low magnification (×100 or ×200) for mobile parasites. The sensitivity of mHCT increases with the number of tubes examined, with an estimated detection threshold of 500 trypanosomes/ml. The optimal number of tubes has not been determined with certainty, but in most programs, six to eight tubes are prepared. This technique is moderately time-consuming, and the concomitant presence of microfilaria in the blood can render the visualization of the much smaller trypanosomes very difficult. Nevertheless, this relatively simple technique can be applied during mass screening by mobile teams.

Quantitative buffy coat:

The quantitative buffy coat (QBC; Beckton-Dickinson), initially developed for the rapid assessment of differential cell counts, has been extended to the diagnosis of hemoparasites including trypanosomes. It has the advantages of concentrating the parasites by centrifugation and, by staining the nucleus and kinetoplast of trypanosomes with acridine orange, allowing a better discrimination from white blood cells. After high-speed centrifugation of the blood in special capillary tubes containing EDTA, acridine orange, and a small floating cylinder, motile trypanosomes can be identified by their fluorescent kinetoplasts and nuclei in the expanded buffy coat. UV light is generated by a cold light source connected by a glass fiber to a special objective containing the appropriate filter. This objective can be mounted on almost every microscope. A darkroom is needed for the procedure. The relative sophistication and fragility of the material prevents its daily transport during active screening sessions.

The QBC is a very sensitive technique that is very appreciated by most field laboratory workers. It also allows the diagnosis of concomitant malaria, which is very useful for patient care. With a 95% sensitivity for trypanosome concentrations of 450/ml, the QBC can detect more patients with low parasitemia than the mHCT when fewer than eight capillary tubes are used. It is as sensitive as the mini-anion-exchange centrifugation technique (mAECT) described below. Production of the QBC kit has been abandoned, but the manufacturing of capillaries was recently resumed.

Mini-anion-exchange centrifugation technique:

The mAECT was introduced by Lumsden et al., based on a technique developed by Lanham and Godfrey. An initial evaluation showed that the mAECT was more sensitive than the thick blood film and the mHCT. An updated version has been described by Zillmann et al. The technique consists of separating the trypanosomes, which are less negatively charged than blood cells, from venous blood by anion-exchange chromatography and concentrating them at the bottom of a sealed glass tube by low-speed centrifugation. The tip of the glass tube is then examined in a special holder under the microscope for the presence of trypanosomes. The large blood volume (300 µl) enables the detection of less than 100 trypanosomes/ml, resulting in high sensitivity, but the manipulations are quite tedious and time-consuming. mAECT columns are now produced with the assistance of the Institute of Tropical Medicine—Antwerp at Kinshasa, DRC. Studies validating this newly produced version of mAECT are under way.

Stage Determination: Cerebrospinal Fluid Examination:

In the absence of sufficiently specific clinical signs and blood tests indicating the evolution from first- to second-stage HAT, staging of patients still relies on examination of CSF obtained by lumbar puncture. It is a vital step in the diagnosis process. Patients with first-stage disease receive daily intramuscular pentamidine for 7 to 10 days, a treatment associated with less than 1% mortality, whereas patients with second-stage disease are still treated in most centers with melarsoprol, an arsenical derivative associated with a 2 to 10% fatality rate. The majority of deaths are due to treatment-related acute encephalopathies. Eflornithine (DFMO) is a safer treatment than melarsoprol, but its complicated schedule (four intravenous infusions per day for 14 days) and cost remain an obstacle to a wide field application.

According to WHO recommendations, second-stage HAT is defined by the presence in the CSF of one or more of the following: (i) raised white blood cell count (>5 cells/µl), (ii) trypanosomes, and (iii) increased protein content (>370 mg/liter, as measured by the dye-binding protein assay). As reviewed below, these criteria are not entirely satisfying and might soon be modified by recent studies' findings.

White blood cell count:

The CSF white blood cell count is the most widely used technique for stage determination. After collecting the CSF sample, the cell count should be carried out as soon as possible to prevent cell lysis. Due to the small number of cells in normal CSF, cell-counting chambers should have a volume of at least 1 µl, such as the Fuchs-Rosenthal and the Neubauer devices. It is not

recommended to dilute the CSF with Türck solution since this solution can lyse trypanosomes. If fewer than 20 cells/µl are counted, it is recommended to repeat the counting procedure and to calculate mean count values. The CSF pleocytosis is of lymphocytic origin, consisting mainly of B cells.

The 5-cells/µl threshold for treatment decision is controversial. Some countries use a threshold of 10 cells/µl (Equatorial Guinea) or even 20 cells/µl (Angola and Côte d'Ivoire) in their national protocol. Patients with 6 to 20 cells/µl in the CSF are sometimes referred to as being in the "early second stage" or "intermediate stage" of the illness. This group is in fact composed of individuals with or without signs of neuroinflammation, as recently demonstrated. This is further illustrated by the effectiveness of pentamidine in HAT patients with 6 to 20 cells/µl of CSF in Côte d'Ivoire, while in a study in Uganda, patients with 11 to 20 cells/µl or with evidence of intrathecal IgM synthesis (a reliable marker of neuroinflammation) had a lower cure rate, suggesting that these patients should be treated with DFMO or melarsoprol. In a smaller study in Angola, the relapse rate after pentamidine treatment was similar in patients with 0 to 5 or 6 to 10 cells/µl. These data support the increase of the CSF cell threshold from 5 to 10 cells/µl. Furthermore, one should take into account the higher normal cell counts in neonates.

There is a general agreement that patients with proven HAT (trypanosomes seen in the lymph node or blood) and with >20 cells/µl in CSF should be treated as having second-stage HAT. In Médecins Sans Frontières and Malteser programs in Sudan and Uganda, serologically suspected individuals (positive CATT of 1:4) with negative parasitological examination and >20 cells/µl in the CSF are treated as second-stage HAT patients. This approach aims at partially compensating the insufficient sensitivity of trypanosome detection but exposes some non-HAT patients to unnecessary treatment for second-stage illness. It can be justified in areas with high HAT prevalence, especially where DFMO, a safer drug than melarsoprol, is used as the first-line treatment. Here again, the availability of more sensitive parasite detection methods and more precise staging tools would solve the controversy.

The morular cells of Mott, which are plasma cells with large vacuoles containing IgM, are reported to be highly indicative of HAT when found in the CSF. Mott cells are rarely observed in the field and can also be found in other neuroinfectious diseases such as neurosyphilis.

Trypanosome detection:

The finding of trypanosomes in CSF allows immediate classification of a patient as being in the second stage of illness. It is important to examine the CSF immediately after lumbar puncture, because trypanosomes in CSF start to lyse within 10 min. Direct detection of trypanosomes (e.g., during cell counting) is a simple and cheap technique but suffers from insufficient sensitivity. Increased sensitivity of trypanosome detection is obtained by centrifugation of the CSF sample, especially when a double centrifugation method is used. The latter method is relatively time-consuming and requires two different types of centrifuges; therefore, it is not applicable in every field setting. A modified and simplified single centrifugation of CSF using a sealed Pasteur pipette has been proposed as an alternative to double centrifugation.

Some authors challenge the value of finding CSF trypanosomes in patients with no sign of CSF inflammation (absence of elevated protein and cell count of ≤20/µl in the CSF) who were shown to respond to pentamidine treatment.

Protein concentration:

In normal healthy individuals, proteins in the CSF consist mainly of albumin (70%) and IgG (30%), both originating from the serum. Protein concentrations in the CSF are elevated in HAT patients and range from 100 to 2,000 mg/liter. Protein concentrations can also be raised in first-stage illness due to the diffusion in the CSF of IgG, which can be present in very high concentrations in the serum. Recent evidence suggests that the protein concentration threshold set by WHO (370 mg/liter) is too low and should be raised to 750 mg/liter to reflect blood-brain barrier impairment, astrocyte activation, and neurodegeneration. Despite its apparent simplicity, accurate determination of the total protein concentration in CSF is rather difficult. CSF protein concentrations obtained by different methods and different standards are not comparable. As a consequence of the sophistication of the methods, the absence of standardization, the instability of reagents, and the limited (if any) added value compared to CSF cell count, total protein measurement for staging HAT is no longer recommended and has been virtually abandoned in field laboratories.

