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Opinion on the diagnosis and treatment of human trichinellosis

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The clinical diagnosis of trichinellosis is difficult because there are no pathogenic signs or symptoms and in diagnosing the infection epidemiological data are of great importance. Trichinellosis usually begins with a sensation of general discomfort and headache, increasing fever, chills and sometimes diarrhoea and/or abdominal pain. Pyrexia, eyelid or facial oedema and myalgia represent the principal syndrome of the acute stage, which can be complicated by myocarditis, thromboembolic disease and encephalitis. High eosinophilia and increased creatine phosphokinase activity are the most frequently observed laboratory features and the parasitological examination of a muscle biopsy and the detection of specific circulating antibodies will confirm the diagnosis. The medical treatment includes anthelmintics (mebendazole or albendazole) and glucocorticosteroids. Mebendazole is usually administered at a daily dose of 5 mg/kg but higher doses (up to 20 – 25 mg/kg/day) are recommended in some countries. Albendazole is used at 800 mg/day (15 mg/kg/day) administered in two doses. These drugs should be taken for 10 – 15 days. The use of mebendazole or albendazole is contraindicated during pregnancy and not recommended in children aged < 2 years. The most commonly used steroid is prednisolone, which may alleviate the general symptoms of the disease. It is administered at a dose of 30 – 60 mg/day for 10 – 15 days.

Keywords: albendazole, clinical diagnosis, eosinophilia, humans, laboratory diagnosis, mebendazole, muscular enzymes, serodiagnosis, steroids, treatment, trichinellosis

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1. Introduction

Trichinellosis (previously referred to as 'trichinosis') is a zoonosis caused by parasitic nematodes of the genus *Trichinella*. The infection has a worldwide occurrence and although most species of *Trichinella* are found in mammals, one species is also known to infect birds and a new genotype has recently been detected in crocodiles in Africa [1]. The main sources of human infection are pork and pork products, game meat and horse meat. With regard to the taxonomy of *Trichinella*, knowledge has increased greatly in the past 30 years and, to date, seven species have been identified (*Trichinella spiralis*, *Trichinella nativa*, *Trichinella britovi*, *Trichinella murrelli*, *Trichinella nelsoni*, *Trichinella papuae* and *Trichinella pseudospiralis*) and four additional genotypes have been identified [2-4]. Although human infection has not been reported for certain genotypes, all *Trichinella* species are pathogenic for humans, although differences have been observed between the species or genotypes in terms of the signs, symptoms and clinical course of infection in humans.

Trichinellosis continues to be a public-health concern throughout the world [1,5]. Specifically, it has been estimated that 10 million people worldwide could be infected [5] and in the past 10 years an increase in the occurrence of infection has been

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Table 1. Glossary for *Trichinella* infection and trichinellosis in humans.

Term	Definition
Chronic phase	Period during which live larvae are present in the striated muscles
Chronic trichinellosis	Refers to people who acquired *Trichinella* infection months or even years before who still show signs or symptoms
Convalescent phase	Period following the acute phase during which signs or symptoms are due to the presence of muscle larvae but during which migrating larvae are no longer present
Incubation period	Period during which infective larvae develop into adults in the intestine without any signs or symptoms
Intestinal (enteral) phase	Period during which the infective larvae develop into adults in the intestine; signs or symptoms present
Muscular (parenteral or systemic) phase	Period during which new-born larvae migrate throughout the circulatory system to striated muscles; signs or symptoms present
Sequelae	Definitive lesions of organs which occurred in the acute phase (e.g., brain, cardiovascular or neuromuscular disturbances)
Trichinella infection	Infection with *Trichinella* with or without signs or symptoms
Trichinellosis	Infection with *Trichinella* with signs or symptoms

reported among domestic pigs and wildlife, with a consequent increase among humans [6]. This review is intended to aid physicians in correctly diagnosing and treating trichinellosis.

2. Parasitic cycle and pathophysiology

The parasitic cycle can be divided into two phases or stages: a GI (enteral) phase and a muscular (parenteral or systemic) phase [7], which can coexist for a period lasting from a few days to weeks. Table 1 is a glossary of terms used to define these different phases.

2.1 GI phase

After the gastric digestion of the infected meat (i.e., meat containing larvae), the larvae are released in the stomach; they then penetrate the mucosa of the small intestine, where they mature into adult worms 4 – 5 days post infection. The larval penetration of the GI mucosa causes modifications in the cells of the epithelium, specifically the brush border of villi, the lamina propria and the smooth muscles of the jejunum. The villi become deformed and there is a proliferation of enterocytes at the villus margins, hyperplasia of the crypts of Lieberkühn and the presence of massive cellular infiltrates in the mucosal sublayer. These lesions may persist for several weeks after infection. After mating in the intestine, females shed new-born larvae into the lymphatic vessels. Mature females release new-born larvae for 3 – 4 weeks; although this estimate was based only on experimental data from pigs, it has been confirmed by the observation of a *Trichinella* female containing embryos on a duodenal section of a person infected 3 – 4 weeks earlier and presenting with fever, myalgia and high eosinophilia [8]. The females then die or are expelled by the immune response.

2.2 Muscular phase

The larvae released in the GI mucosa migrate to the blood vessels, by means of which they spread throughout the body until reaching their final location (i.e., the cells of the striated skeletal muscles). The migration of *Trichinella* larvae into the different organs provokes an immediate reaction, which causes immunological, pathological and metabolic disturbances and the various clinical phenomena observed during the acute stage of the infection [9-11]. The immunological reaction consists of the production on the part of inflammatory cells (mast cells, eosinophils, monocytes and T and B lymphocytes) of cytokines and antibodies, which are important triggers of the cell-mediated and humoral immune responses to the infection. The penetration and permanent presence of larvae in the cells of the striated skeletal muscles cause three major cell modifications: the disappearance of sarcomere myofibrils, the encapsulation of the larvae (in the case of encapsulated species) and the development of a capillary network surrounding the infected cell. Encapsulation consists of the production of a collagen capsule around the larva ~ 18 – 20 days post infection; this occurs in all species and genotypes except for *T. pseudospiralis*, *T. papuae* and the genotype found in crocodiles (these species and genotype are referred to as non-encapsulated). In addition to these three major modifications, the sarcoplasm becomes basophilic, the cell nucleus is displaced to the centre of the cell and the nucleoli increase in both number and size. The cell becomes more permeable, resulting in an increased release of muscle enzymes. The modified cell is referred to as a nurse cell, which is clearly separated from the surrounding host tissue. The length of survival of the nurse cell–parasite complex in the host is known to vary greatly, depending on many factors related to both the parasite and the host. However, it is difficult to accurately estimate the length of survival; it seems to vary from ~ 1 – 2 years to an undetermined number of years, although survival for up to 30 years has been reported [12]. Although *Trichinella* larvae do not mature or become encapsulated in cardiac tissue, their transitory passage can lead to morphological alterations, which consist of focal cellular infiltrates of eosinophils and mononuclear cells. The presence of larvae in the central nervous system causes vasculitis and perivasculitis, which cause diffuse or focal lesions. Moreover, heart and brain lesions are often associated and could result from the conjunction of local prothrombotic effects of eosinophils and vascular injury caused by the migrating larvae [13].

