Neglected Tropical Diseases

Jill E. Weatherhead *Editor*

Neglected Tropical Diseases - North America

Springer

Neglected Tropical Diseases

Series Editor

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Neglected Tropical Diseases - North America

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Preface

Neglected tropical diseases (NTDs) are infectious pathogens that disproportionately affect persons living in extreme poverty in subtropical and tropical regions. Over one billion people are infected with at least one, commonly more, NTDs worldwide. This group of parasites, viruses, bacteria, and fungi has significant geographic overlap, most commonly associated with low- and middle-income countries in Africa, Asia, and Latin America [1, 2]. However, more recent analysis suggests that there is a high prevalence of NTDs in areas of poverty within high-income countries including the United States (US) and Canada. NTDs in G20 nations and Nigeria contribute to 51% of the global disability-adjusted life years (DALYs), a measurement of the "healthy" years lost as a result of premature mortality or disability, due to NTDs [3].

NTDs disproportionately affect persons living in poverty due to poor sanitation and waste management, unstable housing conditions, lack of access to health care, and close living environments with disease vectors[2]. Acquisition of these diseases leads to chronic disability that hinders academic achievement, wage potential, and work productivity significantly impacting economic advancement within communities. This creates a cycle of poverty, in which individuals living in poverty are at increased risk of infection, the infection causes chronic, debilitating disease, and widespread chronic disease among community members impedes the community from economic advancement [1, 4]. As a result of this cycle, NTDs can also be classified as "Infections of Poverty" [5].

Many cases of NTDs in the US and Canada can be contributed to travelers, refugees, or immigrant populations with exposure to NTD endemic regions around the world. However, there is significant poverty in both the United States and Canada that has contributed to the maintenance or emergence of autochthonous transmission of NTDs in North America outside of Mexico. Approximately, 10.5% of the US (34 million people) and 11% of the Canadian populations are living in poverty [6, 7]. Rates of poverty however are higher in Black (18.8%), Hispanics, of any race (15.7%), and foreign-born (12.6%) people living in the US [6]. As a result,

NTDs in the US disproportionately affect underrepresented groups [5]. Furthermore, certain regions of the US specifically the US south have significant-poverty stricken regions with 12.0% of people living below the poverty line [6]. In addition to the pockets of poverty in the US south, particularly in states along the Gulf Coast, the subtropical climate increases the risk of NTDs in these areas [1]. The US has a long history of tropical diseases including malaria which has since been eradicated but several others remain endemic. NTDs such as soil-transmitted helminths continue to be prevalent throughout the US south and Appalachia [8, 9]. In areas in the US south and southwest, Chagas, leishmaniasis, Hansen's disease, vector-borne disease including dengue and murine typhus and cysticercosis remain endemic. Throughout the US, toxocariasis and West Nile Virus can cause significant disease, particularly in urban centers [5, 10].

While it is widely regarded that NTDs exist in the US and in Canada, these diseases remain underdiagnosed and underreported leading to a largely undetermined burden of disease in North America, outside of Mexico. Enhanced surveillance systems, improved accessibility to diagnostic testing and treatment, and increased recognition of these disease among healthcare providers are critical for further understanding the impact these diseases have on the medical and economic health of the US and Canada. The following chapters discuss the implications of NTDs in the US and Canada (NTDS in Mexico will be discussed in a separate edition), both imported and autochthonous transmission, diagnostic and treatment availability, and prevention strategies, in order to reduce the impact of NTDs in this region.

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Soil-Transmitted Helminthiasis

Jannet A. Tobon Ramos, Cesar G. Berto, and Christina Coyle

Abstract Globally, almost one billion people are infected with soil-transmitted helminths (STH). The global prevalence of STH is closely associated with degree of poverty and insufficient sanitation and disproportionately affects children. Most infected individuals are asymptomatic, but children with heavy parasite burdens are at the highest risk for impaired physical development. The most important human STH species are hookworm (*Necator Americanus, Ancylostoma duodenale*), *Ascaris lumbricoides, and Trichuris trichuria*. During the early 1900s, the prevalence of these parasites was higher in rural areas of the Southeast United States; their prevalence has decreased greatly due to the economical and sanitation improvement. However with the increase in international travel and migration, healthcare providers in non-endemic regions like the United States and Canada should be aware of these diseases. The detection of eggs in stool samples by light microscopy is the most common method for diagnosis but has limited sensitivity. Treatment with benzimidazole is effective; however, reinfection can occur frequently in endemic regions. A holistic approach to social determinants of health is needed and recommended by the World Health Organization (WHO), which includes access to appropriate sanitation, hygiene education, and preventive chemotherapy.

Keywords Nematodes · Intestinal · Helminths

1 Introduction

Soil-transmitted helminths (STH) are parasitic nematodes that affect the gastrointestinal tract of humans. They are the most prevalent human parasites with a worldwide distribution, but highly endemic in tropical and subtropical regions.

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The main risk factor for their acquisition is contact with soil contaminated with human feces.

The four most important species: in human disease are "hookworm" (*Necator americanus* and *Ancylostoma duodenale*), *Ascaris lumbricoides*, and *Trichuris trichiura*.

1.1 Global Burden of Soil-Transmitted Helminths

Worldwide, almost one billion people are infected with at least one species of STH. In 2010, an estimated 440 million people were infected with hookworm, 819 million with *A. lumbricoides*, and 464 million with *T. trichiura* [1]. New estimates from the global burden of diseases study in 2017 reported a decrease of infections, with an estimate of 230 million people infected with hookworm, 447 million with *A. lumbricoides*, and 289 million people infected with *T. trichiura* [2]. This reduction is likely due to successful preventive chemotherapy programs. These infections are not a leading cause of death, but they are a cause of important disability during childhood. In 2017, these infections resulted in more than 1.9 million disability-adjusted life-years (DALYs) in all ages [2, 3].

2 *Ascaris* (Roundworm)

2.1 Epidemiology

Ascaris lumbricoides is the most common human helminthic infection worldwide, affecting mainly children living in Asia, Africa, and South America [1] and considered the leading cause of impaired child development in resource limited regions around the world [4]. Socioeconomic factors, particularly sanitation, play a large role in environmental contamination with fertile eggs. The epidemiology of this parasite in the United States (US) has been closely linked to the conditions of poverty and inadequate sanitation with rates of *Ascaris* in children up to 75% in certain rural southeast US counties in the 1930s [5]. This inequity was still reported in 1970 with 14% of schoolchildren infected with ascariasis in another rural county in Eastern Kentucky [5, 6]. In 1974, it was estimated that four million people living in the US were still infected with *A. lumbricoides* [7, 8], but no epidemiologic surveys have been conducted since that time [9]. A systematic review of the published literature on STH in the United States from 1940 to 2010 noted some regions of the Appalachia continue to have sustained significant prevalence of *A. lumbricoides* [10].

2.2 Transmission

Ascaris lumbricoides is acquired via ingestion of water or contaminated food with *Ascaris* eggs. The ingested eggs hatch into larvae in the small intestine and mature into an adult worms in the jejunum after completing their essential larval migration lifecycle. Prior to developing into adult worms, hatched larvae penetrate the intestinal wall and migrate via venous blood through the liver and heart to the lungs. The larvae are then coughed up or swallowed and returned to the intestine to develop into adult worms.

In the intestine, the adult worm (Fig. 1) can grow up to 35 cm in length and can live between 10 to 24 months [11]. Approximately 2 months after the initial ingestion, the adult female worm begins to produce eggs that are eliminated in the human stools. In a warm and humid environment, the fertile eggs become embryonated in the soil and can be ingested by new hosts (Fig. 2) [11]. Adult worms do not multiply within the human host and the intensity of infection will depend on the degree of exposure over time.

Human infection with *Ascaris suum*, a nematode that infects pigs, has also been described. Patients are usually asymptomatic and report excreting worms in the

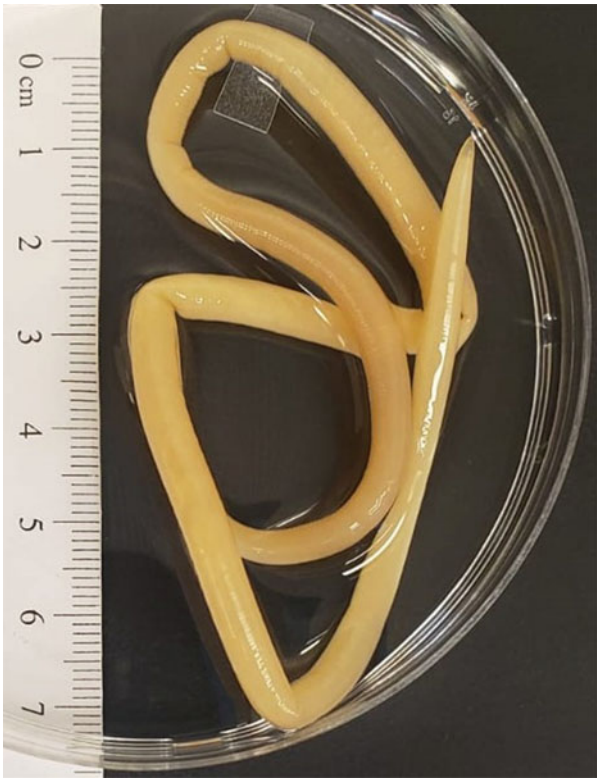

Fig. 1 Adult female *Ascaris lumbricoides* (Courtesy: Cesar Gabriel Berto Moreano, MD)

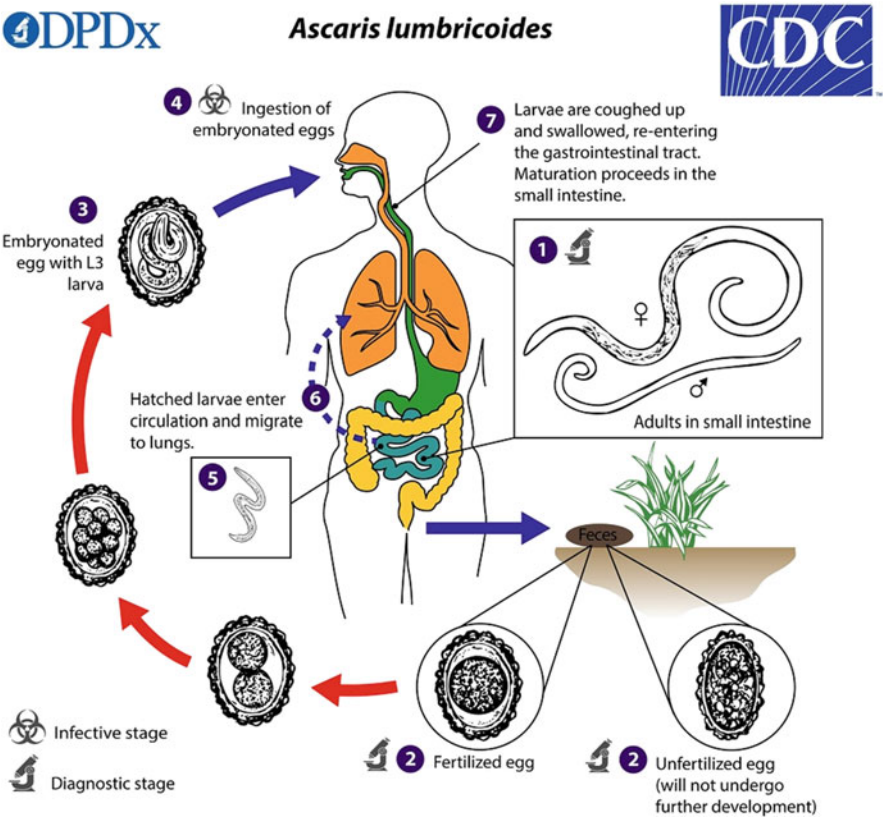

Fig. 2 Life cycle of *Ascaris lumbricoides* (available from: https://www.cdc.gov/parasites/ascariasis/biology.html)

stool. Human infections by this parasite have been increasingly recognized in areas of frequent human-pig interaction, including the United States [12]. Certain practices such as the use of pig manure as fertilizer, use of pig bedding for compost, and location of pig pens where produce is grown seem to contribute to the acquisition of this disease [12].

2.3 Clinical Manifestations

Most infections with *A. lumbricoides* are asymptomatic. The spectrum of illness is broad and ranges from mild abdominal discomfort to pulmonary symptoms to abdominal catastrophes. Children, who have the highest intensity of infection, tend to have more severe clinical manifestations [13].

Pulmonary manifestations are related to inflammation provoked by the migration of the developing larvae through the lung. Pulmonary symptoms usually develop during the second week after the ingestion of eggs as a consequence of both the physical disruption elicited by larvae crossing from blood vessels into the airways and the host response, which is a type 2 mediated immune response. Symptoms range from mild cough with no radiologic changes to Löffler syndrome [14] with transient pulmonary infiltrates, dyspnea, severe cough, and eosinophilia. This is usually a self-limited reaction but tends to be worse in nonimmune hosts [11, 14, 15]. In countries where transmission of *Ascaris* is seasonal (e.g., Saudi Arabia), seasonal outbreaks of pneumonitis are typical [16].

Once adults develop, most individuals with intestinal ascariasis are asymptomatic. Symptoms, if present, include abdominal discomfort, dyspepsia, diarrhea, loss of appetite, or nausea [17]. Moderate and heavy infections may interfere with the absorption of proteins, fats, lactose, and vitamin A, causing impairment of intellectual development, cognitive performance, and growth [11, 18].

Chronic ascariasis complications are one of the main causes of surgical admission in endemic areas. A large bolus of worms can lead to small bowel obstruction in young children. These complications are mainly mechanical given the large size of adult worms. Volvulus, intussusception, and intestinal perforation have also been reported, especially in children [19].

Biliary obstruction results from the presence of an adult worm that entered the biliary tree leading to spasms of the sphincter of Oddi [20]. Adult worms may migrate into the gallbladder and infrequently ascend the biliary tree leading to hepatic abscesses [21]. Pancreatitis, which occurs less frequently than biliary disease, has also been well described [22, 23].

2.4 Diagnosis

The detection of eggs in stool samples by light microscopy remains the cornerstone of diagnosis (Fig. 3); however, the diagnostic accuracy requires well-trained laboratory technicians [24]. A common method of examining a stool sample is the direct method, which involves mixing a small stool sample with a drop of 0.85% NaCl and examining the mixture on a slide under a microscope. Sensitivity can be increased using sedimentation and concentration techniques as well as daily collection of stool samples for 3 consecutive days [25]. Other techniques, such as FLOTAC [26] and McMaster egg counting technique [27], have also been used to a lesser extent.

A clinical diagnosis of *Ascaris* pneumonia is made in a patient with recent exposure to infectious *Ascaris* eggs who present with dyspnea, dry cough, fever, and eosinophilia. In the case of a recent infection, the stool exam may not reveal eggs. Fleeting pulmonary infiltrates, peripheral blood eosinophilia, and Charcot-Leyden crystals in sputum may suggest the diagnosis [28].

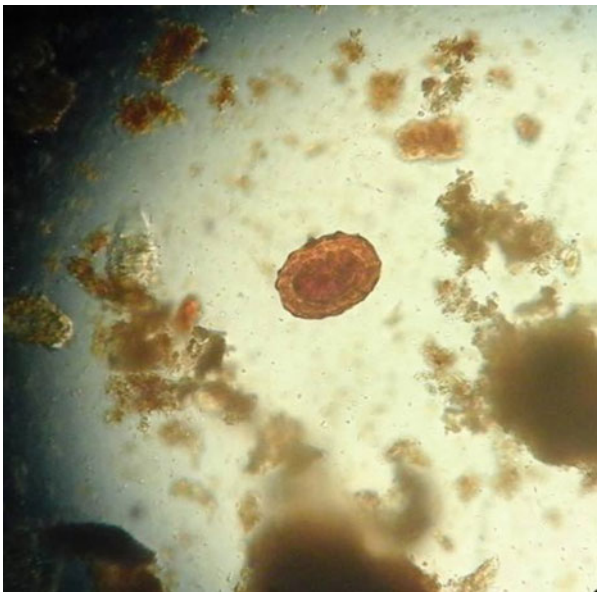

Fig. 3 Fertilized egg of *Ascaris lumbricoides* in a wet mount of stool, 200×. Fertilized eggs are characterized by a thick external mammillated layer and can be measured between 45 and 75 μm in length (Courtesy: Cesar Gabriel Berto Moreano, MD)

2.5 Treatment

Medical therapy with benzimidazoles is highly effective against the adult form of *Ascaris lumbricoides*, but not against the larvae. Several meta-analyses [29–31] have concluded that both albendazole and mebendazole could be used with high efficacy. A single-dose regimen of albendazole has been compared with a repeat-dose regimen and found similar rates of parasitological cure [32]. The current recommendation for treatment is a single dose of albendazole (400 mg orally once) or mebendazole (500 mg orally once), unless in the first trimester of pregnancy. For the latter, a single dose of pyrantel pamoate (11 mg/kg orally once, up to a maximum of 1 g) is recommended [33].

3 Hookworm

3.1 Epidemiology

Hookworm infection is one of the most common chronic infections in tropical areas and an important cause of morbidity due to the deleterious consequences in the nutrition, growth, and development of children [34, 35]. In 2003, it was estimated that more than 740 million cases of Hookworm occurred worldwide with a higher prevalence in sub-Saharan Africa and East Asia [36]. The two major species of hookworm that cause human infection are *Ancylostoma duodenale* and *Necator*

americanus. *Ancylostoma ceylanicum*, which mainly infects dogs, has been recently identified in humans in focal regions of Southeast Asia [4, 37].

In the early 1930s, the southern United States had a high prevalence of *N. americanus* infection, with a rate of hookworm infection as high as 53% [38, 39]. The warm and humid environment of the southern United States combined with the lack of sanitary infrastructure and significant poverty provided a suitable environment for the parasite to be maintained [40, 41].

In response to the high rates of this disease, the Rockefeller Sanitary Commission for the Eradication of Hookworm Disease and its successful control program was established [38, 39]. Thousands of individuals were treated resulting in a reduction of the prevalence to 39% [40]. These interventions had a positive impact on school enrollment, attendance, and literacy, and the cohort that received treatment had substantial gains in long-term incomes [34, 38].

Due to the widespread transmission of hookworm and reinfection rates in the US south, high prevalence have persisted in some areas; a study conducted in the 1950s in rural Alabama still uncovered a Hookworm prevalence of 60% in some counties [42]. After the improvement in sanitation, the prevalence of hookworm infection decreased, but some rural areas with extreme poverty and open sewage systems unfortunately continue to have reported cases. For example, a cross-sectional study recently performed in rural Alabama found the prevalence of *N. americanus* to be as high as 35% using a multiparallel quantitative real-time polymerase chain reaction (PCR) diagnostic method [38, 41].

3.2 Transmission

The three most important conditions for transmission of hookworm are contamination of soil with human feces, favorable soil conditions for larval survival, and contact of human skin with contaminated soil. Eggs are passed in the stool, hatch in the soil, and release rhabditiform larvae that can mature into infective filariform larvae under certain environmental conditions. The filariform larvae can penetrate the human skin, migrate through the blood vessels to the lungs, are coughed up, and finally swallowed into the small intestines (Fig. 4) [11, 35]. In the small intestine, the larvae mature into adult worms and attach to the intestinal mucosa using their cutting apparatus. By secreting hydrolytic enzymes and anticlotting factors [43], the parasite ensures a continuous blood flow that is utilized for its own nutrition [35, 43]. *Ancylostoma duodenale* removes 0.20 ml/day of blood and lives 1–2 years, while *N. americanus* removes less blood (0.03 ml/day) and lives 3–5 years [11].

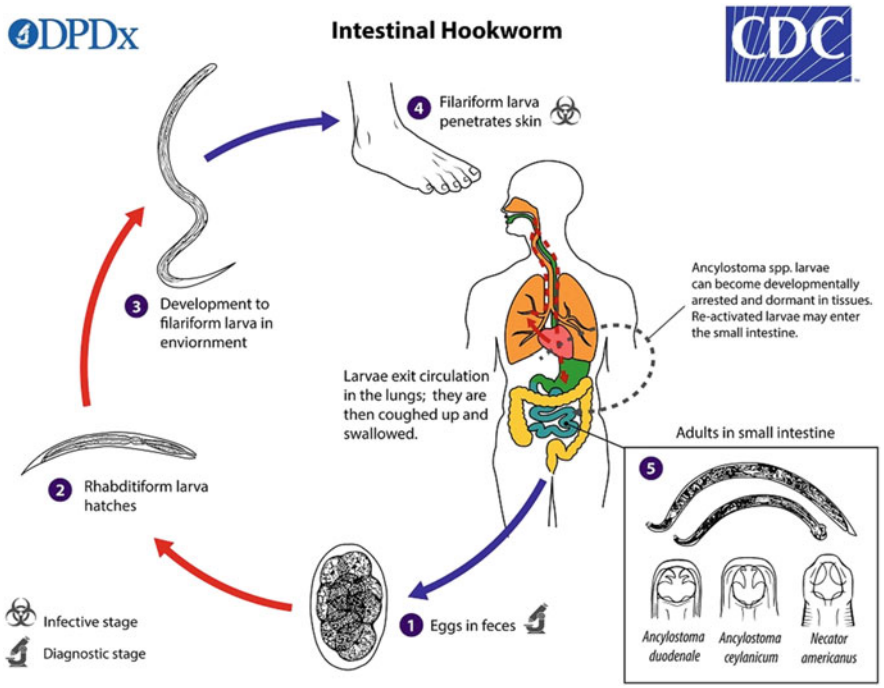

Fig. 4 Life cycle of hookworm (available from: https://www.cdc.gov/parasites/hookworm/biology.html)

3.3 Clinical Manifestations

Similar to those of *Ascaris*, the clinical manifestations of hookworm will depend on the phase of infection. Percutaneous exposure to infective hookworm larvae results in papulovesicular dermatitis referred to as "ground itch." The rash is a self-limited focal pruritic maculopapular eruption at the site of penetration, usually between the toes, and sometimes there is a serpiginous track of subcutaneous larval migration seen on physical examination [11, 15, 37]. A second urticarial rash can occur when the larvae migrate through the lungs. Hookworm pneumonitis occurs as the larvae migrate through the lungs and provoke a mild transient pneumonitis manifested by cough and pharyngeal irritation, similar to *A. lumbricoides* infection, but less severe and less common.

Once the adult worms are established in the small intestine, patients may start experiencing gastrointestinal symptoms. The symptoms caused by hookworm infection in the intestine usually depends on the burden of parasite infection. While light burden infections can be asymptomatic [44], heavy burden infections are usually associated with nonspecific gastrointestinal symptoms [45, 46]. The major clinical manifestations of hookworm infection are caused by chronic intestinal blood loss, leading to severe iron deficiency anemia and hypoalbuminemia [35, 37].

When ingested, *Ancylostoma duodenale* may cause Wakana syndrome, caused by the early migration of third-stage larvae, presenting with nausea, emesis, pharyngeal irritation, cough, and dyspnea [35].

3.4 Diagnosis

Similar to other STH, the detection of the eggs by microscopy in stool sample (Fig. 5) is the main technique for the diagnosis of hookworm infection. However, the sensitivity of the tests is limited by the high variation in egg production. Moreover, the eggs of *A. duodenale* and *N. americanus* cannot be differentiated under light microscopy. PCR can distinguish the two species, although it is only available for research purposes [47]. Recently, Ig-G4 assays have been developed and may help to identify recent infection, but its utility is still not determined [15, 48].

3.5 Treatment

The choice of treatment is a single dose of a benzimidazole, albendazole (400 mg orally once) or mebendazole (100 mg orally twice daily for 3 days or 500 mg orally once). Both are effective at reducing hookworm burden. Pyrantel pamoate (11 mg/kg orally daily for 3 days, up to a maximum of 1 g/day) is an alternative treatment.

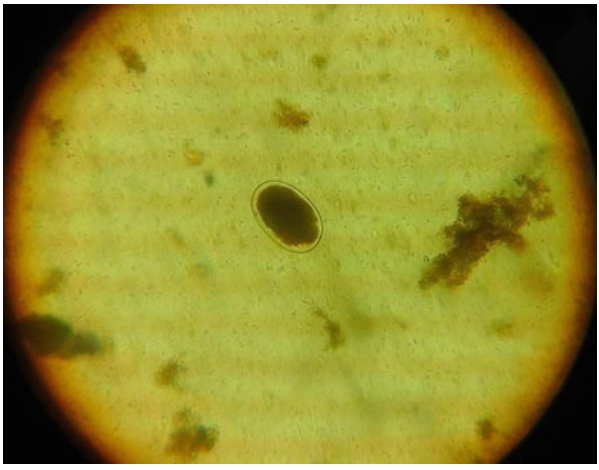

Fig. 5 Hookworm egg in a wet mount of stool, 200×. Hookworm eggs are oval-shaped with a characteristic clear space between the ovum and the shell (Courtesy: Cesar Gabriel Berto Moreano, MD)

4 *Trichuris* (Whipworm)

4.1 Epidemiology

It was estimated that there were approximately 500 million cases of trichuriasis worldwide in 2015 [2]. *Trichuris trichiura* frequently coinfects with other STH such as *A. lumbricoides*, as these parasites thrive under similar conditions, with higher prevalence in tropical and subtropical areas and populations with sanitation deficiencies [4, 49]. Moreover, it mostly affects preschool and school-age children due to their frequent exposure to soil [4] and possibly due to the development of partial immunity after repetitive exposures [50]. Because of these two features, this parasite is widely distributed among the pediatric population from impoverished areas, in which it is an important cause for cognitive-developmental challenges and height and weight restriction [4, 49].

A systematic review [10] of studies done between the 1940s and 1980s reported a prevalence of *T. trichiura* infection between 0.5% and 55.2% in the United States. The highest prevalence was found among school-age children from rural Kentucky in 1965 [51]. The improvement in sanitation impacted the prevalence during the following years but persisted with a prevalence as high as 12.6% in rural areas of Kentucky in 1982 [6]. Subsequent epidemiologic studies are lacking and thus the on-going prevalence in the US remains unknown.

4.2 Transmission

The mechanism of transmission for trichuriasis is fecal-oral. Trichuris eggs are expelled from the intestinal track with human stool and contaminate the soil. In the soil, depending on the environmental conditions, the eggs can embryonate after 15–30 days [52]. The infective eggs are ingested with contaminated food or water. In the proximal part of the large intestine, the eggs hatch and the larvae penetrate the epithelial cells at the crypt base where they develop into adult worms [53]. The posterior end of the adult, which can measure up to 4 cm in length, protrudes into the colonic lumen while the anterior "whip" end is embedded into the colonic mucosa [54]. Approximately 4 weeks after the initial infection, female worms begin to produce between 2000 and 8000 barrel-shaped eggs daily for 1–3 years (Fig. 6) [55].

4.3 Clinical Manifestation

Most of the infections with *T. trichiura* are asymptomatic; peripheral eosinophilia may be present. In individuals with heavy infections, the intestinal mucosa is inflamed, edematous, and friable, causing colitis with loose stools containing

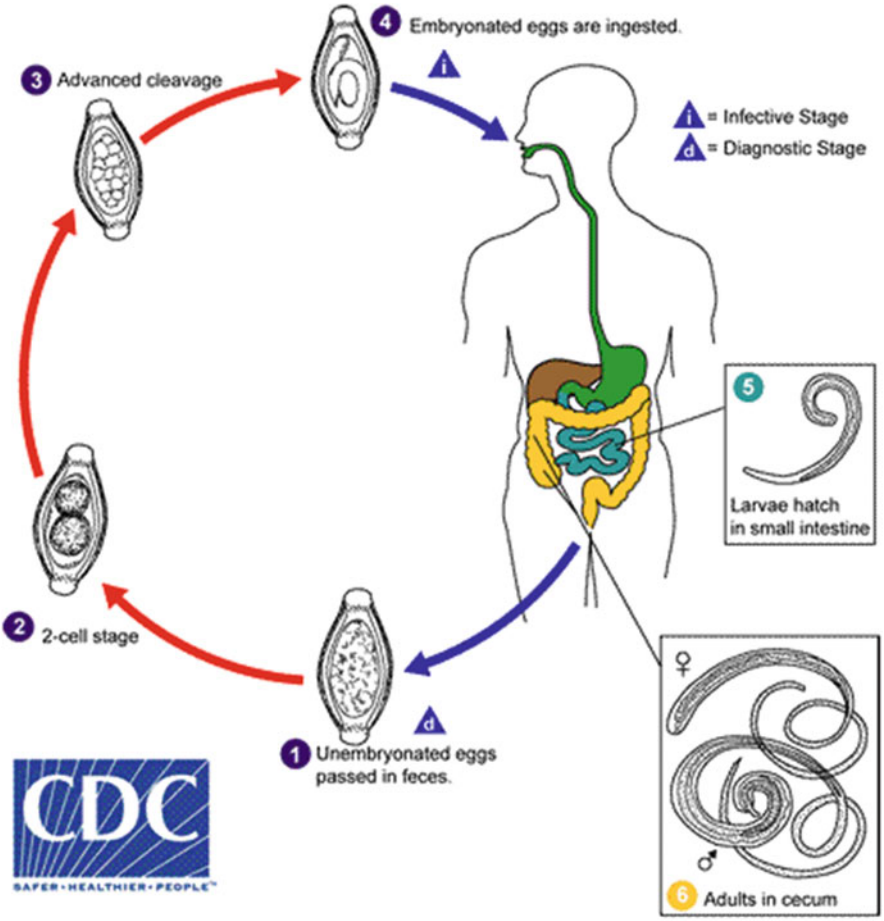

Fig. 6 Life cycle of *Trichuris trichiura* (available from: https://www.cdc.gov/parasites/whipworm/biology.html)

mucus and blood, which resembles inflammatory bowel diseases. Children can complain of abdominal pain, abdominal discomfort, and mucus discharge. Rectal prolapse can also occur, and embedded worms can be visualized in the inflamed rectal mucosa [4].

Trichuris dysentery syndrome has been reported in children with a very high parasite burden and it is characterized by diarrhea, tenesmus, iron deficiency anemia, and growth retardation [56].

4.4 Diagnosis

The diagnosis of trichuriasis relies mainly on direct examination of eggs in stool samples in a quantitative or qualitative manner. The eggs have a characteristic barrel shape with a thick wall and a plug at each end (Fig. 7). Repetitive samples and concentration techniques can help to increase the low sensitivity of direct microscopy diagnostic tests [57]. The detection of a copro-antigen has been used in veterinary diagnostics but has yet to be utilized for human disease [57, 58]. Additionally, the use of polymerase chain reaction is a more sensitive method that is being utilized more frequently for clinical and research purposes [59]. Incidentally, adult worms protruding from the bowel mucosa can be seen during colonoscopy or proctoscopy [60].

4.5 Treatment

Overall, the efficacy of antiparasitic drugs is lower for *Trichuris* sp. than the other STH. Clinical trials have shown a lower cure rate after administering a single dose of antiparasitic medication such as benzimidazoles compared to repeat dosing [32, 61]. Furthermore, meta-analyses have shown higher rates of parasite cure for mebendazole compared to albendazole [29, 31]. One double-blind controlled clinical trial [62] suggested that oxantel pamoate (not available in the United States) in combination with albendazole may be more effective than mebendazole alone for the treatment of *Trichuris* infections. The current recommendation is mebendazole (100 mg orally twice daily for 3 days) as the choice treatment. Alternatively, albendazole (400 mg orally daily for 3 days) can also be employed. In pregnant women, it is advised to defer treatment until after delivery.

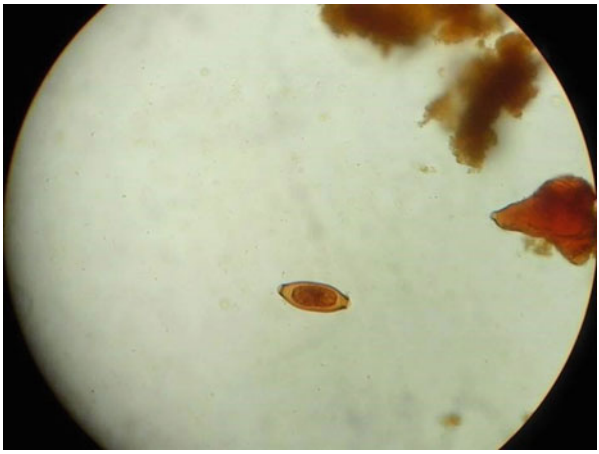

Fig. 7 *Trichuris trichiura* egg. This egg is characterized by a barrel-shaped structure with a pair of polar "plugs" at each end (Courtesy: Cesar Gabriel Berto Moreano, MD)

5 Prevention

To achieve sustained control of STH prevalence, infection intensity, and morbidity, the World Health Organization recommends an integrated approach that includes access to appropriate sanitation, hygiene education, and preventive chemotherapy [63, 64].

A systematic review and meta-analysis in 2012 showed that the availability and use of sanitation facilities were associated with a reduction in the prevalence of infection with STH [65]. Moreover, sanitation not only reduces infection prevalence, but it also prevents reinfection. The effects of improved sanitation on helminth transmission are slow, may take decades, and should cover a high percentage of the population to see a significant impact similar to what was implemented by the Rockefeller Sanitary Commission for the Eradication of Hookworm Disease in the United States [66]. However, further local epidemiologic surveillance to understand the on-going prevalence of STH and the risks of transmission due to on-going poverty in the US and Canada is critical to facilitate control efforts.

Finally, screening for parasitic infections like STH remains a public health priority in the US healthcare settings serving refugees and immigrants from countries where intestinal parasites are endemic. The prevalence of hookworm, *A. lumbricoides*, and *T. trichiura* in a group of 533 refugees at a refugee clinic in California between 2001 and 2004 was 2.1%, 1.3%, and 0.4%, respectively. Hookworm and *A. lumbricoides* were found mainly in refugees from South Central Asia, while *T. trichiura* was found in African and Middle Eastern refugees [67]. The implementation of pre-departure albendazole treatment programs throughout Saharan Africa has helped to reduce the prevalence of these infections among new arrivals to the US and Canada [68, 69].

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Toxocariasis

Eva Clark

Abstract Toxocariasis is the most common clinically relevant helminthic disease in the US and Canada. Humans can be exposed to *Toxocara* spp. in a variety of ways, commonly via household pets. Although most infections are asymptomatic, four main syndromes have been attributed to *Toxocara* spp. infection: visceral larva migrans, ocular larva migrans (ocular toxocariasis), neurotoxocariasis, and covert toxocariasis (common toxocariasis). The symptoms of toxocariasis reflect the quantity and location of migrating larvae and the degree of inflammation that develops in response to the larvae. Diagnosis is based on clinical presentation, serodiagnosis, and supporting laboratory tests. Treatment with either albendazole or mebendazole is recommended for all forms of visceral toxocariasis in both adults and children. There are a number of public health measures that can be undertaken by the populace to prevent *Toxocara* infection, namely, those focused on prevention of infection in household pets and avoidance of contaminated soil.

Keywords *Toxocara* · Toxocariasis · *Toxocara canis* · *Toxocara cati* · Helminth · Pet

1 Introduction

Human toxocariasis is one of the most widespread public health zoonoses. It is caused by inadvertent infection with the larvae of the nematode *Toxocara*, primarily *Toxocara canis* (from dogs) and, to a lesser extent, *T. cati* (from cats) (Fig. 1) [1–3]. Though usually asymptomatic, toxocariasis can manifest in humans in a number

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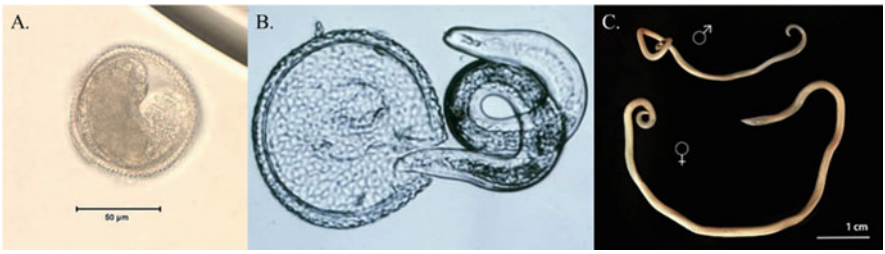

Fig. 1 Life stages of *Toxocara* spp. (from https://www.cdc.gov/dpdx/toxocariasis/index.html). (**a**) *Toxocara* sp. egg teased from an adult worm. The worm was never identified, but the egg size is most consistent with *T. cati*. Image courtesy of the New Jersey State Public Health Laboratory. (**b**) *T. canis* larva hatching. C) *T. canis* adult male and female

of ways. Disease severity ranges from covert with nonspecific asthma-like symptoms to marked eosinophilia, fever, and organ dysfunction in visceral toxocariasis (VLM); retinal scarring and visual impairment in ocular toxocariasis (OLM); and cerebral vasculitis, meningitis, encephalitis, myelitis, and seizures in neurotoxocariasis (NT) [4]. While toxocariasis is prevalent in the tropics and subtropics of low-income countries, it is also associated with socioeconomically disadvantaged communities in middle- and high-income countries where public health interventions are limited. Here we will focus on US and Canadian *Toxocara* data as Mexican data are discussed elsewhere [5].

2 Epidemiology

Globally, the prevalence of *Toxocara* spp. antibodies varies greatly [3, 6, 7] and is typically higher in adults than in children, likely from repeated exposures over time. Seroprevalence varies widely across North America as well and is usually higher in low-resource communities [8]. Although several studies have evaluated parameters such as *Toxocara* spp. egg shedding in the US (Fig. 2), robust US serologic and epidemiologic data are available primarily due to the US National Health and Nutrition Examination Survey (NHANES). In the first NHANES (1971–1973), the seroprevalence of antibody to *T. canis* excretory-secretory antigen (TES-Ag) expressed by infective larvae was 4.6–7.3% among children aged 1–11 years (reaching nearly 30% among 6–11-year-old Black children of lower socioeconomic status) [10]. While NHANES I did not evaluate adults, later, in NHANES III (1988–1994), *T. canis* seroprevalence was found to be 8.6% for children aged 1–5 years and 13.9% for persons (including adults) aged $\geq$6 years [11, 12]. In NHANES III, risk factors for *Toxocara* seropositivity included ages 20–39, non-Hispanic Black race/ethnicity, male sex, living below the poverty level, less than college education, elevated blood lead levels, dog ownership, rural residence, birth outside the US, and residence outside the Western US [11]. These data are

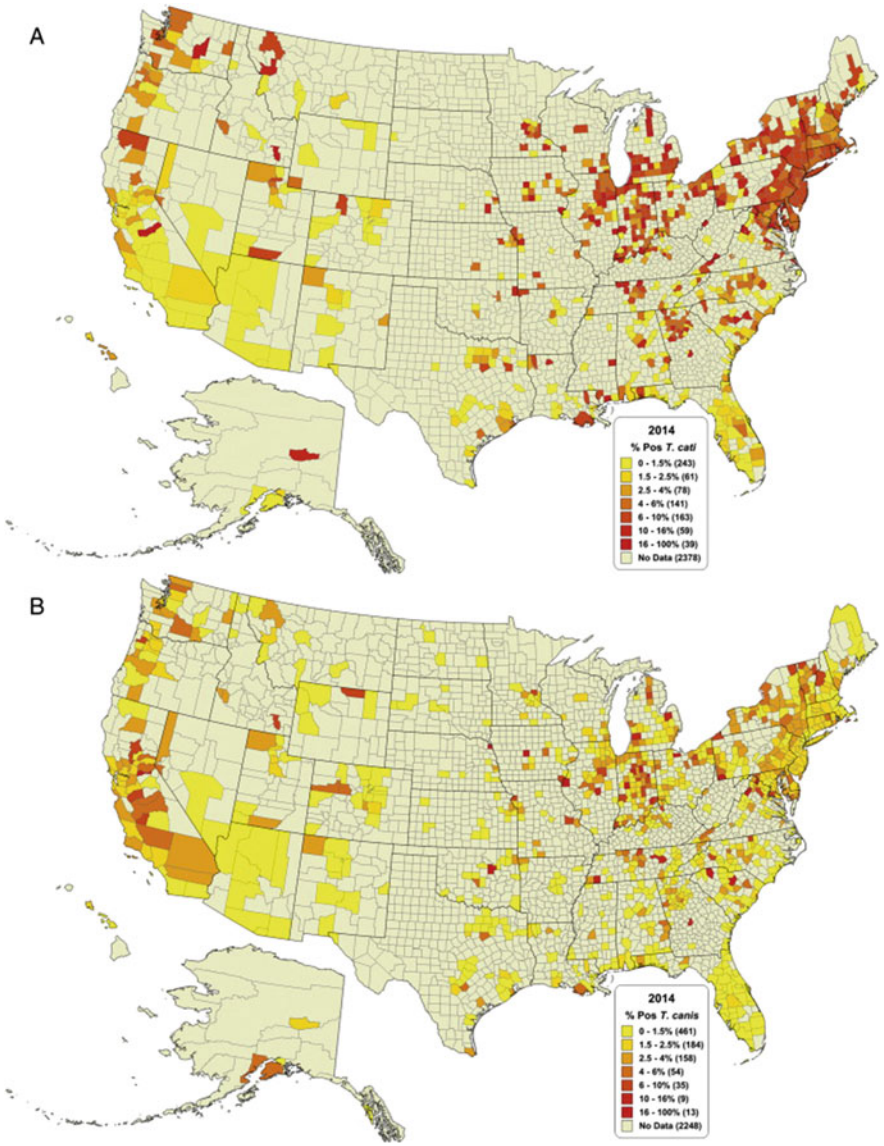

Fig. 2 Fecal egg shedding prevalence of (**a**) *Toxocara cati* and (**b**) *T. canis* per US County (calculated as the number of egg positive tests/total tests performed in that county) in 2014. Adapted from [9]

similar to that of another US study done during the same era [13] and are supported by studies of neighborhood dogs and soil that show increased risk of *Toxocara* exposure in resource-poor US communities, such as Baton Rouge, LA [14]; Baltimore, MD [15]; and rural Alabama [16].

Although insightful, these results must be interpreted with caution. The TES-Ag enzyme immunoassay (EIA) used in NHANES I and III, while reliable, cross-reacts with other helminths (e.g., *Ascaris lumbricoides*). The most recent NHANES (2011–2014) used a more specific multiplex bead-based assay (purified recombinant TcCTL-1 antigen; TcCTL-1MBA) and found an age-standardized estimate of *Toxocara* seroprevalence of 5.1% (95% confidence interval [CI], 4.2–5.8%) [17, 18]. This is lower than previously reported even after adjusting for increased TcCTL-1MBA specificity. In these studies, risk factors for *Toxocara* seropositivity included older age, non-Hispanic Black race/Hispanic origin, male sex, living below the poverty level, households with $\geq$0.5 persons per room, less than college education, and birth outside the US [19]. Most other US studies also support a higher risk of toxocariasis in males [20–24] and in immigrants [25]. An additional risk factor is exposure to environments contaminated by infected animals (such as urban playgrounds) [8, 14, 20].

In Canada, *Toxocara* is also likely more prevalent in rural areas; however *Toxocara* eggs have been identified in the soil of playgrounds and sandboxes in populous, urban areas [26, 27]. Although there is one relatively large Canadian study of *Toxocara* seroprevalence (it evaluated children living in Halifax, Nova Scotia, and found an overall seroprevalence of 17%—higher in children living in rural areas than urban areas) [28], Canadian *Toxocara* seroprevalence estimates have primarily been measured in indigenous groups thought to be at risk for toxocariasis. In those groups, seroprevalence findings range from 0.6% [29] to 13.4% [30] and reflect decreased survival of *T. canis* eggs at colder temperatures [31, 32]. Although male sex may be a risk factor for toxocariasis in Canada, dog ownership and/or occupational exposure to dogs is not a risk factor [29, 30, 33, 34], possibly because dogs that are kept as pets are more likely to be regularly dewormed, live inside, and eat a diet of commercial pet food than are feral canines [8, 35].