Antibody detection:

It has been well known for several decades that the CSF of second-stage HAT patients contains high levels of immunoglobulins, especially IgM. An increased CSF IgM concentration has thus been considered by some as a strong potential marker of second-stage HAT.

The demonstration of intrathecal synthesis of immunoglobulins strongly supports the diagnosis of neuroinflammatory diseases. Immunoglobulins synthesized in the CNS need to be discriminated from blood-derived immunoglobulins by calculation of the intrathecal fraction and antibody index (quantitative approach) or by detection of oligoclonal antibodies (qualitative approach). The origin and composition of the CSF immunoglobulins have been recently studied in experiments with large patients groups. As previously suspected, these studies confirmed that the elevated immunoglobulin concentration in the CSF is due to intrathecal synthesis and that the dominant IgM presence was an early marker of CNS invasion whereas blood-CSF barrier dysfunction was found late in the course of CNS involvement. These results were further confirmed by studies of 272 HAT patients from different areas of endemic infection, where intrathecal synthesis of IgM was found in 95% of patients with second-stage illness. T. b. gambiense HAT can thus be classified among neuroinflammatory diseases with a dominant IgM immune response pattern in the CNS, like Lyme neuroborreliosis and, occasionally, neuro-syphilis.

Despite its relevance to stage determination, IgM detection in CSF has not been carried out in the field, owing to the lack of simple and robust tests. A latex agglutination test for IgM in CSF (LATEX/IgM) has recently been developed. It is designed for field use and remains stable at

45°C for more than 2 years. Following initial promising results, the LATEX/IgM was evaluated with CSF samples from patients from several countries where infection is endemic. CSF end titers obtained by the LATEX/IgM paralleled the IgM concentrations determined by nephelometry and ELISA. At a cutoff value of ≥1:8, the sensitivity and specificity of LATEX/IgM for intrathecal IgM synthesis were 89 and 93%, respectively. Future prospective studies with large numbers of patients are needed for LATEX/IgM validation.

Only a small proportion of the very large amount of IgM produced during HAT is specific anti-trypanosome antibody. Trypanosome-specific antibodies detected in the CSF by indirect immunofluorescence are specific for second-stage illness, whereas a comparison with serum values by calculation of the antibody index is necessary when measurements are performed by ELISA. However, these methods are too sophisticated to be used in remote treatment centers. Unfortunately, the field-designed CATT/T. b. gambiense and LATEX/T. b. gambiense lack sensitivity when used with CSF for detection of anti-trypanosome immunoglobulins.

The CSF of HAT patients also contains antibodies with other affinities. Antibodies against brain-specific components such as neurofilaments and galactocerebrosides (GalC) have been detected and may be promising markers of second-stage illness. These autoantibodies, which might result from the CNS damage and immune activation triggered by trypanosome invasion, are associated with markers of neuroinflammation such as the CSF cell count and protein and immunoglobulin concentrations. Unfortunately, anti-GalC antibodies detectable in the serum are not correlated with neuroinflammation.

Antigen detection tests:

Antigen detection is an attractive concept that would allow, unlike methods detecting antibodies, a distinction between active and cured HAT. By detecting antigens released by noncirculating trypanosomes sequestered in the liver, spleen, lymph nodes, or CNS, antigen detection has the potential to improve the sensitivity of current parasitological methods. Following promising results of specific antigen detection by ELISA, the card indirect agglutination test for trypanosomiasis (TrypTect CIATT; Brentec Diagnostics, Nairobi, Kenya) was developed for field use. A preliminary evaluation of the TrypTectCIATT showed a high sensitivity compared to other parasitological techniques, but the results of subsequent studies raised strong doubts about its specificity.

In vitro culture and animal inoculation:

When inoculated into a suitable medium, viable trypanosomes multiply but are not detected for days or weeks. A ready-for-use kit for in vitro isolation (KIVI) has been developed. After inoculation of 5 to 10 ml of blood, the bottles are kept at ambient temperature. Bloodstream-form trypanosomes transform into proliferating large procyclic forms that can be detected within 3 to 4 weeks. The recorded sensitivity of the KIVI is variable. The yield of isolation of T. b. gambiense in rodents is low unless neonatal, immunosuppressed, or particular (e.g., Mastomys natalensis) rodents are used. The high cost and the long delay before obtaining the result are definite obstacles to routine field use of these techniques.

PCR:

Different assays now exist; however, none of them have been validated for diagnostic purposes. PCRs targeting repetitive sequences are in theory more sensitive than those targeting low-copy or single-copy sequences like the recently developed tests for distinguishing T. b. gambiense and T. b. rhodesiense.

In principle, PCR can be applied to any patient sample that may contain trypanosome DNA, such as whole blood or buffy coat, lymph node fluid, or CSF. Samples should be stabilized in special buffers or on FP. The FTA FP produced by Whatman is particularly convenient since it is easy to handle and it protects the DNA from degradation, unlike common FP. However, the amount of sample that can be applied on filter paper is small, thus limiting the chance to contain enough DNA for detection. Samples should be protected from sunlight to avoid DNA degradation. PCR results are not always unequivocal. Unexplained false-negative and false-positive results were observed in CATT-seropositive but parasitologically nonconfirmed persons and in CATT-negative controls. Also, the significance of a positive PCR on a CSF sample is unclear. True et al. report that PCR is 100% sensitive compared to double centrifugation of CSF, but Jamonneau et al. clearly demonstrate that a number of patients with positive PCR results with CSF were successfully treated with pentamidine, thus showing them to be in the first stage of the disease. Efforts to simplify the PCR amplification method itself, such as using an isothermal amplification reaction and visualization of the PCR product by precipitation or by oligochromatography, may facilitate PCR application in African countries, but PCR is definitely not an option for field diagnosis and for the time being is restricted to research purposes.

Proteomic signature analysis:

Proteomic signature analysis is a promising technology that has been recently used with sera from patients with HAT and other diseases. The accuracy of this experimental method was high (100% sensitivity and 98.6% specificity) but needs to be confirmed in prospective studies. As the authors stated, this method is impracticable in the field but could identify discriminating biomarkers that could lead to the development of more conventional and simpler tests.

Imaging techniques:

Neuroimaging techniques such as computed tomography and magnetic resonance imaging are not available in areas of endemic infection, except in few referral centers. They reveal nonspecific features that are occasionally useful to stage the disease. Brain magnetic resonance imaging may show diffuse white matter abnormalities, ventricular enlargement, hyperintensities in the basal ganglia, and signs of meningitis.

LABORATORY DIAGNOSIS OF T. B. RHODESIENSE INFECTION:

As stated above, T. b. rhodesiense HAT usually presents as an acute febrile illness with rather nonspecific symptoms and signs unless the initial characteristic chancre is present. Biological

parameters are generally more abnormal than with T. b. gambiense illness. Anemia may be severe, at least partly due to decreased red cell survival. Thrombocytopenia may also be severe and can be accompanied by other features of disseminated intravascular coagulation.

There is still no equivalent to the CATT widely available for the screening of T. b. rhodesiense HAT, despite promising results from initial evaluations of simple agglutination tests such as the trypanosomiasis agglutination card test, the procyclic agglutination test for trypanosomiasis, or a modified and more practical version. Immunofluorescence and ELISA-based serological tests exist, but their reported sensitivity is variable and they can be performed only in reference centers with highly trained technicians. Field screening thus still relies on clinical symptoms and signs. The diagnostic confirmation and staging of T. b. rhodesiense HAT are based on the same methods as described above for T. b. gambiense disease. Parasitological confirmation is relatively easy because bloodstream trypanosomes are numerous. Most patients are therefore diagnosed by examination of a stained thin or thick blood smear. Trypanosomes are sometimes detected by chance while searching for Plasmodium spp. Trypanosomes can also be found in the chancre by microscopic examination of an aspirate, but other methods such as lymph node aspiration or blood concentration techniques are needed less often. Unlike T. b. gambiense HAT, the staging of T. b. rhodesiense illness has not been recently studied and thus still relies on WHO recommendations.