Table 2. Algorithm for diagnosing the probability of humans being infected with acute *Trichinella*.*

Group	Characteristics
A	Fever Facial and/or eyelid oedema Myalgia
B	Neurological signs Cardiological signs Conjunctivitis Subungual haemorrhages Cutaneous rash Diarrhoea
C	Eosinophilia (> 1000 cells/mm^3) and/or increased total IgE levels Increased levels of muscular enzymes
D	Positive serology (with a highly specific test) Seroconversion Positive muscular biopsy

Algorithm use: Very unlikely = one A or one B or one C; Suspected = one A or two B plus one C; Probable = three A plus one C; Highly probable = three A plus two C; Confirmed = three A plus two C plus one D; any of groups A or B plus one C and one D.

3. Clinical diagnosis

3.1 Case definition

Although a definitive diagnosis of trichinellosis can only be made with highly specific immunodiagnostic tests or by detecting larvae in a muscle biopsy, the infection can be suspected on clinical grounds. If two or more people in the same household or a number of people in the same community have high fever, periorbital or facial oedema and myalgia, trichinellosis can be suspected. When cases are sporadic or the clinical course is atypical, it is less likely that the infection be suspected. Once infection is suspected, information should be collected on the consumption of raw or undercooked meat or meat products, including the place and time of purchase and consumption. An algorithm for diagnosing acute trichinellosis and for defining suspected, probable, highly probable and confirmed cases is shown in Table 2.

3.2 Severity of infection

The severity of trichinellosis depends on a number of variables which are often interrelated, including the infecting dose (i.e., the number of larvae ingested); the frequency of consumption of infected meat; how the meat was cooked or treated (e.g., whether it was raw or rare or whether it had been smoked or salted); the amount of alcohol consumed at the time of meat consumption (given that alcohol could increase the resistance to the infection) [14]; the specific *Trichinella* species involved (the number of new-born larvae shed by females differs by species); and individual susceptibility. With regard to the infecting dose, the minimum dose necessary for causing symptomatic trichinellosis has been estimated to range from ~ 70 – 150 larvae [9].

Based on disease severity, five clinical forms of trichinellosis can be recognised: severe, moderately severe, benign, abortive and asymptomatic. In the severe form, all of the signs and symptoms are very pronounced (see section 3.4) and there are metabolic disturbances and circulatory and/or neurological complications. In the moderately severe form, all of the signs and symptoms are pronounced, yet complications are rare and, if present, they are benign and transient. In the benign form of trichinellosis, the signs and symptoms are mild and there are no complications; consequently, this form is rarely suspected, unless the infected person is involved in an investigated outbreak. In the abortive form, the clinical signs and symptoms frequently appear individually and not as a syndrome; they are mild and only last for a few days. The asymptomatic form can only diagnosed by means of serology.

3.3 Incubation period

The length of the incubation period depends upon the same variables as disease severity. Furthermore, it has been observed that for the more severe forms of trichinellosis, the incubation period is generally shorter. Specifically, the incubation period lasts ~ 1 week for the severe form, 2 weeks for the moderately severe form and at least 3 – 4 weeks for the benign and abortive forms.

3.4 Acute stage

In most people, the acute stage begins with the sudden appearance of general discomfort and severe headaches, and an increase in fever, chills and excessive sweating. The major syndrome of the acute stage consists of persistent fever, facial oedema, muscle pain and severe asthenia lasting for several weeks. Transient dizziness and nausea can also occur. Though less common, diarrhoea and conjunctival and subungual haemorrhages are also observed. This is the stage during which the adults and the migrating larvae provoke the signs and symptoms of the disease.

3.4.1 Fever

Fever is one of the earliest and most common signs of trichinellosis. Body temperature increases rapidly, usually stabilising at 39 – 40°C. The fever usually lasts from 8 – 10 days, although it can persist for up to 3 weeks when severe.

3.4.2 Periorbital and facial oedema

Periorbital and facial oedema are very typical signs of trichinellosis, although their intensity varies depending upon the intensity of the reaction to the infection. In the severe form of trichinellosis, oedema extends to the upper and lower extremities. The oedema is symmetrical. It usually vanishes rapidly following treatment (within 5 – 7 days), particularly when glucocorticosteroids are used.

3.4.3 Myalgia

Myalgia affects various muscle groups and its intensity is related to the severity of the disease. It most frequently affects

the muscles of the cervix, trunk and upper and lower extremities; it also affects the masseters, although less frequently. The pain usually appears upon exertion, although most people with severe trichinellosis or phlebitis associated with trichinellosis also experience myalgia at rest. Some people with severe disease become disabled with a profound muscle weakness as a result of pronounced angiomyositis-type lesions and neuromuscular disturbances. The restriction of movement due to the pain associated with exertion leads to contractures of the upper and lower limbs, nuchal pseudorigidity and occasionally trismus. Severe myalgia generally lasts for 2 – 3 weeks.

3.4.4 GI signs and symptoms

The most common GI signs and symptoms are diarrhoea (from loose stools to as many as 10 – 15 stools per day, frequently containing mucus but free of blood) and abdominal pain. These signs and symptoms usually precede fever and myalgia by 3 – 4 days and they disappear in < 1 week. It has been observed that the shorter the duration between infection and the appearance of diarrhoea and fever, the longer the duration of both fever and facial oedema [15].