3 Transmission

Humans can be infected with *Toxocara* spp. in a variety of ways, including ingestion of larvae in undercooked infected organ or muscle tissues; infective eggs from soil contaminated with animal feces (e.g., gardens, sandpits, and playgrounds), unwashed hands, or raw vegetables; or by direct contact with pets [36–38]. *Toxocara canis* is transmitted predominantly among canines (dogs, foxes, wolves, and coyotes), and *T. cati* by felines, via a variety of routes (Fig. 3). These include vertical transmission through the placenta (for dogs but not cats) and/or breastfeeding, as well as horizontal transmission through the ingestion of embryonated eggs from the environment or ingestion of larvae (via consumption of smaller *Toxocara* host animals) [39]. The ability of *Toxocara* spp. to survive for years in the tissues of different vertebrate species has facilitated its global distribution. Pet dogs and cats play an important role in the transmission of *T. canis* and *T. cati*, respectively, because they excrete eggs directly into the human environment via defecation, without the involvement of vectors or intermediate hosts [40, 41]. Indeed, in the

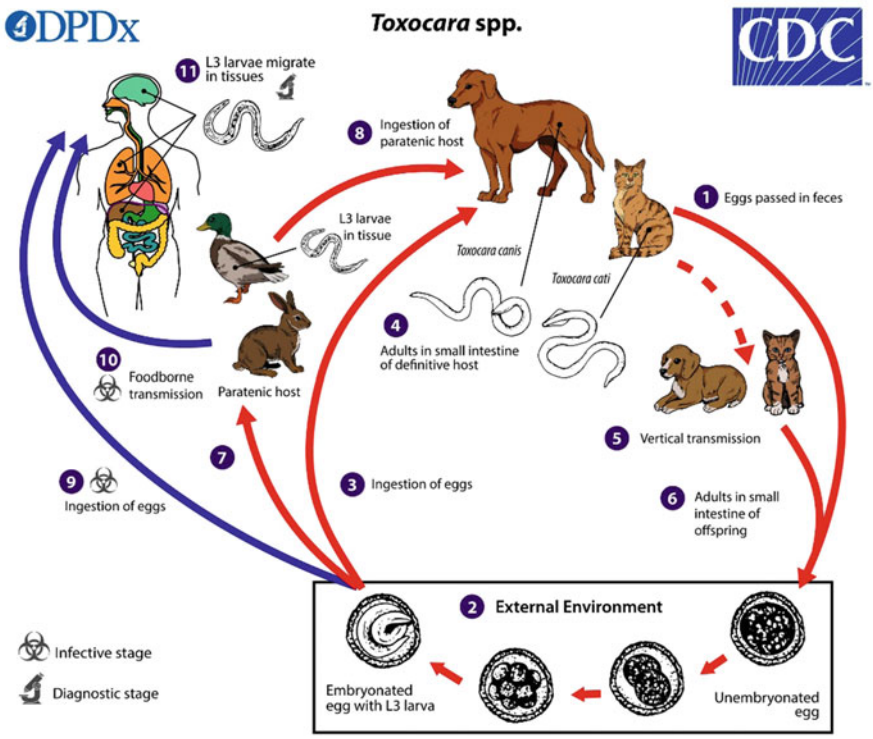

Fig. 3 *Toxocara* spp. life cycle (graphic and text adapted from https://www.cdc.gov/dpdx/toxocariasis/index.html). *Toxocara* spp. can follow a direct (one host) or indirect (multiple host) life cycle. (1) Unembryonated eggs are shed in the feces of the definitive host (canids, *T. canis*; felids, *T. cati*). (2) Eggs embryonate over a period of 1–4 weeks in the environment and become infective, containing third-stage (L3) larvae. (3) Eggs are ingested by a definitive host. (4) The infective eggs hatch and larvae penetrate the gut wall. In younger dogs (*T. canis*) and in cats (*T. cati*), the larvae migrate through the lungs, bronchial tree, and esophagus, where they are coughed up swallowed into the gastrointestinal tract; adult worms develop and oviposit in the small intestine. In older dogs, patent (egg-producing) infections can also occur, but larvae more commonly become arrested in tissues. (5) Arrested larvae are reactivated in female dogs during late gestation and may infect pups by the transplacental (major) and transmammary (minor) routes. (6) In this way adult worms become established in the small intestine of their offspring. In cats, *T. cati* larvae can be transmitted via the transmammary route to kittens if the dam is infected during gestation, but somatic larval arrest and reactivation does not appear to be important as in *T. canis*. (7) *Toxocara* spp. can also be transmitted indirectly through ingestion of paratenic hosts. Eggs ingested by suitable paratenic hosts hatch and larvae penetrate the gut wall and migrate into various tissues where they encyst. (8) The life cycle is completed when definitive hosts consume larvae within paratenic host tissue, and the larvae develop into adult worms in the small intestine. (9) Humans are accidental hosts who become infected by ingesting infective eggs or (10) undercooked meat/viscera of infected paratenic hosts. (11) After ingestion, the eggs hatch and larvae penetrate the intestinal wall and are carried by the circulation to a variety of tissues (liver, heart, lungs, brain, muscle, eyes). While the larvae do not undergo any further development in these sites, they can cause local reactions and mechanical damage that causes clinical toxocariasis

US, 1.8–2.0% of pet dogs excrete *T. canis* eggs into their stool [9, 42], and 4.6–5.1% of pet [9] and 21% of stray [43] cats are infected with *T. cati*. Infection rates are higher for dogs and cats that are left outside and allowed to eat other animals.

4 Clinical Manifestations

After a human ingests *Toxocara* eggs, larvae penetrate the walls of the intestine and travel throughout the body via the circulatory system. *Toxocara* larvae do not multiply within the human host but simply exist in their destination host tissue (s) in a state of arrested development [44]. When the larvae die, the inflammatory reaction produced by the human immune system causes the symptoms of toxocariasis. Thus, the symptoms of toxocariasis reflect the quantity and location of migrating larvae and the degree of inflammation that develops in response to the larvae. Although many infections are asymptomatic, four main syndromes are attributed to *Toxocara* spp. infection: visceral larva migrans (VLM), ocular larva migrans (OLM; also known as ocular toxocariasis), neurotoxocariasis (NT), and covert toxocariasis (CT; also known as "common" toxocariasis in adults). In addition, associations with atopic symptoms are described. Children are more frequently clinically affected than adults. Diagnosis of these syndromes is based on the presence of characteristic signs and history of exposure to a potential source of infectious *Toxocara* eggs or larvae. Immunocompromised patients may have disseminated infection resulting in atypical clinical presentations [45].

VLM is the most advanced form of toxocariasis. It occurs when *Toxocara* larvae migrate to major organs and typically presents in young children as nonspecific signs and symptoms including eosinophilia, coughing, wheezing, abdominal pain, myalgias, rash (e.g., eczema and/or vasculitis), lymphadenopathy, hepatitis with hepatomegaly, headaches, myocarditis, nephritis, or arthritis [46–48]. VLM is uncommon and often difficult to diagnose, especially in immigrants from regions where polyparasitism is endemic [49]. Positive serologic test results, marked eosinophilia, absence of other helminthic infections, compatible clinical signs, and disappearance of symptoms after specific treatment can help establish a VLM diagnosis, especially in areas of low parasitism like the US and Canada. World-wide, severe VLM is mainly seen in young (1–3-year-old) children [50]. While no explicit studies of VLM have been done in North America, this global data showing a higher risk in young children can reasonably be extrapolated to American and Canadian children who play in potentially contaminated soil in yards and sandboxes, put their fingers into their mouths, and/or eat soil.

OLM occurs when *Toxocara* larvae migrate to the eye. It is relatively uncommon and mostly reported in older (3–16-year-old) children [46, 51]. OLM typically manifests as monocular visual impairment and can be accompanied by chronic endophthalmitis, retinitis, and/or granuloma formation (Fig. 4) [46].

Blindness can result from retinal scarring, tractional retinal detachment, vitritis, or macular edema [53]. Several studies of OLM have been done in the US population

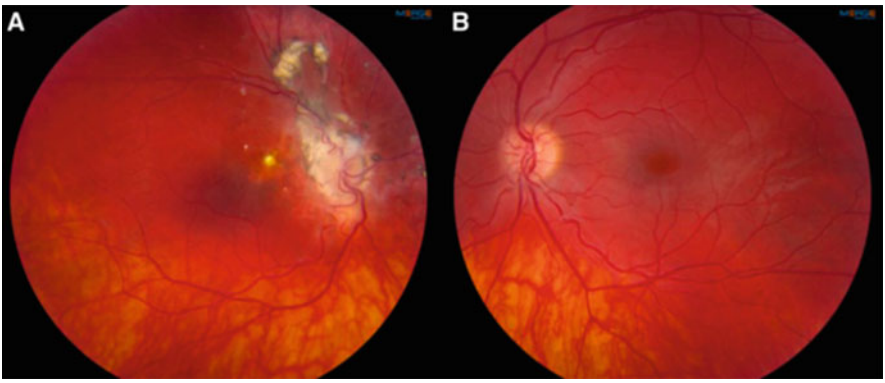

Fig. 4 Granuloma of ocular toxocariasis. (**a**) The standard field fundus photograph shows large white/yellow, posterior pole granulomas superotemporal to the optic disc. Retinal striae from the disc to the fovea were identified but are not clearly seen in the photograph. (**b**) The fundus photo of the left eye is unremarkable. Adapted from [52]

[54]. A study in the US state of Alabama estimated 1 OLM case per 1000 persons (increasing to 11 cases per 1000 persons when ophthalmoscopy was performed) [55]. A 2009 national survey of 68 patients found that the median patient age was 8.5 years (range 1–60 years), 57% lived in the Southern USA, and the most common symptom of OLM was vision loss (83% of patients with clinical data available; of those 68% became blind permanently) [23].

NT has traditionally been thought of as a syndrome that affects adults more often than children and manifests as myelitis, encephalitis, and/or meningitis (including eosinophilic meningoencephalitis) [56, 57]. In addition, various studies have noted possible associations between NT and neurodegenerative disorders, including seizure disorders, schizophrenia, idiopathic Parkinson's disease, and/or dementia [58–62]. More recently, *Toxocara* infection has been associated with cognitive and/or developmental delays in children living in low-resource communities [13]. Specifically, in the US there is one published study that used NHANES III data to measure differences in components of both the Wechsler Intelligence Scale for Children-Revised (WISC-R) and the Wide Range Achievement Test-Revised (WRAT-R) in *Toxocara* seropositive and seronegative children [22]. Seropositive children scored significantly lower on the WISC-R and WRAT-R compared to seronegative children.

CT is thought to be caused by chronic exposure to *Toxocara*. It is challenging to diagnose because symptoms are nonspecific [63]. In adults, the presentation may include weakness, pruritus [64], rash, pulmonary dysfunction, and abdominal pain. In children, the presentation may include fever, anorexia, headache, nausea, abdominal pain, vomiting, wheezing, lethargy, sleepiness, behavioral disorders, pulmonary symptoms, and limb pain. Peripheral eosinophilia and elevated IgE levels are common laboratory findings. Although European and Asian studies have noted an association between CT and atopic disorders (e.g., asthma) [65–67], US studies have

not confirmed these associations [68], and one study suggests that these serological associations may be due to cross-reactivity with *Ascaris lumbricoides* antigens [69]. European studies have also linked chronic urticaria, pruritus, and eczema in adults and children with toxocariasis [70, 71]. A large, systematic, population-based study on skin pathology and *Toxocara* spp. seropositivity is needed [72].

5 Diagnosis

Upon presentation with one of the above-described clinical syndromes, diagnosis of human toxocariasis is based primarily on serologic techniques, since larvae are trapped in tissues and not readily detected morphologically. Although biopsy of affected organs/tissues may be possible, the probability of capturing larvae via any such procedure is low. Because *Toxocara* larvae do not develop into adults in humans, a stool examination will not detect *Toxocara* eggs. Enzyme-linked immunosorbent assay (ELISA) is currently the most reliable tool for detecting antibodies [73]. The serologic test recommended by the US Centers for Disease Control and Prevention (CDC) is an ELISA with larval stage antigens, usually the excretory-secretory antigens that are released when infective *Toxocara* larvae are cultured. This assay has good specificity although cross-reactivity with antibody to *Ascaris lumbricoides* is possible. However, because anti-*Toxocara* spp. antibodies measured by ELISA persist for nearly 3 years in infected adults, their presence alone does not distinguish between current and past infections or between *T. canis* and *T. cati* infections and does not allow a probable or definitive diagnosis of clinically relevant toxocariasis [74]. Therefore, complementary, nonspecific laboratory tests (e.g., peripheral eosinophil count and total serum IgE) must be used in the diagnostic workup of suspected cases [13]. For OLM, serum antibodies are not diagnostic; intraocular antibodies appear more promising as a diagnostic aid [75]. For NT, antibodies should be measured in both the serum and cerebral spinal fluid. Molecular assays such as polymerase chain reaction (PCR)-based techniques exist but are primarily used only for investigational purposes [76]. The major pitfall in the diagnosis of human toxocariasis is the lack of standardized serodiagnostic criteria and case definitions [72].

6 Treatment

In the US, the CDC recommends anti-helminth treatment with one of two drugs approved by the US Food and Drug Administration (FDA), either albendazole (400 mg by mouth twice a day for 5 days) or mebendazole (100–200 mg by mouth twice a day for 5 days) for all forms of visceral toxocariasis in both adults and children [77]. No randomized controlled studies have been conducted to study the treatment of toxocariasis; thus, although most clinicians prescribe a 5-day

treatment course, the optimum dose and duration remain unknown [79]. Some clinicians use longer (e.g., 20-day) courses in patients with severe disease. Because both albendazole and mebendazole have limited efficacy [79] and are no longer cheap, large, well-controlled studies of more effective agents for all presentations of toxocariasis are needed. Note that the safety of albendazole and mebendazole has not been well studied in pregnant/lactating women or in young children. Supportive care, including anti-inflammatory medications (e.g., corticosteroids), may be necessary to treat the various symptomatic manifestations of toxocariasis. No recommendations exist for treating seropositive asymptomatic patients.

7 Prevention

Toxocariasis is not nationally reportable in the USA or in Canada. Even so, there are a number of public health measures that are recommended by the CDC to prevent *Toxocara* infection. It is important to control *Toxocara* infection in pets to reduce the number of infectious eggs in the environment and thereby reduce the risk of human infection. Veterinarians can recommend regular deworming for pet dogs and cats. Owners must keep their pet's living area clean and dispose of pet feces properly. Children should be prevented from playing in and/or eating soil that could be contaminated with animal feces, and sandboxes should be covered when not in use. The practice of good handwashing skills (e.g., after playing with pets and before handling food and eating) is essential for the prevention of *Toxocara* infections. Finally, meat should be well-cooked to avoid *Toxocara* infection via this route.

8 Conclusions

Although uncommon and treatable, disease caused by *Toxocara* infection can be severe and lead to long-term morbidity. Healthcare providers must have a low threshold to evaluate patients who present with typical signs and symptoms of the various *Toxocara* clinical syndromes. Maintenance of high-quality continuing education for healthcare providers including veterinarians and the provision of suitably presented information to pet owners are of priority importance for the success of *Toxocara* prevention strategies.

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Strongyloidiasis

Melody Ren and Andrea K. Boggild

Abstract *Strongyloides stercoralis* is an intestinal nematode estimated to infect at least 100 million people worldwide, predominantly in subtropical and tropical regions. With a unique life cycle and expansion of global travel and migration, strongyloidiasis is increasingly encountered in temperate and even non-endemic regions of North America. Most people harboring the worm are asymptomatic, but if left untreated, and especially in immunocompromised hosts such as people coinfected with HTLV-1/HTLV-2, *Strongyloides* larvae can disseminate and lead to a high-mortality hyperinfection syndrome manifesting as Gram-negative or polymicrobial sepsis and/or meningitis, pneumonitis, and end-organ failure. Diagnosis is based on microscopic examination or PCR of stool or serologic testing. Ivermectin remains the mainstay of treatment, but complicated infections should be treated with the support of a physician expert in tropical medicine.

Keywords Disseminated strongyloidiasis · Helminthiases · Immunosuppression · Migrant health · *Strongyloides stercoralis*

1 Introduction

Strongyloidiasis is caused by the intestinal roundworm (nematode), *Strongyloides stercoralis*. There are over 50 species of *Strongyloides* that infect a wide range of hosts [1]. Two other species, *Strongyloides fuelleborni* subsp. *fuelleborni* and

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Strongyloides fuelleborni subsp. *kellyi*, are known to infect humans but have limited clinical importance and are restricted in their geographic distribution [2]. *Strongyloides fuelleborni* infects primates and has been documented to infect humans in parts of Africa as well as Papua New Guinea [3]. This chapter focuses on the human pathogen *Strongyloides stercoralis*.

2 Epidemiology

Worldwide it is estimated that up to 100 million people are infected with *Strongyloides* [4, 5]. However, many experts believe this is an underestimate as many countries suffer from a lack of reporting and infrastructure to support high sensitivity testing [1, 6–8]. Some estimate the global prevalence as closer to 370 million people infected; however, with a dearth of epidemiological data, *Strongyloides*-related morbidity and mortality remain poorly defined [6].

Strongyloides exists mainly in tropical and subtropical regions with pockets in temperate climates comprising over 70 countries worldwide [1]. It shares a geographic distribution with hookworm [9]. With increasing trends in worldwide travel and northward migration, more cases have been encountered in non-endemic regions including in North America.

In Canada, 2.5 million people are estimated to have simple intestinal strongyloidiasis, mostly reflecting individuals born in endemic countries with a small proportion related to travel [10]. Anywhere from 9–77% of immigrants and refugees in Canada are thought to be infected, and current Canadian guidelines recommend screening for refugees from Southeast Asia, from Africa, and for immigrants from endemic areas including South America, Africa, Southeast Asia, and the Caribbean [11, 12]. Additionally, there have been reports of endemic institutional strongyloidiasis [13].

In the United States, many of the patterns observed in the Canadian context extend including infection in individuals born in endemic countries, travel-related infections, and institutional endemics [14]. There are also pockets in the Appalachia and rural areas in the southeastern United States that are endemic for *Strongyloides* [15–17]. Most people infected in these regions are involved in farming or mining activities, where skin-to-soil contact is presumed to be substantial [7].

The distribution of *Strongyloides* in Latin America is ill defined including in Mexico [18]. Studies in Mexico traditionally have been focused on capturing data on a wide range of intestinal parasites and therefore have used study techniques with a low sensitivity for strongyloidiasis [19, 20]. With these limitations, community-based and health service studies have reported less than 10% prevalence of strongyloidiasis in Mexico [7].

However unclear the specific epidemiological data are regarding strongyloidiasis prevalence; one fact remains clear: *Strongyloides*, as with soil-transmitted helminths, disproportionately affects impoverished peoples without access to adequate water, sanitation, or opportunities for socioeconomic development [21].

3 Transmission

The life cycle of *Strongyloides stercoralis* is unique and allows for host autoinfection (Fig. 1). *Strongyloides stercoralis* exists in four stages: egg, noninfectious rhabditiform larvae (250–300 μm by light microscopy), infectious filariform larvae (measuring 550 μm), and adults, either male and female sexually reproducing in the environment or as parthenogenetic female worms in the intestinal tract (semitransparent colorless worms measuring 2.2 mm). Adult male worms do not exist in the human intestinal tract [22]. Infectious filariform larvae penetrate the host skin from the environment and access the venous or lymphatic systems and then migrate to the lungs. Larvae are able to penetrate alveoli and migrate through to the bronchial system until they reach the trachea and are coughed then swallowed into the host into the gastrointestinal tract. It takes 18–28 days for the larvae to reach the small bowel mucosa from the time of skin penetration. Larvae then develop into adult females that intercalate themselves (hence the moniker "thread worm") into the small bowel epithelium where they produce eggs. The eggs develop into noninfectious rhabditiform larvae in the gastrointestinal tract mucosa before moving into the bowel lumen. By the time rhabditiform larvae reach the end of the gastrointestinal tract they have two potential paths: they are either excreted and become free-living sexually reproductive adult male and female worms which produce eggs that then develop into noninfectious rhabditiform larvae and then infectious filariform larvae or they develop into filariform larvae while still in the bowel lumen and penetrate the intestinal mucosa or perianal skin completing the autoinfection cycle.

The autoinfective capabilities of *Strongyloides stercoralis* enable it to complete its entire life cycle in the human host [23]. This biological imperative has two important implications. First, the number of *Strongyloides* parasites can increase within the human host without exogenous reinfection. This can lead to the clinical manifestations of severe complicated strongyloidiasis, disseminated strongyloidiasis, and hyperinfection, years after the initial infection. Second, *Strongyloides* can be theoretically transmitted from one person to another during close physical contact so infection can occur without the need for travel to an endemic region. For these reasons *Strongyloides* is rather unique among helminths as it can cause disease with significant mortality among persons who might not be easily identified by a history focused solely on the individual risk factors.

Transmission to humans most commonly occurs when filariform larvae in the sand or soil penetrate through intact human skin while walking barefoot. Children are also at risk of exposure when they play in contaminated soil without skin protection. Person-to-person transmission has been reported in institutional settings such as day care centers and psychiatric facilities, among men who have sex with men, and between solid organ donors and recipients via the donated organs [24, 25].

Strongyloides infection elicits a host immune response that is mostly mediated by the Th2 arm. There are also concurrent high levels of IL-4, IL-5, IL-10, IL-13, serum IgE, and often eosinophils. Acquired protective immunity has been demonstrated in

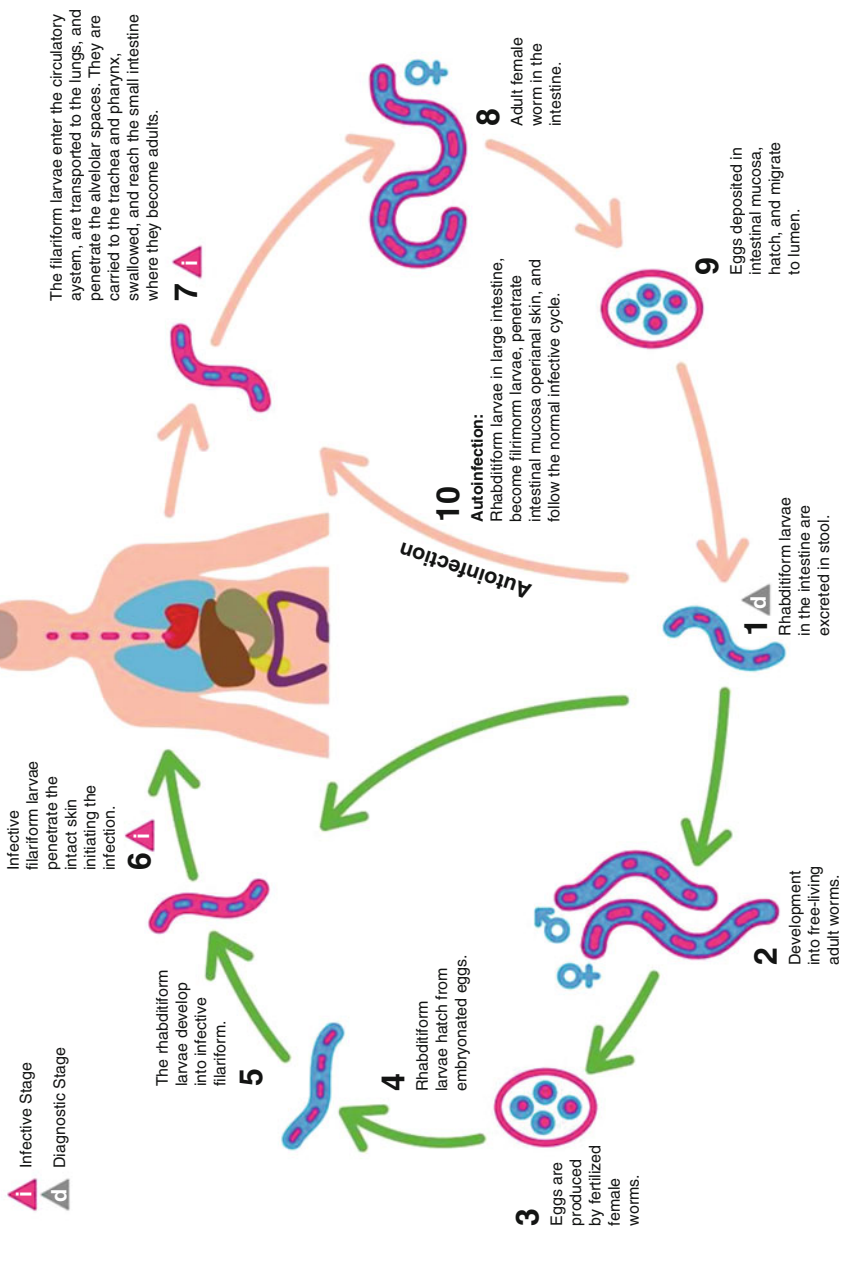

Fig. 1 Life cycle of *Strongyloides* in humans [7]

animal models, though human antibody responses that aid in controlling the worm burden do not lead to worm eradication [26, 27].

The immune response to *Strongyloides* infection is especially important in human T-lymphotropic virus type 1 (HTLV-1) coinfection. HTLV-1 is a retrovirus that typically causes chronic asymptomatic infection [28]. It is most prevalent in Japan, West Africa, focally throughout the Caribbean islands, and certain countries of South America, particularly Peru [29]. HTLV-1 causes an impaired Th2 response, the major immune response to *Strongyloides*, which in turn leads to diminished circulating levels of IL-4 and IL-5, and a suboptimal eosinophil recruitment response, indicated by low peripheral blood eosinophils. This immunopathogenesis leads to increased susceptibility to simple intestinal strongyloidiasis and severe complicated strongyloidiasis as well as poor response to treatment with frequent relapses [30–35]. There is also evidence showing the deleterious relationship between HTLV-1 and *Strongyloides* is mutual, specifically that strongyloidiasis can promote HTLV-1 progression to T-cell leukemia/lymphoma [36].

Human immunodeficiency virus (HIV) and *Strongyloides* have overlapping geographies in low- and middle-income countries with millions of people predicted to be coinfected [37]. When AIDS was first described, there were concerns about an impending outbreak of disseminated strongyloidiasis, and the infection was initially included as an AIDS-defining illness prior to the identification of HIV [38]. At that time, it was hypothesized that the impaired cell-mediated immunity would allow for increased worm proliferation and subsequent dissemination. In 1987, the infection was removed from the revised AIDS classification as it was rarely described [39, 40]. Since then the general consensus has been that HIV is not associated with a higher risk of developing *Strongyloides* hyperinfection [41]. This could be because HIV primarily causes the loss of Th1 activity and may impact Th2 activity to a lesser degree and possibly even augment Th2 activity [42]. There have, however, been reports of severe complicated strongyloidiasis as part of an immune reconstitution phenomenon after initiating anti-retroviral therapy in patients [43, 44].

4 Clinical Manifestations

Strongyloidiasis has three major clinical manifestation profiles:

1. Simple intestinal strongyloidiasis, which can have an acute and chronic phase
2. Hyperinfection, one end of the spectrum of severe complicated strongyloidiasis
3. Dissemination, the other end of the spectrum of severe complicated strongyloidiasis

Simple intestinal strongyloidiasis occurs when *Strongyloides stercoralis* is confined to its typical life cycle in the human body while the host's cell-mediated immunity keeps the worm burden under control. In the acute phase, symptoms can occur that are consistent with the migration patterns of the parasite [23]. After skin penetration, larvae can cause a pruritic papular rash. The specific cutaneous finding

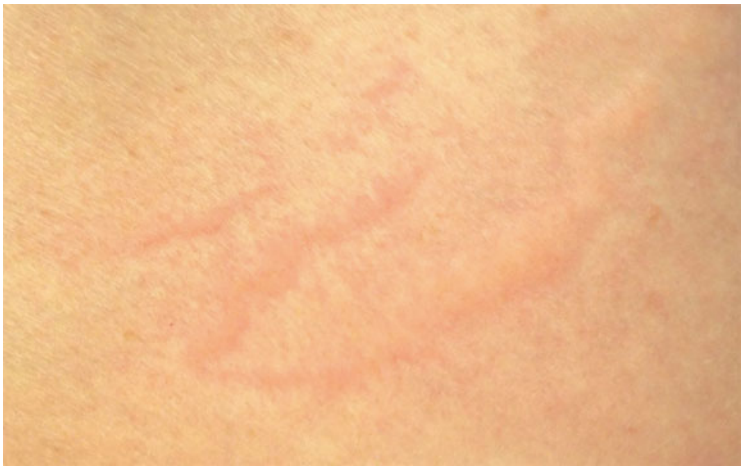

Fig. 2 Larva currens rash of strongyloidiasis [12]. CMAJ has granted permission for reproduction

of larva currens, a serpiginous rash that can move up to 10 cm/h, is pathognomonic for *Strongyloides* infection (Fig. 2). Larva currens is a manifestation of the migrating infectious filariform larvae through the skin and usually occurs on the buttock, groin, or trunk; however, larva currens can affect all areas of the skin and is often one of the manifestations that triggers clinical teams to consider the diagnosis in those who are critically ill with exuberant autoinfection and diffuse serpiginous eruptions. When larvae enter the lungs, they can lead to cough or wheezing, and once in the GI tract, can cause abdominal pain and diarrhea. While penetrating the lungs and other host tissues, larvae incite a high-grade peripheral eosinophilia in 75–80% of cases of acute infection. In the chronic phase of simple intestinal strongyloidiasis, most patients are asymptomatic as the parasite is being regulated by the host immune system. Some patients can have symptoms that are similar to those in the acute phase as *Strongyloides* completes repeated autoinfection cycles in its host. These symptoms include recurrent maculopapular or urticarial rash or larva currens, recurrent asthma or a Loeffler-like syndrome, refractory gastritis, abdominal pain, vomiting, diarrhea, pruritus ani, and an isolated intermittent eosinophilia.

Severe complicated strongyloidiasis represents two states, hyperinfection and dissemination, both of which are usually associated with an impairment of the host cell-mediated immunity arising from corticosteroid use, HTLV-1 coinfection, solid organ and bone marrow transplant, hematologic malignancy, hypogammaglobulinemia, heavy alcohol use, end-stage renal disease, or malnutrition. Eosinophilia is typically absent in cases of severe complicated strongyloidiasis as well as in immunocompromised hosts [45]. In *Strongyloides* hyperinfection, the worm burden is increased from baseline but remains within the organ systems it typically infects including the gut, lung, and skin. Most patients in this stage of illness will remain ambulatory or only intermittently fulfill admission criteria for hospitalization. Gram-negative bacteremia may be detected in a portion of such

patients. Risk factors specific to hyperinfection outside of immunosuppression include prolonged burden of autoinfection [8].

In disseminated strongyloidiasis, the worm burden has increased to the point that larvae and possibly other stages including adults and/or eggs can be detected in off-target organ systems that are not within the typical migratory pattern of *Strongyloides*, such as the central nervous system, renal collecting system, and liver. Specific impairment of the Th2 immune response can lead to dissemination [46, 47]. In these cases filariform larvae are detectable at distant sites and complications include polymicrobial bacteremia, meningitis, and sepsis as a result of fecal flora being tracked throughout the body. The mortality of disseminated strongyloidiasis is quoted to be at least 85% and 100% if left untreated [48].

Due to its ability to autoinfect its host, it is important for clinicians to know that the clinical manifestations of strongyloidiasis can occur over 50 years after the time of presumed exposure [4, 49].

5 Diagnosis

Due to the nonspecific or absent nature of symptoms, clinical diagnosis of *Strongyloides* infection is challenging. Mild eosinophilia that accompanies gastrointestinal symptoms such as abdominal pain, bloating, and diarrhea can occur with many other helminthiases including schistosomiasis, ascariasis, and hookworm infection. The exception is in the setting of larva currens—the rapidly migrating serpiginous skin eruption—which is stereotypical of strongyloidiasis. Laboratory diagnosis of strongyloidiasis is an evolving science, and like all areas of microbiology, molecular techniques are being increasingly utilized in diagnostic algorithms.

The main currently available diagnostic testing for strongyloidiasis includes serology, microscopy- or molecular-based stool ova and parasite testing, stool agar culture, and microscopic ova and parasite testing on other body fluids including sputum, endotracheal aspirates, urine, cerebral spinal fluid (CSF), and tissue. Stool PCR testing remains generally confined to reference laboratories.

There are several high sensitivity enzyme immunoassay-based serologic tests for strongyloidiasis available in North America [50, 51]. The overall reported sensitivity of serologic assays in acute and chronic *Strongyloides* infections are 73% and 98%, respectively; however, this performance can be drastically reduced in situations of immunosuppression such as HTLV-1 infection, immune ablating medications, and hematologic malignancy [52–54]. The sensitivity of serology is also reduced in disseminated infection. The specificity of *Strongyloides* serology is typically limited by a high degree of cross-reactivity with other helminthiases, in particular filariasis. Additionally, a positive serologic test cannot be used to differentiate between simple intestinal infection, hyperinfection, or dissemination.

In contrast to serology, microscopy-based stool ova and parasite testing has a low sensitivity and a high specificity; however, performance can be optimized through collection of multiple stools over the course of several days due to low or intermittent

larval shedding [50, 55]. A single stool specimen can miss up to 70% of cases; however, in some studies >90% sensitivity is achieved if seven or more stool samples are examined consecutively [56–58]. Several techniques have been developed to improve the performance of stool testing such as formalin-ethyl acetate concentration, Baermann concentration, and Harada-Mori filter paper culture. The formalin-ethyl acetate concentration technique increases the larvae yield but kills the larvae rendering them immotile and therefore more difficult to detect at low magnification. The Baermann concentration and Harada-Mori filter paper culture both capitalize on the larval propensity to migrate into warm water; however, neither is commonly used in diagnostic parasitology laboratories. In accordance to the life cycle of *Strongyloides stercoralis*, the long pre-patency generally leads to negative stool testing within the first month of infection while larvae migrate through the human host before reaching the bowel and maturing to reproductive adults [59].

Stool agar culture consists of plating a fresh stool specimen on agar and then incubating in the presence of UV light to help identify and preserve larvae and adult worms. A positive stool agar culture is indicated by gross examination of tracts left by organisms as they crawl across the agar or microscopic examination of agar for different stages. Stool agar culture is highly specific and can be more sensitive than direct stool microscopic examination. However, sensitivity is low in non-endemic settings such as much of North America where microbiological and specialized parasitological testing is usually regionalized, leading to delays between specimen collection by the patient and ultimate inoculation onto agar in the laboratory. Such testing can be plagued with logistical challenges requiring 2–3 days of incubation, expensive equipment, and specialized technical knowledge, which is waning over time with attrition of expert microscopists [51, 57, 60].

Ova and parasite testing on other bodily fluids such as sputum, urine, CSF, and tissue can be used in severe complicated strongyloidiasis when larval burden is high. Prolonged shedding can occur in these fluids and be monitored for parasite stage, density, and drug effect, all of which influence clinical management decisions [50].

The use of stool PCR has generated mixed conclusions across geographic regions and patient populations in which it has been validated. Studies have demonstrated a lack of performance advantage of stool PCR over traditional microscopy or stool agar plate culture, with extremely poor sensitivity when worm burden is low [50, 61–63]. Other studies have indicated that PCR testing of stool offers performance characteristics justifying its implementation including sensitivity and specificity of up to 100% [64, 65]. Ultimately, stool PCR necessitates further validation across regions and laboratory settings but has the potential for far-reaching diagnostic impacts.

5.1 Screening Guidelines

The following approach is recommended to screening for strongyloidiasis in the North American context:

Table 1 Tests for diagnosis of strongyloidiasis. Adapted from [10] (also available as an app on: https://apps.apple.com/ca/app/the-strongy-app/id1260973695)

Clinical scenario	Recommended test
Asymptomatic patient	Serology
Immunocompromised patient	Serology Stool ova and parasite examination
Suspected hyperinfection or dissemination	Serology Stool ova and parasite examination Sputum, urine, CSF, and/or tissue ova and parasite examination

1. Consider screening anyone with a cumulative exposure of greater than 2–-6 months in endemic areas or in anyone with compatible clinical manifestations. It is of particular importance that anyone who fulfills such criteria is screened prior to starting immunosuppression that can lead to severe complicated strongyloidiasis.
2. Screen family members of a positive index cases with common exposures, even if asymptomatic.
3. Use the appropriate tests for diagnosis. See Table 1.

5.2 Differential Diagnosis

It is prudent to consider other migratory helminthiases in the differential diagnosis of gut and/or cutaneous symptomatology suggestive of strongyloidiasis, including toxocariasis, gnathostomiasis, filariasis, and angiostrongyliasis. These other helminths can present with symptoms similar to strongyloidiasis and can also cross-react with *Strongyloides* serological testing.

6 Treatment

6.1 Available Pharmacologic Treatment

The goal of pharmacologic treatment of strongyloidiasis is complete eradication of the parasite (i.e., curative intent) due to the autoinfection process. This is in contrast to other soil-transmitted helminths where decreasing parasite burden is adequate to achieve clinical cure. Three antihelminthics exist to theoretically achieve this goal including ivermectin, albendazole, and thiabendazole.

Ivermectin dosed at 200 mcg/kg orally is first-line treatment for strongyloidiasis. It is safe and well tolerated and imparts its effects on ion channels in the cell membrane causing parasite paralysis. In cases where oral administration is not

feasible—particularly in the setting of disseminated strongyloidiasis—subcutaneous and parenteral ivermectin has been used with success [66–69]. A 2016 Cochrane meta-analysis found no difference comparing one dose versus two doses of ivermectin in simple intestinal strongyloidiasis that was re-demonstrated in a 2019 RCT comparing one versus four doses in immunocompetent patients in non-endemic regions [70, 71]. The two-dose regimen was based on theoretical idea that a 14-day interval between two doses would target the pre-patent infection arising from autoinfection [48]. Prior to ivermectin administration, the risk of microfilaremic loiasis needs to be assessed as ivermectin has been associated with severe fatal encephalopathy in untreated high-microfilaremic *Loa loa* infection [72]. A diagnosis of *Loa loa* should be considered in those who are born or have prolonged residency in countries of the central African rainforest including Cameroon, Equatorial Guinea, Gabon, Central African Republic, the Democratic Republic of the Congo, Nigeria, Chad, South Sudan, and northern Angola. Daytime blood film for microfilaria examination should then be performed in these patients prior to administration of ivermectin.

Albendazole 400 mg orally every 12 h for 7 days is a less effective alternative for strongyloidiasis treatment [10, 48, 70], and based on smaller scale data, thiabendazole has a similar efficacy to ivermectin but with a much worse safety and tolerability profile [73].

Ivermectin and albendazole are pregnancy category C drugs; however, the benefits of treatment likely outweigh the risk in cases of hyperinfection and dissemination. A recent systematic review and meta-analysis of use of antihelminthics in pregnancy noted no signal toward adverse maternal outcomes or safety events following gestational ivermectin use [74].

6.2 Treatment Approach

The following list of interventions should be considered for all patients diagnosed with strongyloidiasis:

1. Consult an expert in migrant health or tropical infectious diseases.
2. Administer pharmacologic treatment based on the patient's clinical manifestations

    a. Simple intestinal or asymptomatic strongyloidiasis: ivermectin 200 mcg/kg orally in two doses separated by 14 days

        If the patients is undergoing imminent immunosuppression with a history of exposure to endemic regions, consider empiric treatment with ivermectin prior to the return of serology.

    b. Mild hyperinfection: empiric ivermectin 200 mcg/kg orally on day 1 and day

14 PLUS albendazole 400 mg orally BID daily for 7 days; or ivermectin 200 mcg/kg orally daily for 7 days

c. Dissemination: empiric ivermectin 200 mcg/kg orally or subcutaneously daily plus albendazole 400 mg orally BID daily until cessation of larval shedding (i.e., repeat sputum and stool ova and parasite testing are negative) and clinical improvement

Also start broad-spectrum antibiotics to cover polymicrobial sepsis.
If possible, lower the degree of immunosuppression in the patient.

3. Consider testing for HTLV-1 in those at risk

   a. If HTLV-1 positive, consider two daily doses of ivermectin every 2–6 weeks to keep larvae suppressed.

4. Arrange for follow-up for repeat serology in 9–12 months after treatment. A greater than 60% reduction in the antibody titer or serologic optical density indicates successful treatment [75, 76].

7 Prevention

In North America, most cases of strongyloidiasis will be encountered in healthcare settings, and prevention measures need to be taken for infection control as nosocomial transmission has been previously described [14, 77–80]. Contact precautions should be instituted for patients with suspected or confirmed strongyloidiasis and the laboratory workers processing their microbiology samples. Agar plates of specimens from patients with disseminated strongyloidiasis should be handled with gloves and sealed with Parafilm® tape [10].

Worldwide, experts agree that strongyloidiasis remains a neglected tropical disease that warrants expanded public health efforts and research coordination in order to manage the disease burden [6, 57, 81–83].

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Taeniasis and Cysticercosis

Elise M. O'Connell

Abstract The majority of cases of *Taenia*, both taeniasis and cysticercosis, seen in the USA and Canada are imported. However, local transmission has been reported. There are three *Taenia* species known to parasitize humans—*T. solium*, *T. saginata*, and *T. asiatica.* The adult stage of these parasites, acquired from ingestion of raw or undercooked meat or viscera, resides in the small intestine of the human host causing taeniasis which is typically asymptomatic. Conversely, cysticercosis develops when a human ingests ova that are shed from a *T. solium* intestinal tapeworm carrier. Infection with the larval stage of the *T. solium* parasite leads to cysts which can be found in any tissue in the human body but most commonly are found in the subcutaneous tissue, muscles, or central nervous system. When cysticerci are found within the central nervous system, it is called neurocysticercosis. Neurocysticercosis is the most common tropical infection causing hospital admissions in the USA. The combined interventions of thoroughly cooking (or freezing) pork, hand hygiene, having a closed system of collection and treatment of human waste, and penning pigs so they do not have the ability to scavenge can aid in prevention strategies.

Keywords Taeniasis · Pork tapeworm · Beef tapeworm · Human cestode infection · Cysticercosis · Neurocysticercosis · Brain calcification · Brain cyst · Parasitic meningitis · Eosinophilic Meningitis · perilesional edema · Single enhancing lesion · Racemose

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1 Introduction

Tapeworms (cestodes) are hermaphroditic helminths that complete their life cycle often alternating between two host species—one that supports the larval form of the disease (intermediate host), and the other supports the adult tapeworm (definitive host). While there are three known *Taenia* species that use humans as the definitive host, only one, *Taenia solium*, also can parasitize humans with its larval stage. It is this larval-associated form of the disease, called cysticercosis (or neurocysticercosis (NCC) when the larvae travel to the brain), that is one of the most disabling and deadly foodborne infections, accounting for an estimated 2.79 million DALYs (disability-adjusted life years), with over 1.5 years of life lost (YLL) worldwide in 2010 [1].

2 Epidemiology

The overwhelming majority of cases of NCC seen in the USA and Canada are imported. However, there have been reports of local transmission [2–4].

A review of cysticercosis-related deaths in the USA from 1990 to 2002 revealed a total of 221 deaths, with 85% occurring in those born abroad. The average age of death was 40.5 years [5]. The highest contributing factors to death were hydrocephalus (26.2%), cerebral edema (10.4%), cerebral compression (7.2%), and seizures (5.4%) [5]. Thus, while the majority of patient presentations are for seizures (parenchymal disease), it is clear that the extraparenchymal forms are the largest contributor to mortality. Another review of US data from 2003 to 2012 documented over 23,000 NCC-related hospitalizations, 57% due to seizures and 18% due to hydrocephalus [6]. NCC admissions surpass all other tropical infections in the USA, including malaria [6]. Texas and California see a majority of cases and have reviewed their individual experiences [7, 8]. However, despite being a costly [6] and persistent problem [9] encountered in hospitals in the USA, there is a lack of knowledge on the topic in the US medical community [10]. It is notable that while the median age to first develop subarachnoid symptoms in 34 US cases was 29 years old, the median time of diagnosis was 6 years later [11]. Whether this discrepancy is due to lack of access to care, lack of physician knowledge or both is not known.

Canada has reported less cases of neurocysticercosis in the medical literature, but reports have increased in recent years [12].

3 Taeniasis

3.1 Background

There are three *Taenia* species known to parasitize humans—*T. solium*, *T. saginata*, and *T. asiatica.* The adult stage resides in the small intestine of the human host (the definitive host), and this type of infection is termed "taeniasis." All are acquired through ingestion of raw or undercooked meat or viscera. *T. solium* is acquired by eating infected and undercooked pork (Fig. 1). Pigs acquire the larval stage of *T. solium* through the ingestion of ova or gravid proglottids released in human stool. The larval form of the cestode then travels to pig muscle where it encysts, and the life cycle can continue. *T. saginata* has a life cycle similar to *T. solium*, with the exception of its being found in beef (Fig. 2). *T. asiatica* is also found in pigs, but unlike *T. solium*, most of the encysted parasites are found in pig liver, though pig

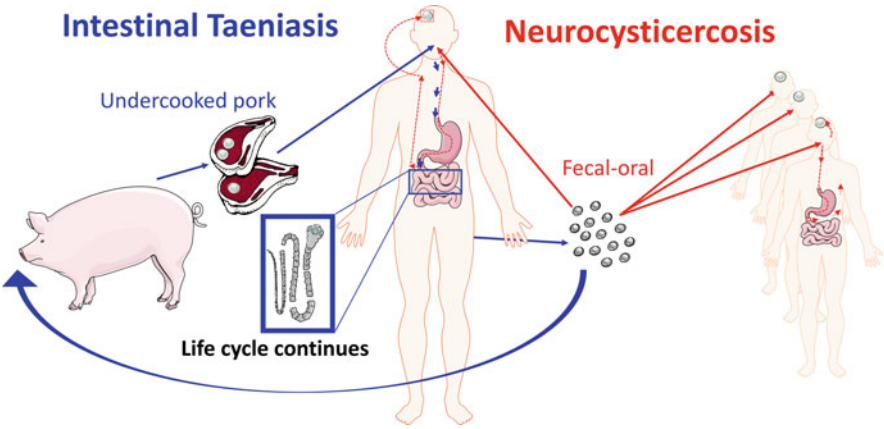

Fig. 1 *T. solium* life cycle of proglottids and fertilized ova are released in human stool, which can contaminate the environment as well as human hands. Ova exit the proglottid uterus and are composed of an embryophore, a thick striated cover, which protects the oncosphere (**a**). The oncosphere (or "hexacanth embryo," named for the six hooklets it contains) is an infectious larva, activated upon encountering gastric secretions. It penetrates the gut wall, travels through the bloodstream, and is capable of depositing in tissue throughout the body, but preferentially distributes to the brain and muscle. A cysticercus forms when the maturing larvae have reached its destination tissue and mature over the course of 2–3 months, developing an invaginated, immature scolex ("protoscolex") (**b**). When this occurs in the pig (intermediate host), the life cycle is completed when humans ingest undercooked pork containing the cysticercus (blue arrows). The cysticercus evaginates upon contact with gastric secretions, and the scolex attaches to the wall of the small intestine in the human (definitive host). Proglottids mature from the neck of the tapeworm, with the most mature gravid proglottids being the most distal. When it is the human that ingests ova released in the stool (red arrows), either by direct fecal-oral transmission from a tapeworm carrier or indirectly through ova-contaminated environment, cysticerci develop in the brain and muscle of humans (illustration made using Smart Servier Medical Art)

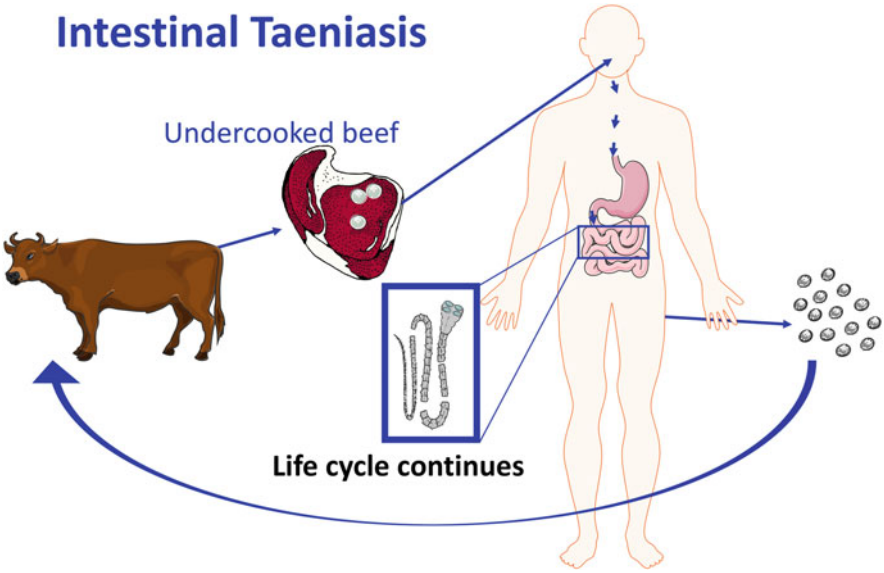

Fig. 2 *T. saginata* life cycle. *T. saginata* cysts are consumed in undercooked infected beef by a human. In the human gastrointestinal tract, the cyst evaginates and scolex adheres to the human small intestine. Proglottids originate at the base of the scolex and mature as they grow more distally. After approximately 2 months, the tapeworm has matured into an adult and proglottids filled with up to 100,000 infectious ova each, and free ova that have been extruded from the proglottid are released in human stool and contaminate the environment. Upon ingesting ova-contaminated vegetation or water, the oncosphere is released by gastric secretions, penetrates the intestinal wall, and travels to the muscles where it develops into a cysticerci (illustration made using Smart Servier Medical Art)

muscle can harbor cysts as well. Thus, the primary mode of transmission in *T. asiatica* is through consumption of undercooked pig liver [13].