T. b. rhodesiense infections have a more chronic clinical presentation at the southern end of their distribution (e.g., in Zambia), with clinical characteristics closer to those of T. b. gambiense illness. A practical and reliable serological test would be a useful screening tool in these areas to detect infected individuals with few or no symptoms, since simple parasitological methods such as the thick blood smear appears to have a more limited sensitivity in these regions.

There are currently no areas of endemicity where T. b. gambiense and T. b. rhodesiense coexist. Endemic foci of both forms exist in Uganda but are geographically separated. Evidence of a northward spread of cattle carrying T. b. rhodesiense has been recently shown. A merger of T. b. gambiense and T. b. rhodesiense foci would create huge problems, since the diagnosis and treatment of these two forms of illness differ considerably. Molecular markers such as the serum resistance-associated gene, present only in T. b. rhodesiense, and a receptor-like flagellar pocket glycoprotein (TgsGP) specific to T. b. gambiense, may play a role in closely monitoring the distribution of both trypanosome species. The development of a simple assay detecting the circulating serum resistance-associated protein would not only be useful for targeting treatment to cattle infected with T. b. rhodesiense but would also be valuable to distinguish the trypanosome species in humans exposed to both forms of HAT.

Over the last few decades, only minor improvements have been achieved in field diagnosis of HAT. Parasite detection remains insufficiently sensitive, and a simple serological diagnostic test for T. b. rhodesiense infection is still lacking. In contrast, extensive research has been conducted on stage determination and molecular diagnosis, which eventually may lead to a new generation of field-applicable tests. Since the prevalence of HAT is expected to decrease as a result of ongoing control activities, new diagnostic tests should be highly specific. In the meantime, major diagnostic challenges for the coming years are to improve the access of the existing tools for the

population at risk and their rational use in the diagnostic strategy. All this will require determined dedication from scientists from countries with endemic infection and Western countries, WHO, nongovernmental organizations, policy makers, and donors. [5]

Toxoplasmosis

Toxoplasmosis (tok-so-plaz-MOE-sis) is a disease that results from infection with the Toxoplasma gondii parasite, one of the world's most common parasites. Infection usually occurs by eating undercooked contaminated meat, exposure from infected cat feces, or mother-to-child transmission during pregnancy.

Toxoplasmosis may cause flu-like symptoms in some people, but most people affected never develop signs and symptoms. For infants born to infected mothers and for people with weakened immune systems, toxoplasmosis may cause serious complications. [(6)]

The disease is caused by Toxoplasma gondii, one of the most successful parasites worldwide, as it can infect all warm blooded animals. It is very small, consisting only of a single cell and is transmitted through ingestion of oocysts shed by infected cats or by the consumption of undercooked infected meat with cysts containing bradyzoites. Contamination of the environment (farmland, gardens, rivers and coastal waters) by Toxoplasma oocysts is widespread and the parasite can survive for more than 18 months in cool and moist conditions.

Toxoplasma gondii is one of the most important causes of abortion in sheep and goats worldwide and in the UK it is the second most frequently diagnosed cause of ovine abortion. More dun was involved in conducting the efficacy testing of the only commercially available vaccine worldwide to protect against ovine toxoplasmosis, using a live attenuated “incomplete” strain of T. gondii.

Disease in humans, due to T. gondii infection, is usually attributed to infections of the foetus if the mother is infected with the parasite for the first time during pregnancy. Immunosuppressed individuals can also suffer serious disease as a result of Toxoplasma infection where problems can arise from a recrudescence of a previous latent infection. [(7)]

The reason that pregnant woman should take special precautions to avoid toxoplasmosis is that it can be very serious, even fatal, for a baby infected in the uterus. For those who survive, toxoplasmosis can have lasting consequences on the:

- brain
- eyes
- heart
- lungs

  They may also have mental and physical developmental delays and recurring seizures. In general, babies who are infected early during the pregnancy suffer from more severe issues than those infected later in the pregnancy. Babies born with toxoplasmosis may have a higher risk of hearing and vision losses. Some children may be affected with learning disabilities. [(8)]

The diagnosis of T. gondii infection or toxoplasmosis may be established by serologic tests, amplification of specific nucleic acid sequences (i.e., polymerase chain reaction [PCR]), histologic demonstration of the parasite and/or its antigens (i.e., immunoperoxidase stain), or by isolation of the organism. Other rarely used methods include demonstration of antigenemia and

antigen in serum and body fluids, a toxoplasmin skin test, and antigen-specific lymphocyte transformation.

Serologic Tests:

The use of serologic tests for demonstration of specific antibody to T. gondii is the initial and primary method of diagnosis. Different serologic tests often measure different antibodies that possess unique patterns of rise and fall with time after infection. A combination of serologic tests is usually required to establish whether an individual has been most likely infected in the distant past or has been recently infected. The clinician and clinical laboratories must be familiar with these problems and consult reference laboratories if the need arises.

A panel of tests (the Toxoplasma Serological Profile [TSP]) consisting of the Sabin-Feldman dye test (DT), double sandwich IgM ELISA [4], IgA ELISA, IgE ELISA, and AC/HS test has been used successfully by our group to determine if serologic test results are more likely consistent with infection acquired in the recent or more distant past. The AC/HS test is interpreted as previously described by comparing IgG titers obtained with formalin-fixed tachyzoites (HS antigen) with those obtained with acetone-fixed tachyzoites (AC antigen).

The TSP has been successfully used in the setting of toxoplasmic lymphadenitis, myocarditis, polymyositis, and chorioretinitis and during pregnancy. For sera with positive results in IgG and IgM tests, the discriminatory power of the TSP to differentiate between recently acquired infection and chronic infection is probably superior to any other single serologic test.

Current interpretation of results in the TSP at the Toxoplasma Serology Laboratory at the Palo Alto Medical Foundation Research Institute (TSL-PAMFRI) is as follows: Sera that are positive in the DT, negative in the IgM, IgA, and IgE ELISAs, and reveal a chronic pattern in the AC/HS test are typically found in patients infected in the most distant past. The combination of high titers in the DT, positive IgM, IgA, and IgE ELISAs, and an acute pattern in the AC/HS test is highly suggestive of a recently acquired infection. In contrast, the presence of positive DT and IgM ELISA results but a negative, low-positive, or equivocal result in the IgA and IgE ELISAs and an equivocal pattern in the AC/HS test is more difficult to interpret. In the latter setting, a follow-up sample is usually obtained, the 2 samples are run in parallel, and the serologic test titer results are compared. If the titers obtained in the 2 samples do not change significantly, the infection is most likely to have been acquired in the distant past. In contrast, significant changes (rise or decline) detected in the titers of the 2 samples are considered to be suggestive of a recently acquired infection.

IgG antibodies. The most commonly used tests for the measurement of IgG antibody are the DT, the ELISA, the IFA, and the modified direct agglutination test. In these tests, IgG antibodies usually appear within 1–2 weeks of acquisition of the infection, peak within 1–2 months, decline at various rates, and usually persist for life.

When two different compounds (i.e., acetone and formalin) are used to fix parasites for use in the agglutination test, a “differential” agglutination test (also known as the “AC/HS test”) results due to the fact that the different antigenic preparations vary in their ability to recognize sera obtained

during the acute and chronic stages of the infection. This test has proved useful in helping to differentiate acute from chronic infections but is best used in combination with a panel of other tests (e.g., the TSP).

Recently, a number of tests for avidity of Toxoplasma IgG antibodies have been introduced to help discriminate between recently acquired and distant infection. It has been observed that the functional affinity of specific IgG antibodies is initially low after primary antigenic challenge and that it increases during subsequent weeks and months by antigen-driven B cell selection. Protein-denaturing reagents including urea are used to dissociate the antibody-antigen complex. The avidity result is determined using the ratios of antibody titration curves of urea-treated and untreated samples.