Conjunctival and subungual haemorrhagic lesions are caused by vasculitis, the leading pathological process of trichinellosis. These lesions are the result of blood extravasations of variable intensity into conjunctivae (uni- or bilaterally) and fingernail beds. In addition, maculopapular rash (after the onset of muscular pain) and formication have been reported for a small proportion of people.

In three major outbreaks involving > 1600 people, which were analysed with the same criteria, the frequency of the main symptoms was: 41 – 50% for diarrhoea, 82 – 93% for myalgia, 81 – 90% for fever and 58 – 84% for facial oedema. Cutaneous rash was observed in 11 – 44% of the individuals [16,17].

4. Major complications

Complications usually develop within the first 2 weeks. They are mainly seen in severe cases but they have also been reported in moderate cases, in people who were improperly treated (including those for whom treatment was begun too late) and, particularly, in the elderly. A positive correlation has been reported between age and the frequency and severity of complications [15]. Encephalitis and myocarditis, both of which are life-threatening, are often simultaneously present [13].

4.1 Cardiovascular complications: myocarditis

Cardiovascular disturbances can occur in moderate or severe cases of trichinellosis, usually later in the infection (between the third and fourth weeks post infection) [18,19]. Myocarditis develops in 5 – 20% of all infected people. The symptoms include pain in the heart region, tachycardia and electrocardiogram (ECG) abnormalities (flattened T-waves, decreased ST, lowered QRS complex, acute Q-waves and disturbances in atrioventricular or interventricular conduction). The persistence of the ECG abnormalities, even if other signs and symptoms of trichinellosis have already subsided, usually reflects hypokalaemia. Compensation of the potassium deficit in such people promotes normalisation of the ECG.

Another cardiovascular complication is thromboembolic disease, specifically deep thrombophlebitis, intraventricular thrombi and/or pulmonary embolism, all of which can lead to death. Cardiovascular complications may be accompanied by oedema of the lower limbs due to hypoalbuminaemia. Sudden death may result from embolism of the pulmonary artery or from tachycardia.

4.2 Neurological complications: Encephalitis

Neurological complications include a variety of signs and symptoms [13,20-22] and have been reported in 3 – 46% of infected people, depending on the specific outbreak. People with severe disease can show consciousness disorders or excessive excitement and frequently somnolence and apathy; some of the people with these symptoms show signs of meningitis or encephalopathy. Dizziness, nausea and tinnitus are transient. Anisocoria, facial nerve paresis and Babinsky's sign have also been observed in severe cases. Brain damage, which is usually observed within a few days of the onset of fever, can result in diffuse encephalopathy or focal signs, such as disorientation, memory disturbances, frontal syndrome, behavioural disturbances, transient hemiparesia or hemiplegia, oculomotor dysfunction, aphasia and cerebellar syndrome. Small hypodensities are seen with the computed tomography (CT) or magnetic resonance imaging (MRI) scans [13,23]. In most people with neurological complications, there is an improvement of focal lesions within 2 – 4 weeks post infection. Most CT scan or MRI brain abnormalities disappear in 4 – 8 weeks post infection Neuromuscular disturbances (decreased muscular strength and tendon reflexes, dysphagia and trismus) usually occur at the beginning of the disease and may persist for a long time. Moreover, sequelae such as confusion, depression or walking problems have been observed in some people. Neurological complications may be less frequent if the infected person is treated early.

4.3 Ocular complications

Ocular lesions appear during the acute stage of the disease and result from disturbances in the microcirculation. The typical traits are oedema and vascular lesions within the conjunctiva, the uvea, the retina and, in some cases, in the optic nerve. Rarely, lesions of the retina may be induced by migrating *Trichinella* larvae, which penetrate ciliary arterioles and the central artery of the retina, leading to irreversible damage to eye sight. An intense invasion of muscles of the ocular bulb provokes pain when moving the eyeballs, muscle paralysis, diplopia or a disturbed accommodation.

4.4 Respiratory complications

Dyspnoea is relatively common and is caused primarily by parasite invasion and subsequent inflammation of respiratory muscles, such as the diaphragm. Respiratory complications

are uncommon. They can occur during both the early and the late stages of trichinellosis. They consist of pneumonia, obstructive bronchitis, Löffler-type infiltrates or ventilature failures [19]. Following glucocorticosteroid treatment, the respiratory disturbances regress within a few days. In the late stages of the disease, pneumonia and pleuritis of bacterial aetiology may appear, as well as lung infarction [24].

4.5 Digestive complications

Digestive complications occur during the acute stage of infection and they consist of:

- Massive proteinic exudation leading to hypoalbuminaemia and localised oedemas.
- Acute GI necrosis.
- Prolonged diarrhoea [25].

In recent outbreaks [16,17], oedema of the limbs was reported in 6 – 8% of infected people. A particular syndrome has been described in people who regularly eat infected meat, for example Inuit populations. In these people, trichinellosis manifests itself as a chronic diarrhoeal syndrome which is due to the strong GI immune reaction; this reaction consists of the expulsion of adult worms from the intestine, thus preventing the muscular phase from occurring [26,27].

4.6 Death

Death from tricinellosis is rare. For example, of the > 6500 infections reported in the European Union in the past 25 years, only five deaths have been reported, all of which were due to thromboembolic disease and reported in people aged > 65 years [16]. Twenty fatalities out of 10,030 cases were reported in a worldwide survey performed by the International Commission on Trichinellosis (January 1995 – June 1997) [5].

5. Convalescent stage

The convalescent stage of trichinellosis begins when the adult females cease to release migrating larvae and the already established larvae have completed their development in the muscle cells. The transition to this stage is characterised by the progressive disappearance of the signs and symptoms of the disease and by the return of laboratory parameters to normal values (see section 10). This stage usually begins between the sixth and the eighth weeks post infection and infected people could still have a severe asthenia for several weeks and chronic muscular pain for up to 6 months. Most people will then be asymptomatic, though larvae will persist or years.