Taeniasis is not endemic in the USA or Canada since the pork and beef industries are highly regulated, and sanitation is such that animals are infrequently in contact with human waste, which is necessary to sustain the life cycle. Clinicians practicing in these areas of North America are likely to encounter taeniasis in immigrants, travelers, and expatriates that have consumed undercooked meat in endemic areas abroad. For *T. solium*, this includes most of sub-Saharan Africa, Central and South America, Mexico, and throughout Asia, in particular India [14]. Prevalence data in *T. saginata* and *T. asiatica* are limited by the lack of speciation in human reports since both antigen testing and most stool exams cannot easily distinguish between the species. As most cases of intestinal tapeworm carriage is not symptomatic, *T. saginata* and *T. asiatica* are not seen as a significant health problems. *T. saginata* has worldwide distribution in cattle, including rare reports in US and Canadian cattle as recent as 2000 [15, 16]. However, the most common geographic areas of human infection are those where consumption of raw beef is common, including parts of Europe, Asia, and Africa, particularly Ethiopia [17]. The full

extent of distribution of *T. asiatica* is not known, but human infection has been identified in recent years throughout Asia [13, 18].

Following ingestion of the cysticercus, it takes about 2 months for *Taenia* tapeworms to mature in the small intestine and start to release proglottids and ova in the stool of the host. The lifespan of the adult *T. saginata* and *T. solium* tapeworms is thought to be on average 2–3 years but can be as long as 6.

Taeniasis is not a nationally reportable disease in the USA or Canada; thus accurate prevalence data is not available. However, a nationwide survey of state health departments in the USA conducted in the 1980s [19] and targeted screening of those from highly endemic areas [20, 21] suggest total prevalence in the USA is low, even among those at highest risk.

3.2 Clinical Manifestations

Taeniasis is most often asymptomatic. Since proglottids from *T. saginata* are motile, patients may experience the sensation of proglottids spontaneously passing from their anus or witness passed proglottids moving in stool. *T. solium* is not motile and proglottids are only passed with bowel movements. Rarely, segments can migrate to the appendix and have been reported to cause appendicitis [22, 23].

3.3 Diagnosis

Ova of *Taenia* spp. are indistinguishable on stool exam. While the ova of *T. saginata* are acid fast and *T. solium* are not, most commonly acid fast staining does not differentiate the two [24]. If only ova are seen in stool, molecular techniques (available only in a research setting currently) are required for definitive speciation. In most clinical settings, visualizing an intact proglottid in the stool and counting primary lateral uterine branches (*T. saginata* and *T. asiatica* 13–30, *T. solium* 7–13) is the only possible way to differentiate. This method, however, is unreliable, due to inconsistent shedding in the stool and degradation of the proglottid as segments are shed. One can also differentiate the species by observing the differences in the scolices of the adult tapeworm. *T. solium* has a hooklet "crown" (or "armed") in addition to four suckers (Fig. 3), whereas *T. saginata* has only suckers (it is "unarmed"). This is clinically an impractical way of differentiating the species, however, as typically one would need to administer anthelmintic treatment in order for the scolex to detach from the small intestine and be shed in the stool. Even then, the scolex shrivels following treatment, making it difficult to observe these differences. Additionally, it is important to know if the patient is infected with *T. solium* prior to treatment, as the primary reason for determining *Taenia* species is to determine whether the tapeworm carrier (or people in their household) is at risk for neurocysticercosis.

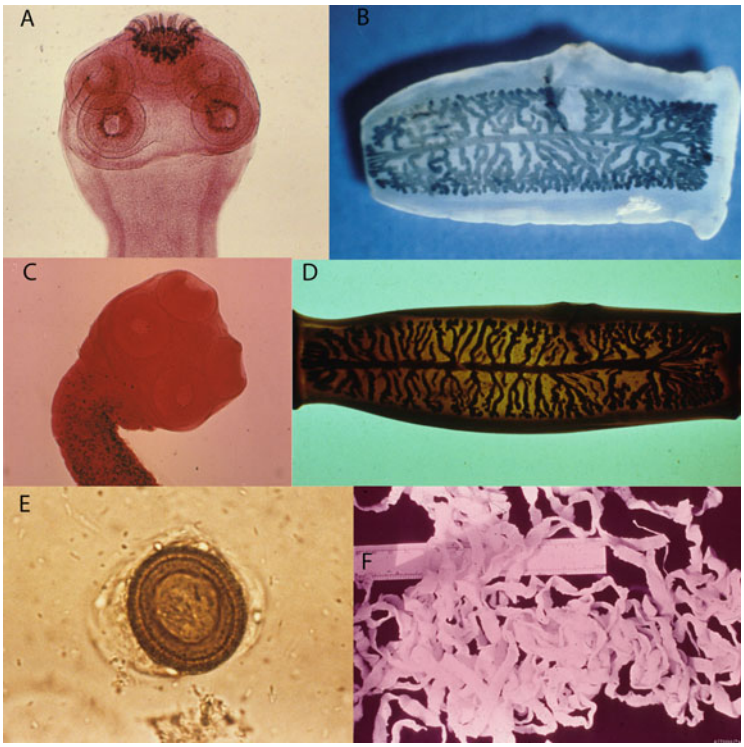

Fig. 3 Features of adult *Taenia* spp. (**a**) *T. solium* scolex with a "crown" of hooklets surrounded by four suckers (**b**) *T. solium* proglottid, (**c**) *T. saginata* scolex with only four suckers (no hooklets), (**d**) *T. saginata* proglottid, (**e**) *Taenia* sp. ova, (**f**) adult *T. saginata* tapeworm, which can be up to 25 m long. Photo credit: From the collection of Herman Zaiman, "A Presentation of Pictorial Parasites"

3.4 Treatment

Praziquantel is the treatment of choice and only treatment available in the USA and Canada for taeniasis, typically given at 5–10 mg/kg as a single dose. When giving praziquantel, one should be aware of the possibility for the rare, but well-documented, occurrence of eliciting neurologic side effects if the patient has asymptomatic, previously undiagnosed NCC [25–27]. Thus, if a patient has risk factors for cysticercosis such as residence in an endemic area, current or previous carriage of a *T. solium* tapeworm, or a household contact with this history, this should be considered prior to treatment.

4 Cysticercosis

4.1 Background

Cysticercosis develops when a human ingests ova that are shed from a *T. solium* intestinal tapeworm carrier (Fig. 1). Ova are immediately infectious once shed; they do not need to undergo further maturation in the soil. Therefore, a tapeworm carrier may be the source of their own cysticercosis and may infect others through fecal-oral transmission, including with food/water as the vehicle for transmission. Acquisition is most common in areas where there is inadequate sanitation and ova can contaminate the environment, thus allowing for completion of the life cycle (Fig. 1). However, there have also been documented occurrences of autochthonous cases of cysticercosis in the USA presumably transmitted from contact with a tapeworm carrier [2, 4].

The term "cysticercosis" refers to any infection with the larval (metacestode) stage of the *T. solium* parasite in any location. Cysts can be found in any tissue in the human body but most commonly are found in the subcutaneous tissue, muscles, or central nervous system. When cysticerci are found within the central nervous system, the term "neurocysticercosis" is used to describe the disease.

The susceptibility and manifestations of cysticercosis likely depend on the genetics of the host [28], genetics of the parasite [29], and the burden of parasite exposure. For instance, it is much more common to see the subarachnoid phenotype in patients from Latin America compared to Asia. Presentations of subcutaneous cysticercosis are much more common in Asia than in Latin America.

4.2 Clinical Manifestations

In general cysts in the subcutaneous (Fig. 4) tissue and musculature are of little clinical significance. Subcutaneous cysts can be seen beneath the skin surface, and the primary goal in the workup of these cases is to eliminate malignancy as the cause of the nodule. Most cysts in the musculature are asymptomatic, but in widespread dissemination of cysticercosis, muscle cysts can cause myalgias. In rare cases where there are very heavy infestations, muscles can become so bulky with cysts they can appear hypertrophic. In these cases, the main concern remains the extent to which cysts also parasitize the brain, and imaging should be performed to assess for neurocysticercosis. Intramuscular cysts are typically suspected when "cigar-shaped" calcifications are seen on imaging performed for other reasons.

Intraocular cysticercosis is an uncommon finding in cysticercosis but when present often leads to blindness unless treated. Cysts can be found in any intraocular area but most commonly are intravitreal or subretinal. Symptoms are most often of unilateral decreased visual acuity. Vitritis is most commonly seen on exam, followed by retinal detachment. Visualization of a cyst on indirect ophthalmoscopy is

Fig. 4 Subcutaneous cysticercosis. Photo credit: from the collection of Herman Zaiman, "A Presentation of Pictorial Parasites"

Table 1 The role of imaging and laboratory tests in diagnosing NCC

NCC type	EITB serology	CSF qPCR/TsAg	CT comments	MRI comments
Single parenchymal	50% positive	PCR—unstudied TsAg—negative	Presence of calcifications can aid diagnosis	Scolex best seen on FLAIR
Multiple parenchymal	~100% positive	PCR—unstudied TsAg—negative	Presence of calcifications can aid diagnosis	Scolex best seen on FLAIR
Calcified	Variable	PCR—unstudiedTsAg—negative	Study of choice	Use to rule out cysts missed by CT
Subarachnoid	~100% positive	~100% positive	Presence of calcifications can aid diagnosis	Scolex not seen. Visualization enabled by FIESTA/BFFE/3D CISS sequences
Ventricular	~100% positive	~100% positive	Presence of calcifications can aid diagnosis	Cysts or obstructing material seen

typically diagnostic, and B scan ultrasonography can confirm with visualization of the scolex. The treatment of intraocular cysticercosis is complete surgical excision, and steroids are given when uveitis is present [30, 31]. If not already diagnosed, the patient should be screened for NCC with brain imaging.

Neurocysticercosis (NCC) is the form of infection with *T. solium* of most clinical consequence. Symptoms and management of NCC vary depending on cyst location, burden, and degree of inflammation in the central nervous system (Table 1). Ninety percent of patients that come to clinical attention have cysts in the brain parenchyma

itself ("parenchymal disease"). The other 10% have "extraparenchymal" disease as their predominant presentation, either in the ventricles and/or the subarachnoid space. There are also reports of intramedullary spinal cord disease, but these are extremely rare (and will not be further discussed here).

4.3 Parenchymal NCC

4.3.1 Background

Management of patients with parenchymal NCC depends on the stage of the cyst(s). The natural history of parenchymal cysticercosis is shown in Fig. 5. Following the migration of the oncosphere (infectious larvae) to the brain, over the course of 2–3 months a well-defined cyst with an invaginated scolex develops. Patients are often asymptomatic for many years at this stage. After a heavy burden exposure, innumerable cysts can be found in the brain ("disseminated cysticercosis"). In this presentation, mental confusion can acutely develop, but these cases are rare and would be unusual to present in the USA or Canada. The vast majority of imported or

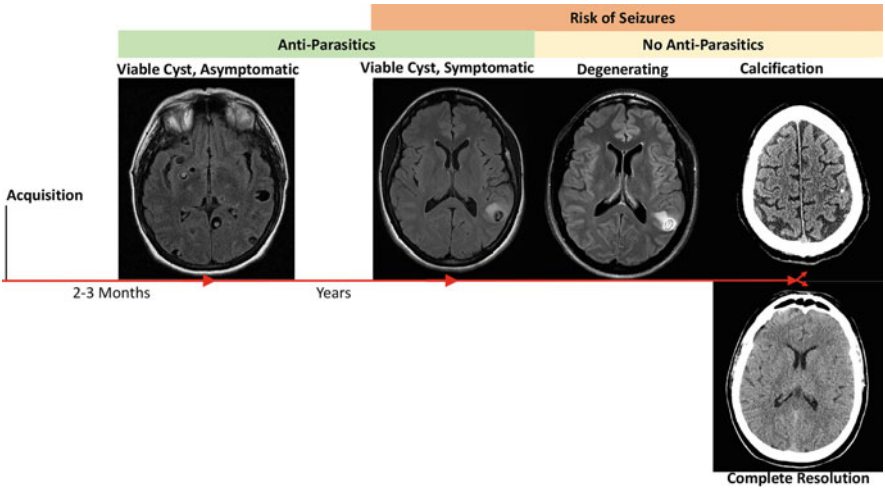

Fig. 5 Natural history of parenchymal neurocysticercosis (NCC). After establishment of the viable cysticercus in the brain parenchyma, it is silent often for years, even decades. MRI reveals a fluid-filled cyst (cyst is the same density as CSF) without surrounding edema. An eccentric "dot" (scolex) can often be seen. As the cyst starts to degenerate, initially it is still fluid filled (viable) but surrounded by inflammation as evidenced by post-contrast enhancement and edema seen on FLAIR sequences. As the cyst continues to degenerate, it becomes a solid nodule and is surrounded by edema and enhances for months, sometimes years, before it completely resolves and nothing is left behind, or calcification occurs. In the about 50% that calcify, seizure risk continues, but not in those that have a complete resolution. Stages when one is at risk for seizures and when one warrants treatment with albendazole and/or praziquantel are indicated (Courtesy of Dr. Elise O'Connell, NIH protocol 85-I-0127)

locally acquired infections are clinically asymptomatic for several years or decades while the cysts live in brain tissue, without inciting an inflammatory response at all.

At some point one of the cysts incites local brain inflammation, likely due to some breakdown in the cyst wall as the parasite becomes less viable, and it is this inflammation that causes seizures. Not all patients have seizures at this stage. In fact, the majority of people living with NCC in endemic areas never have seizures. However, in those that do come to clinical attention, this is the first time they may present with seizures. At this point, MRI often reveals a viable cyst with surrounding edema on fluid-attenuated inversion recovery (FLAIR) sequences. Cysts are considered viable when they are fluid filled (i.e., have the density as CSF on FLAIR or T1 imaging). The scolex can often (although not always) be seen on MRI and is diagnostic when found. When multiple cysts are present, most commonly only one or a few will have inflammation at any given time—thus they are in different stages of degenerating.

With the inflammatory response and death of the parasite, the cyst becomes hyperintense on MRI FLAIR sequences (no fluid component) and over months to 1–2 years either fully resolves or leaves a residual calcification behind. Nearly 90% of degenerating cysts are resolved or calcified after 2 years [32]. Throughout this time until complete resolution, the patient remains at risk for seizure. Whether the cyst is viable, degenerating, or calcified, edema seen around a lesion ("perilesional edema"), is associated with, and often the focus of, seizures. Some patients are completely asymptomatic through the process of the parasite dying and only start having seizures after a calcification has formed. While calcification alone can trigger seizures at any time, the episodic development of edema around calcifications has also been associated with discrete seizure activity. The majority of patients with evidence of NCC do not have clinical seizures, as evidenced by the high number of subjects in endemic areas with otherwise asymptomatic calcifications detected on imaging [33].

In rare cases of "disseminated" NCC, massive exposure leads to innumerable cerebral cysts and is associated with encephalopathy. In some cases, encephalitis with significant intracranial hypertension can occur secondary to the marked inflammation around the cysts. This clinical presentation is more common in children and adolescents than in adults.

4.3.2 Diagnosis

Brain imaging is required to make a diagnosis of NCC. MRI is more sensitive in detecting cysts without inflammation and degenerating lesions than CT. A non-contrast CT is more sensitive in revealing calcified disease than an MRI. Thus, both modalities used together provide the most information in confirming the diagnosis and staging the disease. Diagnostic features of parenchymal cysticerci are a diameter of less than or equal to 2 cm, thin walled, fluid-filled sac with a visible hyperintense eccentric scolex on MRI. The cyst may or may not enhance with contrast, and typically, in the presence of seizures, there is surrounding edema on FLAIR sequences. Sometimes cysts in the convexity of the brain are found in the

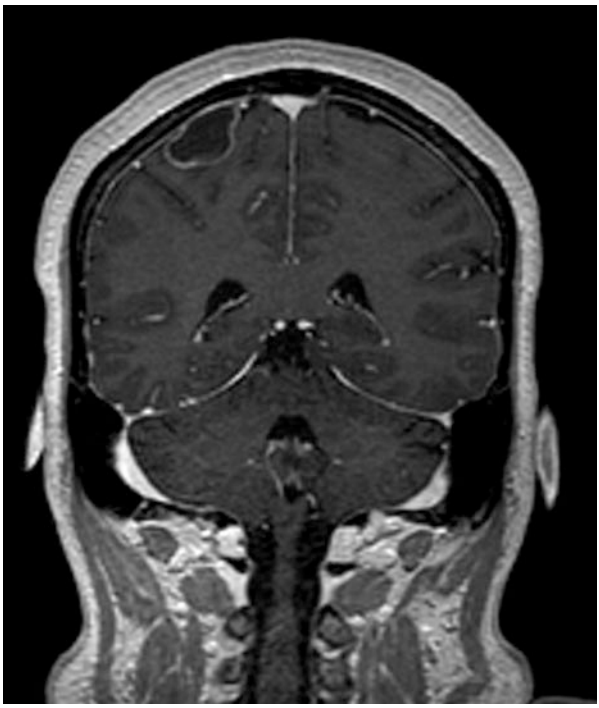

Fig. 6 Subarachnoid cyst in the brain convexity. Cysts in this location may reach over the 2 cm cutoff used for intraparenchymal lesions. This lesion measured 2.4 cm. However, subarachnoid cysts in the brain convexity are not racemose in nature—they do not proliferate or have a relapsing course. They are treated and respond like parenchymal lesions (Courtesy of Dr. Elise O'Connell, NIH protocol 85-I-0127)

subarachnoid space (Fig. 6). These cysts are not proliferative, as is seen in racemose disease (detailed below). In these cases cysts may be technically read as in the subarachnoid space on imaging, however they are managed and respond to treatment similarly to parenchymal cysts.

Enzyme-linked immunotransfer blot (EITB) is the serologic test of choice for diagnosing neurocysticercosis. In the setting of a single viable lesion, EITB sensitivity is only about 50%. However, in the setting of two or more lesions, the sensitivity approaches 100%. Serologic responses fall following the death of the organism. Therefore, in calcified disease the sensitivity of EITB is low. EITB testing is available through the US Centers for Disease Control (CDC). Unfortunately, most commercial laboratories use an ELISA using soluble extracts of *Taenia* parasites. These are both less sensitive and specific than the EITB test. Figure 7 describes the features and reliability of the various testing modalities in making the diagnosis of NCC.

Without a clear scolex on imaging or positive EITB testing, single-lesion disease can sometimes be challenging to diagnose and may require biopsy to rule out malignancy or tuberculoma. Likewise, benign tumors, malignancy, and tuberculomas can occasionally have features that may be cystic or even somewhat mimic the presence of a scolex. In the event of a surgical resection, histopathology reveals an invaginated well-developed scolex with a circular canal inside a fluid-filled bladder (Fig. 7).

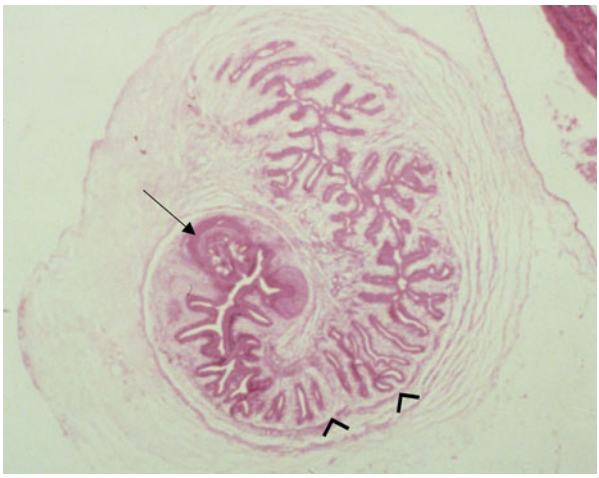

Fig. 7 Histopathology of a cysticercus. Invaginated scolex is seen with suckers (arrow) and spiral canal (arrow heads) on this excised cysticercus. Photo credit: From the collection of Herman Zaiman, "A Presentation of Pictorial Parasites"

4.3.3 Treatment

Detailed guidelines on the management of parenchymal NCC have been published [34]. Antiepileptic medications should be initiated immediately for any patient presenting with a seizure, the main presenting complaint in parenchymal NCC. Anthelmintic treatment is never emergent and should not be pursued until the patient is clinically stable from a seizure standpoint.

Parenchymal cysts typically die over time and do not recur even in the absence of treatment. However, there is good evidence that treatment speeds cyst death and decreases the numbers of generalized seizures [35, 36]. In patients with parenchymal disease, cysts that have edema or enhancement on MRI respond better to a single course of treatment (80%) compared to cysts without these features (60% success) [37]. Overall treatment success (defined as resolution of cyst by 6 months) for one or two viable parenchymal lesions reaches 70–80% with albendazole administration (15 mg/kg/day for 10–14 days), and the addition of praziquantel does not improve this success rate. Thus, albendazole monotherapy is the approach for 1–2 viable lesions. In the setting of three or more viable parenchymal lesions, the addition of praziquantel at 50 mg/kg/day increases the success of cyst resolution from 5–25% with monotherapy to 68% with dual therapy [36]. Notably, these studies include subjects who present with seizures. The treatment of incidentally discovered cystic lesions in patients without a history of seizures has not been studied. This is a more common entity in the USA and Canada given the higher access to imaging techniques than in many endemic areas.

Anthelmintic therapy causes a large localized inflammatory response, thought to be due to rapid parasite antigen release. Thus, any time anthelmintic treatment is given in NCC, corticosteroids must be given prior to anthelmintics to prevent seizures and other intracranial catastrophes. One study demonstrated fewer focal seizures when dexamethasone (8 mg/day $\times$ 28 days followed by a 2-week taper) was used [35]. In the rare cases of encephalitis due to disseminated cysts, treatment

should focus on anti-inflammatories and decreasing intracranial hypertension and not on antiparasitic agents.

The term "single enhancing lesion" originated from the preponderance of this presentation on the Indian subcontinent, where CT with contrast had traditionally been used for diagnosis. Because both viable and nonviable degenerating lesions enhance on CT, this rubric groups viable and nonviable degenerating lesions together. When MRI is utilized, this classification simply does not apply, as viability can be judged based on fluid contents in the cyst.

Patient follow-up has typically focused on a 6-month time frame to repeat imaging studies. If viable (fluid-filled cystic) lesions persist, then repeating treatment until resolution is recommended. At 12 months, nearly 40% of all cysts calcify in those who initially present with seizures, with the other 60% showing complete resolution [38]. Those that have residual calcifications remain at risk for further seizures and are typically managed with antiepileptics for some period of time. Anthelmintics are not used when only calcified lesions are present given the absence of living organisms. In general, steroids are not used to control seizures associated with perilesional edema around calcifications, as withdrawal of steroids can cause a rebound edema and trigger further seizures [39].

4.4 Ventricular NCC

4.4.1 Background

Cysts can be found anywhere throughout the ventricular system and are often clinically silent until they either cause obstruction directly or indirectly through an inflammatory response that in turn leads to obstruction (often at the level of the cerebral aqueduct). In the largest series in the USA, 74% presented with hydrocephalus and 17% were asymptomatic [40]. The majority have another form of NCC concurrently, with 35% accompanied subarachnoid disease [40].

4.4.2 Diagnosis

The salient features of the cyst are the same as in parenchymal disease, and sometimes a scolex can be clearly seen (Fig. 8a). In some situations, the patient comes to medical attention after an inflammatory response has already destroyed the cyst, leaving only parasite membranes and inflammation (Fig. 8b). Without a clear cyst and in the absence of other disease (calcifications, viable parenchymal, subarachnoid), the diagnosis can be easily missed. In someone from an endemic area, the presentation of hydrocephalus or ventriculitis should raise suspicion for NCC. EITB is nearly always positive in ventricular NCC [41]. Additionally, antigen and qPCR for *T. solium* DNA will be positive in the CSF (Fig. 7).

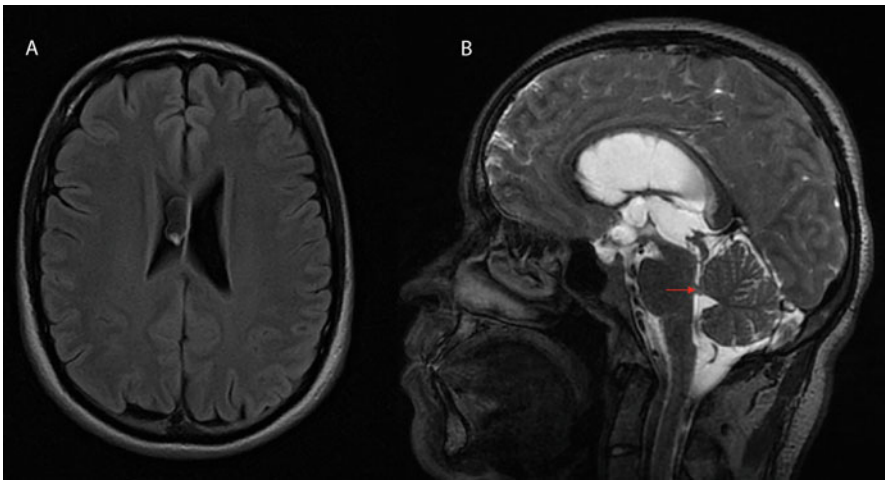

Fig. 8 Ventricular NCC. (**a**) Cysticercus in the right lateral ventricle with scolex visible. (**b**) Obstructing material in the cerebral aqueduct (red arrow). The anterior third ventricle also has a degenerating cyst (Courtesy of Dr. Elise O'Connell, NIH protocol 85-I-0127)

4.4.3 Treatment

When patients present without concomitant subarachnoid disease and the cyst is not adhered to the ventricular wall, the treatment of choice is endoscopic removal. Long-term follow-up suggests good outcomes on these patients if subarachnoid disease has been excluded [40]. Removal is difficult and can cause hemorrhage if inflammation has adhered the cyst to the wall of the ventricle and should be avoided if anthelmintic treatment has been administered since this causes breakdown of the cyst wall. While medical therapy with albendazole and/or praziquantel may be the only option in some patients, the inflammation that develops where the cyst is located can entrap the ventricle or cause permanent obstruction. If the obstructing cyst cannot be removed, a shunt or ventriculostomy is required.

4.5 Subarachnoid (Racemose) NCC

4.5.1 Background

Subarachnoid NCC, often used interchangeably with the term "racemose," refers to the form of NCC where cysts are found in the subarachnoid spaces of the basilar cisterns or Sylvian fissures of the brain or around the spinal cord. For unclear reasons, when found in these spaces the parasite is not a simple single cyst as in parenchymal and ventricular disease. In this location the parasite makes clusters of cysts (hence the term "racemose"). Treatment is prolonged and disease can recur

months or years after presumed successful treatment. Therefore, it is considered the most severe form of disease and the most challenging therapeutically.

Patients may have a history of seizures or parenchymal calcifications seen on brain CT when presenting with symptoms due to subarachnoid NCC. Subarachnoid disease presents during the 5th decade of life, on average later than parenchymal and ventricular disease (4th decade) [42]. Hydrocephalus at presentation is seen in over 50% of patients and nearly always requires a shunt [11, 42]. Hydrocephalus may be obstructive, due to concurrent ventricular disease (seen in ~20%) or communicating due to scarring of the meninges [11]. Other presenting complaints may include severe or recurrent headaches and stroke due to vasculitis of the perforating arteries as they traverse the subarachnoid space (lacunar infarcts); rarely large vessel strokes may be seen. Focal neurologic complaints may be related to inflamed cysts abutting cranial nerves. For instance, patients with cysts in the lateral prepontine cistern may experience unilateral hearing loss and/or tinnitus related to inflammation of the eighth cranial nerve. In the same US series, symptoms began a median of 10 years after emigration from the country of acquisition and as long as 25 years later. In most cases exposure is believed to happen early in life, giving an estimated median incubation of over 20 years before symptoms arise [11].

The prevalence of subarachnoid NCC is not known. Active cases of subarachnoid disease are grossly underreported in the literature. In both endemic and high-income countries, challenges in making the diagnosis include lack of access to adequate medical care and the nonspecific nature of the presenting symptoms. Episodes of severe headaches, including meningitis, are often diagnosed as migraine headaches until non-communicating hydrocephalus eventually develops due to scarring of the meninges. CT scans are insensitive in visualizing subarachnoid cysts unless mass effect has developed. Therefore, diagnosis is typically made only after a patient presents with symptoms of hydrocephalus, meningitis, or stroke. Diagnosis may be missed or delayed in patients due to inadequate imaging, inadequate expertise in interpreting the imaging, the long latency between exposure and symptoms, and the relapsing and recurring nature of subarachnoid disease (see Fig. 9). A recent study from two Northern villages in Peru suggests that there may be highly endemic areas with subarachnoid NCC prevalence as high as 0.8% of the total population [43]. While a small percentage, given the number of people living in highly endemic areas in Latin America (where this phenotype is most common), the absolute number of subarachnoid NCC cases would be staggering.

4.5.2 Diagnosis

Subarachnoid NCC is diagnosed by visualizing cysts in the basilar cisterns, Sylvian fissures, or anywhere along the spine on MRI (Fig. 10a). Unlike parenchymal disease, cysts can get quite large and even push into the brain parenchyma (Fig. 10b). Standard MRI sequences may miss subtle subarachnoid cysts that can be more easily seen with balanced fast field echo (BFFE), three-dimensional constructive interference in steady state (3D CISS), or fast imaging employing steady-

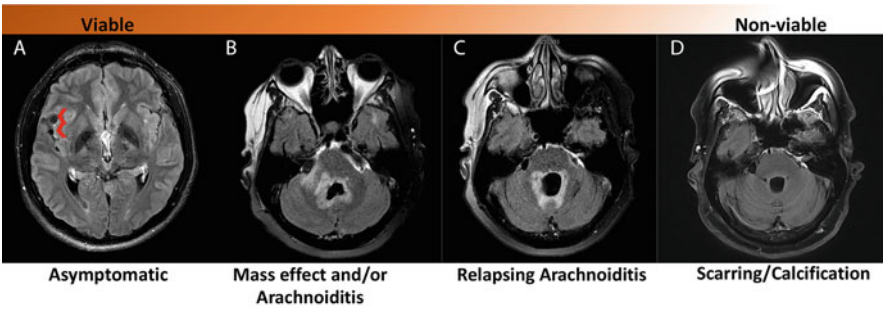

Fig. 9 Natural history of subarachnoid NCC. There is an asymptomatic period of several decades after subarachnoid NCC acquisition. (**a**) Asymptomatic growth in the Sylvian fissure (arrow heads). The parasite grows in the subarachnoid space, eventually causing symptoms when mass effect or inflammation develops. (**b**) Shown are cysts with edema and enhancement in the prepontine cisterns with edema around the fourth ventricle. (**c**) After treatment or a prolonged inflammatory response, the cysts involute but there is ongoing inflammation and potential for cysts to regrow if not fully treated. (**d**) Despite durable cure, imaging remains abnormal with a right prepontine cistern loculation of CSF and enhancement on the left (Courtesy of Dr. Elise O'Connell, NIH protocol 85-I-0127)

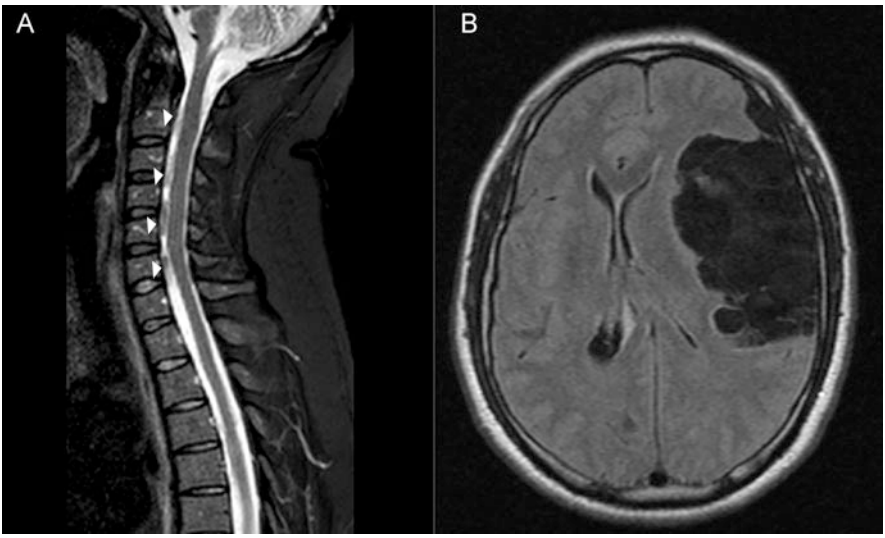

Fig. 10 Other forms of subarachnoid NCC. (**a**) Spinal involvement (arrow heads). (**b**) A giant cluster of cysts in the Sylvian fissure causing mass effect with midline shift (Courtesy of Dr. Elise O'Connell, NIH protocol 85-I-0127)

state acquisition (FIESTA) sequences (Fig. 11). On lumbar puncture the opening pressure is often elevated. The CSF is characterized by low glucose, elevated protein, lymphocytosis, and some presence of eosinophils that can, in some cases, become predominant. Serum EITB sensitivity approaches 100% in those with

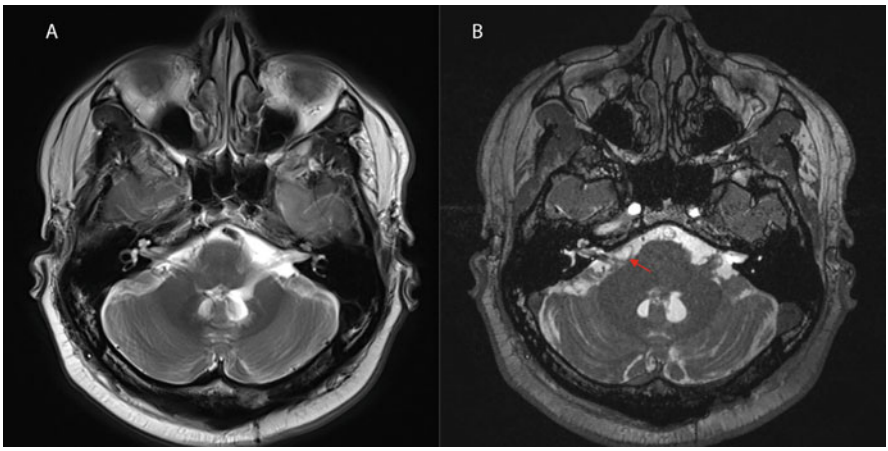

Fig. 11 Improved visualization of subarachnoid cysts with BFFE sequences. (**a**) T2-weighted imaging. (**b**) BFFE sequences reveal a well-circumscribed cyst in the right prepontine cistern (red arrow), which caused auditory disturbances in this patient (Courtesy of Dr. Elise O'Connell, NIH protocol 85-I-0127)

subarachnoid NCC [41]. Diagnostic features of surgically resected cysts are redundant membranes with an absent or degenerated scolex (Fig. 12).

4.5.3 Treatment

While the cyst bulk in the subarachnoid space can cause mass effect and resulting symptoms, the majority of symptoms are a consequence of the exuberant inflammatory response. Therefore, prolonged and high doses of corticosteroids are required initially to relieve symptoms. They must also be used throughout anthelmintic therapy to protect the patient from developing strokes and debilitating symptoms. Anyone with NCC and hydrocephalus upon presentation should have a shunt placed. Patients with hydrocephalus often have improvement of symptoms with high-dose steroids. However, if hydrocephalus (communicating or obstructive) is not corrected with shunting, inevitably symptoms return with tapering of the steroids.

Since up to 60% of patients diagnosed with basilar cistern NCC also have spinal involvement [44], all patients with this type of NCC should have spine imaging to assess for spinal disease.

Once symptoms improve with corticosteroids, therapy is typically started with both albendazole and praziquantel given the high parasite burden in the subarachnoid space, although no trial has compared monotherapy to dual therapy in subarachnoid NCC. While early in treatment there is often some decrease in the bulk of the cysts, imaging infrequently returns to normal [45] and is unreliable in determining cure. Following CSF levels of *T. solium* antigen or DNA has shown promise to

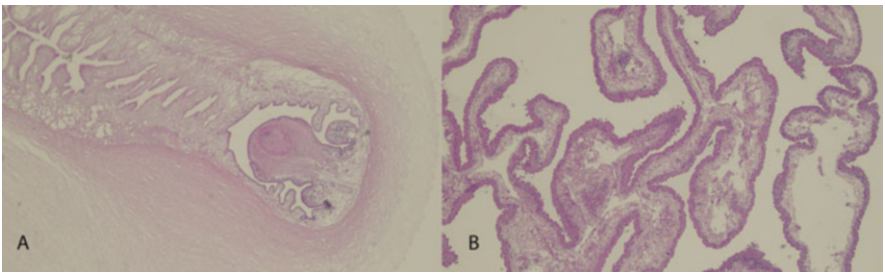

Fig. 12 Histopathology of subarachnoid (racemose) NCC. (**a**) Degenerated scolex, occasionally seen. (**b**) Redundant and disorganized membranes characteristic of racemose disease (Courtesy of Dr. Elise O'Connell, NIH protocol 85-I-0127)

gauge treatment success [46]. In a US series, patients with proven durable cure had a median time on anthelmintic therapy of 1 year [11].

5 Prevention

On a local level, implementing the combination of human and pig mass drug treatment along with pig vaccination can interrupt transmission [47, 48]. How best to use pig vaccination [49] and whether these results can be replicated on a larger scale to sustainably interrupt transmission in endemic areas remains to be seen. The combined interventions of thoroughly cooking (or freezing) pork, hand hygiene, having a closed system of collection and treatment of human waste, and penning pigs so they do not have the ability to scavenge have the ability to interrupt transmission.

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Toxoplasmosis

José G. Montoya and Despina Contopoulos-Ioannidis

Abstract *Toxoplasma gondii* is responsible for significant disease burden in North America and is the etiologic agent of potentially life-threatening toxoplasmosis in congenitally infected offspring, immunocompromised individuals, and patients infected with highly virulent strains. Parasite genetics in the United States reveal the presence of a mixed pool of strains infecting humans including those exhibiting less virulence such as Haplogroup II or those more aggressive such as Haplogroup I, Haplogroup III, and atypical strains. Although congenital toxoplasmosis can have devastating consequences when transmitted vertically, North America is notorious for the absence of coherent screening and treatment policies during gestation. In contrast, in France, Germany, Austria, Lithuania, Slovenia, Uruguay, and Argentina, among other countries, their offspring are protected by systematic screening and treatment of toxoplasmosis throughout gestation. For solid organ transplant patients, the United States did begin to systematically screen donors (D) and recipients (R) for *Toxoplasma* IgG in 2017 with the aim to identify and prophylactically treat high-risk D+/R- pairs. However, for other IC patients in North America, there is still inconsistency in that not every immunocompromised patient is tested for *Toxoplasma* IgG/IgM in order to identify and save the life of those at risk of reactivating. Life-threatening toxoplasmosis in North America is preventable and treatable.

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Keywords Toxoplasmosis · *Toxoplasma gondii* · North America · Parasite · Parasitic infection

1 Introduction

Toxoplasma gondii can be recognized in nature as free moving extracellular tachyzoites, intracellular bradyzoites encysted in the form of tissue cysts, and sporozoites encysted inside oocysts [1, 2] (Fig. 1). Tachyzoites, bradyzoites, and

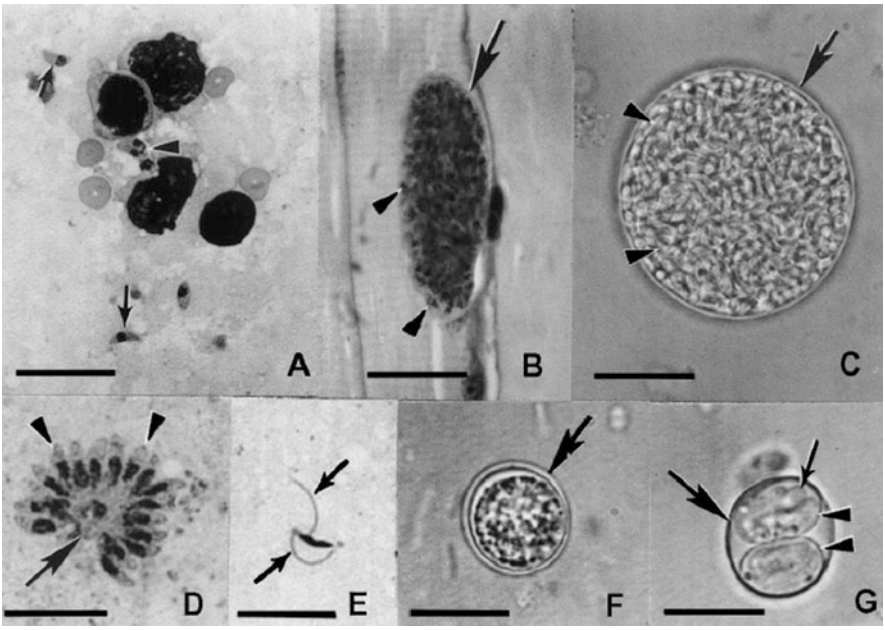

Fig. 1 Stages of *Toxoplasma gondii.* (**a**) Giemsa stain of tachyzoites in impression smear of the lung. Crescent-shaped individual tachyzoites (arrows) and dividing tachyzoites (arrowheads). (**b**) Hematoxylin and eosin (H&E) stain of tissue cysts in section of muscle. The tissue cyst wall is very thin (arrow) and encloses many tiny bradyzoites (arrowheads). (**c**) Unstained; tissue cyst separated from host tissue by homogenization of infected brain. Note tissue cyst wall (arrow) and hundreds of bradyzoites (arrowheads). (**d**) Giemsa stain of schizont (arrow) with several merozoites (arrowheads) separating from the main mass; impression smear of infected cat intestine. (**e**) Giemsa stain of a male gamete with two flagella (arrows); impression smear of infected cat intestine. (**f**) Unstained; unsporulated oocyst in fecal float of cat feces; note double-layered oocyst wall (arrow) enclosing a central undivided mass. (**g**) Unstained; sporulated oocyst with a thin oocyst wall (large arrow), two sporocysts (arrowheads). Each sporocyst has four sporozoites (small arrow). (Obtained from Elsevier Publication, "Toxoplasma gondii: transmission, diagnosis and prevention." Hill D and Dubey JP. Clin Microbiol Infect. 2002. PMID: 12390281. These pictures are not copyrighted, https://www.ars.usda.gov/people-locations/person/?person-id=1472)

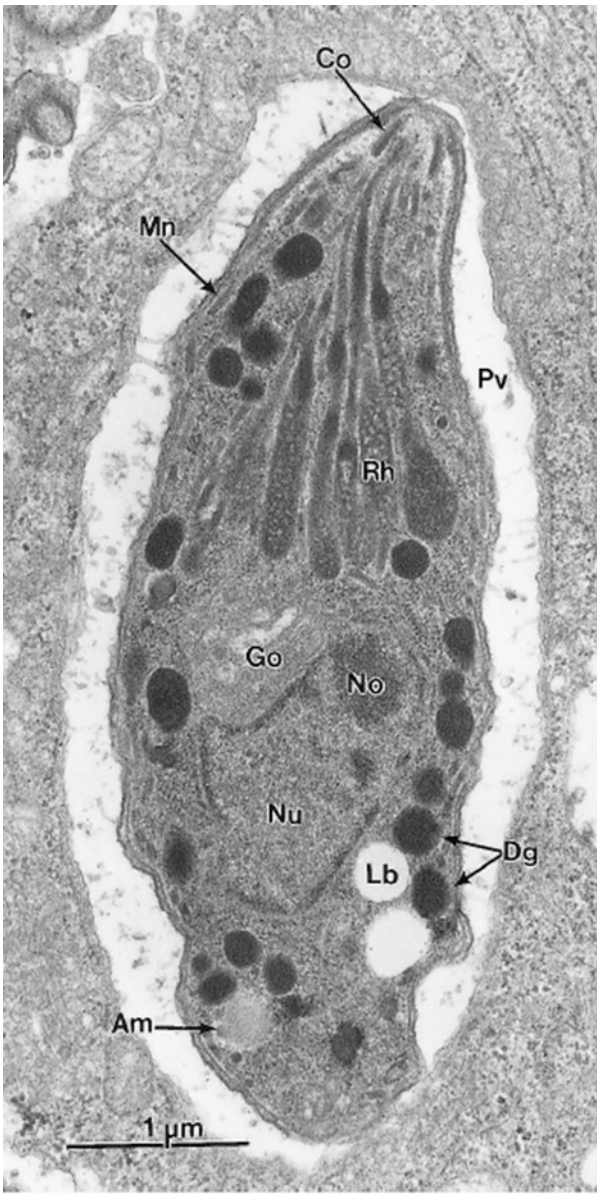

Fig. 2 Transmission electron micrograph of a tachyzoite of the VEG strain of *T. gondii* in a mouse peritoneal exudate cell. Am, amylopectin granule; Co, conoid; Dg, electron-dense granule; Go, Golgi complex; Mn, microneme; No, nucleolus, Nu, nucleus; Pv, parasitophorous vacuole; Rh, rhoptry. (Obtained from ASM Publication, Dubey JP, Lindsay DS, Speer CA. "Structures of Toxoplasma gondii tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts." Clin Microbiol Rev 1998;11:267-99. These pictures are not copyrighted, https://www.ars.usda.gov/people-locations/person/?person-id=1472)

sporozoites of *T. gondii* are very similar at the electron microscopy level and only differ in certain organelles and inclusion bodies [1] (Fig. 2).