IgM antibodies. IgM antibodies may appear earlier and decline more rapidly than IgG antibodies. The most commonly used tests for the measurement of IgM antibody are double-sandwich or capture IgM-ELISA kits, the IFA test, and the immunosorbent agglutination assay (IgM-ISAGA; available from bioMérieux). False-positive results due to rheumatoid factor and antinuclear antibodies in some IgM-IFA tests are not detected by the most commonly used commercial double-sandwich or capture IgM-ELISAs. Despite the wide distribution of commercial test kits to measure IgM antibodies, these tests often have low specificity, and the reported results are frequently misinterpreted.

An IgM test is still used by most laboratories to determine if a patient has been infected recently or in the distant past, and because of the hurdles posed in interpreting a positive IgM test result, confirmatory testing should always be performed.

In patients with recently acquired primary infection, T. gondii— specific IgM antibodies are detected initially, and in most cases, these titers become negative within a few months. However, in some patients, positive T. gondii—specific IgM titers can still be observed during the chronic phase of infection. Some investigators have reported that IgM antibodies can be detected as long as 12 years after the acute infection. The persistence of these IgM antibodies does not appear to have any clinical relevance, and these patients should be considered chronically infected. Further complicating the interpretation of a positive IgM test result is the fact that several methods for its detection still may result in a relatively high frequency of false-positive results. Thus, a positive IgM test result in a single serum sample can be interpreted as a true-positive result in the setting of a recently acquired infection, a true-positive result in the setting of an infection acquired in the distant past, or a false-positive result.

IgA antibodies. IgA antibodies may be detected in sera of acutely infected adults and congenitally infected infants by use of ELISA or ISAGA. As is true for IgM antibodies to the parasite, IgA antibodies may persist for many months or more than a year. For this reason, they are of little additional assistance for diagnosis of acute infection in the adult. In contrast, the increased sensitivity of IgA assays over IgM assays for diagnosis of congenital toxoplasmosis represents an advance in diagnosis of the infection in the fetus and newborn. In a number of newborns with congenital toxoplasmosis and negative IgM antibodies, the serologic diagnosis has been established by the presence of IgA and IgG antibodies.

IgE antibodies. IgE antibodies are detectable by ELISA in sera of acutely infected adults, congenitally infected infants, and children with congenital toxoplasmic chorioretinitis. Their demonstration does not appear to be particularly useful for diagnosis of T. gondii infection in the fetus or newborn when compared with IgA tests. The duration of IgE seropositivity is briefer than that with IgM or IgA antibodies and hence appears useful for identifying recently acquired infections.

PCR

PCR amplification for detection of T. gondii DNA in body fluids and tissues has successfully been used to diagnose congenital, ocular, and cerebral and disseminated toxoplasmosis. PCR has revolutionized the diagnosis of intrauterine T. gondii infection by enabling an early diagnosis to be made, thereby avoiding the use of more invasive procedures on the fetus. PCR has enabled detection of T. gondii DNA in brain tissue, cerebrospinal fluid (CSF), vitreous and aqueous fluids, bronchoalveolar lavage (BAL) fluid, and blood in patients with AIDS.

Histologic Diagnosis

Demonstration of tachyzoites in tissue sections or smears of body fluid (e.g., CSF or amniotic or BAL fluids) establishes the diagnosis of the acute infection. It is often difficult to demonstrate tachyzoites in conventionally stained tissue sections. The immunoperoxidase technique, which uses antisera to T. gondii, has proven both sensitive and specific: It has been used successfully to demonstrate the presence of the parasite in the central nervous system (CNS) of AIDS patients. The immunoperoxidase method is applicable to unfixed or formalinfixed paraffin-embedded tissue sections. A rapid, technically simple, and under-used method is the detection of T. gondii in air-dried, Wright-Giemsa—stained slides of centrifuged (e.g., cytocentrifuge) sediment of CSF or of brain aspirate or in impression smears of biopsy tissue. Multiple tissue cysts near an inflammatory necrotic lesion probably establish the diagnosis of acute infection or reactivation of latent infection.

Isolation of T. gondii

Isolation of T. gondii from blood or body fluids establishes that the infection is acute. Attempts at isolation of the parasite can be performed by mouse inoculation or inoculation in tissue cell cultures of virtually any human tissue or body fluid.

Diagnosis of Specific Clinical Entities

The first step in pursuing the diagnosis of T. gondii infection or toxoplasmosis is to determine whether the individual has been exposed to the parasite. In essentially all cases, any of the tests for the detection of IgG antibodies reliably establish the presence or absence of the infection. In a small number of patients, IgG antibodies might not be detected within 2—3 weeks after the initial exposure to the parasite; however, this is rare. In addition, rare cases of toxoplasmic chorioretinitis and toxoplasmic encephalitis in immunocompromised patients have been documented in patients with negative T. gondii—specific IgG antibodies.

The second step consists of establishing whether the patient has a recently acquired infection or an infection acquired in the more distant past. In general, a true-negative IgM test essentially rules out that the infection has been acquired in recent months. A positive IgM test is more difficult to correctly interpret. One must not assume that a positive IgM test result is diagnostic of recently acquired infection (see above under IgM antibodies in the Serologic Tests section). Confirmatory testing should be done for all cases for whom IgM test results are positive. Serologic tests should not be considered useful for measuring response to therapy.

The third step is to establish whether the patient's condition or illness is due to toxoplasmosis (recently acquired infection or recrudescence of latent infection) or is unrelated to the infection.

Toxoplasmosis in the Immunocompetent Patient:

The vast majority of cases of T. gondii infection in adults and children are asymptomatic. Lymphadenopathy is the most common manifestation in the 10%–20% percent of otherwise immunocompetent individuals whose primary T. gondii infection is symptomatic. Less common presentations in these patients include, but are not limited to, chorioretinitis, myocarditis, and/or polymyositis.

Tests for IgG and IgM antibodies should be used for initial evaluation of these patients. Testing of serial specimens obtained 3–4 weeks apart (in parallel) provides the best discriminatory power if the results in the initial specimen are equivocal. Negative results in both tests virtually rule out the diagnosis of toxoplasmosis. In rare instances early in infection, IgG antibodies may not be detectable, whereas IgM antibodies are present (hence the need for both tests to be performed). Acute infection is supported by documented seroconversion of IgG and IgM antibodies or a greater than four-fold rise in IgG antibody titer in sera run in parallel. A single high titer of any immunoglobulin is insufficient to make the diagnosis since IgG antibodies may persist at high titers for many years and IgM antibodies may be detectable for >12 months. The TSP, performed on a single serum sample, is useful in determining the likelihood that the infection is acute.

Characteristic histologic criteria and a TSP consistent with recently acquired infection establish the diagnosis of toxoplasmic lymphadenitis in older children and adults.

Endomyocardial biopsy and biopsy of skeletal muscle has been successfully used to establish T. gondii as the etiologic agent of myocarditis and polymyositis in immunocompetent patients. Isolation studies and PCR have rarely proven useful for diagnosis in immunocompetent patients.

Ocular Toxoplasmosis

Toxoplasmic chorioretinitis may result from congenital or postnatally acquired infection. In both of these situations, lesions may occur during the acute or latent (chronic) stage of the infection.

Low titers of IgG antibody are usual in patients with active chorioretinitis due to reactivation of congenital T. gondii infection; IgM antibodies usually are not detected. When sera from such patients are examined by use of the DT, titers should be first determined with undiluted serum since in some cases, the conventional initial dilution of 1:16 may be negative.