6. Chronic trichinellosis

Whether or not a chronic form of trichinellosis actually exists is still under debate and chronic trichinellosis could be difficult to distinguish from sequelae of the acute phase. However, its existence is supported by reports of people who complain of chronic pain and a feeling of general discomfort and who show signs of paranoia and a syndrome of persecution months or even years after the acute stage. Persistent formication, numbness and excessive sweating have been observed more frequently in people who have had severe trichinellosis [28]. Impaired muscle strength, conjunctivitis, impaired co-ordination and IgG antibodies have been reported in some people up to ten years post infection [29], whereas live larvae in muscles were detected without clinical signs and symptoms up to 39 years post infection [12]. The existence of a chronic form is supported by the presence of IgG antibodies in the serum, of bioelectric muscle disturbances and of inflammatory cells in the muscles, all due to the chronic presence of live larvae. Moreover, this syndrome can also result from unnoticed brain localisations during the acute phase of the disease.

7. Trichinellosis in children and pregnant women

In children, the signs and symptoms of trichinellosis are the same as those found in adults, although myalgia and diarrhoea are less frequent, the clinical signs and symptoms are less pronounced and regress more quickly and the frequency of complications is lower. The clinical picture is milder possibly because of lower infecting doses and a less intense allergic reaction to the larvae invasion.

In pregnant women, trichinellosis can cause abortion or premature delivery. Although the underlying mechanisms have not been clarified, these complications could be due to modified production of choriogonadotropin, progesterone or cytokines [30]. The existence of congenital trichinellosis has not been clearly established; however, most women infected during their pregnancy have delivered healthy babies [11].

8. Trichinellosis in immunocompromised people

To the best of our knowledge, only three cases of trichinellosis have been reported in immunocompromised people. In a renal graft recipient, the infection was asymptomatic, even in the presence of 1400 larvae/g in the deltoid muscle [31] and in an HIV-positive person the clinical symptoms were not particularly severe [32]. A very severe case was described in a person with chronic myeloid leukaemia [33].

9. Trichinellosis caused by different species

Although clinical differences have been observed among people infected with different species of *Trichinella* [34], it has not been possible to attribute these differences to the species of the pathogen because the number of infecting larvae ingested by each person was generally unknown. However, *T. spiralis* infections could be more severe than those caused by *T. britovi* and this could be due to the fact that *T. britovi* females are less prolific [35]. *T. murelli* seems to be more likely to provoke skin reactions and less likely to cause facial oedema [36]. *T. pseudos-*

piralis, which is non-encapsulated, seems to provoke signs and symptoms that last longer [37,38].

10. Non-specific biological signs

10.1 Eosinophilia

Eosinophilia has been observed in practically every case of trichinellosis, with few exceptions. It appears early, before the development of the general syndrome of clinical signs and symptoms and it increases between the second and fifth weeks of infection [25]. Eosinophilia occurs in various degrees: low (< 1000 cells/µl), moderate (1000 – 3000 cells/µl) and high (> 3000 cells/µl); up to 19,000 cells/µl have been reported. It regresses slowly and can remain at lower levels for several weeks to 3 months. The level of eosinophilia is correlated with the degree of myalgia [39] and is significantly higher in people with neurological complications [13].

During the acute stage of infection, a massive decrease of eosinophils in people with severe trichinellosis can be considered as a predictor of a severe outcome. The mechanism underlying this decrease has not been fully understood, though it could be related to modifications in the levels of certain cytokines and to the massive exit of eosinophils from the vascular system, leading to huge tissue infiltrates.

10.2 Leucocytosis

Polymorphonuclear leucocytosis is typical of trichinellosis and it appears early in the clinical course of infection. At the onset of leucocytosis, leucocyte levels are already quite high and they rapidly increase between the second and fifth weeks of infection; they can reach 15,000 – 30,000 cells/µl. Unlike high eosinophilia, leucocytosis subsides in parallel with clinical signs and symptoms [10].

10.3 Muscle enzymes

The levels of all muscle enzymes increase in serum during the course of trichinellosis: creatine phosphokinase (CPK), lactate dehydrogenase (LDH), aldolase and, occasionally, aspartate aminotransferase. Increased muscle enzyme levels are found in 75 – 90% of infected people. The increase, which is several-fold, occurs between the second and fifth weeks of infection [10,25]. No correlation has been found between increased CPK and the severity of infection, although a correlation has been found with the intensity of muscular pain [39].

10.4 Disturbances in electrolytes and proteins

The main disturbance in electrolytes is hypokalaemia, which decreases muscle power and creates disturbances in cardiac activity, as revealed by ECG. Lower than normal levels of proteins and albumin are usually found at the late stage of severe infection and result in a hydrostatic oedema [10].

10. 5 Bioelectric disturbances

Bioelectric disorders in *Trichinella*-infected muscles are revealed by electromyography (EMG). The disturbances reveal muscle lesions and are characterised by a decreased amplitude of muscle contraction and incomplete interference. However, they are not pathognomonic for trichinellosis. The disturbances regress simultaneously with clinical improvement and with the subsiding of histological lesions in muscle tissue. In most people, bioelectric disturbances at the acute stage of trichinellosis correspond to the severity of the clinical course and to the intensity of the disease. However, disturbances can be observed for several years after the acute stage (i.e., in people considered to be chronically infected), usually in those who have not been adequately treated in the early period of invasion. In these cases, the disturbances are characterised by a mixed type of electrical alterations, revealing a disturbed function of motor neurons and of impulse transmission at the neuromuscular junction [50].

11. Immunodiagnosis

The humoral response consists of the production of anti-*Trichinella* antibodies and the detection of specific antibodies has great diagnostic value. However, antibodies are not usually detectable at the onset of clinical signs and they appear with a distinct time sequence according to the specific class of antibodies [40-42]. IgE antibodies are thought to appear first and are typical of the acute stage of the disease. However, they are seldom detected because their half-life in serum is relatively short, although an amplified ELISA or the use of tyvelose antigen can greatly increase the probability of detection [43]. The levels of antibodies increase during the subsequent 2 – 3 weeks, particularly in people with severe infection. IgG antibodies may persist for many years after infection, even if the disease was benign or asymptomatic [29]. Sometimes the serodiagnosis is negative during the first days of the febrile phase, in which case a second assay performed a few days later is advisable. The use of two techniques can also allow diagnosis to be made earlier [44].