1.1 Parasite Genetics

Multilocus genotyping studies of *T. gondii* demonstrate the presence of a genetic diversity associated with various geographic locales [3] and a spectrum of clinical manifestations. *T. gondii* strains can be categorized into 16 haplogroups belonging to six clades [4]. *T. gondii* type II and III strains appear to predominate in Europe and be associated with toxoplasmosis of lesser severity. Type I and atypical strains appear to predominate in South America and be associated with more aggressive forms of toxoplasmosis.

In North America, *T. gondii* derived from domestic animals are primarily grouped under type II and III strains; in contrast, when isolated from wild game, *T. gondii* usually belong to atypical strains including the Haplogroup 12 [5]. In North America, *T. gondii* genetic diversity is greater in free-roaming animals than in farm-bound animals [5]. In addition, the genotypic composition of parasites in wildlife differs from those in farm-bound and free-roaming animals and tends to be host-specific [5]. Thus, in North America, genotyping studies of *T. gondii* strains in animals demonstrate that genotype distributions are influenced by geographic locale and type of host; parasite diversity has the tendency to decrease in areas closer to human settlement [5]. Genotypic studies in Mexico performed in isolates from dogs [6], pigs [7], feral cats [8], sheep [9], and a wild puma (*Felis concolor*) reveal a high genetic diversity, a number of atypical strains including some apparently exclusively found in Mexico, and some strains shared with South America and the rest of North America. In North America, genotypic studies of *T. gondii* strains infecting humans reveal the genetic diversity found in both domestic and wild animals [10]. Approximately 44% of patients are infected with the type II strain, 44% with atypical strains, and 12% with type III strains [10] (Fig. 3).

1.2 Host Immune Response

Most of the models built to understand immune responses to toxoplasmosis have been derived from murine and in vitro models of infection [11]. Most of these models appear to apply relatively well to human infection. However, studies of innate immunity in mice clearly revealed that crucial discoveries in mice may not be applicable at all to humans [12, 13]. For instance, whereas toll-like receptors (TLRs) TLR11 and TLR12 and the immunity-related GTPase (IRG) proteins were discovered to be essential elements for detection and immune control of *Toxoplasma gondii* in mice, it was clearly demonstrated that TLR11 and TLR12 were not even functionally or structurally present in humans [12].

There is enough information obtained from animal, in vitro, and human studies to conclude that the ultimate outcome in the *T. gondii*-human host interactions is primarily dictated by the immune system capacity to control the infection within a perfect balance that avoids immune deficiency in one direction and immune overdrive (immunopathology) in the other. A well-coordinated immune response,

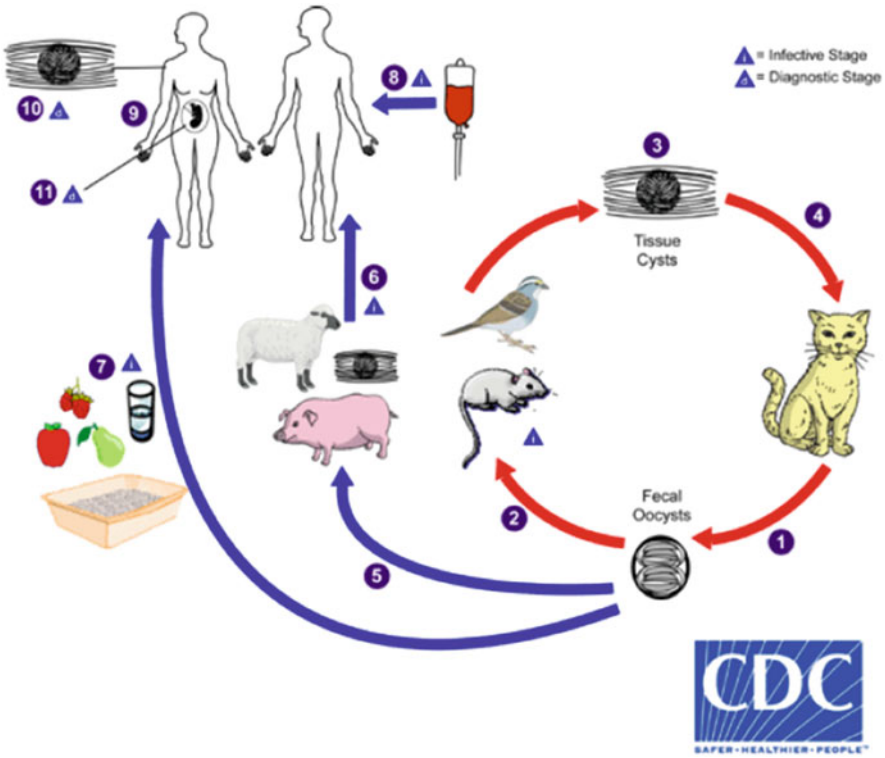

Fig. 3 (1) Unsporulated oocysts are shed in the cat's feces. (2) Intermediate hosts in nature (including birds and rodents) become infected after ingesting soil, water, or plant material contaminated with oocysts. (3) Oocysts transform into tachyzoites shortly after ingestion. These tachyzoites localize in neural and muscle tissue and develop into tissue cyst bradyzoites. (4) Cats become infected after consuming intermediate hosts harboring tissue cysts. Cats may also become infected directly by ingestion of sporulated oocysts. (5) Animals bred for human consumption and wild game may also become infected with tissue cysts after ingestion of sporulated oocysts in the environment. Humans can become infected by any of several routes: (6) eating undercooked meat of animals harboring tissue cysts; (7) consuming food or water contaminated with cat feces or by contaminated environmental samples (such as fecal-contaminated soil or changing the litter box of a pet cat); (8) blood transfusion or organ transplantation; and (9) transplacentally from mother to fetus. (10) In the human host, the parasites form tissue cysts, most commonly in the skeletal muscle, myocardium, brain, and eyes; these cysts may remain throughout the life of the host. Diagnosis is usually achieved by serology, although tissue cysts may be observed in stained biopsy specimens. (11) Diagnosis of congenital infections can be achieved by detecting *T. gondii* DNA in amniotic fluid using molecular methods such as PCR (Source : Pomares C, Devillard S, Holmes TH, et al. Genetic Characterization of *Toxoplasma gondii* DNA Samples Isolated From Humans Living in North America: An Unexpected High Prevalence of Atypical Genotypes. The Journal of infectious diseases 2018;218:1783-91, https://www.cdc.gov/dpdx/toxoplasmosis/index.html)

involving components of the innate and adaptive systems, is required for rapidly replicating pro-inflammatory tachyzoites associated with clinically active acute infection or reactivation, to be converted into metabolically slow encysted bradyzoites associated with lifelong and chronic infection [14].

Macrophages, monocytes, dendritic cells, neutrophils, natural killer (NK) cells, and T cells (both CD8+ and CD4+ T cells) appear to be critical in the cellular immune responses to control toxoplasmosis in humans [11] which may explain why patients with significant cell-mediated immunity compromise are at a high risk for life-threatening toxoplasmosis.

The cluster of differentiation 40 (CD40) is a member of the tumor necrosis factor (TNF) receptor superfamily that is expressed on antigen presenting cells and various non-hematopoietic cells [15]. The CD40 ligand (CD154) is present on the surface of activated CD4+ T cells, activated platelets, and plasma. The CD40-CD154 interaction (between infected macrophages expressing CD40 and CD4+T cells expressing CD154) leads to toxoplasmacidal activity (by autophagy pathways) and TH1-like cytokine responses [15]. Patients who lack functional CD154 (X-linked hyper IgM syndrome) are at high risk of life-threatening toxoplasmosis [16, 17].

Toxoplasmosis associated with distinct humoral immune deficiencies (e.g., common variable immunodeficiency) but without cell-mediated deficiency have seldom been reported [18]. Interferon gamma (IFN-γ) and interleukin 12 (IL-12) appear to be two essential cytokines in the early control of toxoplasmosis and induction of TH1-like successful responses [11]. Thus, in the absence of prophylaxis, life-threatening toxoplasmosis may develop in patients with anticytokine antibodies against IFN-γ and IL-12 [19].

In addition, other cytokines appear to also contribute to the pro-inflammatory [tumor necrosis factor alpha (TNF-α), CCL2, IL-1β, IL-6, IL-13, granulocyte-macrophage colony-stimulating factor (GM-CSF), intercellular adhesion molecule (ICAM)-1] and anti-inflammatory [IL-4, IL-10, IL-27, IL-33, suppressor of cytokine signaling protein 1 (SOCS1), and lipoxin A4 (LXA4)] milieus that ultimately determine health or disease [14, 20] in patients with toxoplasmosis. In North American and Colombian patients, acute and chronic *Toxoplasma* infection have been associated with cytokine levels that are lower than never infected individuals [21]. Lower cytokine levels associated with *Toxoplasma* infection may reflect co-evolutionary and adaptive responses to prevent parasite detection and elimination; it may also in part explain the large number of humans who do not develop symptoms during primary infection.

2 Epidemiology

The overall, age-adjusted, seroprevalence of toxoplasmosis has been steadily declining in the United States over the past several decades (Fig. 4) [22]. According to *Toxoplasma* serological surveys periodically performed in sera collected by the National Health and Nutrition Examination Survey (NHANES) since 1988, the overall seroprevalence has declined from 16% in the 1988–1994 period to 10.4% in the 2011–2014 period (Fig. 4) [22]. For the same periods, Jones JL et al. also demonstrated that the seroprevalence among US-born persons declined from 14.1% to 7.9% [22]. Similar to findings in prior *Toxoplasma* seroprevalence NHANES analyses, those more likely to be infected with *T. gondii* in the United States include

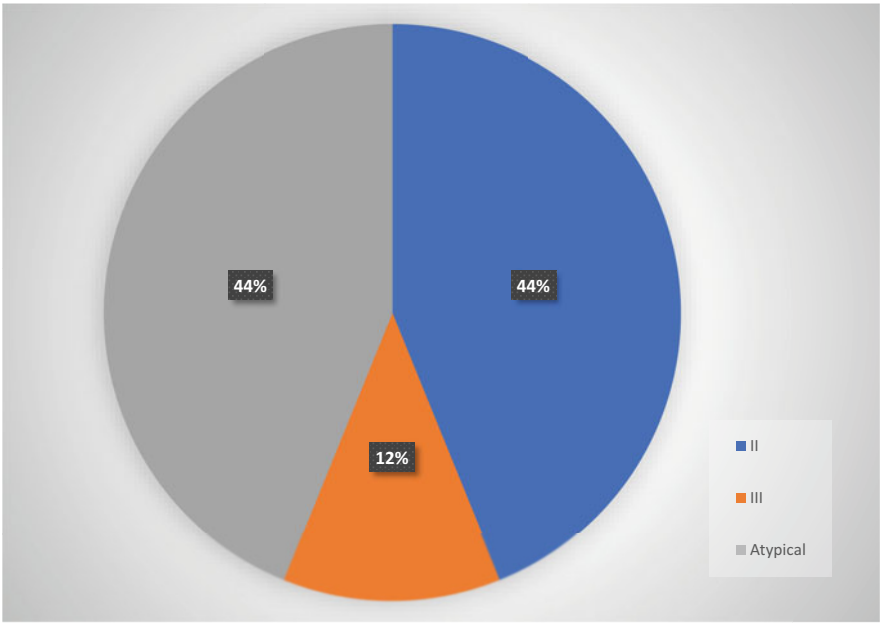

Fig. 4 Distribution of *T. gondii* strains infecting humans in North America (Source : Pomares C, Devillard S, Holmes TH, et al. Genetic Characterization of *Toxoplasma gondii* DNA Samples Isolated From Humans Living in North America: An Unexpected High Prevalence of Atypical Genotypes. The Journal of infectious diseases 2018;218:1783-91)

older persons, males, persons of non-Hispanic Black and Mexican American race/Hispanic origin, non-US-born persons, those living in poverty, those with less education, and those living in more crowded households. Among women of reproductive age, the age-standardized *T. gondii* seroprevalence appeared to decrease from 9.1% (95% CI 7.2–11.1%) in NHANES 2009–2010 to 7.5% (95% CI 6.0–9.3%) in NHANES 2011–2014 ($P = 0.19$). In a separate study, Smith KL et al. also demonstrated that the overall prevalence of infection has been declining for decades earlier in the United States as shown in the decrease of *Toxoplasma* IgG seropositivity among military recruits from 14.4% in 1962 to 9.5% in 1989 [23]. Population-based data estimates that approximately 1.1 million persons are infected in the United States with *T. gondii* each year [24].

This decline of toxoplasmosis in the general population overtime is likely related to changes that have taken place in the way meat is processed in more centralized plants, lower exposure of domestic cats to wild environments, and overall greater observance of hygiene practices in urban and rural populations. Meat is now processed with more controlled and standardized environments with much less access of animals to grazing practices and open soil where they could eventually encounter *Toxoplasma* oocysts. However, since toxoplasmosis is not a uniformly reportable disease in the United States, it is also likely that *Toxoplasma* and toxoplasmosis are more prevalent than what is has been reported. Toxoplasmosis was found to occur in family outbreaks when the index case was asked to submit a

serum sample for testing to a reference laboratory [25]. In 32 families of persons with acute toxoplasmosis in which > or = 1 other family member was tested for *Toxoplasma gondii* infection, 18 (56%) families had > or = 1 additional family member with acute infection [25]. In a similar approach, Contopoulos-Ioannidis D et al. also unveiled new acute and chronic infections among fathers of congenitally infected infants when the fathers were asked to provide sera for testing [26]. In this study, a high prevalence (29 of 81; 36%) of *Toxoplasma* infection among fathers was found, relative to the average seropositivity rate of 9.8% for boys and men aged 12–49 years in the United States between 1994 and 2004 ($P < 0.001$) [26]. In addition, there was a higher-than-expected incidence of recent infections among fathers with serum samples collected by the 1-year visit of their child (6 of 45; 13%; $P < 0.001$) [26].

T. gondii can infect humans as incidental hosts via four routes, oral, mother-to-child involving the placenta, a transplanted organ from an infected donor, or a laboratory accident. In approximately 50% of infections, a risk factor for acute infection cannot be identified and symptoms may not be present. Humans can get infected by ingesting oocysts present in food (including vegetables, fruits, shellfish, or unpasteurized milk) or water or tissue cysts present in undercooked meat from any infected warm-blooded animal including any livestock, poultry, or wild game. A major outbreak of toxoplasmosis associated with municipal drinking water that took place in the Greater Victoria area of British Columbia, Canada, was reported by Bowie WR et al. in the late 1990s [27]. Among 100 individuals reported during the outbreak, 51 had lymphadenopathy, 19 had retinitis, 18 were symptom-free, 4 others had symptoms consistent with toxoplasmosis, 7 had other symptoms, and 1 would not provide information [27]. Mapping studies revealed significant associations between acute infection and habitation in the distribution area of one reservoir supplying water to Greater Victoria (OR 8.27, 1.72–224; $p = 0.025$). The epidemic curve of this water-associated outbreak appeared to have a bimodal behavior, with peaks in December 1994 and March 1995 that were preceded by increased rainfall and turbidity in the implicated reservoir. Municipal water systems using unfiltered water, even it is chlorinated, can be the source of *Toxoplasma* infection [27].

Based on limited population-based data, the Food and Agriculture Organization/World Health Organization estimated that approximately 22% of human *T. gondii* infections are acquired by ingestion of undercooked meat [28]. A systematic meta-analysis to estimate *T. gondii* infection prevalence in food animals produced in the United States revealed that animals raised outdoors or that have outdoor access had a higher prevalence as compared with animals raised indoors. Highest *Toxoplasma* seroprevalence was found in non-confinement-raised pigs (31.0%), followed by goats (30.7%), non-confinement-raised chickens (24.1%), lambs (22.0%), confinement-raised sows (16.7%), and confinement-raised market pigs (5.6%). Viable tissue cysts can be present in processed meat (e.g., cured, dried, or smoked meat) [29]. Recently, outbreaks of toxoplasmosis have been reported in North America among deer hunters [30, 31] including an outbreak in Wisconsin (United States) where an unusual high attack rate and more aggressive clinical presentation was associated with the Haplogroup 12 strain [31].

Vertical transmission essentially takes place in women who become infected for their first time during pregnancy. Whereas the risk of mother-to-child-transmission (MTCT) directly correlates with the gestational age at which the mother acquires the primary infection, the risk of clinical sequelae in the offspring has an inverse correlation with gestational age [32, 33]. The other most important factor determining the risk of MTCT and clinical sequelae in the offspring is prenatal anti-*Toxoplasma* treatment. Anti-*Toxoplasma* treatment promptly initiated following seroconversion has been shown to significantly reduce MTCT and morbidity and mortality in infected offspring [33–37]. Although it is rare, MTCT can also occur in women infected within 3 months before conception [38], reinfected with a second *Toxoplasma* strain [39], or who become seriously immunosuppressed during gestation [40].

The incidence of acute toxoplasmosis during gestation was reported at 1.1 case per 1000 pregnant women several decades ago [41]. No such studies have ever been done afterward. Worldwide, acute toxoplasmosis during gestation is estimated to occur at approximately 11 cases per 1000 pregnant women [42]. Although there is no prenatal screening policy for toxoplasmosis in the United States, several states have been leaders in performing neonatal screening for the parasite. The New England Newborn Screening Program has estimated the incidence of congenital toxoplasmosis (MTCT among women acutely infected during gestation) at 0.82 cases/10,000 live births for the 1986–1992 period [43]. For the 2015–2018 period was estimated at 0.14/10,000 Massachusetts live births (Personal communication with the Massachusetts Department of Public Health, data from Dr. Ho-Wen Hsu).

3 Transmission

Toxoplasma has a sexual cycle that takes place exclusively in the small intestine of animals belonging to the family Felidae (definitive host) [44] and an asexual cycle that takes place in tissues of their incidental hosts (vertebrates including humans) [45] (Fig. 5). Recently, a *T. gondii* microrchidia (MORC) protein has been discovered as a key regulator orchestrating the directionality of the parasite's life cycle [46]. Active MORC expression successfully sends downstream proteins in the direction of the asexual cycle track [46].

Any cat belonging to the 37 species of the family Felidae can shed million of oocysts following ingestion of tachyzoites (from an acutely infected prey or in a laboratory setting), bradyzoites (contained in tissue cysts infecting meat), or sporozoites (walled within oocysts circulating in contaminated soil or water) (Fig. 3). Any of these three infectious forms can penetrate the epithelial cells of cat's small intestine where few asexual cycles take place by schizogony before the initiation of the sexual cycle involving macrogametes (female) and microgametes (males). It is here during the sexual cycle in the intestine of cats where *T. gondii* genetic diversity can expand since wild cats can travel long distances facilitating mating of genetically distant strains [3]. The fertilization of the macrogametes by microgametes produce

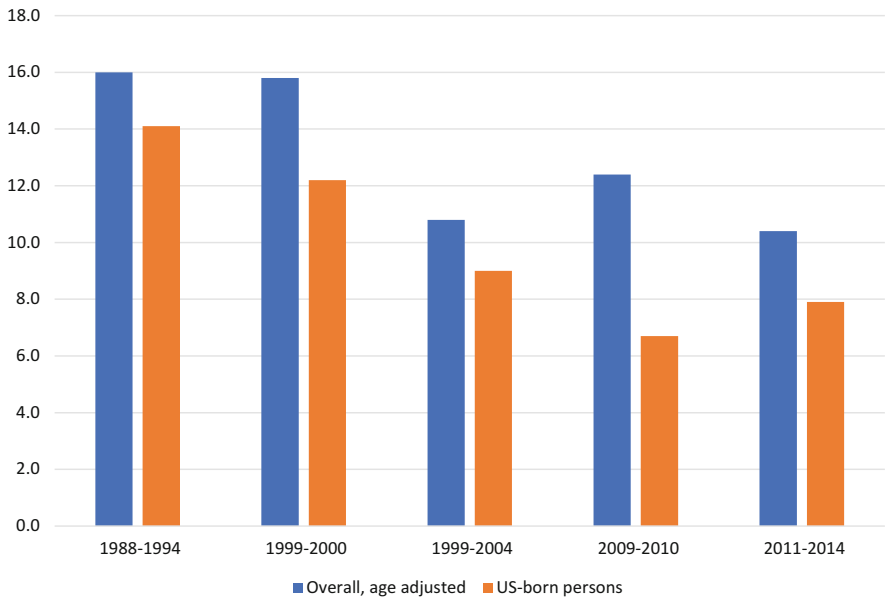

Fig. 5 Overall age-adjusted and US-born persons' prevalence of *T. gondii* infection in the National Health and Nutrition Examination Survey (NHANES, https://wwwn.cdc.gov/Nchs/Nhanes/)

oocysts which are subsequently shed in cat's feces for up to 3 weeks [2]. A single infected cat can shed millions of environmentally resistant oocysts per day. Oocysts can stay potentially infectious in the environment for up to 18 months.

Humans (or any warm-blooded animal) can get infected as incidental hosts by ingesting tissue cysts contained in undercooked meat or oocysts present in soil, water, or food (Fig. 3). Humans can also get infected congenitally or via transplanted organs from infected donors. Laboratory exposure and accidents are extremely rare occurrences. Only the asexual cycle takes place in warm-blooded animals. Once ingested, tissue cysts release bradyzoites and oocysts release sporozoites. Both bradyzoites and sporozoites evolve into becoming tachyzoites. Tachyzoites is the most metabolically active and rapidly dividing form of the zoites forms. Tachyzoites are disseminated throughout the body via macrophages and dendritic cells [47]. The immune response triggered in response to the replication of tachyzoites is responsible for the clinical manifestations of toxoplasmosis. Successful immune responses lead tachyzoites converting into bradyzoites and eventually the congregation of bradyzoites into tissue cyst formation. A new gene, named "Bradyzoite Formation Deficient 1," has recently been discovered as a key regulator for the tachyzoite-to-bradyzoite conversion process [48]. Tissue cysts in the dormant stage are responsible for the chronic infection that usually persists for the life of the host. Certain tissues exhibit predilection for the long-term presence and hosting of tissue cysts, namely, the heart, skeletal muscle, retina, and brain. A full-blown bradyzoite-to-tachyzoite

conversion can occur in the setting of immunosuppression and is usually associated with life-threatening toxoplasmosis.

4 Clinical Manifestations

Toxoplasmosis should not be excluded from the differential diagnosis of a patient because of lack of symptoms during the potential exposure to the parasite or acute or chronic stage of the infection. Any person can be infected with *T. gondii* even though they have not exhibited clinical manifestations attributable to toxoplasmosis. This applies to immunocompetent individuals, pregnant women, the fetus, newborns, infants, children, and immunocompromised patients.

4.1 In Immunocompetent Patients

4.1.1 Acute Infection

Absence of symptoms during the acute infection is more common with less virulent strains (e.g., type II, approximately 80% asymptomatic) than with more virulent strains (e.g., Haplogroup 12, approximately 20% asymptomatic) [31]. When present, symptoms associated with the acute infection include fever, malaise, fatigue, sweats (including drenching night sweats), chills, myalgias, arthralgias, headache, sore throat, anorexia, weakness, neck soreness, dark urine, and lymph node enlargement [31, 49]. Symptoms also include those associated with chorioretinitis, hepatitis, myocarditis, myositis, pneumonia, severe sepsis, brain abscess, and subtle encephalitis including neuropsychiatric manifestations [50, 51]. Physical exam may reveal chorioretinitis, lymphadenopathy, hepatosplenomegaly, and skin rash in cases with mononucleosis-like presentation.

Radiological abnormalities in patients with toxoplasmic pneumonia most often include bilateral and diffuse ground glass opacities in chest CT and in patients with toxoplasmic encephalitis, most commonly present with ring-enhancing space occupying lesions in brain CT or MRI.

Lactic dehydrogenase (LDH) elevation, leukopenia, reactive lymphocytes, thrombocytopenia, and transaminitis have been reported in association with the acute infection, particularly in association with atypical strains (e.g., Haplogroup 12) [31].

4.1.2 Chronic Infection

The chronic stage of infection has been traditionally viewed as not capable of manifesting any symptoms with the exception of chorioretinitis episodes in

immunocompetent patients and reactivation of the parasite in the setting of immunocompromised individuals (see "Clinical Presentation in Immunocompromised Patients). In immunocompetent patients, local reactivation of *T. gondii* in retinal tissue can occur leading to symptomatic chorioretinitis. Chorioretinitis by reactivation can develop months to years later following congenitally or postnatally acquired toxoplasmosis.

Nonetheless, several studies have suggested a possible association between the dormant presence of the parasite in the chronic stage of infection and a higher frequency of motor vehicle accidents [52, 53], schizophrenia and other mental illnesses [54, 55], or self-directed violence [53]. Unpredictably, an association between the chronic infection and cancer appears to be emerging [56]. Although the association between chronic toxoplasmosis and mental disorders, behavioral alterations, or cancer is suggested by a number of epidemiological studies, a causal relationship has not been demonstrated.

4.1.3 During Gestation and in the Offspring

The majority of pregnant women do not exhibit any symptoms or signs of primary *Toxoplasma* infection during gestation [37]. If present, symptoms are similar to those observed in acutely infected immunocompetent individuals (see "In Immunocompetent Patients"). Pregnant women can present with chorioretinitis associated with an acute infection (rare) or, less infrequently, as reactivation of an infection acquired prior to gestation.

During gestation, infection in the offspring can be manifested by anatomical changes detected by ultrasonography. Fetal ultrasound abnormalities associated with congenital toxoplasmosis include brain calcifications, hepatic calcifications, hydrocephalus, ventriculomegaly, hyperechogenic bowel, hepatosplenomegaly, enlarged placenta size, pleural effusion, pericardial effusion, intrauterine growth retardation, and ascites [57, 58]. Toxoplasmosis can tragically result in fetal demise.

At birth, and in subsequent years, infection in the offspring can manifest by, most commonly, retinal and brain inflammatory abnormalities [58]. Clinical manifestations associated with congenital toxoplasmosis after birth include retinal inflammation and/or necrosis, retinal detachment, papilledema, macular edema, optic nerve atrophy, nystagmus, amblyopia, cataract, iritis/leukocoria (associated with retinal lesions), vitritis, strabismus, brain masses, hypotonia, psychomotor retardation, intracranial calcifications, macrocephaly, microcephaly, hydrocephalus, nerve palsies, hearing loss, seizures, spasticity, massive encephalopathy, subtle neurologic signs, jaundice, lymphadenopathy, myocarditis, pneumonitis, rash, sepsis-like syndrome, temperature instability, preterm birth, temperature instability, and sepsis-like illness [58]. Cerebrospinal fluid abnormalities include pleocytosis, elevated protein (including extreme values of more than 1000 mg/dL), and eosinophilia. Other laboratory abnormalities include hyperbilirubinemia, anemia, disseminated intravascular coagulation, transaminitis, and thrombocytopenia [58].

Newborns born without clinical manifestations can develop clinical signs during the first year of life or after [33, 59–61].

4.2 In Immunocompromised (IC) Patients

IC patients, in the absence of an effective immune system, can develop life-threatening toxoplasmosis in three settings, [1] as a primary infection, [2] reactivation of a chronic infection, or [44] via a transplanted organ from an infected donor. Primary infection has been reported and may result in more severe clinical manifestations in IC than in immunocompetent patients [62]. Reactivation is the most common mechanism for toxoplasmosis developing in patients with acquired immunodeficiency syndrome (AIDS), hematopoietic stem cell transplant (HSCT), and primary immunodeficiencies [63]. An infected organ donor, as established by a positive *Toxoplasma* IgG (D+), can transmit the parasite to organ recipients, particularly those never infected (R-); D+/R- or D+/R+ recipients can then develop 100% lethal toxoplasmosis in the absence of prophylaxis. Moreover, an expanding number of IC patients are being added every week to medical care facilities around the world because of the increase use of biologics, including monoclonal antibodies and small molecules, for inflammatory diseases and cancer [64, 65]. This growing number of IC patients can develop toxoplasmosis as primary infection or reactivation of their chronic infection [62, 66, 67].

Toxoplasmosis in IC patients can manifest by brain-occupying lesions, diffuse encephalitis, pneumonia, fever alone or of unknown origin, severe sepsis, chorioretinitis, hepatitis, myositis, myocarditis, skin rash, and hemophagocytic lymphohistiocytosis (HLH) [68]. Most common radiological presentation of toxoplasmic pneumonia in chest CT is bilateral and diffuse ground glass opacities (GGO). The differential diagnosis of GGO in the chest CT of IC patients should include toxoplasmosis, in addition to *Pneumocystis jirovecii* (PJP), viruses, atypical pneumonia, strongyloidiasis, diffuse alveolar hemorrhage (DAH), drug hypersensitivity, and pulmonary edema.

4.3 Ocular Disease

T. gondii is the most common infectious etiology of posterior uveitis worldwide. Toxoplasmosis should always be considered in the differential diagnosis of a patient who presents with chorioretinitis. Ocular disease can be the result of a congenitally or postnatally acquired infections. In congenital infection, active lesions may be present at birth or during the first year of life; lesions are most likely to be bilateral and involving the macula; reactivations are more likely to be seen between the ages of 10 and 30 years. In contrast, in postnatally acquired toxoplasmic chorioretinitis, lesions are more likely to be unilateral and peripheral; patients usually manifest

symptoms associated with the acute infection after the age of 50 years and reactivations are overall rare.

Retinal lesions by reactivation due to toxoplasmosis have a rather typical morphology including existence of a previous scar (usually brownish/greenish in color) with a new whitish/yellowish infiltrate stemming from borders of the scar. Retinal lesions associated with acute infection have more atypical appearances, and their lack of recognizable features may significantly delay diagnosis and treatment.

5 Diagnosis

Toxoplasmosis can be diagnosed by serological and cellular immune responses, polymerase chain reaction (PCR), antigen in tissue detected by immunohistochemistry, histological investigation, direct visualization by light microscopy, or isolation.

The initial step should involve serological evaluation by *Toxoplasma* IgG and IgM to establish whether the patient has never been infected, is acutely infected, or is chronically infected. A positive IgM should always undergo confirmatory testing at a reference laboratory [e.g., in the United States, the "Dr. Jack S. Remington Laboratory for Specialty Diagnostics" (JSRLSD) +1 650-853-4828, www.pamf.org/serology/] [69]. The JSRLSD lab serves as a reference lab for the diagnosis and study of toxoplasmosis in North America and throughout the world. A positive *Toxoplasma* IgM can be observed in the setting of acute or chronic infection and even in never infected patients due to false-positive results in commercial assays or persistent of *Toxoplasma* IgM in some chronically infected individuals [69].

At the JSRLSD, lab timing of the acute infection is possible with the use of confirmatory tests [70] that facilitates linking patient's symptoms to acute *Toxoplasma* infection. In patients suspected of having toxoplasmic lymphadenitis, histological assessment of a lymph node biopsy may be diagnostic. A typical triad of findings in lymph node biopsy may be considered diagnostic: a reactive follicular hyperplasia, irregular clusters of epithelioid histiocytes encroaching on and blurring the margins of the germinal centers, and focal distention of sinuses with monocytoid cells [71]. Langerhans giant cells, granulomas, microabscesses, and foci of necrosis are not typically seen. Rarely, tachyzoites or tissue cysts are found. *Toxoplasma* PCR is seldom positive [71].

5.1 During Pregnancy

In order to successfully prevent and treat congenital toxoplasmosis (CT) as early as possible, pregnant women should be universally screened for *Toxoplasma* IgG and IgM during the first prenatal visit, regardless of the presence or absence of risk factors for acute infection or symptoms. The primary goal of this initial screening is

to establish whether women are at risk or not for CT since most of the risk for CT is observed in women found to be acutely infected or seroconvert during gestation. A negative IgG/negative IgM test result should be followed by IgG/IgM testing at regular intervals to detect whether seroconversion takes place; in France it is performed every month, in Austria every 3 months, and in other places twice during gestation. The monthly approach has shown to reduce MTCT and sequelae in the offspring when compared to every few months during gestation [33]. A positive IgG/negative IgM during the first 16 weeks of pregnancy essentially signifies that the mother was infected prior to conception and is at no risk for CT unless she is or becomes immunosuppressed during gestation [72]. As stated above, although any positive IgM test result should trigger the suspicion of a recently acquired infection, positive IgM test results should routinely be sent for confirmatory testing at a reference laboratory (see above). The following confirmatory tests are available at the JSRLSD lab: IgA, IgE, IgG avidity, and IgG differential agglutination (AC/HS) [70, 73].

Once it has been established that the mother is at risk for CT (serological test results are indicative of an acute infection), an attempt should be made to determine whether MTCT has taken place. Fetal infection can be diagnosed by performing PCR in amniotic fluid (AF) and performing monthly fetal ultrasounds throughout gestation [74]. Assuming absence of laboratory contamination, a positive AF PCR has a 100% positive predictive value for CT.

5.2 At Birth and Later in Life

Newborns and infants born to untreated mothers are more likely to present with severe disease and a higher proportion of positive serological and PCR tests [75–77]. *Toxoplasma* IgG, IgM ISAGA (immunosorbent agglutination assay), and IgA ELISA (enzyme-linked immunosorbent assay) can be used to diagnose CT. A positive IgM ISAGA and/or positive IgA ELISA, after 5 days and 10 days of life, respectively, is diagnostic of CT in the absence of transfusion of blood products. Since the newborn's blood volume is so small, the presence of *Toxoplasma*-specific immunoglobulins in donors of blood products can be detected in the newborn's sera. A positive *Toxoplasma* PCR in cord blood, amniotic fluid, cerebrospinal fluid, urine, and/or peripheral blood is diagnostic of CT. Maternal-infant comparative western blots and IFN-γ assays under *Toxoplasma* antigen stimulation have also been found helpful to diagnose CT [78].

Follow-up of the infant's *Toxoplasma* IgG should be used to definitely establish the diagnosis of CT (a positive result at 12 months of age) or exclude the diagnosis of CT (a negative result at 12 months of age, in the absence of anti-*Toxoplasma* treatment). Infected infants can become falsely negative in their *Toxoplasma* IgG under anti-*Toxoplasma* treatment. Following discontinuation of treatment, a serological rebound, at times quite robust, follows, apparently without any clinical consequences.

Infants suspected or confirmed of having CT should undergo retinal and hearing exams by experienced providers, brain imaging (ultrasound, CT, MRI), and abdominal imaging, to rule out lesions consistent with CT including chorioretinitis, hearing loss, brain and hepatic calcifications, ventriculomegaly, microcephaly, and hydrocephalus (see above).

5.3 Immunocompromised Patients (IC)

IC should be universally screened for a number of infections, regardless of epidemiological history or risk factors. All IC patients should have a *Toxoplasma* IgG and IgM performed at baseline, as soon as the immunosuppressed status is known, or it is known that patients will become immunocompromised. For solid organ transplant patients, both the donor and the recipient should be universally screened for *Toxoplasma* IgG and IgM. The US United Network for Organ Sharing (UNOS) made screening of all solid organ donors mandatory for *Toxoplasma* infection in 2017 [79].

Acute infection in IC patients is primarily diagnosed with serological tests, less frequently with the use of PCR. Toxoplasmosis by reactivation in IC patients is best diagnosed by a positive result in a pathogen-targeted PCR or a next-generation sequencing (NGS) test performed in any body fluid considered to have been affected in the context of a clinical scenario [80, 81]. Positive results in a PCR or NGS test performed in tissue should be interpreted with caution since a positive result cannot distinguish dormant infection from reactivation. Visualization of the tachyzoite form in any body fluid or tissue is considered diagnostic of toxoplasmosis, acute or by reactivation, depending of the clinical scenario [82]. Isolation of the parasite in any body fluid is also considered diagnostic of toxoplasmosis [71]. Identification of tissue cysts in biopsy specimens by hematoxylin and eosin (H&E) should be accompanied by significant inflammation surrounding the cyst(s) in order to ascertain that clinical manifestations are due to toxoplasmosis and not due to an alternative diagnosis. Tissue cysts and extracellular forms of the parasite can be best identified with the use of *Toxoplasma*-specific immunoperoxidase [70].

5.4 Ocular Disease

Toxoplasma IgG and IgM should be performed in all patients presenting de novo with chorioretinitis or in patients with chorioretinitis who have not been tested [83]. Patients with posterior uveitis only are unlikely to have ocular toxoplasmosis in the absence of retinal lesions. Alternative diagnosis should be pursued in *Toxoplasma* IgG/IgM negative patients. The diagnosis of toxoplasmic chorioretinitis should be supported by positive serological test results establishing that the patient is acutely or chronically infected and by adequate response to anti-*Toxoplasma*

treatment. In patients with atypical retinal lesions or inadequate response to anti-*Toxoplasma* treatment, sampling of ocular fluids (vitreous fluid or aqueous humor) for PCR, local production of *Toxoplasma*-specific IgG, or western blot should be considered if clinically indicated and the procedure is considered safe and feasible [83, 84].

6 Treatment

Patients acutely infected with severe and/or persistent symptoms, acutely infected during gestation, congenitally infected presenting during the first year of life, presenting with active chorioretinitis, and who are immunocompromised presenting with acute or reactivated toxoplasmosis should always be treated [85].

Pyrimethamine (PYR) is the best drug against *T. gondii* but should not be used alone. The first line of treatment is PYR in combination with sulfadiazine (SFD) [85]. However, PYR/SFD is only available in the oral form and may not be available because of drug price or manufacturing issues. Folinic acid should always be used in conjunction with PYR-based regimens to decrease likelihood of bone marrow toxicity. If PYR/SFD combination is not available or the oral route is not possible, trimethoprim/sulfamethoxazole (TMP/SMX) can be a good alternative. Other regimens that have been found clinically effective include PRY/clindamycin, PYR/atovaquone, PYR/azithromycin, or SFD/atovaquone [85].

7 Prevention

Toxoplasmosis is a preventable and treatable disease. An effective decrease in the health burden associated with toxoplasmosis in humans is only possible with a "One-Health" approach as proposed by several investigators and organizations [44, 86].

Acute primary infection can be prevented by introduction of basic hygienic and food consumption practices (Table 1). However, it must be recognized that *Toxoplasma* infection can still occur despite implementation and adherence to these measures.

For the prevention and early treatment of congenital toxoplasmosis, pregnant women should be universally screened for *Toxoplasma* IgG and IgM early during their first trimester. Seronegative women should be tested serially throughout gestation with the aim of diagnosing and treating seroconversion as soon as it is detected [33, 72].

For IC patients, in addition to primary infection, reactivation can be prevented by introduction of universal anti-*Toxoplasma* prophylaxis and/or preemptive strategies using weekly or biweekly PCR in whole blood [87].

Table 1 Prevention of toxoplasma infection

Never Infected Individuals
Avoid Ingestion of Cysts
Freeze meat at or below −12 °C (10°F)
Cook meat at or above 67 °C (153°F)
Avoid tasting or eating undercooked or raw meat
Wash hands after touching undercooked or raw meat
Decontaminate surfaces, sartens, and utensils that have been in contact with undercooked or raw meat
Avoid Ingestion of Oocysts
Do not eat undercooked or raw shellfish
Use contact precautions (e.g. gloves, wash hands) while and after gardening or contact with
Wash and peel vegetables and fruits
Decontaminate surfaces and utensils that have been in contact with vegetables and fruits
Chronically Infected Patients
Primary Prophylaxis (e.g. TMP/SMX or pyrimethamine plus dapsone oratovaquone)
HSCT and SOT
SOT (D+/R−)
Hematological malignancies
AIDS
Biologics
Recurrent chorioretinitis
Pre-emptive weekly or bi-weekly whole blood PCR
HSCT
Consider this approach in patients in whom drug prophylaxis is interrupted or not possible

AIDS acquired immunodeficiency syndrome, *HSCT* hematopoetic stem cell transplant, *SOT* solid organ transplant, *D+* Toxoplasma IgG positive donor, infected, *R−* Toxoplasma IgG negative recipient, never infected

8 Conclusions

Although toxoplasmosis can be responsible for serious morbidity and mortality in humans, the Centers for Disease Control (CDC) categorizes toxoplasmosis as a neglected parasitic disease in the United States [88]. This also appears to be the case worldwide [89]. In North America, and worldwide, patients can be become seriously disabled by or die of toxoplasmosis despite that it is a preventable and treatable disease. In the United States alone, toxoplasmosis has been reported to be the second cause of death and the fourth cause of hospitalizations due to a foodborne illness [90, 91]. Toxoplasmosis also appears to disproportionally produce greater mortality among minority groups including Hispanic and African Americans [92].

The primary focus of national policies should be to institute universal *Toxoplasma* IgG/IgM screening for all pregnant women and immunocompromised patients. Unfortunately, North America falls behind in screening and treating toxoplasmosis during pregnancy [77]. In France and other countries in Europe and Latin

America [72], screening and treatment of toxoplasmosis during pregnancy is mandatory, while it is not in the United States [93]. This paradox has resulted in a cohort of congenitally infected children born to untreated mothers in the United States with severe clinical manifestations [75]. In contrast, in countries where pregnant women are routinely screened and treated during gestation, severe presentations of congenital toxoplasmosis have become quite rare [77]. Household or family members of acute or congenitally infected individuals should be serologically tested for toxoplasmosis, particularly those who are pregnant or IC because this strategy has been shown to unveil new acute and chronic infections and family outbreaks [25, 26].

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Chagas Disease

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Abstract Chagas disease is a parasitic infection that has become an emerging public health concern in the United States. There are an estimated 300,000 people living with Chagas disease in the United States; however, many are unaware that they are infected. Many of the complex challenges of addressing this neglected tropical disease in the United States are low awareness among healthcare providers, complicated diagnostics, a lack of access to care by at-risk populations, and gaps in both disease and vector surveillance. This chapter reviews the clinical and epidemiological aspects of Chagas disease and discusses several of the current efforts by researchers and public health programs to improve awareness and expand the knowledge of how this silent killer is impacting our communities.

Keywords Chagas disease · *Trypanasoma cruzi* · American trypanosomiasis · triatomine

1 Introduction

Chagas disease is a zoonosis caused by the protozoan *Trypanosoma cruzi (T. cruzi).* The disease is endemic through much of the Americas, from the southern United States to Argentina and Chile. Globally, estimates indicate over six million people are infected with *T. cruzi* [1–3]. The epidemiological profile for Chagas disease has changed substantially in recent decades; whereas it was originally found primarily in rural areas of Latin America, many people with the disease now live in urban areas, while others reside in the United States, Europe, Canada, Japan, and elsewhere. As with other neglected tropical diseases (NTDs), Chagas disease poses a range of

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complex challenges [4]. Only a small percentage of the estimated population with the disease is able to access diagnosis and treatment [5]. There are also major limitations in diagnostic tools, including the lack of a viable test of cure. Lastly, the only drugs known to be effective against *T. cruzi*, benznidazole and nifurtimox, are decades old and often produce side effects in adults [6]. Moreover, Chagas disease primarily impacts socioeconomically vulnerable people with limited political voice, and investment in improved diagnostic and therapeutic technologies is limited.

1.1 History of Chagas Disease in the United States

In modern times, the Brazilian doctor Carlos Chagas is given credit for the discovery of Chagas disease. He was able to isolate the *T. cruzi* parasite in a young patient and published these results in 1909 [7–9]. Since this discovery, it has been extensively documented that there is widespread transmission of Chagas disease throughout Latin America, which is considered endemic for the disease [10]. There has been a large influx of immigrants into the United States from highly endemic regions in Latin America over the last 60 years, which has increased the number of individuals living in the United States who are at risk for the disease [11]. The United States also has several reports of autochthonous transmission of *T. cruzi* in both humans and animals; however it is considered to be a nonendemic country due to the low number of locally acquired vector-borne cases detected [2, 12].

Triatomines, the insect vector of *T. cruzi*, are not new to the United States and have been reported as far back as the mid-1800s [12, 13]. Evidence of the insects were first reported in California in the 1860s as a result of multiple accounts of allergic reactions. A similar occurrence happened again in 1899, which is when a Washington, DC, reporter gave the name "kissing bug" to the insects in an article discussing the reactions people had after being bitten mostly on the face [12, 14]. The *T. cruzi* parasite was first identified and reported in a triatomine collected in California in 1916 [12, 15]. In 1955, two infants in Texas were the first identified autochthonous cases reported in the United States [12]. With relatively little surveillance of Chagas disease and disparate reporting among states, it is difficult to quantify local transmission. In 2016, Montgomery et al. found 28 documented cases of Chagas disease acquired in the United States between 1955 and 2015 [12]. However, a more recent systematic review conducted by Lynn et al. identified 76 suspected or confirmed cases of autochthonous transmission between the years 2000 and 2018 [13]. The majority of these cases were found in states such as Texas, where there are active research programs and the condition is reportable under state mandates [13]. Chagas disease currently remains one of five neglected parasitic infections in the United States that has been targeted for public health action by the Centers for Disease Control and Prevention (CDC) [2, 12, 16].

2 Epidemiology

2.1 Immigrant Population

While Chagas disease is transmitted by triatomines throughout the southern half of the United States, most people with the disease acquired the infection years or decades ago while living in Latin America. Estimates, based on prevalence rates in countries of origin for Latin American-born individuals, suggest there are over 300,000 people with Chagas disease in the United States [2]. The states with the highest burden of disease are California, Texas, Florida, and New York. However, most of these individuals are undiagnosed and unaware they have Chagas disease. Systematic screening is not widely available in the United States outside of blood donations, where 2462 confirmed positive cases were identified from 2007 to 2019 [17], representing <1% of estimated cases.

Community-based studies have begun to substantiate these estimates. In a study of 4755 Latin American-born individuals who were screened through an outreach program in Los Angeles, 59 (1.24%) were seropositive [18]. People who had previously heard of Chagas disease or lived in housing made of natural materials were significantly more likely to be seropositive. Another Los Angeles study examined the prevalence among 189 family members of 86 patients with Chagas disease at the Center of Excellence for Chagas Disease (CECD) at Olive View-UCLA Medical Center [19]. Among the relatives screened, 14 (7.4%) were seropositive. A community-based study in the Washington, DC, area found a country-adjusted prevalence of 4.27%, with a significantly higher prevalence in Bolivians (25.9%) [11]. Finally, a primary healthcare program conducted through the East Boston Neighborhood Health Center reported 42/4833 (0.87%) patients screened were seropositive for *T. cruzi* [20].