In most cases, toxoplasmic chorioretinitis is diagnosed by ophthalmologic examination, and empiric therapy directed against the organism is often instituted on the basis of clinical findings and serologic test results. In a number of patients, the morphology of the retinal lesion(s) may be non-diagnostic and/or the response to treatment is suboptimal. In such cases (unclear clinical diagnosis and/or inadequate clinical response), detection of a local and increased T. gondii antibody response in ocular fluids (immune load), demonstration of the parasite by isolation or histopathology, or amplification of T. gondii DNA (in both aqueous and vitreous fluids) have been used successfully to establish the diagnosis.

Toxoplasmosis in the Immunodeficient Patient

In contrast to the relatively favorable course of toxoplasmosis in almost all immunocompetent individuals, immunologically impaired patients usually develop a dreadful and often life-threatening disease. Immunocompromised patients at higher risk for toxoplasmosis include those with hematologic malignancies (particularly patients with lymphoma), bone marrow transplant, solid organ transplant (including heart, lung, liver, or kidney), or AIDS.

Toxoplasmic encephalitis is the most common presentation of toxoplasmosis in immunocompromised patients and is the most frequent cause of focal CNS lesions in AIDS patients. It is unclear whether T. gondii penetrates the brain more easily than other organs or whether it is more difficult for the brain, as an immunologically privileged site, to eradicate the organism during the initial acute infection and once residual infection has been established. A wide range of clinical findings, including altered mental state, seizures, weakness, cranial nerve disturbances, sensory abnormalities, cerebellar signs, meningismus, movement disorders, and neuropsychiatric manifestations are observed in patients with toxoplasmic encephalitis. Other organs commonly involved in immunocompromised patients with toxoplasmosis are the lungs, eyes, and heart.

In the vast majority of immunocompromised patients, toxoplasmosis results from reactivation of a latent infection. In contrast, in heart transplant patients and in a small number of other immunocompromised patients, the highest risk of developing disease is in the setting of primary infection (i.e., a seronegative recipient who acquires the parasite from a seropositive donor via a graft).

Because reactivation of chronic infection is the most common cause of toxoplasmosis in patients with malignancies or AIDS or in recipients of organ transplants (other than heart transplants), initial assessment of these patients should routinely include an assay for T. gondii IgG antibodies. Those with a positive result are at risk of reactivation of the infection; those with a negative result should be instructed on how they can prevent becoming infected.

When toxoplasmosis is suspected in immunocompromised patients chronically infected with the parasite (those with documented positive T. gondii—specific IgG antibody prior to the onset of immunosuppression) additional serologic testing adds very little (or may be misleading) to the diagnostic evaluation. In these patients, results indicating apparent reactivation (rising IgG and IgM titers) may be present in the absence of clinically apparent infection. In addition, serologic test results consistent with chronic infection may be seen in the presence of toxoplasmosis. Thus,

for immunocompromised patients in whom toxoplasmosis is suspected, additional diagnostic methods to attempt to establish the diagnosis are strongly recommended. These methods include PCR amplification for detection of T. gondii DNA in blood or body fluids suspected of being infected, isolation of the parasite from blood or body fluids that may contain the parasite, and histologic examination of available tissues with T. gondii—specific stains, such as immunoperoxidase.

When clinical signs suggest involvement of the CNS and/or spinal cord, tests should include computed tomography or magnetic resonance imaging (MRI) of the brain and/or spinal cord. Neuroimaging studies of the brain should be considered even if the neurologic examination does not reveal focal deficits. Empiric anti—T. gondii therapy for patients with multiple ring enhancing brain lesions (usually established by MRI), positive IgG antibody titers against T. gondii, and advanced immunodeficiency (i.e., CD4 cell count of <200 cells/mm3) is accepted clinical practice; a clinical and radiologic response to specific anti—T. gondii therapy is considered as supportive of the diagnosis of CNS toxoplasmosis. Brain biopsy should be considered in immunocompromised patients with presumed CNS toxoplasmosis if there is a single lesion on MRI, a negative IgG antibody test result, or inadequate clinical response to an optimal treatment regimen or to what the physician considers to be an effective prophylactic regimen against T. gondii (e.g., trimethoprimsulfamethoxazole). If T. gondii serologic and radiologic studies do not support a recommendation for empiric treatment or are inconclusive and if brain biopsy is not feasible, a lumbar puncture should be considered if it is safe to perform; PCR can then be performed on the CSF specimen. CSF can also be used for isolation studies, although it is uncommon for T. gondii to be isolated from CSF from immunocompromised patients. Of note, PCR examination of CSF can also be used for detection of Epstein-Barr virus, JC virus, or cytomegalovirus DNA in patients in whom primary CNS lymphoma, progressive multifocal leukoencephalopathy, or cytomegalovirus ventriculitis, respectively, have been considered in the differential diagnosis.

T. gondii Infection in Pregnancy

T. gondii infection acquired during pregnancy may result in severe damage or death of the fetus and long-term sequelae in offspring. Because congenital toxoplasmosis results almost solely in women who acquire the infection during gestation, it is critical to determine whether infection during pregnancy has occurred. The incidence of congenital toxoplasmosis in the offspring of women infected prior to gestation has been shown to be extremely rare unless a woman is immunocompromised (e.g., receiving corticosteroids or immunosuppressive drugs or positive for human immunodeficiency virus [HIV]).

At present in the United States, definitive diagnosis of the acute infection and the time of its occurrence have been compromised by the lack of systematic screening and the fact that only a single serum sample is submitted for testing. When only 1 serum sample is available, tests to detect the presence of IgG and IgM antibodies are most commonly used to determine if a pregnant woman acquired acute infection during gestation.

A negative IgM test result for a pregnant woman in the first 24 weeks of gestation with a low IgG test titer (i.e., DT < 1024) essentially places the acquisition of the infection prior to gestation. In the third trimester, a negative IgM test titer is most likely consistent with a chronic maternal infection but does not exclude the possibility of an acute infection acquired early in pregnancy; this is especially true in those patients who exhibit a rapid decline in their IgM titers during the acute infection. In such cases, the use of other serologic tests (e.g., IgA, IgE, AC/HS, avidity) may be of particular help.

In contrast, a positive IgM test result requires further assessment with confirmatory serologic testing. A false positive IgM test result or its erroneous interpretation canbe misleading and result in unnecessary abortions. Sixty percent of pregnant women with IgM results determined to be positive by nonreference laboratories were found to be chronically infected when tested atTSL-PAMFRI[9]. The potential pitfalls of relying solely on an IgM test as a discriminatory method to allow such distinction and the low reliability of commercial T. gondii—specific IgM kits when positive results are obtained have been reported by our group and others.

Thus, it is recommended that a positive IgM test result should always undergo confirmatory testing at a reference laboratory. In sera with a positive IgM test result, the TSP has been used to help discriminate between recently acquired and distant infection.

A number of tests for avidity of Toxoplasma IgG antibodies have been introduced recently to help discriminate between recently acquired and distant infection. More recently, we reported the usefulness of testing for avidity of IgG in the setting of pregnant women in their first 12 weeks of gestation at TSL-PAMFRI. Measurement of IgG avidity was performed with a T. gondii IgG avidity EIA (Labsystems) method. With this method, a high avidity has been stated to exclude that the infection occurred in the previous 12 weeks. Thus, its greatest value is in sera obtained from pregnant women in their first trimester of gestation.

Whether the avidity test can replace any of the present tests in the TSP or simply be added to that panel requires further evaluation of the avidity tests being marketed. No avidity test has been released by the US Food and Drug Administration for marketing in the United States. We now routinely employ the avidity test as an additional confirmatory diagnostic tool in the TSP for those patients with a positive and/or equivocal IgM test result or acute and/or equivocal pattern in the AC/HS test. Health care providers and clinical laboratories involved in the care of pregnant women should be aware that avidity testing is only a confirmatory test and not the ultimate test for decision making. Its highest value is observed when high IgG avidity antibodies are detected and the serum is obtained during the time window of exclusion of acute infection for a particular method (i.e., 12 weeks for the Labsystems method and 16 weeks for the Vidas immunoassay [bioMérieux]).

Once the diagnosis of acute acquired infection during pregnancy has been presumptively established, diagnostic efforts should then focus on determining whether the fetus has been infected.