Many techniques are used for detecting antibodies against *Trichinella* antigen, such as bentonite flocculation or indirect immmunofluorescence. At present, enzyme-linked immunosorbent assay (ELISA) is the most highly recommended technique and is best used in combination with immunoblotting (western blot) to confirm ELISA-positive samples or to exclude false-positive ELISA results. Although many kits for ELISA are commercially available, only those few that do not produce false-positive results due to crossreaction with other parasitic antigens (e.g., visceral larva migrans and *Loa loa*) should be used. Counterimmunoelectrophoresis or latex agglutination are recommended when a rapid confirmation of infection is required (the result is obtained in < 1 h), yet these tests are not commonly used for the diagnosis of trichinellosis because they are less sensitive and specific than ELISA. Competitive inhibition assay, which detects specific antibodies, is a valuable test but it is used less frequently. The sensitivity and specificity of some commercially available tests have been recently assessed [45].

Four different antigens can be used for serological diagnosis:

- Cryosections of infected muscles or isolated larvae (muscle-larva cuticle antigen), which are generally used for indirect immunofluorescence.
- A crude antigen prepared from muscle larvae.
- An excretory/secretory antigen produced *in vitro* after 18 h of cultivation of the muscle larvae.
- A 3,6-dideoxyhexose sugar (tyvelose), one of the major highly-specific immunodominant epitopes of *Trichinella*.

Tyvelose is highly specific, yet it is less sensitive than crude and ES antigens. Crude and ES antigens can cause crossreactions with non-specific *Trichinella* antibodies [46]. The antigenic pattern is quite similar among all *Trichinella* species and genotypes; thus, the antigen prepared with one species, genotype or strain can be used to detect specific antibodies in people infected with any species.

Although circulating antigens can be detected directly, the techniques (immunoradiometric assay and detection by capture techniques using monoclonal antibodies) are not practical because the results were not reproducible between different studies and no kits are commercially available.

12. Parasitological diagnosis

For parasitological diagnosis, a muscle biopsy must be collected, preferably from the deltoid muscle, although any skeletal muscle could be used. The surgeon should carefully collect 0.2 – 0.5 g of muscle tissue (less than the size of a pea) without fat or skin. One part of the muscle biopsy should be weighed and stored without any fixative, avoiding dehydration; the other part will be processed for histological examination. The sensitivity of the parasitological diagnosis depends on the amount of muscle sample tested.

12.1 Trichinelloscopy

Trichinelloscopy is of great use in diagnosis because it detects *Trichinella* larvae, defines the intensity of infection (i.e., the number of larvae per gram of examined tissue) and allows the collection of individual larvae, which can then be used to identify the parasite at the level of species or genotype [47]. The number of larvae per gram is correlated with the severity of infection: if ~ 1000 larvae/gram are present, the infection is very severe [24]. This technique, like all techniques for parasitological diagnosis, is also useful for diagnosing sporadic cases of the infection, in the diagnosis of doubtful cases (e.g., atypical clinical course, the absence of circulating antibodies, as occurs in immunosuppressed people and retrospective analysis of people) and, frequently, for purposes of compensation claims.

To perform trichinelloscopy, small muscle samples (no larger than a grain) are compressed between two thick slides held together with two screws and examined under a trichinelloscope or a dissection microscope at a magnification of 30 – 40 x or between two microscopy slides and examined under a light microscope at a magnification of 50 – 100 x. The larvae are easier to detect when the muscle biopsy is performed in the late stage of infection, which is characterised by a fully developed nurse cell (encapsulated species). However, trichinelloscopy may fail when the larval density is low, resulting in false-negative results.

12.2 Histology

The histological analysis of muscle tissue reveals fragments of larvae at various stages of development, the presence of the collagen capsule (for encapsulated species) or that which remains of a destroyed capsule, the presence of muscle-cell basophilic transformation and the type and composition of cellular infiltrates. The basophilic transformation of muscle cells represents a valuable diagnostic criterion of *Trichinella* invasion even when no larvae have been detected. This method is more sensitive than trichinelloscopy in the early stage of muscle invasion, when larvae are very small and cannot easily be differentiated from the muscle fibres [48]

12.3 Artificial digestion

The digestion of muscle samples using 1% pepsin and 1% hydrochloric acid digestion fluid [49] is very useful for accurately determining the number of larvae per gram of muscle tissue and for isolating larvae for their molecular identification [47]. However, if the muscle biopsy is taken too early after infection, the larvae can be destroyed by digestion: only muscle larvae that are at least 10 – 12 days of age (from muscle biopsies collected 2 – 3 weeks post infection) are not destroyed by artificial digestion. Non-encapsulated species would necessitate shorter digestion times. The sensitivity of this method depends on the amount of muscle sample tested.

13. Differential diagnosis

It is important that differential diagnosis be performed either before or during the acute stage of trichinellosis so that treatment can be administered in a timely manner, thus ensuring its effectiveness. Since the signs and symptoms of trichinellosis are not pathognomic, misdiagnosis is quite common.

For example, people with high fever and myalgia are often misdiagnosed with flu, particularly in winter. Protracted diarrhoea is often attributed to salmonellosis, shigellosis or other infections of the alimentary tract. Eosinophilia combined with myalgia and an inflammatory response should be differentiated from eosinophilia-myalgia syndromes, such as toxic oil syndrome, trytophan intake and eosinophilic fasciitis. Eosinophilia combined with fever should be differentiated from tissular parasitosis, such as fasciolasis, toxocarosis or invasive schistosomiasis. Periorbital or facial oedema with fever should be differentiated from glomerulonephritis, serum sickness, allergic reactions to drugs or allergens, polymyositis, dermatomyositis and periarteritis nodosa. Intense headaches and stiff neck with confusion, drowsiness, irritability and neurological symptoms should be differentiated from infectious meningitis and encephalopathy. Haemorrhages of the con-

junctiva or haemorrhagic skin petechiae associated with fever should be differentiated from leptospirosis, bacterial endocarditis and typhus exanthematicus. People without periorbital oedema but with high fever and neurological symptoms may be misdiagnosed with typhoid fever.

Only positive serology according to a high quality test or a positive muscle biopsy can confirm the diagnosis.