Other research suggest a significant burden of chronic Chagas cardiomyopathy (CCM) among immigrant populations in the United States. An estimated 30,000–45,000 people in the United States could suffer from CCM [21]. However, Chagas disease as an underlying cause may often go unrecognized. For example, researchers found that 5/39 Latin American patients (13%) with dilated cardiomyopathy in a New York hospital had Chagas disease [22]. Researchers at the CECD in Los Angeles identified a high prevalence among Latin American-born patients with nonischemic cardiomyopathy (25/135, 19%), and Chagas disease was associated with a higher rate of mortality (hazard ratio = 4.46, 95%CI = 1.8–20.8, $p = 0.001$), heart transplantation, and hospitalization for heart failure [23]. Another investigation found 6/80 (7.5%) patients with pacemakers and a previous history of residing in Latin America had Chagas disease [24], while 17 seropositive individuals were identified in a study of 327 Latin American-born patients with abnormalities on electrocardiogram (5.2%) [25].

Finally, many people with Chagas disease may also be managing other chronic comorbidities. A study in Los Angeles of 179 Latin American-born people living with HIV or AIDS identified 2 cases of Chagas disease (1.11%) [25]. Other studies

in Latin America and Europe have found a significant burden of diabetes, depression, hypertension, and obesity among adults with Chagas disease [26–28].

2.2 Autochthonous Populations

Locally acquired cases of Chagas disease have occurred in the United States; however, it is still considered to be rare [6]. It is often stated that improved housing conditions and a mostly sylvatic and peri-domestic population of triatomine species keeps autochthonous transmission low [6, 29]. However, a lack of public health surveillance coupled with gaps in awareness by physicians may contribute to it being under reported [6, 21, 30–32].

Looking at contemporary cases of Chagas disease (since 2000), Lynn et al. reported 76 suspected or confirmed autochthonous cases, which is nearly 10 times the number reported from the prior 50 years of literature [13]. Part of the increase in cases is attributed to the introduction of blood donor screening for *T. cruzi* infection in 2007, which led to the identification of several previously undetected chronic infections [12, 33]. There have been a few studies conducted in collaboration with blood donation agencies looking at transmission sources in blood donors who have tested positive by screening and supplemental tests. One study done in 2015 by Garcia et al. looked at evidence of likely autochthonous transmission in *T. cruzi* seropositive blood donors in southeast Texas and showed 29% ($n = 17$) were likely to be from local transmission. A similar study done in south central Texas found 79% ($n = 14$) were likely to be from local transmission [34, 35].

These blood donor studies and the systematic review by Lynn et al. have helped to shed light on some of the epidemiological risk factors associated with local transmission in the United States. The most common risk factors found include living in a rural area, history of recreational hunting or camping, having a history of working outdoors, and vector sightings in or around homes [13]. To date, we know very little about both risks and prevalence of autochthonous cases of Chagas disease in the United States. Efforts to increase awareness and assess risk in some states may also be helping to improve surveillance. One such example is the Texas Chagas Taskforce that was formed through funding from the CDC to provide education and expert information for healthcare providers. More seroprevalence studies and vector surveillance research is needed to better understand the burden of disease and factors associated with local transmission [12, 13, 29, 34].

2.3 Blood Banks and Organ Donations

Blood donation screening in the United States began in 2007. Prior to this, there were only five *T. cruzi* infections associated with blood transfusions in the United States [30]. As of December 2019, there have been a total of 2462 confirmed positive

cases in 47 states found through blood donation screening (AABB Chagas Biovigilance Network, data through 20 December 2019; www.aabb.org/programs/biovigilance/Pages/chagas.aspx). The state with the highest confirmed positive blood donations was California (890) followed by Florida (325), Texas (199), New York (166), and Virginia (119). The current Food and Drug Administration (FDA) recommendations are to screen all first-time blood donors, and if a sample tests negative using one of the FDA-approved screening tests, no testing of future donations by that donor is necessary [30]. Blood donor screening in the United States has helped public health surveillance efforts to better understand the geographic distribution of people who are chronically infected with Chagas disease. Although these confirmed positives do not indicate where and when an infection has occurred, they can still help to guide public health interventions in communities where a higher prevalence is found [12].

Infections from organ transplantations have occurred in the United States; however, the risk varies depending on the type of organ being transplanted. According to a study looking at data between 2001 and 2011, there was a total of 14 *T. cruzi*-infected donors who had their organs transplanted into 32 different recipients. Of those transplantations, nine recipients from six different positive donors were reported to have been infected [6, 36]. Heart transplants had the highest risk of transmission (75%, $n = 4$) followed by liver (20%, $n = 10$) and kidney (13%, $n = 15$). Many organ procurement organizations have started to conduct selective or universal screening of donated organs and are also partnering with blood banks to assist with the testing. If an infected organ donor is detected, the recipient must be monitored closely as seroconversion may be delayed or never occur in some immunocompromised patients [30].

2.4 Congenital Populations

There are an estimated 40,000 women of childbearing age living in the United States who may have Chagas disease, with an estimated 60–315 infected infants born each year [6, 21, 37]. These estimates are derived from women who would have acquired the disease in an endemic area and are most likely asymptomatic and unaware of their status [21, 37]. Congenital transmission can occur at any point throughout the reproductive years of the mother, but there are some factors associated with an increased risk of congenital transmission [37]. Some of those factors include high maternal parasitemia, younger maternal age, vector exposure during pregnancy, twin pregnancies, and HIV infection [37]. In one study in Texas, researchers collected cord blood post-delivery from a cohort of 4000 women who had lived in endemic regions. Ten women (0.25%) had confirmed *T. cruzi* infection [38]. As of 2016, only two congenital infections have been detected in the United States, and both were treated successfully [6, 39].

Several surveys have been conducted assessing awareness of Chagas disease among obstetrician-gynecologists and pediatric infectious disease physicians. The

majority of respondents in each of the surveys rarely or never considered a diagnosis of Chagas disease among their patients from endemic countries or their offspring or had much knowledge of the disease [40–42]. This low level of knowledge and awareness, along with almost no detection, suggests that targeted education for physicians and an increase in maternal antenatal screening in populations at higher risk should be considered. A pilot study is currently being conducted in Texas to estimate prevalence of maternal infection by using dried blood spots taken from newborns as part of a mandatory screening program. If the methods and results are successful, this could be another low-cost method for screening higher-risk populations and detecting congenital infections early.

3 Transmission

Chagas disease is transmitted through various routes but primarily via triatomine insects, or kissing bugs (known by several names in Latin America including *chinches*, *barbeiros*, and *vinchucas*). *T. cruzi* is found in various species of triatomines in North, Central, and South America. These nocturnal insects feed on the blood of various animals as well as humans. Many North American sylvatic and domestic mammals act as reservoirs for *T. cruzi* [16]. For vector-borne transmission, the infected triatomine must defecate while feeding. When the person who has been bitten (usually while still sleeping) scratches the bite or rubs the area, they may unintentionally introduce triatomine feces contaminated with *T. cruzi* into their bloodstream. It should be noted that in some rare cases the triatomine bite can cause a severe anaphylactic reaction [43]. *T. cruzi* can also be transmitted orally, through consumption of food or drink contaminated with triatomine feces. Mothers with *T. cruzi* infection can transmit the parasite to their infants during pregnancy; the rate of congenital transmission typically varies from 2 to 5% and is usually lower in nonendemic areas [44, 45]. Other potential transmission routes are uncontrolled blood transfusions and organ transplantations, laboratory accidents, and sharing of syringes.

Triatomines transmit the parasite *T. cruzi* to their hosts when taking a blood meal. The parasite is found in the hindgut of an infected triatomine and passes to the host through fecal material that is rubbed into the wound or into a mucosal membrane [29, 46]. Throughout the Americas, there are domestic, peri-domestic, and sylvatic species of triatomines, with the majority of those found in the United States being sylvatic [30, 47]. Peak activity for triatomines in the United States is seasonal and usually occurs during the summer months of June through August; depending on the species, however, they can be active throughout the year in some locations [47]. The prevalence of the parasite in triatomines varies by species and region; however, some data has shown that between 50 and 60% of triatomines tested in the United States are positive for *T. cruzi* [48, 49].

Eleven different species of triatomine insects have been found across the southern two-thirds of the United States, of which all but one species has shown infection with

the *T. cruzi* parasite [13, 50]. The species found in the United States are *Triatoma gerstaeckeri*, *T. incrassata*, *T. indictiva*, *T. lecticularia*, *T. neotomae*, *T. protracta*, *T. recurva*, *T. rubida*, *T. rubrofasciata*, *T. sanguisuga*, and *Paratriatoma hirsute* [6, 30]. There have been reports of triatomines in 29 out of 50 states, with the two species having the largest geographic distribution being *T. sanguisuga* and *T. protracta* [6, 30]. Although *T. gerstaeckeri* is limited mostly to the southern and central regions of Texas and southeastern New Mexico, it is one of the most reported species as it tends to infest dog kennels and other structures found near human dwellings [6, 47]. Each species of triatomine has a particular geographic range and preferred ecological niche [6]. For example, *T. sanguisuga* has a wide geographic range across approximately 23 states and has a diverse range of ecological associations such as woodrat nests, chicken coops, horse stalls, and even human dwellings [6]. Texas has the greatest diversity of triatomines, with seven different species found across the state [30, 47, 51].

Multiple publications have indicated a well-established zoonotic cycle of the parasite in the United States [30, 34, 50, 52–54]. Researchers have identified at least 24 species of wild mammals in the United States as hosts for the parasite [6, 48, 55]. The seroprevalence rates in the different species varies; however, racoons, skunks, and woodrats have been documented to have the highest rates [6, 56]. There have been several reports from Texas and Louisiana of nonhuman primates from research laboratories being infected [57, 58] as well as domestic dogs [59–62]. From 2013 to 2015, the Texas Department of State Health Services listed canine Chagas disease as a reportable condition and confirmed 439 positive cases of canine Chagas disease during this short period of time [49]. A recent study by Meyers et al. assessed working dogs from across 41 different states and found that 7.5% ($n = 120/1610$) of those tested were infected [61]. Although more research needs to be done on the peri-domestic life cycle of triatomines in the United States, dogs may be an important reservoir to consider when looking at relative risk to humans [9, 63].

4 Clinical Manifestations

Chagas disease has both an acute and chronic phase. The acute phase, which usually begins several days after infection, is often asymptomatic. Others experience symptoms resembling common viral illnesses, yet, in some cases, meningoencephalitis, severe myocarditis, and other life-threatening conditions may develop [10]. In the acute phase, *T. cruzi* can be observed in the bloodstream in its flagellate form. Subsequently, the parasite penetrates deep organ tissue, especially the heart and gastrointestinal tract, to evade the body's immune response, and the disease enters an indeterminate (asymptomatic) chronic phase. Without treatment, the chronic phase is lifelong, but most people remain without symptoms. However, 30–40% progress to a more severe form of chronic Chagas disease, several years to decades later [10]. The most common manifestations are cardiac symptoms, including conduction

disease, arrhythmias, heart failure, and sudden death [10, 64, 65]. Gastrointestinal complications, including megacolon and megaesophagus, are more commonly observed in South America. Chagas disease causes an estimated 10,000 deaths annually [66].

5 Diagnosis

Most people with Chagas disease are unaware of the infection, yet once noticeable chronic symptoms develop, antitrypanosomal treatment is apt to be less effective. Therefore, the challenge is to identify people with the infection as early as possible so they may receive proper care. This necessitates proactive, provider-initiated testing. However, thus far, systematic screening for Chagas disease only occurs for blood donations and organ transplants, to prevent transmission through these routes. Screening via primary healthcare is likely the best way to address the current underdiagnosis of the disease in the United States, since screening of blood donations may underrepresent the socioeconomically vulnerable segment of the population with Chagas disease [67]. Further, widespread screening of at-risk pregnant women to control vertical transmission has not been implemented as part of obstetric care, although research suggests this would be highly cost-effective [68].

Confirming *T. cruzi* infection remains challenging. Direct detection of the parasite is only recommended during the acute phase of the disease, but the vast majority of cases that providers in the United States will encounter are in the chronic phase, when *T. cruzi* DNA is very difficult to detect. Polymerase chain reaction (PCR) is typically not sufficiently sensitive for routine clinical use during the chronic phase [69] but has value in certain acute cases such as reactivation due to immunosuppression. Identifying *T. cruzi* infection during the chronic phase, therefore, depends on serological testing.

Because no test is sufficiently accurate to act as a stand-alone, current guidelines recommend using two assays based on different principles [70]. In case of discordance, a third assay (again of a different type) should be used as a tiebreaker. Several *T. cruzi* assays are in use throughout the world, including enzyme-linked immunosorbent assays (ELISAs) and rapid tests, but they have varying sensitivity and specificity, often depending on the location and context in which they are used [6]. Manufacturer inserts also tend to overestimate performance characteristics, making it challenging to predict how tests will perform in a real-world scenario [69]. Additionally, test performance varies in different populations, possibly because of different immune responses to *T. cruzi* and/or the genetic diversity of the parasite itself [71, 72], which includes seven major subtypes described by DTUs classifying different lineages that cause human infection (TcI-Tcbat) [73]. Although TcI predominates in North America, infection with other strains or multiple strains has been documented [74, 75]. Geographically patterned variations in test performance mean an assay which performs well in Brazil, for example, may be less accurate when employed in Mexico or the United States. Finally, due to individual variations in

immune response, a small percentage of individuals cannot be definitively diagnosed. According to 2007–2019 data from US blood donor testing of 15,653 initially reactive donations with known status for confirmatory diagnosis, 77% were negative, 15.7% were confirmed positive, and 7.3% were classified as indeterminate [17].

In the United States, only four assays have FDA clearance for clinical use as of July 2020: the Wiener Chagatest ELISA recombinante v.3, the Ortho *T. cruzi* ELISA, the Hemagen *T. cruzi* ELISA, and the InBios Chagas Detect Plus (a point-of-care test). Only two of these are readily available within the health system. Because demand for testing in the clinical setting remains low (due to a host of factors, including limited awareness of Chagas disease), conditions are not conducive to companies obtaining regulatory approval and marketing new or existing tests. For practical purposes, most provider-initiated testing is handled by a few laboratories, while confirmatory testing is available through the CDC.

Recent research has examined the performance of the assays cleared for clinical use in the United States. Whitman et al. evaluated the 4 assays in 800 plasma samples from blood donors, where 500 were previously confirmed seropositive and 300 seronegative [76]. Results were compared to previous blood donation testing results and a consensus of two or more positive results with the assays evaluated in the study. Latent class analysis was also performed. Sensitivity was highest for InBios (97.4–99.3%) and lowest for the Hemagen ELISA (88.0–92.0%), whereas specificity was highest for the Hemagen (99.0–100.0%) and lowest for InBios (87.5–92.3%). Specificity was also >95% in both the Wiener and Ortho ELISAs. Interestingly, there was a trend toward higher sensitivity and specificity in samples from South America and lower values for Mexican samples, while Central American samples fell between the two. This suggests an important limitation for the currently available assays for testing Mexicans and Central Americans, who form the bulk of the population needing Chagas testing in the United States.

Confirmation of positive results is currently provided by the CDC, without cost for patients, but providers are required to send samples to state health departments, who route them to the CDC. States have different requirements regarding processing of samples.

6 Treatment

Only two drugs have proven effectiveness against *T. cruzi*: benznidazole and nifurtimox. Both were developed half a century ago. Treatment currently has two key limitations: the lack of an effective marker of cure and safety and tolerability issues, especially in older adults. Treatment is effective at eliminating the parasite in the acute phase, which includes congenital infections [77], and reactivations due to immunosuppression [78]. However, the value of treatment in the chronic phase has been the subject of debate. Historically, complications from advanced chronic disease were attributed to an aggressive immune response, and antiparasitic treatment was not thought to provide any benefit to adult patients [79]. In many countries,

only children were offered treatment, while parents were told their Chagas disease could not be cured [80]. However, by the 2000s, increasing evidence suggested that the parasite itself, hidden in the tissue of the heart and other organs, caused direct damage and acted as a trigger for damage caused by the immune response [81]. Furthermore, long-term observational studies have shown that chronically infected adults who receive treatment are less likely to develop cardiac complications and have improved mortality compared to patients receiving no treatment [82–84]. Furthermore, treatment of girls and women of childbearing age prevents future congenital transmission [85, 86]. Treatment does not appear to prevent morbidity or mortality once patients have developed moderate to severe complications but can reduce parasitemia. In the BENEFIT trial, which included 2854 patients with moderate to severe cardiomyopathy, the incidence of death and other indications of cardiac clinical deterioration were not significantly different in patients who took benznidazole compared to placebo [87]. A CECD cohort study observed that Los Angeles patients with normal baseline electrocardiograms who underwent treatment did not develop conduction abnormalities during 4 years of follow-up, while those with baseline abnormalities continued to experience progression even after being treated [88].

The results of the BENEFIT trial indicated that the best window for providing antitrypanosomal treatment to chronically infected patients is before the onset of severe complications. However, the effectiveness of treatment in the chronic phase is difficult to measure. It usually takes years to decades before chronically infected individuals who have received treatment see negative results on serological tests, and for adult patients, the portion that seroreverts even 20 years following treatment remains under 50% [82, 83]. Nonetheless, clinical trials in the last decade, which have used serial PCR results to assess the efficacy of treatment, do indicate a strong antiparasitic effect for benznidazole, with around 80% of treated patients exhibiting sustained negative PCR results throughout follow-up periods of 6–12 months [89–91]. This evidence should be interpreted with caution, as many chronically infected patients regularly test negative on PCR regardless of whether they are treated.

Benznidazole and nifurtimox are well tolerated in infants and children, but adult patients are more likely to experience side effects, whose frequency and intensity increase with the age of the patient. Historically, 15–20% of patients have discontinued treatment due to side effects [92], although some programs have been able to reduce this rate using a system of careful management and follow-up [93]. For benznidazole, the most common side effects are dermatological, while nausea and vomiting are also frequent. Most are mild and resolve spontaneously. However, a smaller percentage of patients experiences peripheral neuropathy, bone marrow depression, and, very rarely, Stevens-Johnson syndrome [92].

Nifurtimox produces more frequent side effects than benznidazole, although most are mild. Neurological and gastrointestinal manifestations commonly result from nifurtimox; anorexia, nausea, headache, amnesia, insomnia, and anxiety are frequently reported. Among patients treated in Los Angeles, moderate or severe adverse effects, or more frequent adverse effects per 30 days of treatment, were significantly associated with treatment withdrawal [94].

The possibility of side effects means patients should undergo periodic laboratory testing during treatment to monitor liver and kidney function, white blood cell counts, and other factors. This necessitates extra medical appointments and potentially additional costs for patients. As well as being unpleasant in themselves, side effects could entail further appointments and costs.

In the United States, few patients have been treated with benznidazole or nifurtimox. Prior to 2018, both drugs were only available through an investigational new drug (IND) protocol administered by the CDC. From October 2011 until May 2018, 365 patients received benznidazole through the IND, which is about 55 annually [95]. Others were treated with nifurtimox during this period, but the total number treated with either drug typically remained below 75 annually. In August 2017, benznidazole was approved by the US Food and Drug Administration, and since May 2018 it has been distributed through an online pharmacy (https://www.benznidazoletablets.com/en/). The FDA indication is for children 2–12 years old, based on the available evidence and endpoints from clinical research. Treatment of infants and adolescents or adults with benznidazole is considered off-label. In August 2020, the FDA subsequently issued approval for the use of nifurtimox in pediatric patients, children less than 18 years old weighing at least 2.5 kg.

Following the commercial marketing of benznidazole, the number of patients treated increased to over 150 in the first year [96]. However, dramatic expansion is needed to reach the entire patient population who would benefit from treatment in the United States. Following commercial availability, several barriers were identified to further increase access to benznidazole in the United States. These include the limited indication, a lack of treatment guidelines, high medical costs for uninsured patients, and the limited availability of providers familiar with offering treatment [96]. Meanwhile, a percentage of individuals do not respond to treatment with benznidazole or discontinue due to side effects. For these individuals, nifurtimox continues to be available through the CDC [95].

Finally, because it is difficult to measure treatment success with current tools, patients continue to need periodic monitoring following treatment to check for development of cardiomyopathy. Treated patients do not receive a definitive assurance that they are cured, and even in the most optimistic scenario, treatment fails in about 20% [92]. For another subset of patients, etiological treatment is not recommended due to advanced complications, but other interventions including pacemakers or heart transplants may be required. These interventions are much costlier than etiological treatment and could impose crippling expenses on uninsured or underinsured individuals.

New drug treatments for Chagas disease are still years away from being available for patients. In the last decade, two new chemical entities, fosravuconazole and posaconazole, were evaluated in clinical trials but did not demonstrate adequate efficacy against *T. cruzi* [89–91]. A third (fexinidazole) is under evaluation at the time of this writing (https://clinicaltrials.gov/ct2/show/NCT03587766). Other research are assessing whether adjustments to dosage and timing of benznidazole treatment might improve the safety profile while maintaining efficacy compared to the current standard treatment (for adults, 300 mg daily for 60 days). Both an

intermittent scheme and a shortened 2-week regimen of benznidazole have shown promising results, with >80% of patients maintaining negative results in PCR testing after 12 months (short regimen) to 3 years (intermittent scheme) of follow-up, although further confirmatory studies are needed. Another ongoing trial in Bolivia is assessing alternative regimens of both benznidazole and nifurtimox (https://clinicaltrials.gov/ct2/show/NCT03981523). It is important to note that the current underdiagnosis of affected people in the United States means there is not a large pool of patients who could participate in clinical research. New treatments should ideally be evaluated in Mexico and Central America to ensure they are also effective against North American *T. cruzi* strains. Development of improved drugs for Chagas disease cardiomyopathy also remains an urgent need.

7 Prevention

There are significant barriers to an appropriate public health response to Chagas disease in the United States. Limited awareness by healthcare providers and the general public, systemic problems with access to adequate healthcare in lower-income populations, diagnostic complications, and a lack of data are just a few of the challenges. However, awareness of the disease in the United States seems to be one of the biggest barriers.

7.1 Physician Awareness

Physician awareness of Chagas disease in the United States is limited. Several surveys have been conducted to assess levels of awareness among different practices, and almost all show that the majority have limited knowledge and/or rarely consider Chagas disease as a possible diagnosis [41, 42, 97–99]. A survey conducted by Stimpert and Montgomery in 2010 showed that 44% of cardiologists, 27% of infectious disease specialists, 68% of OB/Gyns, and 47% of primary care physicians were not at all confident that their Chagas disease knowledge was up to date [42]. In 2018, Pacheco surveyed physicians in Texas and found that 38% of primary care physicians were not at all confident in identifying risk factors of Chagas disease, while 58% of infectious disease specialists were somewhat confident [99]. Additionally, this same study conducted interviews with physicians to assess perceived barriers preventing diagnosis and management of Chagas disease patients. The responses included limited access to medical care, lack of insurance, no clear up-to-date guidelines or protocols, lack of a patient risk profile, complicated or poor diagnostics, lack of epidemiological evidence, and limited knowledge or expertise [99]. In 2020, Stigler Granados et al. conducted an online survey of physicians before they participated in online education sessions on Chagas disease. Table 1 shows the results of questions regarding knowledge levels, confidence, and

Table 1 Self-reported knowledge, confidence, and willingness to screen for Chagas disease from physicians ($n = 57$), nurse practitioners ($n = 1$), and physicians assistants ($n = 5$) who registered for an online educational session on Chagas disease [97][a]

How would you describe your level of knowledge about Chagas disease?	
Excellent	7.5%
Good	47.8%
Limited	32.8%
Very limited	11.9%
I don't know anything about Chagas disease	0.0%
How confident are you that your knowledge on Chagas disease is up to date?	
Very confident	13.4%
Confident	26.9%
Somewhat confident	35.8%
Not at all confident	23.9%
As a healthcare provider, would you be interested in screening patients for Chagas disease?	
Yes	62.5%
No	1.6%
Maybe	18.8%
I don't know	3.1%
Does not apply	14.1%
Do you think people in the community you serve would be interested in getting tested for Chagas disease?	
Yes	42.2%
No	18.8%
Maybe	29.7%
I don't know	9.4%

[a]Unpublished data provided by the author

willingness to screen patients. Overall primary care providers indicated they were limited in their knowledge regarding Chagas disease (44%) yet were just as likely to be interested in screening their patients as the infectious disease specialists (56%) [97].

7.2 Patient Awareness

Awareness issues not only affect healthcare providers in the United States but also the general public and, more importantly, the populations most at risk. Although public awareness has grown over the last several years, it is still difficult for patients to access testing and to find physicians who are knowledgeable on the topic [12]. The barriers faced by patients are complicated by systemic issues with access to care meaning that less than 1% of the Chagas disease cases in the United States ever receive a diagnosis or treatment. Interviews conducted with Latin American immigrants in southern California in 2014 showed that 86% had never heard of Chagas

disease, even though many reported having seen triatomines in their countries of origin [100]. The majority of patients who are unaware of their diagnosis are already marginalized with many living below the poverty line, lacking health insurance, or fearing to seek care due to immigration status [101].

7.3 Public Health Surveillance

As of July 2020, there are seven states (Arizona, Arkansas, Louisiana, Mississippi, Tennessee, Texas, and Utah) and one county health department (Los Angeles County, California) that list Chagas disease as a reportable condition [102]. Massachusetts discontinued its requirement in 2014. The majority of the surveillance activities in these states/county takes place at blood donation centers; however, physicians and laboratories may also provide some reports. All cases that are reported are followed up with investigations to determine the source of the transmission and where the exposure most likely happened [102]. Each of the states, with the exception of Arkansas and Utah, have published reports of locally acquired infections [6]. Many of the state health departments in these states also offer free triatomine identification and testing in coordination with the CDC. Texas, for example, provides online guidance for healthcare providers, materials for the general public, triatomine testing, and downloadable data on Chagas disease for the state. The Texas Department of State Health Services (DSHS) has reported a total of 156 cases of Chagas disease between 2013 and 2018. Of those positive cases, there has been one acute infection, 121 chronic asymptomatic, and 34 chronic symptomatic cases. Twenty six of these cases were locally acquired, 92 were imported cases, and 38 were of unknown origin [49].

There have been several important projects and efforts across the United States to increase surveillance and awareness of Chagas disease. In California, the CECD conducts routine community screening and has tested over 8000 residents of Los Angeles County since 2008 [18]. The East Boston Strong Hearts Pilot Project provides screening for high-risk populations as well as providing continuing education on Chagas disease for local healthcare providers [103, 104]. The Baylor College of Medicine's National School of Tropical Medicine in Texas has multiple ongoing research studies on Chagas disease and conducts regular screening for local residents in Harris County. The US Chagas Disease Providers' Network was launched in 2019 as a result of collaborations between researchers calling for more streamlined information and resources to be made available for providers. The Latin American Society of Chagas (LASOCHA) is an organization developed to provide not only awareness but also access to care for patients in the Washington, DC, area. The Texas Chagas Taskforce was formed in 2015 and came about as a result of a 5-year cooperative agreement with the CDC to help raise awareness of Chagas disease in Texas. This group is a multidisciplinary collaboration that consists of universities, local and state health departments, veterinarians, physicians, public health professionals, military partners, and citizens. They have recently partnered with Project

ECHO (Extension for Community Healthcare Outcomes) to provide online continuing education on Chagas disease for physicians and community healthcare workers across the United States.

These are just a few of the activities focusing on Chagas disease in the United States that are currently underway. Each of these efforts is helping to improve awareness of Chagas disease in the United States with the hope of better understanding the prevalence of the disease and how best to address any gaps or barriers to accessing care and treatment.

7.3.1 Barriers to Diagnosis and Treatment

Access to healthcare for Chagas disease in the United States has remained persistently low, due to a wide range of factors. As stated earlier, less than 1% of the estimated population with Chagas disease has been diagnosed, and far fewer have been treated [5]. Aside from blood and organ donations, which are routinely screened, there is a lack of widespread testing for Chagas disease in the healthcare system. Proactive screening is essential, since due to low awareness of the disease and the long asymptomatic period, most people with Chagas disease do not realize they are infected. Identifying people with the infection is important in order to direct them to proper healthcare services and prevent morbidity and mortality. In the absence of effective public health actions, Chagas disease exacts a heavy economic and social toll in the United States, estimated annually at over $130,000,000 in healthcare costs (in 2020 dollars) and 27,687 disability-adjusted life years [101, 105].

Manne-Goehler et al. described four major barriers in the United States: low provider awareness, limited diagnosis and follow-up of patients, low investment in research and education, and lack of stable financing mechanisms for patient care [5]. The authors noted some patients could incur costs for treatment, including management of side effects, depending on their insurance status. Providers incurred significant demands on their time both from arranging financing for patients and for administrative processes to acquire medication via the CDC.

Another analysis proposed a multidimensional framework with four main types of barriers: structural, systemic, clinical, and psychosocial [101]. Structural barriers consist of political and economic inequalities impacting people with Chagas disease, including repression of migrants and restrictions on their access to healthcare. Systemic barriers are gaps in the health system, such as the lack of diagnostic tools with FDA clearance or low awareness among healthcare providers. Clinical barriers involve challenges in detecting and eliminating *T. cruzi*, such as the lack of reliable biomarkers of disease progression or therapeutic success. Stigma, anxiety, or worry about the disease, language differences, and different cultural approaches to healing are examples of psychosocial barriers. These dimensions overlap and reinforce each other, such that design and implementation of public health programs for Chagas disease should take these multiple dimensions into account and leverage diverse actors, including not only researchers and healthcare providers but community organizations, social scientists, and mental health specialists.

7.4 Access Challenges for People with Chagas Disease

The majority of people with Chagas disease in the United States are likely to have been born in Latin America and are highly diverse in terms of national origin, racial/ethnic identity, social class, insurance coverage, and immigration status. There is an extensive literature on challenges in access to healthcare in the United States for immigrants from Latin America and elsewhere, which continues to be a dynamic and politicized issue. Migrants living in the United States represent a marginalized group that face significant barriers to access to care [106, 107]. They are less likely to have health insurance than natural-born citizens; migrants often work in industries which do not offer employee-sponsored health insurance and are excluded from coverage under most privatized and government health insurance plans [108]. Other identities/axes including race, gender, and socioeconomic status intersect with migratory status to determine access to and quality of care.

These barriers have major repercussions for migrants with Chagas disease. A study of 50 patients at the CECD in Los Angeles found that the majority lived in households earning below the federal poverty line and 60% lacked a high school education [104]. While this largely reflects the broader patient population of the safety net hospital where the CECD is located, it suggests many patients with Chagas disease face significant social and economic hardships. Patients typically lacked financial resources with which to pay for healthcare expenses and in some cases did not have their own transportation to go to appointments. The majority relied on local insurance programs for low-income people, something which would be largely unavailable to patients in other high-burden states including Texas and Florida. Patients also described challenges in scheduling appointments due to not having paid time off from their jobs.

People with Chagas disease could face challenges in communicating with healthcare personnel in the United States due to differences in language, social class, and concepts of health and healing. In the Los Angeles study, the majority of patients noted language was the most difficult aspect of adapting to life in the United States [104]. Communicating about a complex disease such as Chagas disease, especially given low provider awareness, would be challenging even without language barriers. Los Angeles patients described a range of reactions from healthcare personnel when attempting to inquire about Chagas disease (usually after receiving a letter about a positive screening test when donating blood). Oftentimes the encounter ended up being confusing or embarrassing for patients when providers did not recognize what Chagas disease was or said it was nothing to worry about. This often discourages patients from continuing to seek help. This response from physicians has also been documented anecdotally by patients in Texas (especially in rural areas) who have asked the Texas Chagas Taskforce for assistance in communicating with their physicians after being turned away.

Furthermore, while the healthcare system treats Chagas disease as principally a biomedical issue, people with Chagas disease are concerned with the disease's impact on their daily lives and emotional well-being. For patients, Chagas disease

represents one among several issues that have to be confronted in a day-to-day struggle for economic survival. As long as they are not feeling symptoms from the disease, people with Chagas disease may prefer to focus their time and energy on other, more pressing priorities, such as work and family. On the other hand, patient narratives reveal a diagnosis with Chagas disease can often be traumatic, leading to anxiety and depression [104]. Research in Brazil and Europe suggests a significant burden of depression in people with Chagas disease [27, 109].

8 Conclusion

A broad range of short- and long-term actions are needed to improve the lives of people with Chagas disease in the United States [5, 6, 31, 96, 101, 110]. Guidelines for providers on diagnosis and treatment of Chagas disease should be strengthened and disseminated in conjunction with awareness-raising activities in the medical community and greater incorporation of Chagas disease into medical school curricula. To address the bottleneck in diagnosis, improved availability of testing through primary healthcare providers is needed, along with easily accessible information in English and Spanish on where to get tested. Treatment, including related laboratory testing and monitoring, needs to be accessible and affordable for patients, perhaps through the creation of national and/or state financial mechanisms. There is a strong cost-effectiveness argument (both in terms of savings for the health system and economic savings from reduced morbidity and mortality) for detecting and treating Chagas disease early, in order to prevent the development of debilitating complications. Improved flow of information to the community at risk for Chagas disease is also an urgent need; such programs should be integrated with healthcare activities while making use of communication formats, including social media, that are relevant for affected people. Other recommendations include creation of a patient registry and provider referral network [5] and integration of mental health services into Chagas disease care [101].

These programs could be jump-started and/or coordinated by a national task force on Chagas disease [5], working in close coordination with the CDC, and with development of reference centers in high-burden states. Such efforts would need to be accompanied by strong investment in research on biomarkers, diagnostic tools, and new drugs and interventions. Improved coordination between different research groups (and areas) is needed to make the most of the available funding and to prevent redundancy and fragmentation. A supportive policy framework, such as the STOP Neglected Diseases of Poverty Act proposed to Congress in late 2019 (https://www.congress.gov/bill/116th-congress/senate-bill/2675/text), would be key to implementing the solutions listed above.

Finally, Chagas disease and other neglected diseases can no longer be viewed as isolated biomedical problems requiring purely technical solutions. Conversations about controlling neglected disease should be part of a broader dialogue on addressing the structural political and economic inequalities which, in intersection

with race/ethnicity, gender, sexuality, migratory status, and other factors, allow these diseases to persist. Managing Chagas disease as a public health problem in the United States is fundamentally about assuring the healthcare rights of the diverse people and communities which currently endure the disease in silence.

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Leishmaniasis

Divya Bhamidipati and Laila Woc-Colburn

Abstract Leishmaniasis is a protozoan zoonotic disease caused by the protozoa *Leishmania* with more than 24 species implicated in human disease. *Leishmania* spp. are transmitted by the *Phlebotomus* and *Lutzomyia* species of sand fly. Rates of leishmaniasis are increasing around the world, including in the United States. More recently leishmaniasis has been noted in the Southern United States, primarily in Texas with case reports of autochthonous transmission. Leishmaniasis has a varied clinical presentation ranging from localized cutaneous disease, mucocutaneous disease, to disseminated visceral disease. Treatment for leishmaniasis is complex and depends on the region of the world, the *Leishmania* spp. as well as the disease presentation. Vaccine development is critical for control and prevention efforts.

Keywords Leishmania · Zoonosis · Vector-borne · Parasite · Tissue protoza

1 Introduction

Leishmaniasis is a protozoan zoonotic disease caused by the protozoa *Leishmania* with more than 24 species implicated in disease manifestations in humans. However, leishmaniasis varies in clinical presentation, from cutaneous to visceral forms, and in severity, from asymptomatic to fatal disease [1]. Various factors including host defenses, pathogen factors, and inflammatory response dictate the presentation of disease in both humans and animals [2]. Leishmaniasis was not considered endemic to the United States in the past and was primarily noted in returning travelers, in refugees and immigrants, and in returning military personnel from endemic regions [3, 4]. However, rates of leishmaniasis are increasing around the world, including in the United States.

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2 Epidemiology

Leishmaniasis is endemic around the world, with the Middle East, East Africa, and Southeast Asia considered ecological hotspots for leishmaniasis [5]. Historically, most cases in the United States were imported cases seen in foreign travelers, recent immigrants, and military personnel returning from an endemic area [3, 4, 6]. However, in recent years, increasing numbers of animal reservoirs have been noted, increasing risk of transmission in the United States, particularly in Texas [1, 6, 7]. In North and South America, the most common species include *Leishmania braziliensis*, *L. peruana*, *L. mexicana*, and *L. panamensis*, of which *L. mexicana* has been shown to be endemic to South Texas [4, 6]. In general, cutaneous leishmaniasis is more frequent in this area than visceral leishmaniasis which is usually associated with imported cases.

Animal reservoirs of *Leishmania* have been increasing in recent years as well. As of 2015, the WHO has classified the United States as an endemic region for animal-related leishmaniasis, although this same recognition has not been observed in human disease to date. However, in addition to rising animal host reservoirs in Texas, recent case studies in Texas have also highlighted cases of autochthonous transmission [4, 6]. Additional, epidemiologic surveillance is necessary to determine the true degree of leishmaniasis burden particularly in the southcentral and southwest regions of the United States.

3 Transmission

Leishmaniasis is transmitted by the *Phlebotomus* and *Lutzomyia* species of sand fly. In North America, it is primarily the *Lutzomyia* species that bites and transmits *Leishmania* to multiple mammalian reservoirs such as rats, other rodents, opossums, and armadillos [1, 7–9].

In the human transmission cycle (Fig. 1), infected *Lutzomyia* sand flies bite and transmit *Leishmania* to the human host. The sand flies inoculate the skin with flagellated promastigotes when they bite. These promastigotes then go on to invade or are phagocytosed by host cells such as dendritic cells and neutrophils. Surviving promastigotes within these cells can then transform and replicate as amastigotes. Amastigotes go on to infect additional macrophages locally or distally after dissemination. Incubation can take weeks to months, leading to slow development of disease [2]. Varying vector biology, host factors, and parasite factors result in variable presentation of disease. Across the spectrum of disease, however, it has been noted that inflammation and macrophage activation regulate disease expression initially with persistence of parasites noted even in subclinical or asymptomatic disease [10, 11].

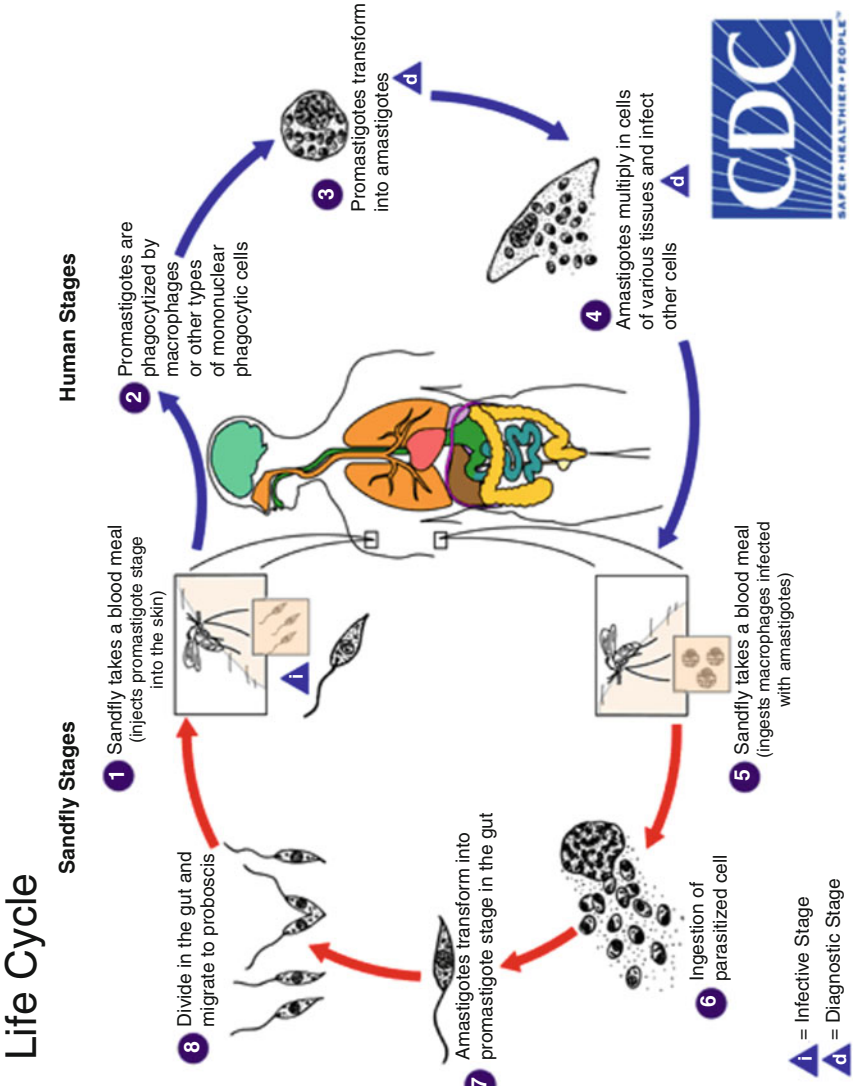

Fig. 1 Life cycle of *Leishmania* spp (source: https://www.cdc.gov/parasites/leishmaniasis/biology.html)

3.1 Host Factors

The ability of the promastigote to establish infection is dependent on its ability to evade host defenses. In order to progressively infect tissues and macrophages, the parasite needs the host macrophages to remain inactive. If the host is able to mount an inflammatory response, disease expression can be moderated and thus results in potentially asymptomatic or self-healing disease. The inflammatory response is driven mainly by an intact helper T-cell response. In cases where this T-cell response is impaired, this can result in nonhealing, more clinically apparent disease. In those that are subclinically infected, it is theorized that these patients have a robust, tightly regulatory T-cell response as there is minimal evidence of inflammation though there is detectable parasitemia. Brazilian studies have shown that subclinically infected humans are also less likely to transmit disease [12] though this mechanism is not fully understood. Studies have shown that even when provoked, humoral antibody responses are not protective, and in fact chronic nonhealing disease has the highest immunoglobulin titers noted.

3.2 Parasite Factors

Leishmania is ingested in the amastigote form when female sand flies ingest a blood meal from an infected human or animal. The amastigotes then replicate into the promastigote form in the sand fly gut and are regurgitated when the sand fly feeds on a host and is injected into the skin directly. Vector transmission is dependent on the parasite's ability to resist the proteolytic enzymes in the sand fly gut in addition to ability to avoid excretion by binding to gut epithelium. The ability to bind to the midgut epithelium is dependent on protein expression which can vary between species, thus affecting the rates of transmission across species as well [2]. Additionally the *Leishmania* parasites can affect intracellular enzymes which in turn can disrupt and downregulate immune system activating pathways in the primary host. This, in turn, can affect macrophage activation and leishmanicidal activity either promoting or hindering disease progression.

4 Clinical Manifestations

Leishmaniasis has a varied clinical presentation (Table 1), ranging from localized cutaneous disease to disseminated visceral disease. In the United States, there is evidence of autochthonous cutaneous leishmaniasis (CL) but no evidence of autochthonous visceral leishmaniasis [4–6, 13, 14]. Presentation of disease depends largely, as noted above, on host immune response and parasite virulence factors.

Table 1 Clinical features of leishmaniasis

Mucosal leishmaniasis	Cutaneous leishmaniasis	Visceral leishmaniasis (kala-azar)
Destruction of nasal cartilage, can lead to airway compromise	Chronic nonhealing ulcerative or nodular skin lesions, can be associated with bacterial superinfection of the lesions	Hepatosplenomegaly and bone marrow suppression pancytopenia renal failure, liver failure
Develops months to years after primary cutaneous infection	Develops over months	Insidious onset
Difficult to treat	Depending on species can heal spontaneously	High mortality (>90%) without treatment
Mostly seen in patients returning to the United States from South America particularly Brazil, Peru or Bolivia	Documented transmission in United States including in Texas and Oklahoma as well as in patients returning to the United States from Mexico, Central and South America, the Mediterranean region, the Middle East and Central Asia	Mostly seen in patients returning to the United Staes from Brazil, India, Sudan, South Sudan, Ethiopia, China, Iraq, Kenya, Nepal, and Somalia

4.1 Cutaneous Leishmaniasis

Cutaneous leishmaniasis (CL) is the least severe form of the disease and is caused by various species of *Leishmania*, including *L. mexicana*, the species that is endemic to South Texas and Oklahoma. Disease can present as a singular ulcerative or nodular lesion near the site of the sand fly bite. The lesions start as a papule at the site of inoculation and then evolve to a nodule before ulcerating over the course of 1–-3 months. These lesions can usually heal over months spontaneously. In New World leishmaniasis (such as that seen most frequently in the United States), ulceration is more common than the nodular form seen in Old World leishmaniasis. Lesions due to *L. mexicana* can heal spontaneously, though there is risk of bacterial superinfection with ulcerative disease. If non-healing lesions are present (Fig. 2), especially in those who have epidemiological risk factors, it is prudent to consider that another strain of *Leishmania*, such as *L. braziliensis*, may be causing the disease [7].

4.2 Visceral Leishmaniasis

Visceral leishmaniasis (VL), also known as kala-azar, is a more severe form of disease. It is caused when parasites disseminate from the initial site of cutaneous infection to the reticuloendothelial system and infect the local phagocytes. In the United States, there is no endemicity reported of visceral leishmaniasis. Cases generally are seen in returning military personnel, immigrants, and returning travelers. VL is caused primarily by *Leishmania donovani* and *Leishmania infantum* in the Old World and by *L. infantum* (*chagasi*) in the New World (mostly in Brazil).