Congenital Infection in the Fetus and Newborn

Prenatal diagnosis. Prenatal diagnosis of fetal infection is advised when a diagnosis of acute infection is established or highly suspected in a pregnant woman or an abnormality in the fetus suggests congenital toxoplasmosis. Methods to obtain fetal blood, such as periumbilical fetal blood sampling, have been largely abandoned because of the risk involved for the fetus and the delay in obtaining definitive results with conventional parasitologic tests.

Prenatal diagnosis of congenital toxoplasmosis is currently based on ultrasonography and amniocentesis. PCR on amniotic fluid for detection of T. gondii—specific DNA performed from 18 weeks onwards of gestation should be used in all cases of established acute maternal infection or cases with serologic test results highly suggestive of acute acquired infection during pregnancy. In a recent report, the overall sensitivity of PCR on amniotic fluid was estimated to be 64%, the negative predictive value was estimated to be 87.8%, and specificity and positive predictive value were estimated to be 100%; in this study, marked differences in sensitivity were observed depending on the gestational age at the time of the amniocentesis. The reliability of the PCR test before 18 weeks of gestational age is unknown. PCR on amniotic fluid is not recommended for HIV-infected women because of the risk of transmitting the HIV virus to the fetus during the amniocentesis procedure.

Diagnosis in the newborn. Maternal IgG antibodies present in the newborn may reflect either past or recent infection in the mother. For this reason, tests for detection of IgA and IgM antibodies are commonly employed for diagnosis of infection in the newborn. Serum samples obtained from peripheral blood are preferred. Samples from umbilical cord should not be used as they may be contaminated with maternal blood. Demonstration of IgA antibodies appears to be more sensitive than detection of IgM antibodies for establishing infection in the newborn. T. gondii—specific IgA may be present when there is no T. gondii—specific IgM, and the converse may also occur. If IgA antibodies are detected in the newborn, the test should be repeated at ~10 days after birth to make certain that what is being measured is not contaminating maternal IgA antibodies. In addition, if the newborn has received a blood transfusion, serologic tests may measure exogenously administered rather than endogenous antibody.

Additional diagnostic methods that have been used successfully to diagnose the infection in infants include direct demonstration of the organism by isolation of the parasite (e.g., mouse inoculation or inoculation in tissue cultures of CSF, urine, placental tissue, or peripheral blood) and amplification of T. gondii—specific DNA (e.g., PCR in CSF, peripheral blood, or urine). Evaluation of infants with suspected congenital toxoplasmosis should always include ophthalmologic examination, non-contrast computed tomography or ultrasound of the brain (to determine whether hydrocephalus or calcifications are present), and examination of CSF. Detection of calcifications in the brain of a newborn by x-ray, ultrasound, or computed tomography should heighten the suspicion of T. gondii as the cause of the disease. In severely affected infants with congenital toxoplasmosis, unilateral or, more often, bilateral and symmetric dilatation of the ventricles is not an uncommon finding. Persistent CSF pleocytosis and elevated protein content should lead the physician to consider a diagnosis of congenital toxoplasmosis even in subclinical cases.

Although not clinically available, antigen-specific lymphocyte transformation and lymphocyte typing in response to exposure to T. gondii antigens has been used successfully to diagnose the congenital infection in infants' ⩾2 months of age. Specific lymphocyte anergy to the organism may also occur in congenitally infected infants. [8]

Cysticercosis

Cysticercosis (i.e., infection caused by eggs of the pork tapeworm) is an increasingly common medical problem in the United States, especially in the Southwest and other areas where large populations migrated from endemic areas and among populations that often travel to these areas.

Cysticercosis is caused by the metacestode, or larval, stage of Taenia solium, the pork tapeworm. The clinical syndromes caused by T solium are categorized as either cysticercosis (cysts in various tissues including the brain) or taeniasis (intestinal tapeworm infection).

Neurocysticercosis refers to CNS infection with T solium. Neurocysticercosis, which is probably the most common parasitic infestation of the CNS, has gained increased recognition in the last two decades because of the development of MRI and CT scanning in the United States and in countries where neuro cysticercosis is endemic.

Neurocysticercosis is further divided into parenchymal and extraparenchymal disease. Parenchymal disease is characterized by infection with cysticerci within the brain parenchyma. Extraparenchymal disease develops when cysticerci migrate to the CSF of the ventricles, cisterns, and subarachnoid space or within the eyes or spinal cord.

Pathophysiology:

When humans ingest undercooked pork that contains cysticerci of T solium, the scolex evaginates from the cyst and develops into an intestinal tapeworm (taeniasis). The tapeworm grows to a length of up to 10 meters and has hundreds of proglottids. Mature proglottids contain approximately 50,000 eggs each. Free eggs or whole proglottids are released periodically into the stool of the carrier and can survive in the environment for many months.

When pigs ingest the proglottids or eggs, the eggs hatch, penetrate the pigs' intestinal wall, and spread to skeletal muscle, especially the neck, tongue, and trunk. There, the larvae mature into encysted cysticerci over 2-3 months. The cysticerci suppress the host inflammatory response and can survive in tissues for months to years. The life cycle is completed when humans ingest inadequately cooked pork that contains viable cysticerci. [(10)]

Cysts, called cysticerci, can develop in the muscles, the eyes, the brain, and/or the spinal cord. Symptoms caused by the cysts depend on the location, size, number, and stage of the cysts.

Cysts in the brain or spinal cord:

•Cause the most serious form of the disease, called neurocysticercosis

•May cause no symptoms

•May cause seizures and/or headaches (these are more common)

•May also cause confusion, difficulty with balance, brain swelling, and excess fluid around the brain (these are less common)

•May cause stroke or death

Cysts in the muscles:

•Generally do not cause symptoms

•May cause lumps under the skin, which can sometimes become tender. [11]

The varied clinical presentations of neurocysticercosis (NCC) result from a series of factors, which include the number, stage, size, and location of parasites in the nervous system of the human host, factors which also influence case management and prognosis. In this context, the utility of immunodiagnosis as a tool on which to base clinical decisions by itself is quite limited. Diagnosis and characterization of human NCC should be based on a brain imaging examination to observe the characteristics of the lesions, accompanied by a serological test result to confirm the etiology. In the best possible scenario, the immunological test should not only be highly sensitive and highly specific for etiological confirmation but also be able to discriminate infections with living parasites from inactive infections, and correlate the characteristics of the infection with parasite load, for patient management and follow-up. A century of serological assay development for Taenia solium cysticercosis has provided some tests which fulfill several of the above requirements, albeit the ideal assay has yet to be developed.

Antibody Detection Tests for Cysticercosis:

Early in the twentieth century, the laboratory diagnosis of tissue parasites was limited to non-specific findings of increased white cell counts, strongly valuing the presence of increased eosinophil numbers. The first serological assays for parasitic infections were complement precipitation and fixation techniques. In 1909, Weinberg used complement fixation with cystic fluid from cysticerci to demonstrate specific antibodies in the sera of a group of cysticercotic pigs. This test became known as 'Weinberg's reaction' and was used until a few decades ago. In 1911, Arthur Moses reported the use of an aqueous cysticercal extract to demonstrate the presence of antibodies in the serum of three patients with subcutaneous cysticercosis and in the cerebrospinal fluid (CSF) of a patient with cysticercosis encephalitis, thus demonstrating for the first time, the presence of anti-cysticercal antibodies in CSF.