14. Anthelmintics

Although drug treatment during the early stages of infection has been shown to be effective, the treatment of trichinellosis with drugs has been debated for years, in part because none of the data in the literature have been provided by well-conducted case-control studies. The drugs used to treat trichinellosis include anthelmintics and glucocorticosteroids.

To be completely effective in treating trichinellosis, anthelmintics must act against the parasite at all stages of development and thus in various locations of the body. No real drug of choice exists, in that no drug is completely effective against all stages of development or is very well absorbed in the GI lumen.

14.1 Experimental studies in animals

In animal models, benzimidazoles have been shown to be effective against new-born larvae in the lymphatic and blood vessels and against larvae and young adults in the intestine (i.e., only larvae in the first 4 – 5 days post infection). Their effectiveness against muscle larvae is variable and it depends on both the specific drug and the time of administration. The longer the time between the invasion of muscle cells and treatment, the lower the effectiveness of the therapy. Thienpont *et al.* [51] showed an important activity of mebendazole on the GI or muscular stages of *T. spiralis* in rats. McCracken [52] thoroughly studied changes in the sensitivity of *T. spiralis* to anthelmintic treatment during the first three days of infection in mice. Oral administration of either mebendazole or albendazole at 6.25 mg/kg 2 h after exposure to infection eliminated 95 – 100% of the worms, as determined at necropsy 7 days post infection. Beyond the first day of infection, the sensitivity of the parasite to benzimidazole therapy was greatly reduced and an oral dose of 50 mg/kg was only partially active against the adult worms. Despite decreases in drug sensitivity during the enteral phase, administration of either mebendazole or albendazole at 50 mg/kg for 5 consecutive days in mice infected 2 weeks previously significantly reduced (by 96 and 67%, respectively) the number of larvae recovered from host muscles 45 days post infection. The activity of mebendazole against muscle larvae could act in co-operation with the local inflammatory response [53]. Other effective compounds in animals have been extensively reviewed by Campbell and Denham [54]. Flubendazole has been shown to have a high efficacy on GI larvae and some efficacy on muscle larvae in pigs [55]. Several trials that included ivermectin did not show any effect on encapsulated larvae in mice or pigs [56,57].

14.2 Comparative studies in humans

To date, only three comparative studies on different types of treatment have been published. In a prospective study, Fourestié *et al.* [58] compared two regimens (albendazole versus thiabendazole followed by flubendazole) in 117 people infected in a single outbreak of *T. spiralis* infection related to horsemeat consumption. The infected people were treated with either albendazole alone or a regimen consisting of thiabendazole followed by flubendazole (Table 3). Disease activity was evaluated at days 1, 7, 15 and 45 post infection. No difference was found between the two groups with regard to the evolution of myalgia, fever, fatigue, new clinical manifestations or laboratory and serological data. Both treatment regimens were well-tolerated. Sixteen months post infection, 30 of the people treated with albendazole and 29 of those treated with thiabendazole and flubendazole were re-evaluated. Serology was negative for 70% of the albendazole group and for 34.5% of the thiabendazole-flubendazole group. Moreover, of the four muscle biopsies performed on people treated with albendazole, one was positive and it revealed a low number of larvae, whereas three of the five biopsies performed on people receiving thiabendazole plus flubendazole were positive and they revealed a high number of larvae. In other words, although no difference was observed between the two regimens in the early response to therapy, albendazole could be more effective against residual larval infestation.

Cabié *et al.* [59] compared the immediate and midterm efficacy and tolerability of thiabendazole and albendazole as therapy for 46 people infected in a horsemeat-related outbreak due to *T. spiralis* (Table 4). A total of 26 people received thiabendazole and 18 received albendazole. All infected people were also treated with prednisone. Eight relapses occurred (seven in the thiabendazole group and one in the albendazole group). Side effects were reported by seven people, all of whom were treated with thiabendazole. Six months after treatment, 16 of the 31 people who responded to a questionnaire still had symptoms, the most frequent of which were myalgia (81%) and fatigue (69%), with no significant differences between the two treatment groups. The authors concluded that the immediate efficacy of thiabendazole and albendazole as therapy for trichinellosis was comparable but that albendazole was better tolerated

In a recent study,Watt *et al.* [60] conducted a double-blind, placebo-controlled comparison of three antiparasitic drugs during an outbreak of trichinellosis in northern Thailand (Table 5). Forty-six adults were randomised to receive 10 days of of oral treatment with either mebendazole (200 mg b.i.d.), thiabendazole (25 mg/kg b.i.d.), fluconazole (400 mg initially, then 200 mg/day) or a placebo. The people receiving fluconazole or placebo were also treated with pyrantel for 5 days. All of the infected people were seropositive; 19 people (41%) had a positive biopsy. Improvement was observed among a significantly higher proportion of people treated with mebendazole (12 of 12) and thiabendazole (7 of 7) compared to those receiving the placebo (6 of 12; $p < 0.05$) or fluconazole (6 of

Table 3. Randomised trial comparing albendazole and thiabendazole-flubendazole, used to treat 117 people with *T. spiralis* infection.*

	Albendazole‡	Thiabendazole and flubendazole§	p value
Infected people	59	58	
Mean age (years)	39.4	38.1	
Mean time between infection and treatment (days)	22	20	
Mean length of symptoms (days)			
Myalgia	23.4	20.8	
Fever	3.6	5.4	
Fatigue	27.9	25.4	
New signs/symptoms during treatment (% patients)	57.6	44.8	< 0.005
16 months post infection			
Enrolled people	30	29	
Residual myalgia	7 (23.3%)	6 (20.7%)	
Negative serology	21 (70%)	10 (34%)	< 0.01
Positive biopsies	1/4 (1 larvae/g)	3/5 (mean 220 larvae/g)	

* Fourestié *et al*., 1988. ‡ 400 mg/day for 3 days, then 800 mg/day for 15 days. § Thiabendazole 25 mg/kg/day for 5 days then flubendazole 400 mg/day for 15 days.