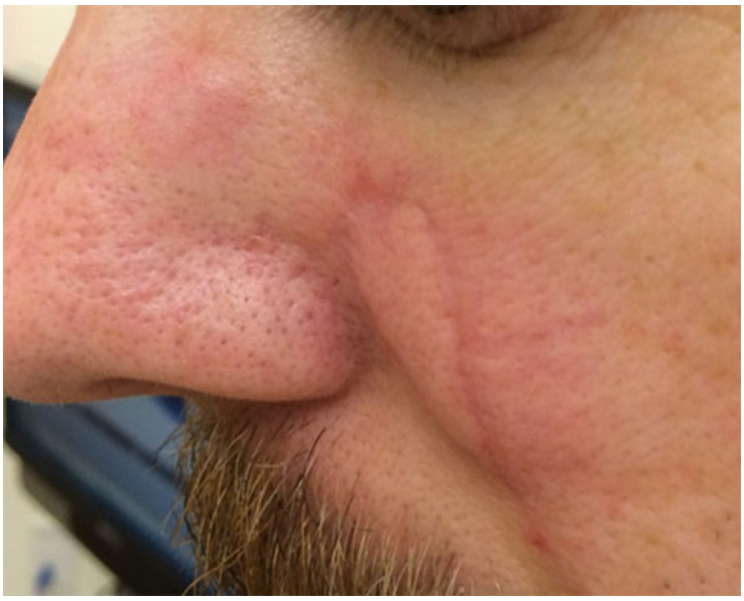

Fig. 2 Chronic non-healing lesion on nose that was eventually diagnosed as cutaneous leishmaniasis. Courtesy: Laila Woc-Colburn, MD, patient permission provided

Hallmarks of VL infection include progressive hepatosplenomegaly and bone marrow suppression. This is due to the rapid proliferation of amastigotes within the liver, spleen, and bone marrow. Patients have insidious onset of disease over months with fever, malaise, weight loss, and splenomegaly. As the disease progresses, they develop severe cachexia, thrombocytopenia, severe anemia, hepatic dysfunction, and hemorrhagic complications. Renal impairment has been seen in both adults and children with VL. If untreated, patients will also eventually develop significant pancytopenias that place them at risk for superinfection with other bacteria, fungi, or parasites. Mortality from VL is due to this immunosuppression and superinfection as well as progressive liver failure in most cases.

In contrast to other parasitic infections, patients with VL have eosinopenia rather than eosinophilia. Other lab abnormalities include elevated liver enzymes, bilirubin, neutropenia, and severe anemia.

4.3 Mucosal Leishmaniasis

Mucosal leishmaniasis, or mucocutaneous leishmaniasis (MCL), can be seen in patients with previous or concurrent cutaneous leishmaniasis. It is generally due to *L.Viannia* subgenus (particularly *L.[V.] braziliensis* and less commonly by *L.[V.] panamensis*, *L.[V.] guyanensis* and *L.[V.] amazonensis*) and presents months to

years after the primary infection. It is the result of parasites invading from the cutaneous site of infection into mucosal tissues, often resulting in destruction of tissue and cartilage that can hamper respiration. As a result, patients can develop severe disfigurement. Unfortunately this iteration of leishmaniasis is often difficult to treat, and patients die of secondary infections and malnutrition. Most cases of MCL are found in South America, primarily in Brazil, though there are cases reported in the surrounding region and as far north as Ecuador.

5 Diagnosis

Diagnosis of leishmaniasis in the United States requires a high degree of clinical suspicion. While officially the United States is not endemic for leishmaniasis, multiple studies have shown that *L. mexicana* is endemic to the southern regions of the United States with case reports of autochthonous transmission in Texas. The presence of this organisms in select regions in the southern United States requires physicians to keep leishmaniasis in their differentials for patients with skin lesions, especially those with the appropriate epidemiological risk factors.

5.1 Cutaneous Leishmaniasis

Cutaneous leishmaniasis can be diagnosed with multiple methods including serology, pathology, or nucleic amplification tests, though usually multiple tests are used.

Diagnosis is usually made by identification of amastigotes in biopsy or scrapings of the cutaneous lesion (Fig. 3). It is possible to culture the *Leishmania* parasites as well with combination of microscopy and culture increasing sensitivity of testing.

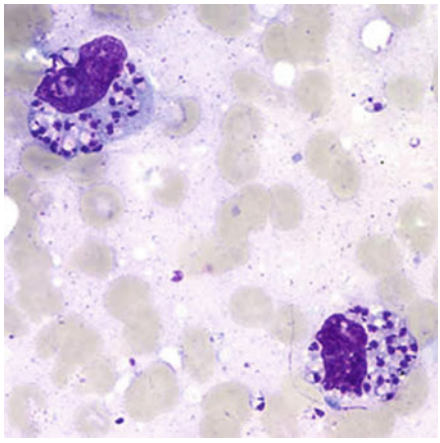

Fig. 3 *Leishmania* sp. amastigotes; touch-prep stained with Giemsa (source: https://www.cdc.gov/dpdx/leishmaniasis/index.html)

Nucleic acid PCR testing further increases the sensitivity of diagnosis and is recommended when available.

When obtaining tissue for direct visualization, the base (the area furthest from the center) of the ulcer is considered to have the highest yield. It is important to make sure the tissue is obtained from an ulcerative lesion without evidence of superinfection. For non-ulcerative lesions, samples may be obtained via injection and withdrawal of saline into the lesion at multiple sites. This aspirate can then be sent for culture and PCR testing. Culture of the parasites can take time, however, and is sometimes not available in a timely manner for diagnosis.

If a biopsy is to be pursued, it is recommended to obtain a full-thickness punch biopsy at the edge of the raised border in an ulcerative lesion. Excision biopsies are not recommended as there can be recurrence along the margins and at the suture line.

While culture and smear can be helpful, studies have shown that sensitivity for these methods varies depending on age of the lesion and appearance of the lesion. Of all the methods noted, PCR is the most sensitive, irrespective of lesion appearance or age [15].

Serological testing is not used routinely in clinical practice for diagnosis of CL. The antibody testing is unable to distinguish between past and present infection, making it less helpful in clinical practice. There is also a skin test available to help diagnose leishmaniasis. The Montenegro skin test is used in South America but is not approved for use in the United States. The skin test is similar to tuberculosis testing, where killed promastigotes are injected into the skin and the injection site is read 48–72 h later for induration. However, like serological testing, this test cannot distinguish between active and resolved infection.

5.2 Visceral Leishmaniasis

Diagnostic methods for VL are similar to CL in that visualization of the parasite (from the bone marrow or spleen in this case) provides a definitive diagnosis. Serological testing is usually utilized when other diagnostic tests are inconclusive. Peripheral blood testing for smears or culture is generally more sensitive when there is a high level of parasitemia.

When obtaining tissue for direct visualization, splenic aspiration appears to have better sensitivity than bone marrow [16]. However, splenic aspirations are difficult and carry the risk of bowel perforation or splenic hemorrhage. Bone marrow biopsies are generally safer than splenic aspiration and recommended if an experienced physician is not available for splenic aspiration. PCR molecular testing continues to have greater sensitivity in this setting; however the sensitivity itself varies depending on the sample used. In general, splenic tissue and bone marrow have higher sensitivity than peripheral blood [17].

While resource-limited settings use more serologic testing in VL diagnosis, in the United States serologic testing is reserved when all other testing is inconclusive or

negative in someone where there is high suspicion of disease. The leishmanin skin test has no role in VL diagnosis as it is uniformly negative in visceral disease.

The Centers for Diseases Control Division of Parasitic Diseases and Malaria (DPDM) maintains the laboratory identification of parasites for public health concerns (DPDx https://www.cdc.gov/dpdx/index.html) is a good resource for obtaining testing materials, sending serological or molecular testing, and for expert review of results. Culture material for parasite culture can be obtained from them as well.

6 Treatment

Treatment of leishmaniasis depends on both the region of the world as well as disease presentation. Historically, pentavalent antimony was used to treat leishmaniasis, but this reagent has significant toxicities associated with it, and more recently there is evidence of increasing parasitic resistance.

In the United States, guidelines set forth by the Infectious Diseases Society of America can help guide treatment and therapy for the various clinical syndromes that can be encountered. In immunocompetent patients with CL, watchful waiting to monitor lesions that are already healing can be an option but if the patient meets criteria for more complicated disease per guidelines [18], then systemic therapy with antimonials, pentamidine, liposomal amphotericin, and miltefosine should be considered. In more simple cutaneous disease, there are options for local therapy such as topical paromomycin, cryotherapy, and intralesional pentavalent antimony that can be used. In general the goal of treatment with CL is to reduce scarring, reduce recurrence, and prevent MCL.

Visceral disease always requires oral or systemic parental therapy for treatment with liposomal amphotericin B or miltefosine as first-line treatment in the United States. In general, risk of MCL based on where patient acquired the disease can help guide therapy in patients as well [18].

6.1 Amphotericin B

Amphotericin B comes in two formulations—amphotericin B deoxycholate which is associated with high rates of toxicity and liposomal amphotericin B. In general amphotericin B has the greatest antileishmanial activity of the treatment modalities. However, the toxicity profile and cost of amphotericin have limited its use in South Asia, Africa, and the Middle East. The development of liposomal amphotericin B has reduced some of the side effects seen (renal toxicity, electrolyte disturbances), but cost remains a barrier outside of the Americas and Europe.

AmBisome, or liposomal amphotericin B, is one of a few drugs approved by the FDA for VL in the United States. European trials have shown cure rates up to 98% in

those treated with AmBisome, while trials in Africa and India have shown rates of cure from 88 to 100% [19, 20]. Per WHO guidelines, a cumulative dose of up to 20 mg/kg/day is recommended. AmBisome is the preferred monotherapeutic agent in Europe, North America, and South America for VL.

Non-liposomal formulation of amphotericin (amphotericin B deoxycholate) has antileishmanial activity in cutaneous leishmaniasis but as noted above, carries significant side effects with use. Liposomal amphotericin B additionally is more widely used now for CL but more research is needed for optimal duration and dose. Efficacy of this medication varies depending on the species being treated with highest efficacy seen in *L. infantum* infections.

6.2 Antimony

Pentavalent antimony, also known as sodium stibogluconate, can be used for both cutaneous and visceral leishmaniasis treatment. The exact mechanism of action is not fully understood. It is available in IV, IM, and intralesional forms. Varying regimens are available with some species of *Leishmania* requiring adjunctive therapy for efficacious treatment. However, for VL, monotherapy is no longer recommended.

Side effects of antimonial regimens include cardiac toxicity (arrhythmias, sudden death), elevation of liver enzymes, pancytopenias, and electrolyte abnormalities. It is also worth noting that pentavalent antimonial resistance is rising, particularly in areas of Northern India.

Pentavalent antimony therapy can be considered for patients with CL and for patients with VL who cannot tolerate AmBisome or miltefosine.

6.3 Topical Paromomycin

Topical therapy can be considered for Old World cutaneous leishmaniasis with agents such as paromomycin [18]. Paromomycin is a topical aminoglycoside that is available in ointment or cream form. It has been shown to be most effective for ulcerative infections caused by *L. major* without lymphocutaneous involvement and in cases with only a few CL lesions noted. Studies have shown that paromomycin has efficacy against both Old World CL (OWCL) and New World CL (NWCL). In OWCL, paromomycin has been shown to be just as efficacious as intralesional pentavalent antimony, while in NWCL, it was inferior to parental antimonial therapy [21].

6.4 *Pentamidine*

Pentamidine is an alternative parenteral agent for both CL and VL though it is not FDA approved for either indication. Due to many adverse effects from its mechanism of action as a disruptor of DNA synthesis, it has been relegated to second-line treatment in many areas of the world. Data overall is limited in its use as an agent, but studies have shown that it has the highest activity against NWCL (particularly *L. guyanensis*). Side effects include pancreatitis, QT prolongation, electrolyte disturbances, and nephrotoxicity [22–24].

6.5 *Miltefosine*

Initially an anti-neoplastic agent, miltefosine was approved for use for leishmaniasis by the FDA in 2014 for MCL, VL, and CL. Miltefosine is a well-tolerated oral drug that has good efficacy. Studies done in India have shown comparable cure rates to amphotericin B deoxycholate in patients with VL (94% vs 97%). However, notably there is now evidence of increasing failure rates with miltefosine monotherapy in India. In CL, miltefosine efficacy varies depending on the species of *Leishmania* being treated with cure rates from 50 to 90%. Overall, it is considered to have good efficacy against many NWCL species, especially compared to other therapies such as the antimonials. Side effects are primarily gastrointestinal related with nausea, vomiting, and diarrhea the most common side effects noted. Of note, miltefosine is teratogenic and contraindicated in pregnant or breastfeeding women [2, 24–28].

7 Prevention

Given the various factors involved in *Leishmania* transmission, prevention strategies are multifactorial, targeting the human reservoir, the animal reservoir, and the vector population [29]. The use of insecticides including indoor residual spraying and the use of insecticide-impregnated bed nets and fabrics (curtains, clothing, bed sheets) have been effective in reducing the incidence of cutaneous leishmaniasis [30, 31]. Although the impact of each individual insecticide intervention is largely unknown, other vector-based reduction strategies include destruction of sand fly breeding grounds as well as plastering of cracks in house walls and latrines [29, 31]. Dogs are a known reservoir of *Leishmania infantum*, and current public health strategies engaging veterinarian control efforts have been promising such as the use of insecticide-impregnated dog collars. Canine *Leishmania* vaccines are also effective measures in human *Leishmania* control and prevention strategies [32, 33]. There are currently four licensed veterinary vaccines available in endemic regions. Used on a large scale in Brazil, canine vaccination programs lead to

decreased incidence of human leishmaniasis [34]. Development of human vaccines, however, is lagging behind the veterinarian counterpart. While human vaccine development programs are an area of ongoing research, currently there is only one licensed human vaccine which has been available in Uzbekistan [34, 35]. Vaccine development is critical for control and prevention efforts. Clinical trials for human leishmaniasis vaccine development should be a *Leishmania* research priority in order to enhance control and prevention public health strategies [29].

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Trichomoniasis

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Abstract *Trichomonas vaginalis* is an extracellular parasite that primarily infects the squamous epithelium of the genital tract and is sexually acquired. It is highly prevalent in women, can cause poor perinatal outcomes and infertility, and is associated with increased risk of HIV acquisition. In the United States, there are about 3.1 million *T. vaginalis* infections each year with women and African Americans having the highest rates. While most *T. vaginalis* is asymptomatic, women may have vaginitis and cervicitis, and men can have urethritis and epididymitis**.** There has been a recent proliferation of diagnostic tests, including nucleic acid amplification and point-of-care tests which are far more sensitive than the commonly used wet mount microscopy. Despite the predicted high prevalence of disease and important sequelae, *T. vaginalis* is not currently a reportable disease in the United States, and there are no recommendations for general screening making the true prevalence of this neglected infection unknown. The preferred treatment among women for *T. vaginalis* is 500 mg of twice-daily metronidazole (MTZ) for 7 days and among men is 2 g of single-dose MTZ. Tinidazole (TDZ) (2 g single-dose) is another treatment option for both sexes. 5-Nitroimidazole drug resistance occurs in up to 5–10% of cases, and treatment can be difficult, many times requiring expert consultation. Because of high repeat infection rates, sex partners should also be treated, and infected women should be retested 3 months after initial treatment. Condoms can help prevent *T. vaginalis***.** More research on the importance of asymptomatic infection and infection in men is needed.

Keywords *Trichomonas vaginalis* · Epidemiology · Treatment · Sexually transmitted infection

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1 Introduction

T. vaginalis is a member of the eukaryotic supergroup Excavata that includes other parasites such as *Leishmania*, *Trypanosoma*, and *Giardia* [1]. It thrives in anaerobic environments although minimally elevated levels of O_2 can boost growth [2]. It is remarkable in that it has one of the biggest parasitic genomes sequenced, encoding 60,000 proteins on 6 haploid chromosomes, 30,000 of which are expressed [1]. Up to 65% of the genome consists of repetitive sequences; genes relevant to infection and pathogenicity are expanded. Transcriptional analyses have found that *T. vaginalis* genes are differentially regulated upon environmental changes, in particular oxygen exposure. This results in a change of expression of hundreds of genes within minutes, reflecting the ability of the parasite to rapidly adapt to its environment [1].

Free-swimming *T. vaginalis* organisms (Fig. 1) are pyriform with four anterior flagella and a fifth recurrent flagella that is associated with the cell's surface, running toward the posterior end and attached to the cell through an undulating membrane [1]. The organism is known to invade the squamous epithelium of the urogenital tract leading to multiple adverse health outcomes including vaginitis [3], nongonococcal urethritis [4], prostatitis [4], infertility [5], increased risk of HIV acquisition [6], and perinatal morbidity [7]. It experiences rapid morphogenesis during host infection (i.e., its flagella is internalized, and it becomes an adherent amoeboid within minutes of exposure to host epithelial tissue; subsequent adherence is cytotoxic and results in lysis of the host cell) [8], secretes exosomes critical for mediating host/parasite interactions [9], manipulates the vaginal microbiota through phagocytosis (for nutrient uptake as well as neutralization of host defense proteins) [1], and has a rich strain-dependent diversity [1].

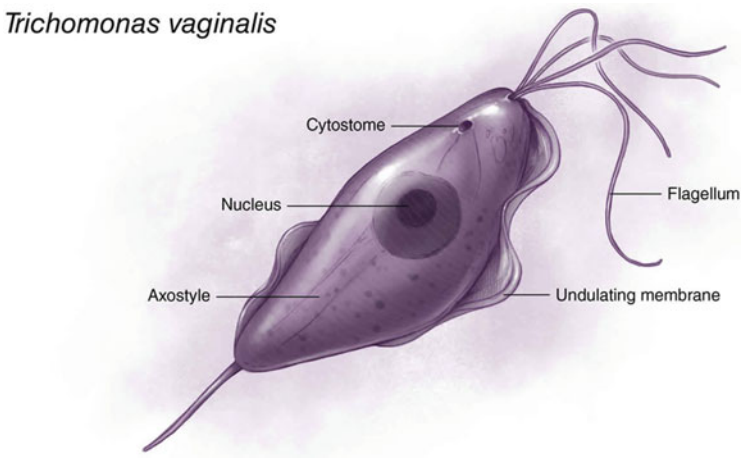

Fig. 1 Trichomonas vaginalis parasite (source: https://healthjade.net/trichomonas-vaginalis/)

As a consequence of its anaerobic lifestyle, the aerobic mitochondria of *T. vaginalis* have evolved into anaerobic hydrogenosomes (semi-functional mitochondria) present in the cytoplasm of the organism [1]. These double-membrane-bound organelles do not require oxygen (O_2) and instead produce molecular hydrogen (H_2) and adenosine triphosphate (ATP) through several energy production pathways. The first pathway uses pyruvate:ferredoxin oxidoreductase (PFOR) and hydrogenase to produce ATP from pyruvate, generating H_2 as a by-product. The second pathway utilizes malate dehydrogenase (MDH) and nicotinamide adenine dinucleotide:ferredoxin oxidoreductase (NADH:FOR) to decarboxylate malate and produce pyruvate and CO_2. Pyruvate subsequently enters the first pathway to produce acetyl-CoA and ATP [10, 11]. These metabolic pathways inside the hydrogenosome are one of the primary targets of the drug class used to treat *T. vaginalis*, the 5-nitroimidazoles [i.e., metronidazole (MTZ) and tinidazole (TDZ)] [1]. 5-Nitroimidazoles enter *T. vaginalis* in an inactive form by passive diffusion and must be activated to cause cell death [12]. This activation occurs through a nonenzymatic reduction inside the hydrogenosome, generating nitro-radicals, leading to disruption in DNA synthesis and repair and cell death [13, 14].

In addition to the energy production pathways that occur in the hydrogenosomes, *T. vaginalis* also has an antioxidant defense pathway that takes place in its cytoplasm [1]. This pathway may also be a target of 5-nitroimidazole medications [1]. In this pathway, the radical oxygen species, superoxide (O_2-), is reduced to O2 and hydrogen peroxide (H_2O_2) by superoxide dismutase. The O_2 is then reduced by either nicotinamide adenine dinucleotide (NADH) oxidase to H_2O or by flavin reductase 1 to H_2O_2. The H_2O_2 must be further reduced to avoid its cytotoxic side effects. This is accomplished indirectly by flavin enzyme thioredoxin reductase (TrxR) and its accompanying protein and enzyme thioredoxin (Trx) and thioredoxin peroxidase (TrxP), respectively. TrxR facilitates the reduction of Trx by nicotinamide adenine dinucleotide phosphate (NADPH). Reduced Trx activates TrxP, which reduces H_2O_2 to H_2O; it can also activate MTZ [15]. Upon activation, MTZ then forms a covalent adduct with TrxR and other enzymes and proteins in the antioxidant defense pathway. The inactivation of TrxR by MTZ prevents Trx activation which blocks TrxP from reducing H2O2, which is cytotoxic, leading to death of the organism [1].

Drug resistance in *T. vaginalis* is classified as either aerobic (clinical resistance) or anaerobic (laboratory-induced, in vitro resistance). Aerobic resistance arises due to deficiencies associated with oxygen-scavenging mechanisms of the antioxidant defense pathway [13, 16, 17]. Anaerobic resistance has mainly been observed in vitro and induced under laboratory conditions rather than arising clinically. It is characterized by the disruption of enzymes that participate in the energy production pathways in the hydrogenosome [18, 19]. *T. vaginalis* isolates that exhibit anaerobic resistance tend to have much higher in vitro minimal lethal concentration (MLC) values compared to aerobically resistant isolates. Aerobic resistance is more common and may be the first step in development of anaerobic resistance. The true prevalence of aerobic resistance is not known due to a lack of recent surveillance

studies. However, prior studies suggest it is present in approximately 5–10% of *T. vaginalis* infections [20] and may be rising [1].

Complicating the picture of 5-nitroimidazole resistance in *T. vaginalis* is that resistance may be relative and not absolute. For example, *T. vaginalis* infections unresponsive to currently recommended doses of MTZ (i.e., 2 g single oral dose or 500 mg orally twice daily for 7 days) [21] may be treated by increasing the dosage and duration of treatment (i.e., 2–4 g of MTZ daily for 3–14 days) [22]. This may be because the enzymes that activate MTZ are important in other critical cellular functions and complete loss of these enzymes would result in parasite death. In addition, some *T. vaginalis* isolates have been found to be clinically more resistant than others despite similar MLC values to MTZ [22]. This suggests that complex interactions between drug levels in the vaginal mucosa, the intra-vaginal redox potential (which may regulate the amount of drug taken up by the parasite), and the composition of the vaginal microbiota (which may modify the amount of available drug) may contribute to the level of drug resistance [22]. A major limiting factor of high-dose MTZ treatment is the amount of drug that patients can safely tolerate, as significant side effects including nausea, metallic taste, sensorium changes, and peripheral neuropathy have occurred in patients receiving high-dose MTZ for extended periods of time [22]. Thus, the risks and benefits of treating patients with MTZ-resistant trichomoniasis with daily doses of MTZ exceeding 3 g should be carefully considered; alternative treatments outside of 5-nitroimidazoles should be considered in these cases [21]. Additionally, the continued use of MTZ and TDZ can lead to cross-resistance to other 5-nitroimidazoles (i.e., secnidazole, currently under study for treatment of *T. vaginalis*) (NCT03935217), as they share the same mechanism of action [23].

2 Epidemiology

T. vaginalis is estimated to be the most common nonviral STI in the world [24]. General screening is not recommended, and it is not currently a reportable disease in the United States, but modeling has shown that, worldwide, there are approximately 156 million new cases per year [24]. Among women, the global prevalence of *T. vaginalis* is more common than *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and syphilis combined [24]. In the United States, the prevalence of *T. vaginalis* in a population-based study was 1.8% among women and 0.5% for men [25] with an estimated 3.1 million persons infected each year [26].

Women are 6 times more likely to have a prevalent *T. vaginalis* infection than men, and non-Hispanic Black people are 8 times more likely to be infected than non-Hispanic White people, constituting a dramatic health disparity [27]. Other risk factors for *T. vaginalis* include older age, two or more sex partners in the past year, having less than a high school education, living below the poverty level, smoking, and history of incarceration [26, 27]. The prevalence of *T. vaginalis* in men who have sex with men (MSM) is very low [28]. It is currently unclear if extragenital

(oral, rectal) *T. vaginalis* infection occurs; however, a few studies have detected it in these regions by NAAT testing but is much less common than genital *T. vaginalis* [28, 29]. Oral and rectal testing or screening is not currently recommended for women or men [21].

T. vaginalis is an extracellular parasite that primarily infects the squamous epithelium of the genital tract. It commonly infects the female lower genital tract (vagina, urethra, and endocervix) and the male urethra and prostate [30]. *T. vaginalis* is transmitted among humans, its only known host, primarily by sexual intercourse. Infection in women may persist for long periods of time, possibly months or even years [31] but may be shorter (e.g., less than a month) for men [32].

The *T. vaginalis* parasite does not appear to have a cyst form and does not survive well in the external environment but can survive outside the human body in a wet environment for more than 3 h [33]. There may be, however, a pseudocyst form [34] which has been found to be more virulent in animals and could have relevance for humans, particularly in the case of cervical neoplasia [34, 35]. While thought to be rare [30], evidence of nonsexual transmission via fomites and possibly water has been described [36–38]. *T. vaginalis* can be infected with one of four double-stranded RNA (dsRNA) viruses (*T. vaginalis* virus or TVV), of which TVV2 has been previously linked to more severe genital symptoms [39]. However, in a recent study of 355 *T. vaginalis* isolates, of which 40% were positive for TVV, there was no association between TVV positivity and genital symptoms [40].

3 Clinical Manifestations

The majority of women (85%) [41] and men (77%) [42] with *T. vaginalis* are asymptomatic. Half of the asymptomatic women may become symptomatic within 6 months [30]. Less is known about the natural history of *T. vaginalis* in men. Symptomatic women can have vaginal erythema, dyspareunia, dysuria, and vaginal discharge (which is often frothy, diffuse, malodorous, and yellow-green in color) (Fig. 2), as well as pruritus in the genital region. The normal vaginal pH is 4.5, but with *T. vaginalis* infection, this may increase markedly, often to >5 [30]. However, *T. vaginalis* may still be present in the setting of a normal vaginal pH. *Colpitis macularis* or strawberry cervix is seen in about 5% of women on pelvic exam, though with colposcopy this rises to nearly 50% (Fig. 2) [43]. Other complications of *T. vaginalis* among women include infection of the adnexa, endometrium, and Skene and Bartholin glands. One study of HIV-infected women found an association between *T. vaginalis* and pelvic inflammatory disease [44]. In men, it can cause urethritis, epididymitis, prostatitis, and decreased sperm motility [45].

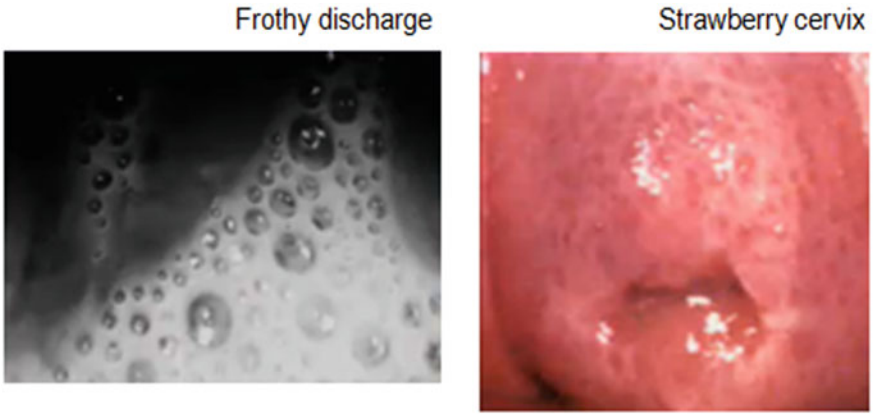

Fig. 2 Symptoms of *T. vaginalis*

3.1 T. vaginalis *and HIV Transmission*

Previous investigations show that *T. vaginalis* infections are associated with the risk of HIV acquisition [46]. A meta-analysis of 19 peer-reviewed studies found that persons infected with *T. vaginalis* were 1.5 times more likely to acquire HIV than individuals not infected [47]. This greater susceptibility is biologically plausible for three reasons: (1) the inflammatory response to *T. vaginalis* infection results in an increased appearance of HIV target cells in the genital tract mucosa [48]; (2) *T. vaginalis* infection can impair the mechanical barrier to HIV via punctate mucosal hemorrhages [49]; and (3) *T. vaginalis* infection may change the normal vaginal microbiota rendering it more permissive for the development of bacterial vaginosis (BV) [50], which, in turn, can increase the risk of HIV acquisition [51]. The influence of *T. vaginalis* on HIV transmission in the era of HIV pre-exposure prophylaxis (PrEP) needs further study.

There is less direct evidence that HIV-infected persons with *T. vaginalis* are more likely to transmit HIV. A review paper found that 7 of 14 studies demonstrated higher likelihood of shedding of HIV in genital fluids if a person was co-infected with *T. vaginalis* compared to HIV-infected persons without co-infection [46]. HIV vaginal shedding was decreased after *T. vaginalis* treatment in a cohort of women from Kenya, diagnosed by wet mount microscopy and culture [52], and another cohort of women in New Orleans, Louisiana, diagnosed by culture [53].

A study by Sorvillo et al. estimated that in a community with a high prevalence of *T. vaginalis*, as much as 20% of HIV could be attributed to *T. vaginalis* infection [54]. Chesson et al. estimated that 6.2% of all HIV infections among US women may be attributable to *T. vaginalis* infection [55]. Control of *T. vaginalis*, therefore, may provide a cost-effective strategy for reducing HIV transmission, especially in settings where *T. vaginalis* is common [56, 57] or among subgroups who have higher

rates of *T. vaginalis*, such as African Americans [58]. These data underscore the importance of screening for and treatment of *T. vaginalis* among women.

3.2 T. vaginalis *and Other STIs*

T. vaginalis appears to have a similar bidirectional association with herpes simplex virus type 2 (HSV-2) as it does with HIV-1. Concomitant infection with *T. vaginalis* has been associated with HSV-2 shedding [59], and *T. vaginalis*-infected women have a higher incidence of HSV-2 [60]. *T. vaginalis* has been associated with the presence of other STIs including *C. trachomatis*, *N. gonorrhoeae*, and human papillomavirus (HPV) [61, 62].

3.3 T. vaginalis *and Bacterial Vaginosis (BV)*

Up to 40–60% of women with *T. vaginalis* also have BV [58–60], and women with BV are at higher risk for acquiring *T. vaginalis* [63]. While vaginal dysbiosis has been associated with increased pathogenicity of *T. vaginalis*, it is not clear if BV interferes with *T. vaginalis* treatment. In randomized trials, BV was found to increase MTZ treatment failure among HIV-infected women [64] but not among HIV-uninfected women [65]. This difference may be due to impaired immunity among HIV-infected women, altered pharmacokinetics and pharmacodynamics of MTZ, or inadequate power in the studies conducted [66].

3.4 T. vaginalis *and Neoplasia*

A study of women in Tanzania found that women with *T. vaginalis* were 6.5 times more likely to have high-risk HPV, suggesting an indirect link between *T. vaginalis* and cervical neoplasia [62]. A meta-analysis also found that *T. vaginalis* was associated with a 1.9-fold risk of cervical neoplasia [67]. Studies of Finnish, Dutch, Belgian, and Chinese women have all found elevated odds (1.4–2.0) of cervical neoplasia among women who have *T. vaginalis* or vice versa [68–71]. The association between *T. vaginalis* and prostate cancer in men has been inconclusive to date, and additional data are needed [72, 73].

3.5 T. vaginalis *and Perinatal Outcomes*

T. vaginalis has been associated with poor birth outcomes such as premature rupture of membranes, preterm delivery, low birth weight, and pelvic inflammatory disease among HIV-infected women [7, 74]. One study also showed an association between maternal *T. vaginalis* infection and intellectual disability in children born to infected mothers [15]. Although rare, *T. vaginalis* infection can be transmitted perinatally [75] and cause vaginal and respiratory infections in neonates [76, 77].

4 Diagnosis

There has been a proliferation of *T. vaginalis* molecular diagnostics over the last decade which have greatly decreased cost, compared to culture (the prior gold standard), and the number needed to test for one positive *T. vaginalis* case [78].

A wet mount examination of vaginal fluid has traditionally been the most common method for diagnosis of *T. vaginalis* in women [79]. Direct observation of pear-shaped trichomonads on wet mount with their characteristic tumbling motility is considered 100% specific for diagnosis [80]. The benefits of wet mount are that it is inexpensive and can be performed at the point of care. However, it requires access to a microscope as well as appropriate training. In addition, it has a relatively low sensitivity (44–68%) compared to culture [80], as it is dependent on inoculum size (fewer than 10^4 organisms/mL are unlikely to be seen) and the experience of the reader [81].

Prior to the recent availability of *T. vaginalis* molecular diagnostic tests (such as nucleic acid amplification tests [NAATs]), *T. vaginalis* culture in Diamond's medium was the gold standard for diagnosis, with a sensitivity of 81–94% [81]. The inoculum size for culture is much lower than that for wet mount, in the range of 10^2 organisms/mL [81]. Contamination of Diamond's media with vaginal bacteria is common, even when broth cultures are spiked with antibiotics to eliminate bacterial growth [81]. Because of this, culture systems such as the InPouch® system (BioMed Diagnostics, White City, OR) have been developed to avoid this issue by placing the specimen in a two-chambered bag [82]. *T. vaginalis* is an anaerobic organism that grows more slowly under aerobic conditions; thus, CO_2 incubation at 37 °C has been recommended for optimal recovery of the organism in culture [81]. Beyond availability of an incubator, the major drawback of culture is that it is time-consuming, as the InPouch system requires reading a number of times over several days. A prior study of 2499 InPouch® *T. vaginalis* cultures found that daily examination over 3 days would only detect 82.8% (95% CI 79.0%, 86.2%) of positive specimens [83]. Of the remaining positive cultures, 17.2% (95% CI 13.8%, 21.0%) were detected with reads spanning 4–7 days. Based upon these data, it is recommended that the InPouch® culture be examined daily for 5 days over a 7-day period to reduce the possibility of false-negative test results [83]. Because of the

need for incubation and multiple reads over a period of time, *T. vaginalis* culture is categorized by the Clinical Laboratory Improvement Amendments (CLIA) as a moderately complex test [81].

The OSOM® Trichomonas Rapid Test (Sekisui, Framingham, MA) is a qualitative antigen detection immunochromatographic (IC) assay for the rapid diagnosis of *T. vaginalis* within 10 min [84]. This test uses color IC capillary flow dipstick technology to detect the presence of *T. vaginalis* antigens from vaginal swab specimens in women and is CLIA-waived. It has not been evaluated in urine specimens from women. Compared to a composite reference standard of wet mount and culture, the sensitivity and specificity of the OSOM® test are 97.9% and 99.4%, respectively [84]. Although this test is more expensive than wet mount, microscopy access and training are not needed; the test can be performed on-site by laboratory personnel, nurses, and clinical providers with minimal training. It is most practical in settings where a rapid point-of-care test is needed and wet mount microscopy and culture are impractical or unavailable [84]. Huppert et al. have reported high acceptability and accurate results when adolescent women were allowed to self-perform this test [85, 86]. Of note, compared to the APTIMA *T. vaginalis* NAAT (Hologic Gen-Probe, San Diego, CA), the OSOM® test has poor sensitivity (37.5%) for *T. vaginalis* diagnosis in men when male urine specimens are used [87].

T. vaginalis may also be incidentally visualized on a Papanicolaou (Pap) smear for women undergoing cervical cancer screening; however, this is not an optimal diagnostic test. Although Pap smear is highly specific (99.4%) for *T. vaginalis* diagnosis compared to culture, it is poorly sensitive (61.4%) [88]. Because the parasite is predominately found in the vagina, sampling of the endocervix during the Pap smear is not the optimal location for diagnosis [81]. Nevertheless, if *T. vaginalis* is noted on a Pap smear, the test should be considered accurate, and treatment is warranted without further testing [88].

The first molecular assay for *T. vaginalis* detection was the Affirm VPIII test (Becton Dickinson) [89], a moderately complex test which also detects *Gardnerella vaginalis* and *Candida albicans*. It uses synthetic nucleic acid capture probes and color development detection probes that are complementary to unique genetic sequences of the target organisms. Approximately 45 min is required to obtain test results [90]. However, when this assay was subsequently compared to a NAAT test for *T. vaginalis* detection, its sensitivity was only 46% [91].

Over the past several years, a relatively large number of highly sensitive and specific NAAT tests for *T. vaginalis* diagnosis have become commercially available. NAAT tests are now considered the diagnostic method of choice for *T. vaginalis* [21, 90]. The tests only require a small number of alive or dead organisms for detection [92]. The APTIMA *T. vaginalis* NAAT (Hologic Gen-Probe, Bedford, MA) was the first *T. vaginalis* NAAT to be approved by the FDA for use in asymptomatic and symptomatic women [93]. This assay detects an rRNA target via transcription-medicated amplification (TMA), with a sensitivity and specificity ranging from 88 to 100% and 98 to 100%, respectively, depending upon the specimen type (clinician-obtained vaginal and endocervical swab specimens, urine

specimens, and ThinPrep PreservCyt specimens). This test has not been FDA approved for use in men and must be internally validated prior to use [80]. The Becton Dickinson ProbTec *T. vaginalis* Q^x amplified DNA assay (BD Diagnostics, Baltimore, MD) was the second *T. vaginalis* NAAT FDA approved for use in female urine, endocervical swab specimens, and patient- or clinician-obtained vaginal specimens [94]. Similar to the APTIMA *T. vaginalis* assay, this test is only FDA approved in women and must be internally validated prior to use in men [95]. Both of these tests are able to yield results within 8 h.

The Xpert® TV assay (Cepheid, Sunnyvale, CA) was the first *T. vaginalis* NAAT to be FDA approved for use in both male and female urine specimens as well as endocervical specimens and patient- and clinician-collected vaginal specimens [96]. The sensitivity and specificity of this assay range from 99.5 to 100% and 99.4 to 99.9%, respectively, for female genital specimens (when compared to patient infected status (PIS) results derived from *T. vaginalis* broth culture and bidirectional gene sequencing of amplicons) [96]. For male urine specimens, the sensitivity and specificity are 97.2% and 99.9%, respectively [96]. This assay can provide on-demand results in 63 min or less, with early termination for positive results within 40 min. It is important to note that coinfection with *C. trachomatis* and *N. gonorrhoeae* can be detected from the same genital specimen used for *T. vaginalis* NAAT testing for each of the above mentioned assays (Hologic, BD, and Cepheid).

The Roche Cobas® *T. vaginalis*/*Mycoplasma genitalium* NAAT test recently became available for use on the cobas® 6800/8800 systems [97, 98]. This test can be performed on self-collected vaginal swab specimens (collected in a clinical setting), clinician-collected vaginal swab specimens, and endocervical specimens in women. It can also be performed on urine and meatal swabs in men. Compared with a composite reference, sensitivity is 100% for all specimen types; specificity ranges between 99.2 to 100%, respectively, depending upon the specimen type [97, 98]. The BD Max™ CTGCTV2 assay for use on the BD MAX system is also currently in development. Once available, it will be used for detection of *T. vaginalis* in patient- or clinician-collected vaginal swab specimens (in a clinical setting) and male and female urine, with sensitivity and specificity ranging from 81.1 to 100% and 98.7 to 100%, respectively, depending upon the specimen type (https://www.accessdata.fda.gov/cdrh_docs/reviews/K182692.pdf).

The rapid Solana® Trichomonas assay (Quidel, San Diego, CA) is another relatively new molecular test FDA approved for the qualitative detection of *T. vaginalis* DNA in female vaginal and urine specimens from asymptomatic and symptomatic women [99]. It has not, however, been studied in men. This assay uses helicase-dependent amplification (HDA) technology on the Solana® instrument and is classified as a moderately complex test. To detect *T. vaginalis* directly from female genital specimens, the assay targets a conserved repeat sequence of the *T. vaginalis* genome. It can produce results within 45 min of specimen collection. Compared to wet mount and culture, its sensitivity is 99.2% for vaginal specimens (regardless of symptom status) and 95% for female urine specimens (regardless of symptom status) [99]. However, it did not detect more positive test results than the APTIMA

T. vaginalis NAAT [99]. Nevertheless, this platform can be useful in many clinical situations, including situations where a rapid diagnosis is needed.

The AmpliVue® Trichomonas Assay (Quidel, San Diego, CA) is another relatively new rapid molecular test providing qualitative detection of *T. vaginalis* in women [100]; similar to Solana®, it has not yet been studied in men. This test is FDA approved for use on vaginal specimens obtained from asymptomatic and symptomatic women. Similar to Solana®, AmpliVue® uses HDA technology, and results are available within 45 min. However, testing can be performed in a small handheld cartridge with no additional equipment requirements. AmpliVue® has performed as well as wet mount and culture combined and has comparable sensitivity and specificity to the APTIMA *T. vaginalis* NAAT, at 90.7% and 98.9%, respectively. It is currently unknown, however, if the Solana® and/or AmpliVue® assays are more accurate than the OSOM® Trichomonas Rapid Test in women as this has not yet been studied [99, 100].

5 Treatment

In recent years, optimal treatment strategies, particularly among women, have been a hot topic in the field of trichomoniasis research. Treatment of *T. vaginalis* is essential both to reduce the burden of signs and symptoms for patients and to reduce transmission as well as prevent and/or minimize associated adverse outcomes. As previously mentioned, the mainstay of *T. vaginalis* treatment is the 5-nitroimidazole class of antibiotics. The two drugs from this class currently approved by the FDA for the treatment of trichomoniasis are MTZ and TDZ [101]. Both drugs, when given as single 2 g oral doses, have demonstrated similar cure rates, both in terms of resolution of symptoms and achievement of parasitological cure, in randomized clinical trials [102–107]. Gastrointestinal side effects such as a metallic taste in the mouth and nausea and vomiting are most commonly reported with MTZ. TDZ has a similar side effect profile, but the gastrointestinal side effects are less common [102]. Therefore, it is often better tolerated by patients than MTZ. TDZ also achieves higher serum levels and has a longer half-life than MTZ in the genitourinary tracts of both men and women [108–110]. It is, however, generally more expensive than MTZ [3]. A disulfiram-like reaction in the setting of 5-nitroimidazole administration and concurrent alcohol use is theoretically possible, and refraining from alcohol use for 25 h after taking these medications is currently recommended [21]. However, in a systematic review of the literature on this topic, no in vitro studies, animal models, reports of adverse effects, or clinical studies have provided any convincing evidence that a disulfiram-like interaction between alcohol and MTZ occurs. MTZ does not inhibit acetaldehyde dehydrogenase as disulfiram does; ethanol alone or ethanol-independent side effects of MTZ may explain the suspicion of disulfiram-like effects [111].

Other 5-nitroimidazoles such as secnidazole (SEC) and ornidazole are used for the treatment of trichomoniasis in other countries, but are not currently FDA

approved for this indication in the United States [112, 113]. A recent multicenter, prospective, randomized, placebo-controlled, delayed treatment, double-blind study to evaluate the effectiveness and safety of a single oral dose of 2 g of SEC for the treatment of trichomoniasis in women found that, compared to placebo, SEC was 92–95% effective [23]. This medication was also well tolerated; the adverse events observed (vulvovaginal candidiasis (2.7%) and nausea and vomiting (2.7%)) were consistent with the prior labeling for SEC in the treatment of BV. MTZ vaginal gel is sometimes used to treat BV, but it does not achieve therapeutic levels in the urethra and perivaginal glands to effectively treat trichomoniasis in women. In addition, multiple treatment trials have demonstrated it to be less effective than systemic MTZ [114, 115]. Therefore, its use in the treatment of *T. vaginalis* is not recommended.

Currently, per the 2015 Centers for Disease Control and Prevention (CDC) sexually transmitted disease (STD) treatment guidelines, first-line regimens for HIV-uninfected women with *T. vaginalis* include a single oral 2 g dose of either MTZ or TDZ, with oral metronidazole 500 mg twice daily for 7 days recommended as an alternative [101]. The only group for which the CDC currently recommends the 7-day regimen of MTZ as the first-line regimen is the HIV-infected women. This recommendation is based upon a randomized controlled trial (RCT) which demonstrated single-dose therapy with 2 g of MTZ to be less effective than 500 mg twice daily for 7 days [116]. More recently, a meta-analysis of trichomoniasis treatment trials was conducted and found that women receiving the 7-day MTZ dose were 50% less likely to have a positive test of cure compared to women who received the single 2 g MTZ dose [117]. In addition, a recent RCT was performed in HIV-uninfected women with *T. vaginalis* comparing these two treatment regimens, which also demonstrated single-dose MTZ to be less effective than the 7-day regimen in this population [118]. Given these new and compelling data, it is likely that the 7-day regimen of metronidazole will be recommended for all women in future CDC STD treatment guidelines [119]. In fact, this change has already occurred in the 2020 American College of Obstetrics and Gynecology (ACOG) Treatment Guidelines [120].

For men, the single 2 g dose of oral MTZ or TDZ is the currently recommended therapy. Some observational data suggest that the single oral MTZ dose provides suboptimal cure rates; however, there has never been a head-to-head comparison between the single oral MTZ dose versus the 7-day regimen [121, 122]. More studies are needed to determine which regimen provides the greatest efficacy among men infected with *T. vaginalis*.

In addition to receiving treatment, all individuals with *T. vaginalis* should abstain from sexual activity until they and all sexual partners complete therapy and all symptoms have resolved (if present) [101]. Following completion of their initial treatment regimen, all sexually active women with *T. vaginalis* should be retested within 3 months of therapy given reportedly high rates of reinfection in this population [123]. Data are currently insufficient to recommend retesting in men following treatment for *T. vaginalis*.

5.1 Treatment in Pregnancy

In pregnant women infected with *T. vaginalis*, associations have been found with several adverse birth outcomes, including preterm delivery and premature rupture of membranes [7]. Currently, the CDC recommends that all symptomatic women, regardless of stage in pregnancy, be tested and, if positive, treated for *T. vaginalis* with a single 2 g dose of oral MTZ [101]. The benefits of treatment of symptomatic pregnant women include relief of symptoms, reduced likelihood of transmission to partners, and, though uncommon, prevention of respiratory or genital infections with *T. vaginalis* in newborns [76, 124]. Emphasis on the importance of partner treatment and condom use is also cornerstone in the management of trichomoniasis in pregnant women [101].

Although it crosses the placenta, data suggest that MTZ is safe in pregnancy and is currently designated as a Class B drug [125]. As demonstrated through multiple cross-sectional and cohort studies including pregnant women, there is no evidence of MTZ treatment resulting in teratogenicity when given at any stage of pregnancy [126–129]. MTZ is secreted in breast milk, and, therefore, breastfed infants receive low doses and metabolites of the drug when the mother is taking it [130]. There have been case series suggesting no evidence of adverse events in infants exposed to MTZ in breast milk, but many clinicians still recommend deferring breastfeeding for 12–24 h following completion of maternal treatment with MTZ [130]. Data on treatment with TDZ in pregnancy are limited, but animal studies suggest moderate risk (Class C). Therefore, it is not recommended for pregnant women or for those breastfeeding [101].