In the following decades, many attempts to develop better diagnostic tests focused on indirect, antibody detection assays. Antibody detection does not distinguish active from inactive infections, and is not useful to monitor changes over short periods; however, its diagnostic efficacy is much higher than that of antigen detection assays. These indirect assays include indirect hemagglutination, immunoelectrophoresis, double immunediffusion, precipitation, indirect immunofluorescence, and skin reaction, among others, and are comprehensively described in Flisser et al.18 by 1971, Engvall and Perlmann described the enzyme-linked immunosorbent assay (ELISA) technique. The ELISA is a quite simple technique, is sensitive, quantitative, and can process many samples at the same time, thus it soon became the most frequently used antibody detection assay (ELISA-Ab). It was initially applied in 1978 for the diagnosis of NCC by Arambulo et al. in cases with high suspicion for NCC, reporting better sensitivity than the indirect hemagglutination, the test in use at that time. Coker-Vann et al. then

applied the ELISA technique to detect T. solium antibodies in epidemiological studies. Many other laboratories adopted the ELISA-Ab with varied sensitivities according to the antigen and serum panels used. It was evident, however, that the ELISA performed better than the previous techniques. Initial assays used crude metacestode antigens. Better results were obtained using cystic fluid as the antigens, but not with membrane or scolex antigens.

Unfortunately, a series of factors affected the diagnostic capacity of these early techniques: the sensitivity and specificity of each technique, the difficulties in defining appropriate reference sera sets, and the use of crude or minimally purified antigens, leading to non-specific reactions mainly with echinococcosis, schistosomiasis, angiostrongyliasis, sparganosis, and fasciolasis. Case definitions and reference serum batteries were greatly improved with the advent of computed tomography (CT) in 1977 and magnetic resonance imaging in 1986. Cases of NCC could then be differentiated in terms of number of lesions, stage, and location (intraparenchymal or extraparenchymal NCC), variables, which strongly influence the host's humoral immune response.

Antigen characterization became then the objective of researchers looking for improved serological tests. One of the more studied antigens was antigen B, described by Flisser et al. in 1980 as the antigen more frequently recognized by sera from NCC-infected patients, producing a strong antibody response. Use of antigen B in an antibody detecting ELISA in serum as well as in CSF, did not demonstrate much advantage over other antigen sources. Grogl et al. in 1985 characterized a series of antigenic proteins from the total metacestode extract as suitable candidates for immunodiagnosis, using for the first time the EITB technique, originally used for immunodiagnosis of schistosomiasis. A series of other antigens were then purified using chromatographic techniques and were reported to perform with high sensitivity in cysticercosis in 1989, the EITB (also known as western blot or immunoblot) using the LLGP fraction was developed and quickly became the assay of choice for serodiagnosis. The LLGP-EITB combines the specificity of using antigens previously purified by chromatography plus the resolution capacity of polyacrylamide gel electrophoresis with sodium dodecyl sulfate coupled with the sensitivity of enzyme-based immunodetection. Seven antigenic glycoproteins (GP) were isolated from a total metacestode homogenate and then purified using lentil-lectin chromatography, namely GP50, GP42-39, GP24, GP21, GP18, GP14, and GP13, where the numbers referred to their molecular mass in kilo Daltons. The presence of any one of the seven antibody bands defines a positive test, with an initial sensitivity and specificity reported to be 98 and 100%, respectively. No cross-reactions were found in 376 sera from 18 heterologous infections. Further comparative testing demonstrated the superiority of the LLGP-EITB over ELISA for the diagnosis of human and porcine cysticercosis.

Antibody-detecting techniques in general do not have the capacity to distinguish between exposure, inactive infection and active infection, have a low positive predictive value in cases with viable cysticercosis (due to positive antibody reactions in individuals with calcified cysticerci and a high background of seroprevalence in the general population in endemic areas), and have low sensitivity in cases with a single brain lesion. Even though the LLGP-EITB is currently considered the test of choice for serodiagnosis of cysticercosis, it has its own

drawbacks, which include the source of antigen (the method requires fresh cysts from infected pigs), and is also a complicated procedure. Thus, more recent research efforts have focused on the characterization and synthesis or production of recombinant forms of the seven LLGP diagnostic antigens to produce simpler and more reproducible assays.

Molecular studies showed that the seven LLGP diagnostic antigens comprise three protein families: GP50, T24/T42, and the 8 kDa family. GP50 is the largest of the LLGP antigens. Although no defined cross-reactions have been reported to any of the LLGPs, a 'bogus' band can appear slightly above GP50 and generates a problem of interpretation when reading the strips, even in patients without evidence of exposure to T. solium, thus its presence as a single reactive band should be taken with caution. GP50 and GP39–42 are the more immunodominant antigens, inducing vigorous IgG-response, both are membrane proteins. Studies have demonstrated that GP24 is a monomeric form and GP42–39 is a homodimeric form of the same protein. Both have already been produced as recombinants (rGP50 and rT24H) in a eukaryotic expression system, with good diagnostic performances in EITB and in ELISA as well as in a novel proprietary technique, the Quick ELISA. In general, rT24H performs slightly better than rGP50, but neither antigen alone reaches the sensitivity and specificity of the combined native LLGP set.

The remaining LLGPs, of lower molecular weights, correspond to a complex group of 8 kDa peptides, which can be found alone or in oligomeric structures, which have molecular masses as large as 42 kDa. Similar antigens have been reported in other taeniid cestodes, such as T. hydatigena, T. multiceps, and Echinococcus granulosus. These small peptides have been described as excretory/secretory (E/S) products and have been associated with immune evasion functions. In some cases, the immunogenic activity of synthetic and native forms correlate well and thus do not seem to depend on secondary structure resulting from post-translational modifications such as a glycosylation; in some cases, there seems to be a component of the immune response that depends on conformational epitopes. The presence of low molecular bands in the LLGP-EITB is rarely seen in absence of reactivity to the higher LLGP antigens and seems to be associated with more severe infections. The use of these smaller 8 kDa LLGP peptides as serodiagnostic tools has been proposed because of their capacity to discriminate between active and inactive infections and their availability as synthetic peptide. TsRS1 and Ts18var1 are two peptides in this family, with reasonable sensitivity and specificity in ELISA, which greatly improve when used in EITB format. They however, show lower diagnostic utility when compared to the native forms to detect single lesion cases.68 Other diagnostic candidates in the 8 kDa family have been expressed and produced as recombinant proteins including Ts8B1, Ts8B2, and Ts8B3. Among these, the Ts8B2 antigen was better able to discriminate between cases of active and inactive NCC, although some cross-reactions with echinococcosis and schistosomiasis were observed. Splitting the Ts8B2 in smaller synthetic peptides greatly affected the antigen performance. Yang et al. described a 10 kDa antigen from cyst fluid, also belonging to the 8 kDa family, reacting mainly to IgG4 and IgG1.This 10 kDa antigen was also produced in recombinant form and showed a good performance to differentiate active from inactive NCC.

Other native antigens under research include parasite proteases. In 2005, Baig et al. described a protease from the T. solium metacestode with L-cathepsin activity and able to degrade IgG

(suggesting a role in immune evasion). A second similar protease was also identified by a different group soon after. This protease, produced in recombinant form, had antigenic activity recognized by sera from patients with NCC. Two other protease fractions highly abundant in cystic fluid have been isolated and evaluated in ELISA and EITB with promising results, and in dot blot form, with lower sensitivity, albeit higher specificity.

Synthetic peptide production is appealing for its ease of production and inherent reproducibility. However, to date no synthetic peptide has performed at the level of native antigens for diagnostic purposes. A possible alternative is to use more than one synthetic peptide in the same assay, as in the multiantigen print immunoassay, in which several recombinant or synthetic antigens are printed at different positions along a single strip and thus obviating the need for electrophoretic separation. Recombinant proteins perform better than synthetic peptides, most likely because part of the response is directed towards conformational epitopes.69 Recombinants to several of the seven LLGP described by Tsang et al., have shown better results than related synthetic peptides in EITB as well as in ELISA.

Other attempts to develop immunodiagnostic tests include the lymphocyte transformation test (LTT) described by Prasad et al., in 2008, who found 94% of sensitivity and 96% of specificity. This assay requires lymphocyte separation, long incubation time, and a radioactive developer. The authors proposed their use in patients with a single brain lesion but further evaluations are still missing. LTT seems to offer a good alternative to evaluate a host's exposure to a given antigen but will likely not differentiate active from inactive cases because the assay is based on the presence of memory T cells.