12). Muscle tenderness resolved in more people treated with thiabendazole and mebendazole than in those treated with placebo ($p < 0.05$). Although the results of this study may have been greatly affected by bias, they show that 77% of the people treated with pyrantel still had myalgia 10 days after beginning treatment, compared to 16% of the people treated with mebendazole or thiabendazole. Moreover, as reported by Cabié *et al.* [59], the people treated with thiabendazole showed side effects: intolerable dizziness, urticarial rash, generalised maculopapular rash, hand rash [61], tinnitus and GI disturbances. The presence of side effects associated with thiabendazole has led to this drug no longer being considered a drug of choice. In light of the results of these three studies, mebendazole and albendazole should be considered as the first-line drugs for treating the acute phase of trichinellosis.

14.3 Mebendazole

Mebendazole, an anthelmintic benzimidazole, is poorly absorbed in the GI lumen. The plasma concentrations differ considerably from individual to individual, ranging from 17.5 – 500 ng/ml [62], although concentrations have been shown to increase when cimetidine is administered concomitantly [63]. Mebendazole is available in tablets (100 mg) or as a suspension (30 ml bottle at a concentration of 100 mg/5 ml) and should be administered at a daily dose of 5 mg/kg administered in two doses (e.g., in adults 2 tablets twice-daily) for 10 – 15 days. The whole treatment cycle may be repeated after five days. In some countries (Germany, Italy and Lithuania), higher doses are recommended (20 – 25 mg/kg/day administered in three doses for 10 – 14 days) and in this case 500 mg tablets are used. However, compared to lower doses, this dose has been more frequently associated with adverse effects, such as allergic reactions, increased liver enzymes values, alopecia and bone marrow depression. People receiving high doses should be supervised closely, monitoring blood counts and liver function [64]. People with liver failure should receive reduced doses [62]. Since mebendazole is teratogenic in rats, it is contraindicated in pregnant women and in children aged < 2 years. However, a recent study showed that mebendazole 200 mg/day for 3 days during pregnancy was not associated with a significant risk for major congenital defects when used during the second and third trimesters, though some results indicated that it should be avoided during the first trimester [65]. Thus, during pregnancy, especially in the first trimester, mebendazole should be used only when the infection is severe and treatment must begin no later than 1 – 3 weeks from infection, given that at the recommended dose for pregnant women, it is not effective after this period [64]. Regarding treatment in children, the use of mebendazole has been reported aged < 2 years in situations where it was deemed necessary [66].

Table 4. Comparative study of albendazole versus thiabendazole in 46 people infected with *Trichinella*.*

	Albendazole‡	Thiabendazole§	p value
Infected people	18	26	
Mean age (years)	53.3	38.9	< 0.01
Days between onset of illness and treatment (mean)	15.1	7.2	
Days of recovery (mean)	8	8	
Relapses	1 (5.5%)	7 (27%)	< 0.1
Side effects	0	7 (27%)	< 0.01

* Cabié *et al*., 1996. ‡ 13 mg/kg/day for 8 days. § 45 mg/kg/day for 6 days.

Several studies have reported that mebendazole is effective against trichinellosis [60,67-69]. The efficacy of mebendazole against larvae in muscle tissues depends on the time between infection and treatment and could be dose-dependent. For example, when using a cumulative dose of 7.5 – 15 g of mebendazole for 10 – 13 days started 1 month after infection, the treatment failed to kill muscle larvae [70]. A larvicidal effect was obtained only when using a cumulative dose of 77 g of mebendazole for 56 days, started 4 months after infection [71].

Table 5. Placebo-controlled trial for myositis in 47 people infected withTrichinella.*‡

	Placebo	Fluconazole§	Mebendazole¶	Thiabendazole#	p value
n	12	11	12	12	
Muscle pain at day 10 of treatment	9	8	2	1	< 0.05
Unable to walk at day 10 of treatment	6	3	1	0	< 0.05
Mean CPK value at day 7 of treatment (% of day 0)	150	69	78	72	< 0.05
n 1 – 3 months after treatment	6	6	12	5	
Myalgia at 1 month	5	5	6	1	
Myalgia at 3 months	4	1	4	0	
Positive muscle biopsies	2/3	2/2	0/1	0/2	

* Watt *et al.*, 2000. ‡ All people enrolled in the placebo or fluconazole groups were treated with 11 mg/kg for 5 days of pyrantel. § 200 mg/day for 10 days. ¶ 400 mg/day for 10 days. # 50 mg/kg/day for 10 days. CPK: Creatinine phosphokinase.

14.4 Albendazole

Albendazole, an anthelmintic benzimidazole carbamate, is absorbed in the GI lumen relatively quickly. After oral administration of a single dose of 400 mg, peak plasma concentrations of the sulfoxide metabolite (between 0.04 – 0.55 µg/ml) were obtained after 1 – 4 h [72]. When the drug was administered with a fatty meal, a two- to fourfold increase in plasma concentration was observed, although large intra- and inter-individual variability in the plasma concentration has been reported [73]. Concentrations of 0.45 – 2.96 µg/ml were obtained in people treated with 15 mg/kg and the half-life of the active sulfoxide metabolite was between 10 – 15 h [74]. It is not clear if these higher concentrations of albendazole, compared to those obtained with mebendazole, are correlated with a higher antiparasitic activity. Albendazole is well-tolerated in people with trichinellosis [59,60,75]. In addition, when high doses were used to treat people with echinoccocosis, GI side effects were observed in 4%, dizziness and headaches in 2.4%, urticaria and itching in 1.2%, leucopenia in 2.4% and elevated serum transaminases in 16.6% (repeated sequences of 800 mg/day for 4 weeks) [76]. Alopecia has also been reported [77]. Albendazole is available in tablets (200 mg) or as a suspension (20 ml bottle at a concentration of 100 mg/5 ml). In adults, it should be used at a dose of 800 mg/day (15 mg/kg/day) administered in two doses for 10 – 15 days; in children aged > 2 years the drug is given at 10 mg/kg. For severe infection, the treatment may be repeated after 5 days. Blood cell counts and liver function should be regularly monitored. Albendazole is contraindicated in pregnant women and not recommended in children aged < 2 years, although offspring of pregnant women accidentally receiving albendazole at high dosages did not show any damage at birth [78-80].