As mentioned above, however, there is mounting evidence that a 7-day course of oral metronidazole 500 mg twice daily is more effective than the single-dose therapy in women. It's unclear at this time whether or not this will now be recommended to pregnant women since a head-to-head comparison of the two regimens has not been performed in this population. However, given the relative safety of MTZ in pregnancy and the potential for adverse birth outcomes, the 7-day regimen may soon be recommended [131].

Regarding asymptomatic screening (and subsequent treatment) of pregnant women, there is currently a paucity of data to recommend this practice. In HIV-infected women, screening at the initial prenatal visit and treatment as needed is recommended given that *T. vaginalis* is a risk factor for vertical transmission of HIV [101, 132]. It remains controversial whether or not screening and treatment of asymptomatic *T. vaginalis* is beneficial or harmful for HIV-uninfected pregnant women. One study in the late 1990s suggested an increased risk in preterm birth among asymptomatic women who were infected with *T. vaginalis* after treatment with MTZ [133]; however, study limitations make it difficult to draw definitive conclusions. More recent studies have shown no definitive negative association between MTZ administration in pregnancy and adverse birth outcomes [127, 134, 135].

5.2 Treatment in Patients with 5-Nitroimidazole Hypersensitivity

Given that 5-nitroimidazoles offer the most effective treatment option for trichomoniasis, patient reporting allergies to this drug class are difficult to manage. It is important to first clarify the reported reaction with the patient, as gastrointestinal and other side effects are often perceived by patients as allergies. In such patients, 5-nitroimidazoles can still be considered. In patients who report reactions suggestive of IgE-mediated hypersensitivity (i.e., anaphylaxis), the recommended management strategy is MTZ desensitization using a validated protocol with the assistance from an allergy specialist [101, 136]. Should desensitization not be possible, there is anecdotal data for treatment with alternative regimens, many of which have to be compounded and can be costly. Studies of boric acid have demonstrated in vitro activity against *T. vaginalis* [137]. Given these data, a regimen of intravaginal boric acid 600 mg twice daily for 60 days has been used in 5-nitroimidazole-intolerant patients with some success [138–140]. Intravaginal paromomycin has also been used effectively in patients unable to receive 5-nitroimidazoles. Case series report success with a 14-day course of daily 6.25% paromomycin intravaginal pessaries [141]. An important side effect of intravaginal paromomycin is vaginal ulcerations, which usually spontaneously resolve once treatment is complete; these can be mitigated by applying lubricant such as Vaseline to the affected area while treatment is occurring [142]. Several other alternative treatment regimens, including intravaginal furazolidone [143], nonoxynol-9 intravaginal suppositories [144], and povidone-iodine (betadine) douches [145], have also been used; however their efficacy is limited. Dosing regimens for these alternative agents are described in detail in Table 1. Ideal dosing regimens for these agents for both efficacy and tolerability are not known. Their use in patients should be done in consultation with an infectious disease specialist.

5.3 Management of Persistent Trichomoniasis

The most common reason for a patient to experience persistent infection with *T. vaginalis* despite treatment is re-infection from an untreated sexual partner(s). Obtaining a detailed sexual history from the patient is essential to determine the likelihood of re-infection. Another reason for persistent infection is failure of the patient to complete their treatment course, which is often related to side effects they may experience. Less common is 5-nitroimidazole resistance. MTZ resistance occurs in 5–10% of *T. vaginalis* isolates and $< 1\%$ of TDZ isolates [146, 147].

Once re-infection has been ruled out, persistent trichomoniasis often requires longer courses and higher doses of standard therapy (i.e., MTZ, TDZ), as previously described in the Biology of *T. vaginalis* section [101]. In the United States, if drug resistance is suspected, isolates can be sent to the CDC for drug resistance testing

Table 1 Currently recommended treatment of trichomoniasis in the United States

	Recommended treatment[a]	Alternative treatment
HIV-uninfected women		
First episode	MTZ 2 g PO ×1 dose TDZ 2 g PO ×1 dose	MTZ 500 mg PO BID × 7 days[b]
Persistent infection[c]	MTZ 2–3 g PO daily × 7 days TDZ 2 g PO daily × 7 days	TDZ 2–3 g daily plus intravaginal TDZ[d] 500 mg BID × 14 days TDZ (1 g TID) plus 4 g of 6.25% intravaginal paromomycin cream[d] nightly × 14 days Intravaginal furazolidone[d] 100 mg BID ×12 days Intravaginal boric acid 600 mg alternating nightly with intravaginal clotrimazole cream × 1–5 months Intravaginal povidone-iodine (betadine) douches, 20 mL of 10% solution BID × 2 days per week × 2 weeks[e] Nonoxynol-9, 100 mg intravaginal suppository
5-Nitroimidazole hypersensitivity[f]	MTZ desensitization[g]	Intravaginal boric acid[d] 600 mg BID × 60 days 4 g of 6.25% intravaginal paromomycin cream nightly × 14 days Intravaginal furazolidone 100 mg BID × 12 days Intravaginal boric acid 600 mg alternating nightly with intravaginal clotrimazole cream × 1–5 months Intravaginal povidone-iodine (betadine) douches, 20 mL of 10% solution BID × 2 days per week × 2 weeks[e] Nonoxynol-9, 100 mg intravaginal suppository
Pregnancy[h]	MTZ 2 g PO ×1 dose	MTZ 500 mg PO BID × 7 days
HIV-infected women	MTZ 500 mg BID × 7 days	n/a
Men	MTZ 2 g PO ×1 dose TDZ 2 g PO ×1 dose	MTZ 500 mg PO BID ×7 days

BID twice daily, *CDC* Centers for Disease Control and Prevention, *HIV* human immunodeficiency virus, *MTZ* metronidazole, *PO* orally, *STD* sexually transmitted disease, *TIN* tinidazole

[a]Per 2015 CDC STD treatment guidelines

[b]This dose will be the first choice in the 2020 CDC STD treatment guidelines

[c]Should consider reinfection, either by an untreated sexual partner or a new sexual partner, prior to treating as persistent infection

[d]Must be made at a compounding pharmacy

[e]Must be left in the vagina for 10 min

[f]IgE-mediated hypersensitivity reaction such as anaphylaxis

[g]Should be done in consultation with allergy specialist

[h]TIN is class C drug and cannot be used in pregnancy or during lactation

(https://www.cdc.gov/laboratory/specimen-submission/detail.html?CDCTestCode=CDC-10239). If 7 days of high-dose MTZ or TDZ is unsuccessful, combination therapy with 2–3 g of oral TDZ (in divided doses) and intravaginal TDZ twice daily for 14 days may be used [148]. Another option is high-dose oral TDZ (1 g three times daily) plus intravaginal paromomycin for 14 days [149]. This regimen is thought to be effective because not only does paromomycin have a different mechanism of action than MTZ (destruction of ribosomal RNA as opposed to inhibition of nucleic acid synthesis by DNA disruption), there may also be a synergistic effect between these two agents [150]. As with treatment of trichomoniasis in the setting of 5-nitroimidazole hypersensitivity, there are several other regimens that have been anecdotally successful and should be considered only with the assistance of an infectious disease specialist [151]. The details of these regimens are summarized in Table 1.

6 Prevention

One important element in preventing transmission and re-infection of individuals with trichomoniasis is concurrent treatment of all of the infected person's sexual partners [101]. Since *T. vaginalis* is not a nationally reportable disease is the United States [152], adequately treating all contacts for trichomoniasis is difficult. In the face of this struggle, expedited partner therapy (EPT) has emerged as a promising prevention tool for trichomoniasis [153]. EPT involves the treatment of sexual partners of a patient diagnosed with an STI by providing a prescription to the patient without performing a clinical assessment of the partner(s) [154]. Based on a large study demonstrating its efficacy, the CDC recommends EPT as an option for partner treatment in women and heterosexual men with gonorrhea and chlamydia [155], but the data for trichomoniasis are not quite as clear.

Two RCTs have been conducted to assess the use of EPT for the partners of women infected with *T. vaginalis*. One found that receiving EPT did not increase uptake of treatment by partners and lower follow-up rates were noted compared to standard partner referral [156]. The other found EPT to be well accepted and safe with rates of repeat infection in the EPT arm lower than those who were randomized to standard partner referral [157]. Given these mixed results, it is difficult to know how effective EPT truly is. However, since re-infection rates are so high [158], up to 70% of male sex partners are also infected [42], and men infected with *T. vaginalis* are often asymptomatic [42]. EPT is still recommended by the CDC as a valid and acceptable means of prevention for trichomoniasis [101]. While EPT is allowable in most states (https://www.cdc.gov/std/ept/legal/default.htm), its implementation for STI prevention has been limited as insurance often will not pay for partner treatment [159]. As mentioned previously, re-screening of *T. vaginalis* infection in women is also recommended 3 months after treatment [21].

Prevention of *T. vaginalis* is much like prevention of other STIs (i.e., reduce the number of partners and use barrier protection). Condoms impregnated with

nonoyonol-9 are protective against *T. vaginalis* [160]. There is some evidence that circumcision in men can prevent *T. vaginalis* in female sex partners [161].

7 Conclusion

T. vaginalis is a highly prevalent sexually transmitted parasitic infection in the United States and Canada that causes important perinatal morbidity, can amplify HIV acquisition, and may cause infertility in both men and women. While testing options have increased, the infection is not reportable, and there remains no recommendations for universal screening specifically in the United States. Until public awareness is raised, this parasitic STI is likely to remain neglected in the United States and Canada [162].

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Chikungunya, Dengue, Zika, and Other Emerging Mosquito-Borne Viruses

David M. Vu and A. Desiree LaBeaud

Abstract The past two decades have seen an explosive increase in emerging and reemerging infections, ranging from SARS and Ebola viruses, to epidemics of arthropod-borne viruses (arboviruses), including chikungunya and Zika viruses. Dengue and St. Louis encephalitis viruses have emerged from areas of the United States where they had been absent for over a decade. This alarming increase in number and frequency of outbreaks of vector-borne diseases, in particular, stems from the convergence of several factors. Abrupt changes in land use have brought humans closer to transmission cycles between vectors and non-human vertebrate hosts that previously had been strictly sylvatic. Rapid and unplanned urbanization due to spread of poverty has created opportunities for insect vectors, like *Aedes albopictus*, to establish urban endemicity by adapting breeding habits to thrive in man-made containers. Global warming has expanded the habitable range of vectors like *Aedes aegypti*. This chapter focuses on viruses transmitted by mosquitoes to highlight the importance of these emerging diseases. Only by learning from the past can we anticipate and prepare for the future.

Keywords Dengue · Zika · Chikungunya · Mosquito · Emerging · Virus

1 Introduction

Written records of human health among indigenous populations of North America prior to the European invasion of the Americas starting in the fifteenth century are largely unavailable, either due to lack or loss. While modern paleopathologic studies of human bony remains have aided in reconstructing the histories of certain chronic infections, such as syphilis or tuberculosis [1, 2], our understanding of the impact of most infectious diseases on human history over the previous millennia has been

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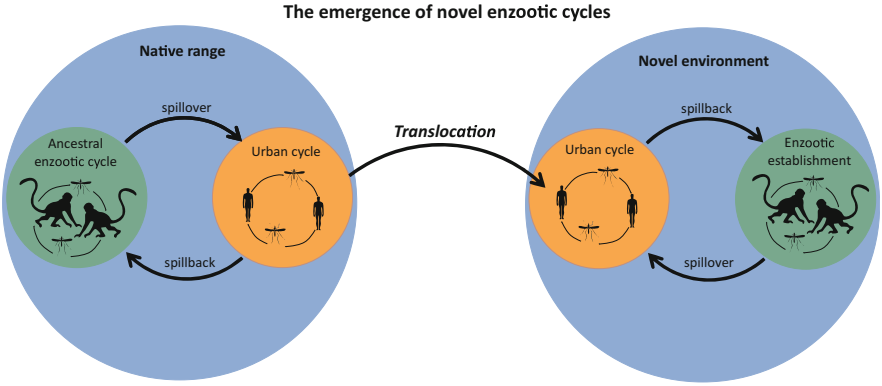

Fig. 1 A diagram of the processes by which novel enzootic cycles emerge. "Ecological processes underlying the emergence of novel enzootic cycles: Arboviruses in the neotropics as a case study" by Guth et al. [8] is licensed under CC BY 4.0

inferred mostly from written historical descriptions. Consequently, our knowledge of infectious diseases and their ramifications among indigenous peoples of North America prior to the sixteenth century is limited.

European colonization of North America in the sixteenth century began introducing foreign infectious diseases into the indigenous population, frequently with catastrophic consequences. The earliest documented epidemic in North America was the "plague" of 1616–1619, the cause of which is still debated today, which decimated over 90% of the indigenous American population from modern-day Massachusetts to Maine [3]. Ships crossing the Atlantic Ocean carried human passengers who carried with them smallpox, while rodent stowaways brought *Yersinia pestis* with them causing the plague. Ships that trafficked human slaves from Africa also transported mosquitoes. *Aedes aegypti*, one of the most important mosquito vectors presently affecting human health worldwide, is believed to have been introduced into the Americas in the seventeenth century [4] by ship. *Ae. aegypti* and its genus-mate *Ae. albopictus* transmit chikungunya virus, dengue virus, and Zika virus, the three arbovirus focuses of this chapter.

The term "arbovirus," a conjunction for arthropod-borne virus, refers to any of a number of primarily RNA-based viruses transmitted by insect vectors [5]. By definition, these viruses must replicate in the arthropod vector [6]. While some viruses can be transmitted vertically from infected female vector to offspring [7], most arboviruses require amplification in a vertebrate host, transmitted through the infected saliva of a biting vector during a blood meal. Viral transmission from infected host to susceptible vector occurs when the vector takes a blood meal. Viral amplification ensues in the vector's midgut and is followed by viral secretion in saliva, which perpetuates the enzootic cycle upon the vector's next bite (Fig. 1, green circles). Maintenance of this sylvatic transmission cycle relies not only on the

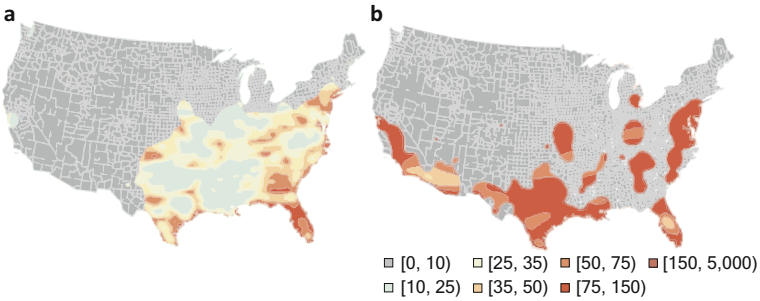

Fig. 2 Reconstruction of *Ae. albopictus* (panel **a**) and *Ae. aegypti* spread (panel **b**) in the United States. Estimates of speed of spread in km per year are based on thin spline regression on mosquito observations since their earliest detection in each continent. Red indicates fast dispersal, whereas yellow and white indicate slower spread velocity measured in km per year (see legend below **b**). "Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*" by Kraemer et al. [11] is licensed under CC BY 4.0

ability of the virus to replicate in both host and vector [9] but also on vector behavior and preference for biting that particular host.

Historically, mosquito-borne viral infections in humans are incidental zoonoses. Human arboviral infections occur as spillover events when humans invade the sylvatic cycle space. Human infection of urban vectors allows for establishment of an urban cycle (Fig. 1, orange circles). Both *Ae. aegypti* and *Ae. albopictus* thrive in the urban environment. These species have evolved to rely on human-stored water for breeding, and some subspecies of *Ae. aegypti* have even been shown to have a preference for biting humans [10]. The ability of these species to be infected by and to amplify chikungunya, dengue, Zika, and yellow fever viruses imported by an infected human traveler makes possible the establishment of autochthonous transmission in new locations. The past decades have seen the expansion of the geographic range of *Ae. aegypti* and *Ae. albopictus* (Fig. 2) related to global warming, heralding the growing potential for both epidemic and endemic arboviral infection [12]. *Ae. aegypti* and *Ae. albopictus* have been on both coasts of the United States and as far north as Ontario, Canada [13]. Autochthonous transmission of chikungunya, dengue, and Zika viruses in the continental United States in the past decade has raised awareness of the threat of *Ae. aegypti* and has prompted calls for greater efforts toward vector surveillance and control [14].

In North America, West Nile virus is the most prevalent and well-described arboviral infection affecting humans and is described separately. This chapter focuses on chikungunya, dengue, and Zika viruses, three mosquito-borne viruses emerging or re-emerging in North America as important human infections. Recent explosive epidemics of chikungunya and Zika virus infection have highlighted the epidemic potential of arboviral infections. All three can cause a self-limited acute febrile illness, with variable symptomatic manifestations including arthralgia and rash, which makes distinguishing between acute clinical disease caused by the individual viruses challenging. But the differences in potential short and long-term

disability and/or mortality between the three viral infections are striking and highlight the vast gaps that remain in our collective knowledge of arboviral pathogenesis.

2 Chikungunya Virus (CHIKV)

2.1 Epidemiology

In October 1952, an epidemic of fever, rash, and severe arthralgia broke out among residents of all ages living in villages on the Makonde Plateau in the Southern Province of Tanganyika (present-day Tanzania). The people called it "chikungunya," which meant "that which bends up" in the local dialect, to describe the contorted positions of those who were afflicted by the severe arthralgia. Investigators observed that while the illness shared many clinical similarities to dengue fever, there were notable differences. The attack rate was higher than dengue, affecting 60–80% of a village's population, and the outbreak spread rapidly within villages, over a 2–3-week period [15]. In the lab, attempts to isolate the virus by inoculating mouse brain also led to high mortality among the mice, which was not characteristic of dengue virus [16]. Subsequent characterizations found the virus to be closely related to Semliki Forest virus [17].

Over the next four decades, small CHIKV outbreaks were reported throughout Central, Southern, and Western Africa. In Africa, CHIKV is transmitted by arboreal *Aedes* mosquitoes (*Ae. furcifer-taylori*, *Ae. africanus*, *Ae. luteocephalus*, and *Ae. neoafricanus*) in an enzootic cycle with non-human primates as the principle reservoir [18]. In Southeast Asia, however, CHIKV outbreaks were reported in larger cities, with transmission attributed primarily to *Ae. aegypti*, which is adapted to thrive in urban human environments.

In 2004, a CHIKV outbreak erupted in Kenya and swept down the coast onto the islands on the Indian Ocean (Comoros, Mayotte, Seychelles, Réunion, Madagascar, Sri Lanka, and the Maldives) (Fig. 3). The epidemic continued onto India, Southeast Asia (Malaysia, Singapore, Thailand), and China. European travelers infected with CHIKV returning home infected local populations of mosquitoes, resulting in the first observed autochthonous transmission of CHIKV in Italy in 2007 [20, 21] and in France in 2009 [22]. In December 2013, autochthonous transmission of CHIKV was documented for the first time in the Americas in St. Martin [23]. By October 2014, 11 cases of autochthonous transmission of CHIKV had been identified in Dade County, Florida [24].

Outbreaks of febrile arthralgia in the Americas have been described since the eighteenth century, attributed to dandy (dengue) fever. However, re-examination of clinical descriptions of the outbreaks have raised questions about whether the outbreaks could have been misclassified CHIKV epidemics. One report of an epidemic from 1827 to 1828 on the islands of St. Thomas and Santa Cruz of "breakbone fever," referring to modern-day dengue fever, was remarked on its high attack rate, affecting "almost every individual in the town." It was associated with "pains in the

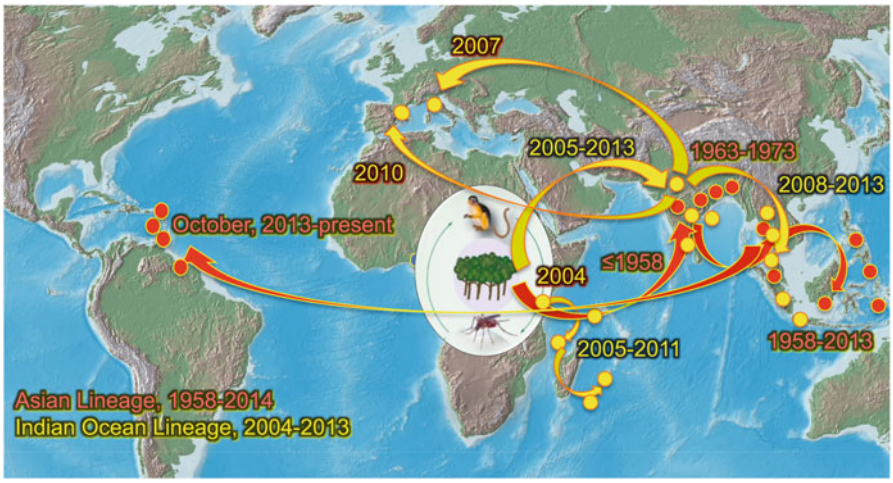

Fig. 3 Map showing the distribution of chikungunya virus enzootic strains in Africa and the emergence and spread of the Asian lineage (red arrows and dots) and the Indian Ocean lineage (yellow arrows and dots) from Africa. From "Arrival of Chikungunya Virus in the New World: Prospects for Spread and Impact on Public Health" by Weaver [19], licensed under CC BY 4.0

Table 1 Cases of CHIKV in the United States, 2015–2020

Year	United States		US territories (American Samoa, Puerto Rico, US Virgin Islands)	
Transmission	Travel	Local	Travel	Local
2014	2799	12 (Florida)	51	4659
2015	895	1 (Texas)	0	237
2016	248	0	1	180
2017	156	0	0	39
2018	116	0	0	8
2019	171	0	0	2
2020	11	0	NA	NA

Cases reported to the US Centers for Disease Control and Prevention ArboNET available at https://www.cdc.gov/chikungunya/geo/united-states.html
NA = not available

joints for weeks after recover" but had a low mortality [25], characteristics more reminiscent of chikungunya fever than dengue fever. Given our present-day knowledge of the overlapping non-specific aspects of chikungunya and dengue fever, along with the lack of knowledge of the etiologies of the two syndromes and diagnostic testing, the potential for misclassification of previous CHIKV epidemics is high and raises doubts whether the 2013 CHIKV outbreak truly was the first presentation of CHIKV transmission in North America [26].

Table 1 summarizes the number of cases of CHIKV infection in the United States between 2015 and 2020. The majority of cases were in travelers returning from areas experiencing the epidemic, but autochthonous transmission of CHIKV observed in Florida [24] and Texas highlights the potential for CHIKV outbreaks within the United States. CHIKV is not nationally notifiable in Canada, so limited data are available. However Canada did report nearly 500 confirmed or suspected cases related to travel in 2014 [27].

2.1.1 Virology and Ecology

CHIKV belongs to the genus *Alphaviridae* within the *Togaviridae* family. It is closely related to other arthritogenic alphaviruses from the Semliki Forest antigenic group, including O'nyong nyong, Ross River, and Mayaro viruses [28, 29]. The mature virion is spherical with a diameter of approximately 70 nm and consists of a host cell-derived lipid bilayer embedded with E1 and E2 envelope protein heterodimers surrounding a nucleocapsid formed by 240 copies of the capsid protein and the ~12 kb positive sense single-stranded RNA genome [30]. The genome has two open reading frames that encode four nonstructural proteins and one structural polyprotein that is cleaved into the capsid and envelope proteins.

Based on phylogenetic analyses, CHIKV strains isolated over the past two decades can be grouped into one of four lineages: West African, East/Central/South African (ECSA), Indian Ocean (IOL), and Asian. Strains from the Americas are most closely related to strains in the Asian lineage (Fig. 4) [31]. While CHIKV has been found in several mosquito genera, *Ae. aegypti* is most efficient in transmitting the virus to humans. However, a novel single amino acid substitution of alanine for valine at position 226 (A226V) in the E1 envelope protein increased infectivity of CHIKV in *Ae. albopictus* contributing to the 2006 La Reunion outbreak [32]. Since *Ae. albopictus* has a wider distribution than *Ae. aegypti*, its role in spreading CHIKV infection has caused great concern [33].

3 Clinical Manifestations

Disease caused by CHIKV infection can be divided chronologically into three phases: acute (up to 14 days), post-acute (15–90 days), and chronic (>3 months) [34]. Acute CHIKV infection is characterized by abrupt onset of high fever that can occur within 2 days of a bite by an infected mosquito. Onset of fever coincides with peak viremia, which can be up to 10^9 plaque-forming units equivalents per milliliter of blood [35]. Infected individuals also may experience fatigue and develop myalgias, nausea, vomiting, conjunctivitis, and a maculopapular rash. The characteristic hallmark of chikungunya fever, however, is severe, debilitating arthralgia, a manifestation less frequently observed in infected children [36, 37] but reported in up to 90% of infected adults. The arthralgia is frequently polyarticular and tends to affect

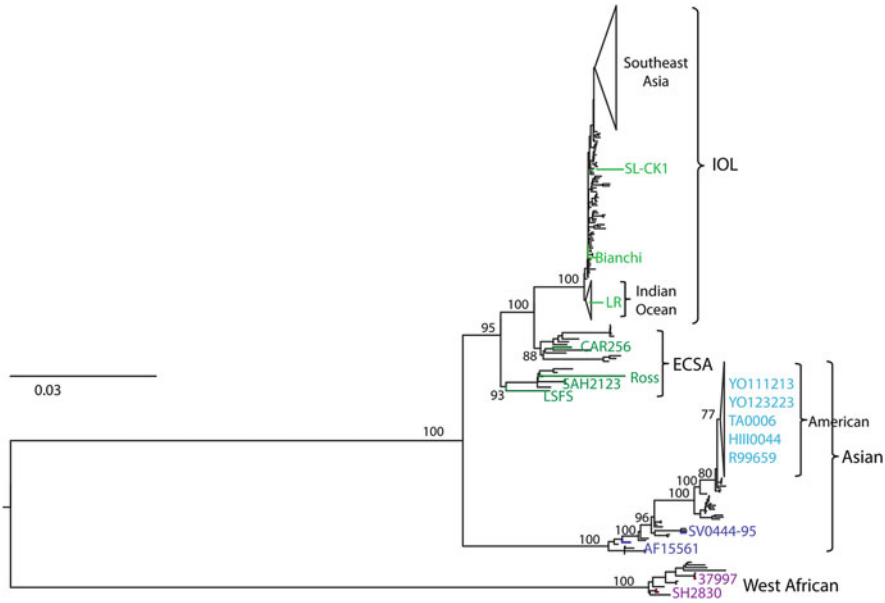

Fig. 4 Evolution of the major CHIKV lineages. Maximum likelihood tree based on chikungunya virus open reading frames with bootstrap values for the most prominent chikungunya virus clades. From "Chikungunya Virus Strains Show Lineage-Specific Variations in Virulence and Cross-Protective Ability in Murine and Nonhuman Primate Models" by [31], licensed under CC BY 4.0

joints symmetrically. The intensity of the pain often impairs activities of daily living. Patients have difficulty walking, picking up objects, and performing other activities of daily living [34].

Atypical manifestations and severe outcomes, including death, are more frequently observed in elderly individuals, particularly those with co-morbid illnesses including diabetes and hypertension [38, 39], and neonates [40]. Neonatal infections can present with severe dermatologic manifestations, including bullous lesions [41]. Neurologic manifestations include seizures and meningoencephalitis [42, 43], often associated with poor outcome [44]. Maternal to child transmission of CHIKV is most likely to occur during the intrapartum period and can lead to long-term neurologic deficits in the newborn [45]. Myocarditis [46], hepatitis [47], and fatal pneumonia [48] also have been reported.

Resolution of fever, rash, and nausea heralds the post-acute phase, characterized by persisting arthralgia, most frequently of the wrists and ankles with or without arthritis [34]. While some patients recover during the post-acute phase, many with symptoms persisting for 3 months or more enter the chronic phase of chikungunya disease. Some have reported symptoms persisting for years. Long-term rheumatic sequelae contribute to the largest disability-adjusted life year (DALY) burden for CHIKV [49]. Thus, the burden of disease can be devastating to local economies. The 2014–2015 CHIKV outbreak in the US Virgin Islands costed $14.8–$33.4 million

Table 2 WHO chikungunya case definitions

(i) Acute clinical case	1) Clinical criterion: fever >38.5 °C (101.3 °F) and joint pain[a] (usually incapacitating[b]) with acute onset AND 2) Epidemiological criterion: resident or visitor in areas with local transmission of chikungunya in the last 15 days ("suspect case" for epidemiological surveillance) OR 3) Laboratory criterion: confirmation by laboratory: PCR, serology, or viral culture ("confirmed case" for epidemiological surveillance)
(ii) Atypical case	Clinical case of laboratory-confirmed chikungunya accompanied by other manifestations: neurological, cardiovascular, dermatological, ophthalmological, hepatic, renal, respiratory, or hematological, among others
(iii) Severe acute case	Clinical case of laboratory-confirmed chikungunya presenting dysfunction of at least one organ or system that threatens life and requires hospitalization
(iv) Suspect and confirmed chronic cases	Suspect chronic case Person with previous clinical diagnosis of chikungunya after 12 weeks of the onset of the symptoms presenting with at least one of the following articular manifestations: pain, rigidity, or edema, continuously or recurrently
	Confirmed chronic case Every chronic case with a positive chikungunya laboratory test

[a]Usually accompanied by exanthema, myalgia, back pain, headache and, occasionally, vomiting, and diarrhea (pediatric age group)
[b]In children aged <3 years, joint pain is expressed as inconsolable crying, irritability, rejection to mobilization, and/or walking

when accounting for direct medical costs, lost wages due to absenteeism, and years lived with disability (YLD), which was estimated to range from 599 to 1322 years [50].

One non-arthritic complication of chikungunya fever is development of Guillain-Barre syndrome (GBS) [51, 52]. A case-control study of GBS during the 2014 CHIKV outbreak in the French West Indies calculated that CHIKV infection increased the odds of GBS by eightfold [53].

Due to the wide range of presentations of CHIKV infection, the WHO convened a panel of experts in 2015 to develop consensus case definitions (Table 2) [54].

3.1 Pathogenesis

CHIKV cellular tropism likely contributes to why arthritis and arthralgia are so prominent in chikungunya disease. CHIKV displays tropism for epithelial cells and fibroblasts, whereas lymphocytes, monocytes, and dendritic cells appear to be relatively resistant to CHIKV infection [55]. Staining of skeletal muscle, joint

capsule, and dermis biopsy specimens from patients with CHIKV infection showed CHIKV antigens mostly in fibroblasts [56]. MXRA8, an adhesion molecule on the surface of human fibroblasts, osteoblasts, chondrocytes, and skeletal muscle, was recently identified as an important cellular receptor for CHIKV as well as for other arthritogenic alphaviruses (Ross River, Mayaro, and O'nyong nyong). Blocking of chikungunya infection in a mouse foot arthritis model by antibody to the mouse homolog of MXRA8 reduced foot swelling in a CHIKV mouse model of arthritis [57]. Thus, CHIKV binding of MXRA8 appears to be an important event early in CHIKV infection.

Pathogenesis of chronic chikungunya arthritis remains poorly understood. Over 40% of people infected with CHIKV report symptoms of arthralgia and/or arthritis that persist for over 3 months, contributing to the large burden of chikungunya disease. Chronic chikungunya arthritis clinically mimics rheumatoid arthritis. Both are associated with similar biomarkers, including elevated serum inflammatory markers and cytokine profiles [58]. Both can demonstrate joint and bony erosion [59]. Thus autoimmune mechanisms may play a large role in pathogenesis of chronic chikungunya [58].

One important question is whether persistent chronic infection may contribute to the chronic symptoms. Chang et al. investigated joint fluid in patients with chronic chikungunya arthritis, but did not find evidence of CHIKV RNA by PCR [60]. Thus, the reasons underlying why and how some people develop chronic symptoms after CHIKV infection while others do not remain a mystery.

3.2 Diagnosis

In adults, CHIKV infection frequently causes disproportionately painful arthralgia in addition to the more nonspecific symptoms of fever and rash. Intense arthralgia is less prevalent in infected children, making clinical diagnosis of CHIKV more difficult, particularly since most CHIKV outbreaks have occurred in areas also endemic for dengue virus circulation.

Gold standard viral isolation of CHIKV from patient samples is impractical and unsafe. In addition to being labor-intensive and having poor sensitivity for detecting infection, CHIKV also is considered a BSL-3 pathogen, due to its potential for aerosol transmission in the laboratory. In the acute phase of the illness, detection of CHIKV RNA in viremic blood samples using reverse-transcriptase polymerase chain reaction (RT-PCR) is highly specific and can also be fairly sensitive. Viral copy numbers peak at the onset of fever, so testing of samples obtained later in the course of illness may reduce the sensitivity of RT-PCR. It is typically recommended that testing be performed on samples obtained within 5 days of fever onset [61].

Both IgM and IgG can be detected within days after onset of symptoms. IgM is expected to last for up to 4 months [62]. Serum antibodies to CHIKV can be detected using commercial ELISA kits. There is no FDA-approved kit; however FDA approval currently is not required for their clinical use. Thus, many commercial

labs are offering chikungunya IgM and IgG testing. Although they are not FDA approved, all testing of clinical samples must be validated according to CLIA standard before use. Acute infection can be defined by a positive IgM or a fourfold rise in IgG antibody to CHIKV between acute and 1-month convalescent visits [54].

3.3 Treatment

There is no licensed antiviral therapy. Treatment for acute CHIKV infection consists largely of symptomatic pain management and supportive care. Frequently, during the acute disease, intense arthralgia is not responsive to non-steroidal anti-inflammatory drugs. Management of persistent musculoskeletal pain after CHIKV has varied widely. Clinicians have treated patients with chronic chikungunya arthritis with agents used for chronic rheumatoid arthritis. The use of methotrexate has yielded some encouraging results [63], while the use of chloroquine has not [64]. Paradoxically, while chloroquine has been observed to inhibit CHIKV replication in vitro, it enhanced viremia when used as prophylaxis in a non-human primate model of CHIKV infection [65]. Guidelines for management of persistent musculoskeletal symptoms after CHIKV have been produced by the Brazilian Society of Rheumatology to aid clinicians in treatment of chronic chikungunya arthritis while potential therapies continue to be explored [34]. A human monoclonal against MXRA8 has been shown to neutralize CHIKV in vitro and in vivo in a mouse model of chikungunya arthritis [66].

For children with intrapartum or neonatal infection with CHIKV, intense neurodevelopmental screening and therapeutic intervention are suggested to optimize developmental outcomes [67, 68].

3.4 Prevention

There is currently no licensed vaccine for the prevention of CHIKV infection. However, efforts to produce a CHIKV vaccine have been ongoing since the 1960s [69, 70], and many candidates are in clinical trials [71].

Prevention of CHIKV infection is dependent on prevention of mosquito bites. Integrated vector management programs to target the vectors of CHIKV (*Ae. aegypti* and *albopictus)* coupled with public health education campaigns to increase personal protective behaviors are paramount to prevention of CHIKV [14].

The past decade has seen explosive viral epidemics, from severe acute respiratory syndrome to Ebola to arboviruses including Zika virus and CHIKV. For some diseases, the human toll is acutely evident in the form of mortality or acute morbidity. For CHIKV and others, the long-term sequelae from infection are yet ill-defined. The prolonged debilitating arthralgia associated with CHIKV infection has tremendous potential for impacting the global economy and should be considered when

evaluating the human burden of disease and the allocation of resources [49]. There is much still unknown about CHIKV and the illnesses that it causes. Developing a better understanding of the pathogenesis of CHIKV infection is a priority and forms the basis for developing effective strategies at infection prevention and disease control.

4 Dengue Virus (DENV)

4.1 Epidemiology

Like CHIKV, dengue virus (DENV) also is an arthritogenic single-stranded RNA virus transmitted by aedine vectors. It also can cause a nonspecific febrile illness with rash, headache, and joint pains. Unlike CHIKV infection, which nearly always manifests with symptoms, acute DENV infection can be asymptomatic or subclinical [72]. Symptomatic DENV infection, called dengue fever, typically self-resolves in 4–5 days. However, a number of patients progress to severe dengue, formerly referred to as dengue shock syndrome or dengue hemorrhagic fever, and develop capillary leak syndrome that can lead to hypovolemic shock. Mortality due to severe dengue is high [73].

DENV is transmitted by *Aedes aegypti* and *Ae. albopictus.* The virus likely arrived in North America after *Ae. aegypti* had been imported via the trans-Atlantic slave trade and has established endemicity [74]. The first description of an epidemic of febrile arthralgia in the United States compatible with DENV fever chronicled the 1780 outbreak of a "bilious remitting fever" in Philadelphia, which people also referred to as "breakbone fever." This term likely was translated from the Spanish *quebranta huesos*, which was documented by a physician, José Sabater, in 1771 in Puerto Rico as one of a series of indications for which he would recommend the use of rum [75]. The term "dengue" first appeared in a letter by Queen Luisa of Spain in 1801 in which she described experiencing a febrile illness with jaundice and bleeding "that they call dengue." These terms, along with "dandy fever" and "dinga" were used interchangeably to describe outbreaks of febrile arthralgia in the 1800s [76–78]. However, as many arthritogenic viruses cause illnesses with overlapping symptoms, it's uncertain whether the outbreaks were caused by modern-day DENV or if CHIKV or another arthritogenic virus could have played a role. DENV was not isolated until 1922 [79]. And its association with *Aedes* mosquitoes was not established until the 1940s [80].

After World War II, population growth, unplanned urbanization, and increasing travel facilitated geographic expansion of DENV endemicity leading to more frequent outbreaks and development of hyperendemicity, where multiple virus serotypes co-circulate in the community [81]. Now, DENV causes nearly 400 million infection per year worldwide. A quarter of those infections may be clinically symptomatic, while the remainder are subclinical [82]. Only a fraction of cases are

Table 3 DENV in the United States, 2014–2020, total cases (severe dengue cases)

Year	United States	Puerto Rico	US Virgin Islands
2014	668 (0)	8664 (2)	19 (0)
2015	945 (0)	1876 (0)	3 (0)
2016	990 (0)	204 (0)	11 (0)
2017	453 (0)	10 (0)	1 (0)
2018	331 (0)	2 (0)	0 (0)
2019	1158 (21)	30 (1)	0 (0)
2020	186 (0)	240 (10)	0 (0)

Cases reported to the Pan American Health Organization available at https://www.paho.org/data/index.php/en/mnu-topics/indicadores-dengue-en.html

identified and reported, in part due to lack of robust and affordable diagnostic testing. Thus, the true burden of DENV is grossly underestimated.

In North America, most reported dengue cases are in travelers returning from areas endemic for DENV. However, global warming has allowed both *Ae. aegypti* and *Ae. albopictus* to spread into many areas of the United States (Fig. 2). The ease and rapidity of modern-day travel facilitates importation of DENV by infected humans into areas now endemic with *Ae. aegypti* and *Ae. albopictus*. Between 2010 and 2017, 5387 DENV cases were reported to ArboNET, of which 5009 (93%) were travel related. However, 378 cases were locally acquired: 250 in Hawaii, 103 in Florida, 24 in Texas, and 1 in New York [83] (Table 3).

4.1.1 Virology and Ecology

DENV is a member of the *Flavivirus* genus within the *Flaviviridae* family of viruses, which includes the genus' eponymous yellow fever virus, as well as Japanese encephalitis virus, West Nile virus, and tick-borne encephalitis virus [84]. Flaviviruses are single-stranded positive-sense RNA viruses. Their ~10.8 kb genomes encode single polyproteins, which are processed post-translation into the capsid, pre-membrane/membrane, envelope structural proteins, and seven nonstructural proteins that are important for viral replication (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The capsid protein complexes with the RNA genome to form the virus core. The virus envelope is composed of host cell lipid bilayer, with 90 antiparallel homodimers of the envelope protein anchored to the membrane protein [85, 86].

Phylogenetic analyses demonstrate four distinct viral "serotypes," designated DENV-1 through DENV-4. Most analyses suggest that DENV-4 diverged first, then DENV-2, followed by the split between DENV-1 and DENV-3 [87]. A fifth serotype has been described but is believed to be mainly a sylvatic strain [88]. Viruses within the same serotype share 97% amino acid identity, whereas viruses from different serotypes share 60–75% identity [89].

Humans are the major hosts for DENV; however a sylvatic cycle involving non-human primates does persist [87]. Based on clustering of sylvatic and human

strains in the phylogenetic trees of DENV-2 and DENV-4, human DENV infection is believed to have emerged from sylvatic strains that still continue to circulate among nonhuman primates in the forests of Southeast Asia and West Africa [90].

4.2 Clinical Manifestations

Most DENV infections result in minimal symptoms. Patients frequently do not seek care or may not even realize that they have an infection. About a quarter of patients develop dengue fever. Onset of fever can occur between 4 and 10 days after a bite from an infected mosquito. Dengue fever should be suspected if there is fever with temperature $\geq$ 40 °C/104 °F plus any two of the follow symptoms: severe headache, retro-orbital pain, myalgias and/or arthralgias, nausea, vomiting, adenopathy, or rash. The fever usually starts to resolve after 3–7 days; however this constitutes the beginning of critical phase of observation, where warning signs can help identify patients at higher risk for developing severe dengue. Warning signs, such as severe abdominal pain, protracted vomiting, tachypnea, bleeding gums or hemoptysis, and fatigue or restlessness, should prompt close observation over the following 24–48 h to detect signs of plasma leakage, fluid accumulation, respiratory distress, severe bleeding, or organ impairment [73] (Fig. 5).

Maternal to child transmission of DENV has been reported [91–94]. A systematic review linked DENV in pregnancy with poor birth outcomes including miscarriage, still birth, preterm birth, and low birth rate [95]. Neonates born to DENV-infected mothers can present with rash, thrombocytopenia, and transaminitis [96–99].

Neurologic manifestations are rare. Guillain-Barre syndrome has been reported to present in the acute febrile setting [100].

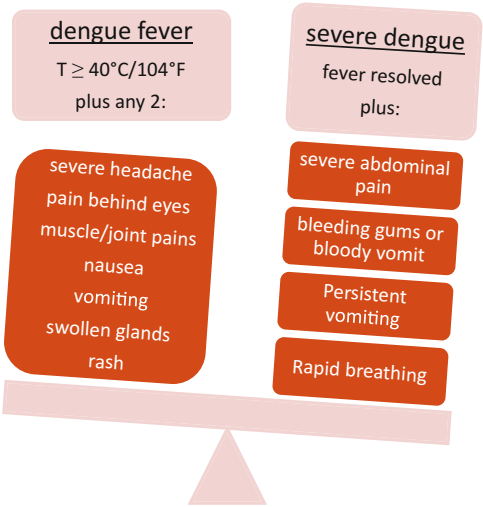

Fig. 5 Signs of dengue fever versus warning signs for severe dengue

4.2.1 Pathogenesis

Progression to severe dengue disease represents the greatest risk for patients, as it is associated with high mortality. Mortality in severe dengue is a result of hypovolemic shock. Shock results from intravascular volume depletion. The principal mechanism for sudden intravascular volume depletion is oncotic leak into the extravascular space, followed by volume shift. Thus, the key process during the pathogenesis of severe dengue is development of vascular leak, which can be referred to as dengue vascular permeability syndrome [101].

Beatty et al. demonstrated that recombinant dengue nonstructural protein 1 (NS1) increased permeability of human pulmonary microvascular endothelial cell monolayers, whereas NS1 from a different flavivirus, West Nile virus, did not. Using a mouse model of dengue infection (interferon-α/β receptor deficient C57BL/6 mice), the authors demonstrated that mice injected with NS1 leaked intravenously administered Evans blue dye into lung, liver, and small intestinal tissue to a greater extent than a control protein. Thus, NS1 alone could compromise the integrity of the endothelial cell layer in both in vitro and in vivo models. Higher levels of secreted NS1 associated with higher viremia may raise the risk of endothelial dysfunction in severe dengue [102].

Higher levels of viremia are observed in patients who had been previously infected with DENV of a different serotype (heterotypic infection). This is due, in part, to antibody dependent enhancement (ADE) of infection. Antibodies that develop after infection with one serotype can cross-react with DENV of a different serotype. This cross-reactivity provides some temporary protection against heterotypic DENV infection but wanes over time. Upon infection with a heterotypic DENV strain, these cross-reactive antibodies bind but do not neutralize the heterotypic DENV. Instead, the antibody-bound virus infects the host cell more efficiently due to binding of the antibody Fc portion with the host cell ligand, DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin, CD209). Infants aged 6 months or less are also at greater risk of severe dengue because transplacentally transferred maternal antibody in the infant mimics previous dengue infection and can lead to ADE when the infant has its first DENV infection [101].

Patients with severe dengue have higher levels of circulating cytokines, termed cytokine storm. Cytokines such as tumor necrosis factor-alpha (TNF-α) can play an important role in mediating endothelial dysfunction. Endothelial cell apoptosis has been associated with elevated tissue TNF-α. Additionally, direct infection of endothelial cells by the virus may contribute to vascular compromise [103]. Platelet dysfunction and high levels of platelet-activating factor observed in patients with severe dengue also can contribute toward endothelial dysfunction [104].

Overall, these particular findings are but a fraction of the vast efforts made by a multitude of investigators to understand the pathophysiologic processes that result in the complicated manifestations of DENV infection [105].

4.3 Diagnosis

As with chikungunya, RT-PCR detection of viral RNA is the most sensitive and specific method of detection within the first 3–4 days of fever. Once fever resolves, DENV viremia decreases, along with the sensitivity of the PCR assay [106]. Development of loop-mediated isothermal amplification (LAMP) assays holds the promise of greater accessibility of nucleic acid detection in resource restricted areas [107].

IgM is detectable days after primary DENV infection and is the target of many point-of-care rapid diagnostic tests (RDTs). IgG is detectable by 14 days after fever onset [108]. Many RDTs detect both IgM and IgG. However reports of sensitivity of individual RDTs are variable [109, 110]. The ability to detect existing IgG at acute presentation is important because a positive IgG indicates a non-primary DENV infection, heightening the risk for severe dengue due to ADE [111].