Phage display peptide selection was reported as early as in 1999. Almost 10 years later, Hell et al. produced a synthetic peptide against a scolex antigen with this technology.89 Initial promising results have been reported for two other peptides produced with this technique. As proposed by Esquivel-Velazquez et al., in 2011, new tools like phage display peptide selection, production of synthetic, and recombinant antigens, could permit us to shorten the path to identify specific antigens capable to distinguish not only the stages of the parasite, but also exposure from viable and non-viable infection. In this way, a good alternative to distinguish exposure from infection could be the use of oncospheral antigens, which to date have mostly been used as vaccine candidates. The 8 kDa antigens seem to be promising candidates to distinguish viable from non-viable NCC; however, the sensitivity of these assays needs improving.

Advantages and disadvantages of antibody detection in cysticercosis

Sound use of serological assays goes beyond the choice of a test and greatly depends on appropriate interpretation of results in the context of a given patient or a given population. Antibody detection with a sensitive and specific assay is the best alternative to diagnose whether somebody has been infected with cysticerci. Antibody detection however does not discriminate between active and inactive infections and thus its clinical utility is restricted to etiological confirmation (although strong antibody reactions suggest severe infections and, unlike total IgG8, IgG4 detection can be associated with active infection as well as provide a good monitoring marker for cure).

In field conditions, antibody seroprevalence overestimates the actual prevalence of infection because persons with antibodies from exposure and from past infections are also detected. Even more, there is evidence that almost 40% of the positive results in an endemic area are produced by transient antibodies, which become undetectable within 1 year. Detection of parasite specific antibodies in asymptomatic individuals has limited clinical use. Antibody prevalence, however, can provide valuable information on exposure to the parasite, transmission dynamics, risk factors, and incidence calculations, thus it should still be considered a tool for control programs.

In summary, a positive antibody test associated with a suggestive brain image strongly supports the diagnosis of NCC, while in endemic regions where no CT or magnetic resonance imaging is available, a positive antibody test should be mainly used to refer patients with neurological symptoms to a more equipped center for imaging diagnosis and etiological case management.

Antigen Detection Tests for Cysticercosis

Direct immunodiagnosis (detection of products of the infective agent in the host) has the advantage of demonstrating active infection and in most cases, the antigen levels are associated with the infective burden and thus the severity of the infection, so this type of test can be used to determine therapeutic decisions and guide the prognosis of the patients. Cure is frequently associated with negative antigen results, and on the other hand, relapses, reinfections, or complications result in increases in circulating antigen levels. Unfortunately, in most cases, the sensitivity of antigen detection assays is inferior to that of indirect, antibody-detecting assays.

The initial reports on finding T. solium antigens in the CSF of patients with NCC used ELISA assays with rabbit polyclonal antisera raised against crude cysticercal extracts. Their results were promising, particularly in terms of specificity (likely resulting from the use of CSF instead of serum, as detailed above). As expected, circulating antigen cannot be demonstrated in the CSF of all NCC patients. Also, only a fraction of all antigens present in the cyst fluid can be detected in the patient's CSF. Circulating antigen can also be detected in serum, as initially demonstrated for T. saginata cysticercosis in cattle and later in human samples.

Monoclonal antibody (MoAb)-based antigen detection greatly improved the performance of these assays. The initial tests for T. solium antigen detection originated from assays developed against T. saginata and performed well thanks to an unexpected interspecies cross-reaction. In 1989, Harrison et al. developed a MoAb against a repetitive epitope from excretory/secretory glycoprotein products of the T. saginata metacestode, HP10. In an ELISA format, HP10 detected circulating antigen in cattle with 200 or more live cysts, with levels detectable in cattle serum as early as 4–5 weeks post-infection. No cross-reactions other than the above described with T. solium were reported. The sensitivity of the HP10 ELISA in CSF of confirmed NCC cases was 72%. A similar method was pursued by Brandt et al. in 1992. They found eight MoAbs of IgM isotype, which when used in combination, had a lower detection limit of 88 live cysts in infected cattle, and also were able to detect antigens as late as 5 weeks post-infection. These MoAbs were also directed against repetitive glycoprotein epitopes. Further studies generated IgG MoAbs, which improved the antigen assay performance, reaching 92% sensitivity and 98.7% specificity in sera from cysticercosis-infected cattle. They also showed that the target antigen was

thermostable. Heat treatment of samples prior to testing gave better results, in particular fewer non-specific reactions.

MoAbs against T. solium were first described in 1991. The initial report concludes that the antigen detection test performed well for diagnosis of T. solium cysticercosis, but was not 100% sensitive; the test worked better on CSF than on serum, and antigen levels dropped to undetectable levels after successful treatment. Another anti-T. solium MoAb targeted cyst fluid (1F11, IgG1 isotype) and had a diagnostic sensitivity of 82%, mainly missing cases with fewer lesions or only calcifications. This same group also developed MoAb 4F8. A 4F8-based ELISA was used by them to demonstrate that patients with subcutaneous nodules had higher levels of circulating antigen, likely because subcutaneous cysticercosis is found in patients with higher parasite burdens.

Interesting and very promising tools are nanobodies (Nbs), single domain antibodies that are produced in immunized camelids. These molecules are, highly stable and soluble, devoid of light chains, and capable of binding to antigens with high affinity and specificity. Their small size (12–15 kDa) allows detection of hidden epitopes and expression in various microorganisms. Nbs directed against an 8 kDa antigen of the metacestode have been developed and proven specific for T. solium, without cross-reactions with T. hydatigena, T. saginata, T. crassiceps, and Trichinella spiralis. Further work is needed to determine the utility of these reagents for antigen detection in T. solium cysticercosis.

Advantages and disadvantages of antigen detection in cysticercosis

Currently available antigen capture assays do not reach the same sensitivity and specificity as antibody-detecting assays, so they are not the best option for initial diagnosis of clinical cases. They, however, provide an exceptional tool for clinical management and follow-up of confirmed cases. As described above, the levels of circulating antigen correlate with the severity and type of NCC infection. In intraparenchymal brain cysticercosis, the higher the number of viable cysts, the higher the antigen level, both in humans and in pigs. In cases of extraparenchymal NCC, which carry a poorer prognosis, the levels of antigen are much higher, particularly in patients with basal subarachnoid NCC10, and also correlate with the volume of the lesions except when hydrocephalus is present. (Unpublished data, the Cysticercosis Working Group in Peru 2012) Very high antigen levels should thus raise the suspicion of extraparenchymal NCC or massive intraparenchymal NCC. Since the levels of antigen drop quickly in cured NCC patients, serum antigen monitoring is of great help in the follow-up of clinical cases. Detectable levels of circulating antigen do not always correlate with neurological symptoms. A study in India followed 42 antigen-positive individuals for up to 5 years, and none of them developed neurological symptoms associated with NCC. It follows that the isolated finding of circulating antigen without neurological symptoms or compatible neuroimage should not be interpreted as an indication for therapeutic intervention.

The usefulness of population-based antigen detection surveys is yet unclear. From the available data, the expected proportion of asymptomatic individuals with viable brain cysticerci and thus positive antigen levels should be quite small. Its use would likely be more productive if focused

in patients with compatible symptoms such as seizures or intracranial hypertension. In field studies, one would expect to find a higher prevalence of antibody-positive individuals than antigen-positive individuals, since antibody responses occur in non-viable infections and individuals with exposure only, and for an unknown time after resolution. However, population-based studies show discordant results. In a community in Mexico, 1% of all people were positive to circulating antigen versus 4.8% seropositive to antibodies. In Ecuador, 2.25% of 800 participants in a rural community were positive for antigen, while the antibody seroprevalence measured in a subset of 100 randomly selected samples was 40%. On the other hand, a study in South Africa found more antigen-positive than antibody-positive cases, and another study in Burundi found higher prevalence of antibodies in patients with epilepsy than in controls but no differences in the frequencies of antigen-positive cases between these groups. [(12)]

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