14.5 New formulations of benzimidazoles

The effectiveness of benzimidazoles is limited by their poor water solubility and the consequent poor bioavailability in GI fluids and poor absorption through the GI lumen. To increase the rate of absorption and the concentration of these drugs in plasma, certain formulations have been developed and used in animal models. Specifically, mebendazole and albendazole have been supplemented with polyvinylpyrrolidone to create solid dispersions and albendazole in liquid solutions has been supplemented with absorption promoters (diethylene glycol monoethyl ether [Transcutol®]) or mixed with crystalline complexes of cyclodextrin [81-83]. As a result of their increased bioavailability, these formulations have shown an increased antiparasitic activity against GI and encapsulated muscle larvae. Methimazole, an inhibitor of microsomal oxidases, shows increased bioavailability when administered with albendazole, inhibiting oxidation of its main active metabolite (albendazole-sulfoxide) to sulfone and consequently increasing its antiparasitic activity [84]. With regard to the most suitable dosage for these formulations, the study of albendazole formulations has shown that treatment with repeated doses allows the drug metabolites to remain in plasma for a significantly longer period of time compared to single doses. Since the effectiveness of three doses against encapsulated larvae has been shown to be significantly higher than that achieved with a single dose, the effectiveness of these anthelmintics is thought to be correlated with pharmacokinetic parameters [84]. Although these results are promising, studies have only been conducted among mice and the use of these formulations for humans needs to be evaluated in clinical trials.

15. Glucocorticosteroids

Though no real controlled studies have been performed, glucocorticosteroids are used by most physicians to treat the signs and symptoms of immediate-type hypersensitivity. They must always be used in combination with anthelmintics and never alone, since they could increase the larval burden by delaying the expulsion of worms from the intestine. Klein *et al.* [67] showed that steroids used in combination with mebendazole would significantly shorten the duration of fever. They could also provoke a prolonged eosinophilia resulting from a delayed encapsulation process of the muscular larvae [75]. Glucocorticosteroids could also be

used to treat acute vasculitis and myositis, which could also help to prevent complications by inhibiting eosinophil activation, degranulation and cytotoxicity for endothelium [13]. Moreover, dexamethasone administered with albendazole has been reported to increase the serum levels of albendazole sulfoxide by ~ 50% [85]. The most commonly used glucocorticosteroid is prednisolone, which is available in tablets of 1 or 5 mg and is administered at 30 – 60 mg/day in multiple doses for 10 – 14 days.

16. Expert opinion on treatment

The principal anthelmintics used for trichinellosis are mebendazole (Vermox®, Janssen Pharmaceuticals) and albendazole (Zentel®, GlaxoSmithKline). Thiabendazole is no longer used because of its side effects. Pyrantel (Combantrin®, Pfizer) has been proposed for children and pregnant women [30] and flubendazole has been used in some countries. However, the efficacy of these two products is doubtful.

To eliminate larvae from the GI lumen, thus preventing muscle invasion and the development of trichinellosis, anthelmintics must be used at the stage of GI invasion, that is, < 1 week after infection. However, this is rarely possible and treatment is usually begun during the beginning of their development in muscle cells. Since it has not been clearly established how long the adult females survive and produce new-born larvae in the human intestine, it is recommended that anthelmintics should be administered to all people with trichinellosis during the 4 – 6 weeks after infection. Mebendazole was shown to prevent the occurrence of clinical disease when given to people 48 h after the consumption of meat highly infected with *Trichinella* [68]. The later the treatment is prescribed, the higher the probability that the infected person will harbour viable larvae in their muscles for years [69], with possible persistent myalgia. The different options for treatment are summarised below.

16.1 Treatment of severe and moderately severe disease

- Hospitalisation is compulsory for severe forms and debatable for moderately severe forms.
- Administration of anthelmintics (albendazole or mebendazole).
- Monitoring of the pharmacokinetics of anthelmintics (if possible).
- Administration of glucocorticosteroids (prednisolone).
- Compensation of fluid and electrolyte deficits.
- Administration of pain killers.

16.2 Treatment of benign, abortive and asymptomatic disease

- Administration of anthelmintics (albendazole or mebendazole).
- Administration of NSAIDs if necessary.

16.3 Treatment of children

- Administration of anthelmintics (albendazole or mebendazole) for children aged > 2 years; the use of these drugs in younger children is contraindicated in principle.
- Administration of glucocorticosteroids if necessary.

16.4 Treatment of pregnant women

- Hospitalisation is compulsory for symptomatic forms.
- Only anthelmintics that are poorly absorbed by the GI lumen should be used (i.e. pyrantel 10 mg/kg for 1 – 3 days), although the efficacy of these drugs has not been evaluated in humans or is doubtful. However, for severe infection, mebendazole could be considered under the physician's control and responsibility.
- In the acute stage of trichinellosis, with severe disease, prednisolone may be administered at a dose of 20 – 30 mg/day for 10 – 12 days, gradually tapering the dose (particularly for women in the third trimester of pregnancy). Salicylates are not administered because of their negative effects on the fetus.

16.5 Treatment of sequelea and of chronic trichinellosis

At this stage, anthelmintics are useless; however, glucocorticosteroids or NSAIDs prescribed for short periods can lead to some transient improvement of myalgia. Physiotherapy and psychotherapy could certainly alleviate muscular and neurological sequelae.

17. Conclusions

Any physician who observes a case of trichinellosis should alert public-health and veterinary authorities so that other cases and the source of infection can be identified and so that treatment can be begun as soon as possible. Additional information on all aspects of human trichinellosis or assistance with specific problems can be obtained by contacting the International Commission on Trichinellosis [101]. Although it has not been clearly proven by case-control studies, early treatment with anthelmintics and glucocorticosteroids must be used to alleviate the general syndrome of the disease, to prevent complications and to reduce persistent muscular pain. Anthelmintics are effective in the GI stages of the parasite and should be prescribed in all occurrences, although efficacy against muscle larvae decreases as the time between infection and treatment increases. But there are still several important unresolved questions about the treatment of trichinellosis: whether or not to treat with benzimidazoles a pregnant woman with a severe disease; what is the anthelmintic drug of choice for severe, life threatening disease; would higher doses of benzimidazoles have a higher effect on encapsulated larvae; and could new formulations or absorption promoters increase the efficiency of benzimidazoles? Information from prospective, controlled trials is urgently needed.

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