The latest addition to the DENV RDT is detection of NS1 antigen [109]. NS1 is secreted so it can be detected in serum/plasma of acutely infected individuals [112]. The levels of NS1 in serum roughly follow the pattern of viremia [113] and have similar specificity to PCR assays [114]. However, NS1 RDT detection via lateral flow immunoassay offers advantages over PCR as it is more portable and less costly [115].

Identification of second episode non-primary DENV infections early in the course of infection assists the clinician to make decisions regarding close monitoring for development of severe DENV due the elevated risk of progression to severe disease in these patients. Thus, accurate diagnosis of DENV early during infection has important utility that can alter clinical decision-making [73].

4.4 Treatment

Management of DENV infection is largely supportive, focusing mainly on fluid management. Due to concern for the potential of NSAIDS to inhibit platelet aggregation, the use of NSAIDS to control fever or pain symptoms is typically discouraged. Fluid management during severe dengue becomes tricky. Initially, the patient suffers from hypovolemic shock due to intravascular depletion associated with capillary leak, so they require fluid support [73]. Reversal of the capillary leak then places the potentially over-hydrated patient at risk for fluid overload [116]. Thus, careful monitoring of fluid status in patients with severe dengue is essential to reduce risk of mortality.

4.5 Prevention

A vaccine to prevent dengue (Dengvaxia®) is licensed and available in some countries for people ages 9–45 years old. People without prior dengue virus

exposure who receive the vaccine may be at risk of developing severe dengue if they get dengue after being vaccinated [117]; therefore, the World Health Organization recommends that the vaccine only be given to persons with confirmed prior dengue virus infection (WHO). In May 2019, Dengvaxia® was approved by the US Food and Drug Administration (FDA) in the United States for use in children 9–16 years old living in dengue endemic areas (the US territories of American Samoa, Puerto Rico, and the US Virgin Islands), with laboratory confirmed prior dengue virus infection. Other dengue vaccines are in various stages of development [118].

Like CHIKV, prevention of DENV infection is also dependent on prevention of mosquito bites. Integrated vector management programs to target the shared vector (*Ae. aegypti*) coupled with public health education campaigns to increase personal protective behaviors are paramount for prevention of DENV. *Aedes* mosquitoes bite during the daytime as well as at twilight, and they breed preferentially in standing water (particularly manmade containers); therefore, larval source reduction is critical to reduce vector abundance. Domestic water tanks should be covered so that mosquitoes cannot enter and containers or drains that allow standing water should be eliminated.

5 Zika Virus (ZIKV)

5.1 Epidemiology

Like DENV, ZIKV also is a member of the *Flaviviridae* family of viruses. It too can cause a relatively mild acute febrile illness, which clinically may be difficult to distinguish from other clinical syndromes caused by DENV, CHIKV, or other arthritogenic viruses. However, recent epidemics of ZIKV have been associated with severe microcephaly.

The first reported isolation of ZIKV was in April 1947, from the blood of a febrile sentinel rhesus monkey (Rhesus 766) that was being monitored as part of a Rockefeller Foundation yellow fever virus sylvatic transmission study in the Zika Forest of Uganda. Using a mouse brain challenge model, the authors found that serum from Rhesus 766, obtained 31 days after fever, could neutralize the unknown virus but not DENV or yellow fever virus. Similarly, DENV and yellow fever hyper-immune sera could not neutralize the novel virus, which authors named after the Zika Forest. Collection of mosquitoes led to isolation of a virus from an *Aedes africanus* mosquito several months later that demonstrated similar serologic reactivity to the isolate from Rhesus 766 [119].

The first report of human ZIKV infection described three patients with febrile illness during an outbreak of jaundice in Nigeria, 1952. A virus was isolated from blood of one of the patients, a 10-year-old girl with fever and headache, that was susceptible to neutralization by serum from a monkey that had been immunized with ZIKV but was resistant to neutralization by monkey antisera to variety of other viruses, including members of the same *Flaviviridae* family as ZIKV: yellow fever

virus, West Nile virus, Ntaya virus, and Uganda S virus [121]. However subsequent studies of this virus isolate identified it as Spondweni virus, a flavivirus closely related to ZIKV, that had not yet been identified at that time [122–124]. Due to the cross-reactivity of the ZIKV hyper-immune serum with the 1952 isolate, and the cross-reactivity of the study subjects' serum samples for the reference ZIKV strain, the first documented human Spondweni virus infection was misidentified as ZIKV [125]. The discovery of DNA and RNA in the 1960s, and the subsequent development of methodology to sequence viruses in the 1970s, brought a new tool that raised the specificity of viral identification. Invention of PCR in the 1980s made molecular diagnostics broadly accessible to scientists. In the past two decades, development of technologies to quickly sequence whole viral genomes has brought resolution to our ability to distinguish between individual viral isolates, facilitating studies of viral evolution that provide important inferences on disease epidemiology.

Reports of human ZIKV infections were relatively sparse before the 2007 outbreak in the island state of Yap, Federates States of Micronesia [126]. Prior to the outbreak, reports of cases and serologic surveys offered evidence of ZIKV circulation in sub-Saharan Africa [124, 127] and parts of Asia (Fig. 6). Isolation of virus from mosquitoes and primates confirmed circulation of the virus.

In 2007, an epidemic of rash, conjunctivitis, and arthralgia, suspected to be due to DENV based on positive DENV rapid diagnostic tests, broke out on the Yap Islands. None of the samples sent to the CDC for confirmation were positive for DENV by RT-PCR [126] but rather yielded viral sequences with 90% homology to ZIKV [128]. The outbreak was explosive, with most cases presenting between May and July of 2007 [126]. Approximately 75% of the island was infected.

In 2013, a larger outbreak occurred in French Polynesia affecting two-thirds of the population, resulting in approximately 32,000 infections [129]. By February 2014, ZIKV was detected on Easter Island, Chile [130]. Its subsequent spread to Brazil in May 2015 [131] resulted a massive epidemic that spread up through Central and parts of North America.

Prior to the emergence of ZIKV in the western hemisphere in 2014, very few travel-associated cases of Zika virus disease were identified in the United States. The large outbreaks of Zika virus in the Americas in 2015 and 2016 led to an increase in travel-associated cases in US states, widespread transmission in Puerto Rico and the US Virgin Islands, and limited local transmission. As with DENV and CHIKV, autochthonous ZIKV infections in the United States have occurred in Florida and Texas (Table 4).

5.1.1 Virology and Ecology

ZIKV shares the genomic and viral structures characteristic of other flaviviruses. It is a positive-sense single-stranded RNA virus with a genome of ~10.8 kb encoding a polyprotein consisting of three structural and seven non-structural proteins [132]. ZIKV is closely related to Spondweni virus (Fig. 7) [133], which was misidentified as ZIKV in MacNamara's report of the first human case of ZIKV

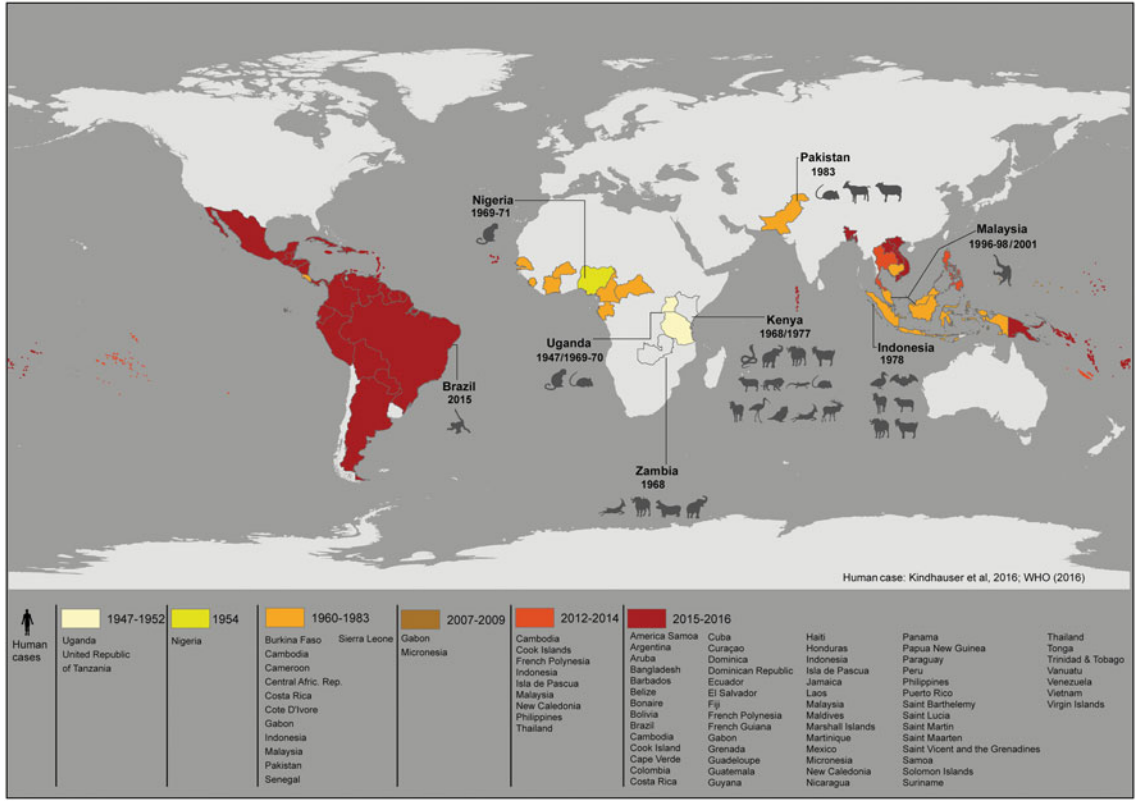

Fig. 6 Historical timeline of ZIKV spread in humans and animals in the world. Colored countries have reported autochthonous vector-borne human cases, and those labeled with specific years and animal silhouettes have reported diagnosed cases of ZIKV in naturally infected animals. From Bueno et al. [120], licensed under CC BY 4.0

Table 4 Cases of ZIKV infection in the United States, US territories, and Canada

Year	United States	US territories	Canada
2015	62	10	19
2016	5168	36,512	468
2017	452	666	74
2018	74	148	14
2019	22	71	NA
2020	1	13	NA

Cases reported to the United States Centers for Disease Control and Prevention ArboNET available at https://www.cdc.gov/zika/reporting/index.html
Provisional data as of August 6, 2020
Cases reported by the Canada National Microbiology Laboratory https://www.canada.ca/en/public-health/services/diseases/zika-virus/health-professionals.html#_Zika_cases_in

infection in 1952 [121, 125]. Alignment of complete genomes of ZIKV and other flaviviruses demonstrated 58–60% nucleotide identity and 55–58% amino acid identity to Japanese encephalitis virus, West Nile virus, DENV, and St. Louis encephalitis virus [132].

ZIKV is classified into two lineages: African and Asian. The phylogenetic analyses of envelope gene sequences of ZIKV isolates during the epidemic indicate that the strains that spread through the Americas originated from the Asian lineage [132]. Comparing the canonical MR766 strain from Uganda to a strain from the French Polynesia outbreak in 2013 revealed only 50 lineage-specific differences in amino acid sequences [134–136]. The differences are mainly in the NS1 or NS5 proteins [134, 135]. In vitro, African lineage strains appear to cause higher viral replication and cause great cell death than the Asian ZIKV strains, leading to a hypothesis that perhaps the lower viremia and cell death caused by the Asian strains might facilitate establishment of more chronic infection [134]. Overall, the search for potential virulence factors that might explain the rapid spread of ZIKV during the epidemic remains ongoing.

5.2 Clinical Manifestations

The first as yet uncontested report of human ZIKV infection occurred in a 28-year-old European male conducting entomological studies in the Zika Forest of Uganda in 1962–1963. The clinical syndrome described was that of a mild febrile illness accompanied by a generalized maculopapular rash [124]. Subsequent intermittent cases of ZIKV in Africa were not well described, and it was not until the 2007 ZIKV outbreak on the Yap Islands, that reports of detailed symptomatology was available. During the 2007 outbreak, confirmed cases of ZIKV infection most frequently reported macular or papular rash often with pruritis (90%), followed by fever (65%), arthritis or arthralgia (65%), conjunctivitis (55%), myalgia (48%), and

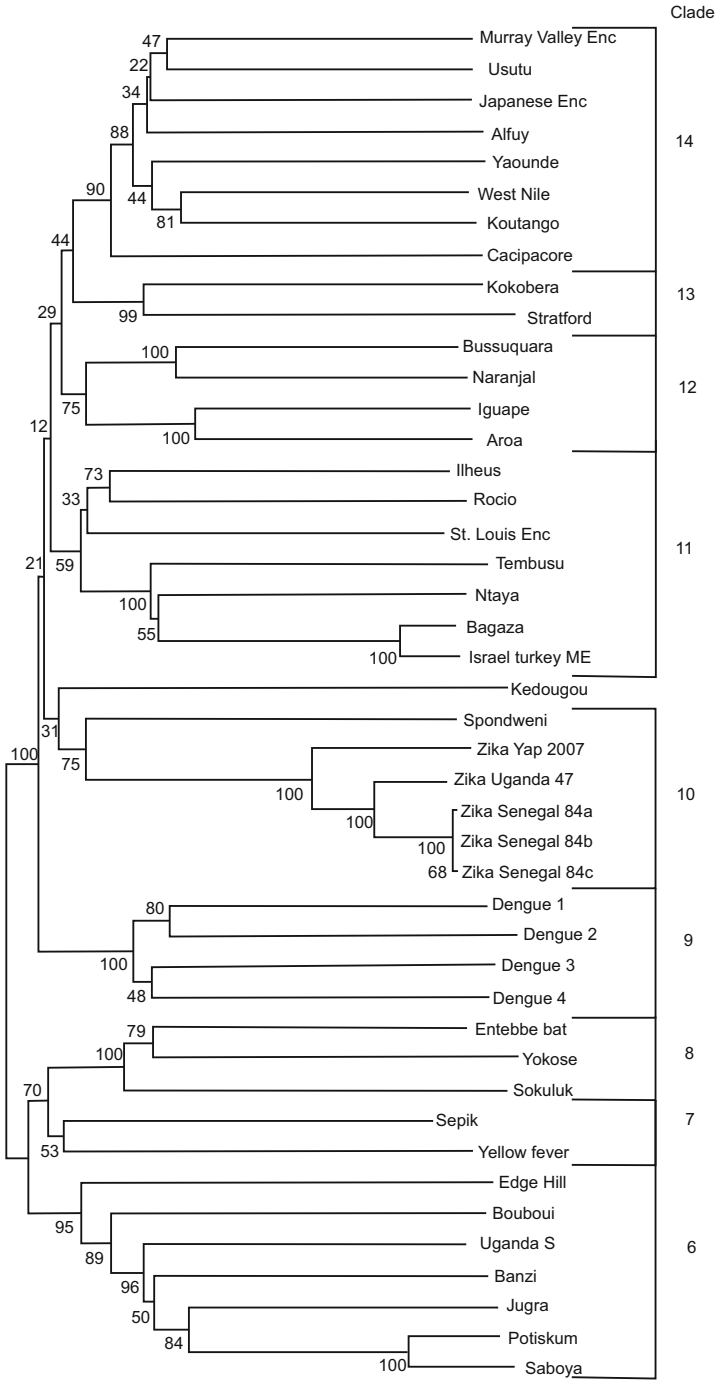

Fig. 7 Phylogenetic relationship of Zika virus to other flaviviruses based on nucleic acid sequence of nonstructural viral protein 5. Enc, encephalitis; ME, meningoencephalitis. From Hayes [133], licensed under CC BY 4.0

headache (45%). Retro-orbital pain, a classic symptom of DENV infection, was reported in 39% [126]. Less commonly observed symptoms and signs include abdominal pain, nausea, diarrhea, and mucous membrane ulcerations. Thrombocytopenia has also been observed. Other manifestations including facial puffiness, palatal petechiae, uveitis, transient hearing impairment, myocarditis, and pericarditis have been described in case reports [137–147].

Like DENV, most ZIKV infections are subclinical or asymptomatic [148]. Symptomatic disease is typically self-limited, starting with abrupt onset of fever and rash, with arthralgia, myalgia, conjunctivitis, and headaches lasting between 4 and 7 days. Most cases fully recovered without apparent sequalae until the first case of Guillain-Barre syndrome (GBS) was reported during the 2013/2014 French Polynesia outbreak [149]. Subsequent observations from the ensuing epidemics in South America (Fig. 8) [150] led to an alarming association of GBS with ZIKV infection. Additional neurologic manifestations, including meningoencephalitis and myelitis, were also reported [151]. Risk for GBS appears to increase with age [152].

Clinical presentation of ZIKV infection in pregnant women did not appear to be different than in non-pregnant adults [153]. However, neonatal complications were observed with alarming frequency (Fig. 9). Although perinatal transmission had previously been reported [155], an dramatic increase in number of reported cases of microcephaly in Brazil coinciding with the eruption of the ZIKV epidemic raised concern for the effect of congenital infection on fetal brain development. Neurologic manifestations reported in neonates born to women infected with ZIKV during pregnancy included microcephaly, craniofacial disproportions, joint contractures, pyramidal/extrapyramidal symptoms, and epilepsy [156, 157]. Initial epidemiologic associations between ZIKV infection during pregnancy and neonatal microcephaly were confounded by inconsistent case definitions and variable surveillance infrastructure. However subsequent systemic reviews and meta-analyses have confirmed the heightened risk of congenital neurologic disorders associated with ZIKV infection during pregnancy, particularly during the first trimester [158–161]. Currently, congenital Zika syndrome (CZS) is characterized by severe microcephaly in which the skull has partially collapsed; decreased brain tissue with a specific pattern of brain damage, including subcortical calcifications; damage to the back of the eye, including macular scarring and focal retinal pigmentary mottling; congenital contractures, such as clubfoot or arthrogryposis; and hypertonia restricting body movement soon after birth [156].

In contrast to DENV and CHIKV, ZIKV is readily transferred sexually [162], and the duration of viral persistence in semen and in female genital tract secretions may be prolonged [163, 164]. The first documented sexual transmissions of ZIKV occurred when an arbovirologist who was infected by mosquito in Senegal in 2008 infected his wife upon returning home to Colorado before his symptoms began [165]. Sexual transmission of ZIKV has been documented to occur as late as 41 days after a partner's onset of symptoms has been described [166], and infectious ZIKV in semen has been detected in culture as late as 69 days after onset of illness [167].

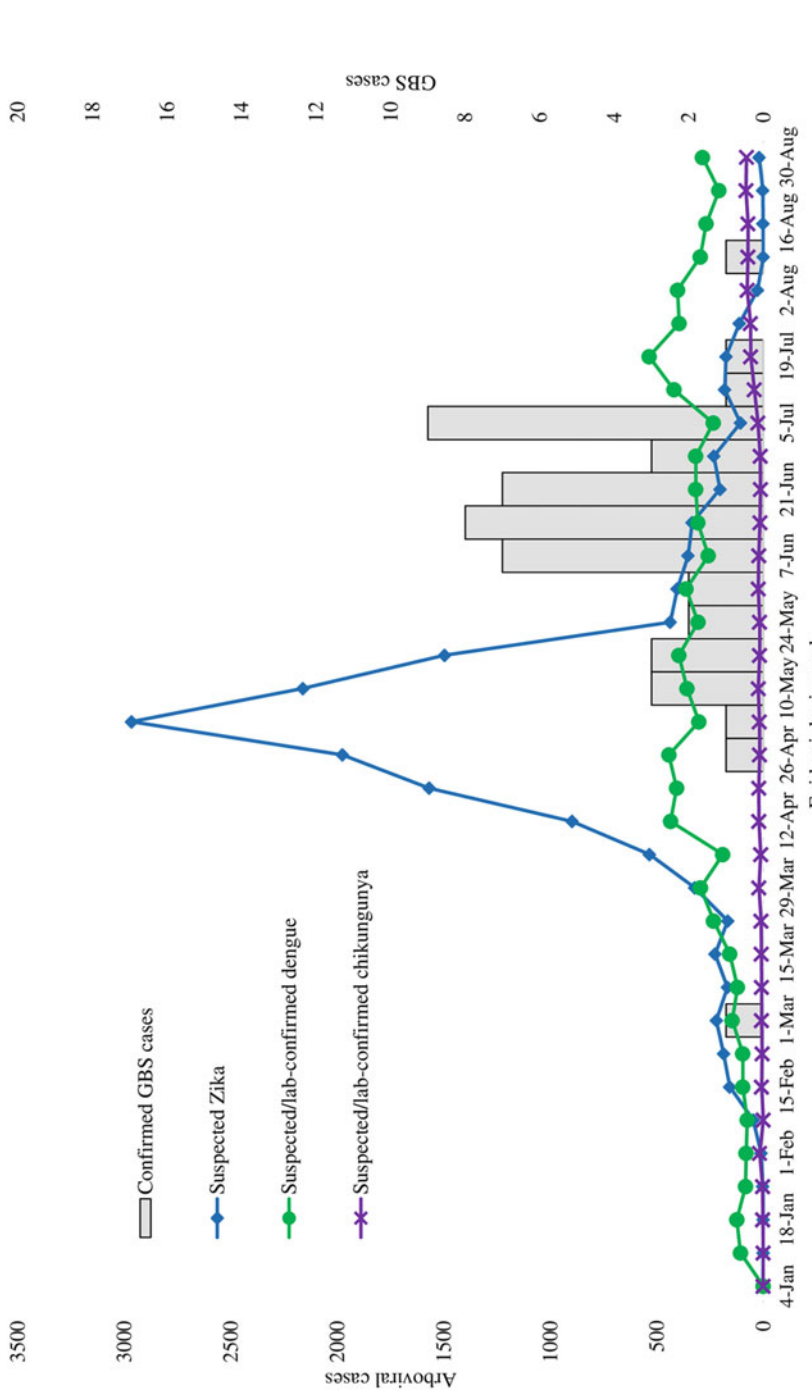

Fig. 8 Confirmed GBS and reported Zika, dengue, and chikungunya cases, Salvador metropolitan area, Brazil (2015). Epidemiologic curve of incident Guillain-Barré syndrome (GBS) cases was juxtaposed with reported symptomatic ZIKV, dengue, and chikungunya infections in the Salvador metropolitan area, Brazil, during January 1–August 31, 2015. From Styczynski et al. [150], licensed under CC0 public domain dedication

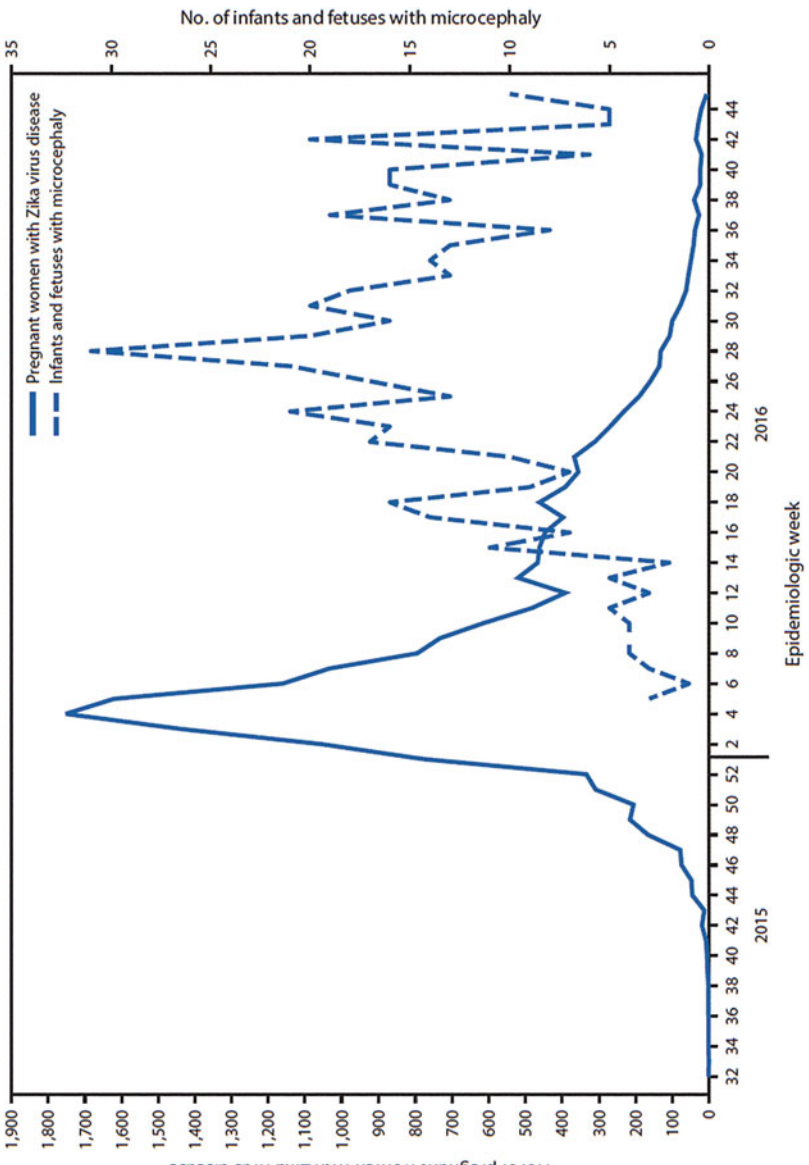

Fig. 9 Date of symptom onset of reported cases of Zika virus disease among pregnant women and date of birth of infants or of pregnancy loss for fetuses with reported microcephaly – Colombia, August 9, 2015 (epidemiologic week 32)–November 12, 2016 (week 45). From Cuevas [154], licensed under CC BY 4.0

5.2.1 Pathogenesis

Substantial efforts continue to be made to understand the pathogenesis of ZIKV infection, particularly the mechanisms that lead to the severe manifestations of CZS. As with other flaviviruses, the principal mediator of cell entry is the envelope protein. Also like other flaviviruses, incomplete proteolytic maturation of other viral structures, including the pre-membrane protein, can result in the production of heterogeneous immature particles that affect susceptibility of cells to infection [86]. Human cells susceptible to ZIKV infection include skin [168] and uterine fibroblasts [169], placental trophoblasts [170] and macrophages (Hofbauer cells) [171], endometrial stromal cells [172], Sertoli cells [173], and cortical neural progenitor cells [174]. Although human Sertoli cells are permissive to ZIKV infection and exhibit dysregulation, they did not display cytopathic effect [175] and continued to support high levels of ZIKV replication for at least 6 weeks [176]. This may help explain the persistence of ZIKV in human sperm [177].

A causal link between ZIKV and brain malformations has been established [157], because of the pathological impact of ZIKV on neural progenitor cells [174], immature neurons [178], and the neurovasculature [179] of the developing brain. A link between ZIKV-associated progenitor cell dysfunction and microcephaly is established in mice [180, 181]. ZIKV infects human pluripotent stem cell-derived neural progenitor cells in vitro, which induces apoptotic cell death [174].

5.3 Diagnosis

Diagnosis of Zika fever by clinical symptomatology is unreliable, since many areas that experienced the outbreak also were endemic for dengue fever, which can present with similar symptoms. Viral isolation led to discovery of ZIKV as a novel virus in 1947 by inoculating serum from a febrile rhesus monkey into mouse brains. Today cell culture is rarely used as a means of diagnosis in human infections. Instead the diagnosis of Zika virus infection is established using PCR or serology. As with other arboviruses, for those presenting early in their disease (within 7 days), PCR is recommended in order to establish a definitive diagnosis (CDC). Serology can cross-react with other related flaviviruses and is therefore less specific [182]; however Zika virus IgM with confirmatory PRNT is indicated in those presenting later in illness (after 7 days) (CDC).

5.4 Treatment

Like many arboviral infections, specific antiviral treatments are not available. The majority of Zika fever cases are self-limited. Aspirin and other nonsteroidal anti-inflammatory drugs should be avoided until dengue infection has been ruled out, to reduce the risk of hemorrhage.

Optimal neurodevelopmental monitoring for infants born with CZS is unknown. Interim guidance from the CDC suggests establishing a medical home to coordinate care [183]. The World Health Organization has issued guidance on psychosocial support for patients and families affected by congenital Zika virus infection (World Health Organization).

5.5 Prevention

As with the other arboviruses, prevention depends on understanding the many factors that contribute to emergence of the disease (Fig. 10). Interventions are possible at levels ranging from the individual to the community to national action. Aggressive vector management is critical in preventing establishment of local transmission. Education is important to improve community awareness of household mosquito breeding sites. Personal protection includes encouragement of the use of clothing that covers the skin, as well as mosquito repellants. For ZIKV in particular, due to the risk of sexual transmission, education about sexual transmission of the virus and providing and encouraging the use of condoms or other barrier methods of protections is important. No vaccine is currently licensed for ZIKV; however, many vaccines are in development [185].

6 Other Emerging Mosquito-Borne Viruses

6.1 Introduction

The rising tally of mosquito-borne viruses of epizootic importance in North America that have been reported to cause disease in humans highlights the emerging threat of vector-borne infections. In addition to chikungunya, dengue, Zika, and West Nile viruses (discussed separately), the CDC's National Notifiable Diseases Surveillance System tracks the following mosquito-borne viruses: California serogroup virus (La Crosse encephalitis and Jamestown Canyon viruses), eastern equine encephalitis virus, St. Louis encephalitis virus, western equine encephalitis virus, and yellow fever virus. Autochthonous transmission of Western equine encephalitis virus and yellow fever virus has not been observed in the United States for many years. The remaining viruses are emerging mosquito-borne viruses with neuroinvasive potential currently circulating in North America (Fig. 11): La Crosse encephalitis virus (LACV), Jamestown Canyon encephalitis virus (JCV), eastern equine encephalitis virus (EEEV), and St. Louis encephalitis virus (SLEV) [186]. Cache Valley virus is briefly mentioned. Usutu virus, not yet known to be circulating in North America, has the potential to emerge as an important pathogen based on presence of known vectors and climatic permissiveness.

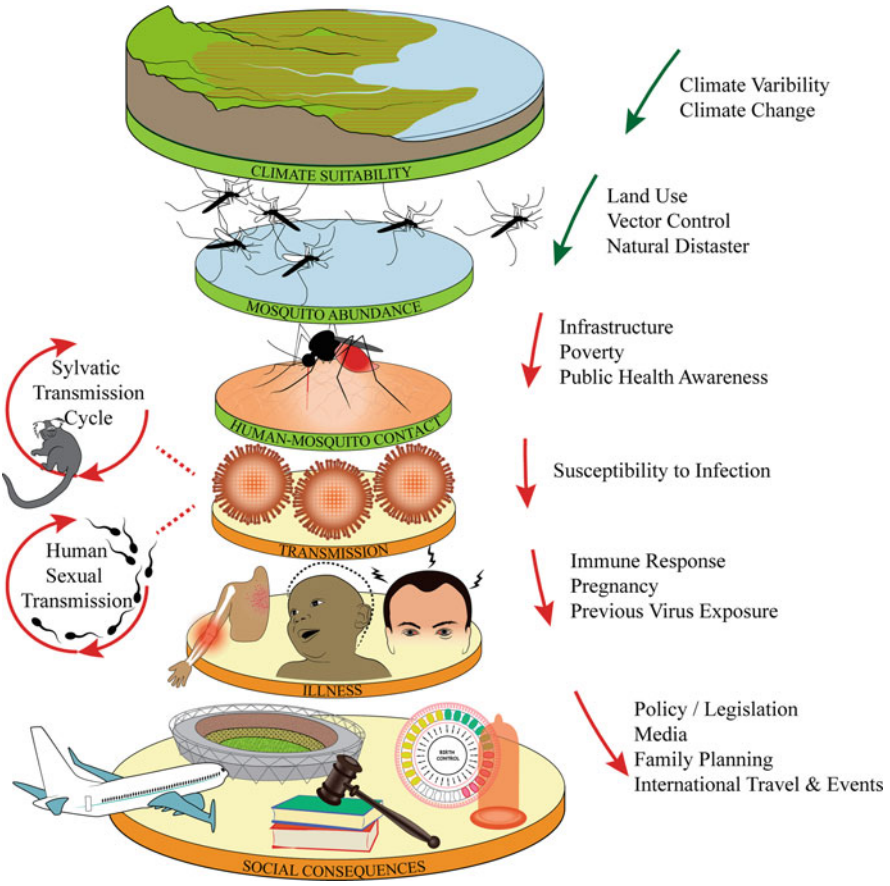

Fig. 10 "Hierarchy of factors that influence ZIKV transmission, illness, and social consequences" by Ali et al. [184] is licensed under CC BY 4.0

6.2 *La Crosse Encephalitis Virus*

La Crosse encephalitis virus (LACV) belongs to the California serogroup of mosquito-borne orthobunyaviruses, which includes Jamestown Canyon virus. It is transmitted primarily by *Aedes triseriatus*; however other *Aedes* species may also serve as vectors (*Ae. canadensis* and *Ae. albopictus*). LACV infects a large variety of small mammals (squirrels, chipmunks, hares, rodents) [187] but also exhibits transovarial transmission in its mosquito vector [188].

LACV is the leading cause of pediatric arboviral encephalitis. Although most cases occur in children, adult cases have been reported that have been associated with greater morbidity such as need for long-term rehabilitation [189]. Most cases of pediatric encephalitis resolve with supportive care; however at least two fatal cases have been reported [190]. No antiviral treatment is available.

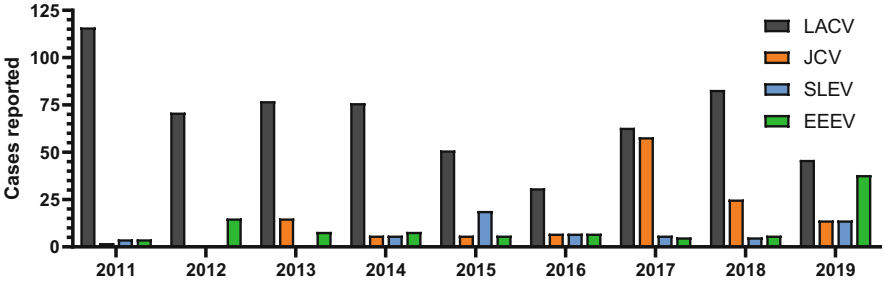

Fig. 11 Yearly cases in the United States reported by CDC. La Crosse encephalitis virus, LACV; Jamestown Canyon encephalitis virus, JCV; St. Louis encephalitis virus, SLEV; eastern equine encephalitis virus, EEEV

Human infections occur as spillover events. Cases tend to occur during the summer. While historically, most cases of LACV have been reported in the upper Midwestern United States, changes in land use and climate may be contributing to increasing numbers of infections in the Appalachian region [191–195].

6.3 *Jamestown Canyon*

Jamestown Canyon virus (JCV) is an orthobunyavirus closely related to La Crosse encephalitis virus within the California serogroup. It was first identified in Jamestown Canyon, Colorado, in 1961. Its mammalian host is primarily the white-tailed deer; however it has been found in other large mammals. Its principal vector is believed to be *Ochlerotatus canadensis* [196], but it has been found in many *Aedes* species as well [197].

Human infection can be asymptomatic or can manifest as disease ranging from mild febrile illness to meningitis and encephalitis. Diagnosis is by detection of IgM; however antibodies against California serogroup viruses tend to cross-react with other viruses within the group, so frequently additional confirmation is required [198]. PCR has been developed but has not yet been tested on a large number of clinical samples [196]. There are no antiviral treatments. Care is supportive.

6.4 *St. Louis Encephalitis Virus*

St. Louis encephalitis virus (SLEV) is a flavivirus transmitted in an enzootic cycle between birds and mosquitoes, primarily *Culex* species. It was first isolated from brain specimens of patients who died from an outbreak of 548 cases of "encephalitis lethargica" in St. Louis, Missouri, in 1933 [199]. It has wide geographic range and has caused infections a far north as southern Canada and as far south as Argentina [200]. The overall incidence of SLEV had been declining since the introduction of

West Nile virus into the United States in 1999. Both are flaviviruses that infect birds and are transmitted primarily by *Culex* species mosquitoes. However, after having disappeared from the Southwestern United States for about a decade, in 2015, an outbreak of SLEV in Maricopa County, Arizona, coincided with an outbreak of West Nile virus [201]. Since then, several cases have occurred in Southern California. Phylogenetic analyses indicate that recent outbreak strains are related to the strains that caused the outbreak in Argentina in 2005 [202].

6.5 *Eastern Equine Encephalitis Virus*

Eastern equine encephalitis virus (EEEV) is a highly virulent mosquito-borne virus that belongs to the alphavirus family of *Togaviridae* [203]. EEEV causes an encephalitis that is associated with high mortality, in both humans and horses. It had been suspected as the cause of an outbreak that killed 75 horses in 1831 and was first isolated from the brain of a horse in 1933 [204]. It was recognized to infect humans during an outbreak of encephalitis in Massachusetts between August and October 1938 that affected 38 children, killing 25. During the same time period, 240 cases of horse encephalitis were reported to the Massachusetts Division of Livestock Disease Control. EEEV was isolated from eight of the children post-mortem.

EEEV is maintained in an enzootic cycle between birds and ornithophilic mosquitoes, primarily *Culiseta melanura*. Infection of humans, horses, and other mammals occur as spillover events, as infection in mammals does not cause viremia levels sufficiently high to infect other mosquitoes [205].

Most people who are bitten by an infected mosquito do not develop disease [203]. Those who do develop EEEV disease can have fever, myalgias, and headache progressing to encephalomyelitis. EEEV encephalitis is associated with 50–70% mortality [206]. The majority of cases are reported from the southeastern regions of the United States. In the United States, an average of 11 human cases of EEE are usually reported annually. In 2019 however, 39 cases were reported. There are no antiviral treatments. Care is supportive.

6.6 *Cache Valley Virus*

Cache Valley virus (CVV) is an enzootic infection affecting small ruminants with zoonotic potential. But it also has been implicated as the cause of several cases of meningoencephalitis in humans [207–210]. It belongs to the Bunyamwera antigenic group of orthobunyaviruses within the *Peribunyaviridae* family of *Bunyavirales*. Since it was first isolated from *Culiseta inornata* mosquitoes in 1956 near Wellsville, in the Cache Valley of northern Utah [211], CVV has been isolated from at least 44 species of mosquitoes, including *Aedes*, *Anopheles*, *Coquillettidia*, *Culex*, *Mansonia*, *Ochlerotatus*, and *Psorophora* species of mosquitoes [212].

Among non-human hosts, reports of CVV infection have largely centered on outbreaks among sheep and goats. Outbreaks among sheep have been reported primarily between December and February, during lambing season, resulting in large numbers of stillbirths and fetal deformities [212]. Very few cases of Cache Valley virus disease have been reported in the United States, and fewer than 10 human cases have been reported. All have occurred in late spring through early fall.

6.7 Usutu Virus

Usutu virus is a flavivirus classified within the Japanese encephalitis virus serogroup and closely related to Murray Valley encephalitis, West Nile, and St. Louis encephalitis viruses. It has been documented in South Africa, Uganda, Nigeria, Central African Republic, Senegal, and Cameroon. While it has not yet been documented in the Americas, the abundance of its principal transmission vector, *Ae. aegypti*, raises concern for its pandemic potential.

6.8 Conclusions

Rampant destruction and urbanization of previously wild habitats continue to provide ever-increasing opportunities for zoonotic infections to emerge. Vector-borne infections are restricted by their host habitat and behavior, so those pathogens that have not adapted to infect an urban vector may have less pandemic potential. Unfortunately, the geographic expansion of vectors that have adapted to human living conditions, including *Ae. aegypti* and *Ae. albopictus*, implies spread of the pandemic potential of vector-borne diseases. Both vectors already are present in states lining the southern border of the United States from California to Florida and up the east coast to New York. Development of vaccines after emergence of novel arboviruses can be useful to mitigate and management epidemics but require time, effort, and luck to be successful. Preventing emergence or re-emergence of arboviral infections with pandemic potential requires vector control. At the global level, this requires concerted effort to combat global warming. At the community level, the most immediate and effective interventions are to educate and encourage citizens to make efforts to identify and eliminate potential local mosquito breeding sites and to use personal protective strategies including the use of insect repellants and clothing that covers the skin.

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West Nile Virus

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Abstract Since its introduction to North America in 1999, West Nile virus (WNV) has established itself as an endemic pathogen with regular seasonal outbreaks. The single-stranded RNA flavivirus is primarily transmitted by mosquitoes in the genus *Culex* and maintained in an enzootic transmission cycle by a diverse assemblage of avian hosts. Humans, equines, and other mammals serve as incidental or dead-end hosts. WNV is a significant threat to public health, with estimates indicating that more than seven million individuals have been infected. Although the majority of these individuals are asymptomatic, approximately 20% develop a febrile illness or neuroinvasive disease, the latter associated with high rates of mortality in the elderly and immunocompromised. Disease-associated pathology of the central nervous system is prevalent not only during the acute phase of WNV infection but also as significant long-term sequelae. Although vaccine and therapeutic research progressed over the last 20 years, no agents are licensed for use in humans, and treatment depends on supportive care. Mitigation efforts are instead directed towards the elimination and control of mosquito vectors. Future research will need to leverage technological and epidemiological advances to overcome a host of challenges in order to alleviate the immense economic and human costs of this endemic zoonotic disease.

Keywords West Nile virus · West Nile neuroinvasive disease · West Nile fever · Arbovirus · Epidemiology · Immunology · Clinical features

Bonnie E. Gulas-Wroblewski and Miguel A. Saldaña contributed equally to this work.

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1 Introduction

West Nile virus (WNV) is a virus of the family *Flaviviridae* that primarily circulates between avian reservoirs and mosquito vectors. The virus has a broad host range, and new studies suggest that ticks may be another possible vector, with reptiles and small mammals acting as additional reservoirs or amplifying hosts [1–3]. Spillover events in equines and humans occur annually, establishing WNV as an endemic pathogen in North America [4–9].

WNV is an enveloped RNA virus, with a single-stranded positive-sense genome of approximately 11 kb in length flanked by stem-loop noncoding regions (NCR) at the 5′ and 3′ ends [10]. The viral genome contains a single open reading frame (ORF) encoding a single polyprotein that undergoes posttranslational modification to generate 10 proteins. Three of these proteins are structural, while seven are nonstructural (NS) and do not form part of the virion [2, 11–13]. The three structural proteins are the capsid (C), the premembrane (prM) protein, and the envelope (E) protein, a glycoprotein with three functional domains critical to cell attachment and fusion. The E protein is strongly antigenic and a major target for neutralizing antibodies [13, 14]. The seven NS proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) play a variety of roles in viral replication and immune response suppression. NS1 is an essential cofactor for viral replication, antagonizing complement activation, and inhibiting toll-like receptor signaling [13]. Interestingly, WNV can produce variant proteins via a ribosomal frameshift [15–17]. For example, a variant of NS1 is longer than the standard NS1 and may relate to neuroinvasive disease [18]. NS2A plays a role in viral particle assembly [19] but more importantly functions as an immunomodulator by inhibiting interferon (IFN)-α and β production [20]. Substituting an amino acid in NS2A leads to viral attenuation [20, 21]. Similarly to NS2, NS4A and NS4B interfere with IFN signaling [22–24], while cleavage of NS4A produces a signal that translocates NS4B to the endoplasmic reticulum for viral replication [25–27]. NS2B is an important cofactor for the multifunction viral protease NS3, which is responsible for cleaving the WNV polyprotein into 10 individual proteins [28, 29]. NS5 is the largest protein with two essential functions: the N-terminus generates the mRNA cap to prevent degradation of viral mRNA [30–32], while the C-terminus encodes the RNA-dependent RNA polymerase (RdRp) to replicate the viral genome, critical for proliferation within humans since mammalian cells do not contain the appropriate polymerase to replicate single-stranded RNA viruses [33, 34]. In addition, NS5 is a major antagonist for IFN signaling [35].

Strains of WNV can be divided into nine lineages, of which the majority of cases are linked to strains within lineages 1 and 2. Viruses from the remaining seven lineages are primarily restricted to Europe and Africa [3]. Lineage 1 exhibits the widest global distribution and is responsible for most of the WNV cases in North America. Outbreaks in the United States and Canada reveal genetic divergence in the currently circulating strains when compared to the emergent New York strain. In fact, two strains from lineage 1 that emerged during the 2002 and 2003 outbreaks, the North American genotype WNV 2002 (NA/WN02) and the Southwestern

genotype (SW/WN03), now replace the emergent lineage 1 strain from 1999 as the dominant circulating forms of WNV in North America [36–41]. These strains have remained stable since their introduction nearly a decade ago, but research continues to evaluate the diversity of circulating strains and their epidemiological and clinical significance.

2 Epidemiology

Since its original identification in Uganda in 1937 through the 1990s, WNV was primarily restricted to the continents of Africa and Asia, with occasional introductions into Europe [42]. Cases of neuroinvasive infections occurred less frequently prior to 1996 and were recorded in younger populations [42]. In the late summer of 1999, an infectious disease physician at a hospital in Queens, New York City, United States, identified an unusual cluster of viral encephalitis cases. The New York City Department of Health discovered additional cases in neighboring hospitals and requested investigative assistance quickly from the Centers for Disease Control and Prevention (CDC). The response team initially suspected St. Louis encephalitis virus on the basis of positive enzyme-linked immunosorbent assay (ELISA) tests of cerebrospinal fluid (CSF) collected from encephalitic patients [43, 44].

Concurrently, unexplained die-offs were observed in wild avian populations, with the most significant population losses experienced by corvids, especially American crows. Deaths attributed to encephalitis were also observed in exotic birds housed at the Bronx Zoo [45]. The US Department of Agriculture's (USDA) National Veterinary Services Laboratory evaluated samples from these birds for encephalitic viruses and other common wildlife pathogens. Despite the inconclusivity of these tests, the USDA isolated the virus for molecular sequencing. The mystery virus was genetically similar to the Israeli strain of WNV isolated in 1998 [43, 46].

Shortly thereafter on August 31, 2001, Health Canada detected WNV during routine surveillance of a pool of *Culex* spp. vectors in Ontario [47]. In 2002, Canada identified their first human index patient in Ontario [48]. WNV has since established itself as an endemic pathogen throughout the United States and Canada, with regular seasonal outbreaks that are typically associated with unusually dry and hot weather patterns [48–50].

Humans are not the only incidental host for this flavivirus. WNV infections in equines were first reported following the human cases of New York's 1999 outbreak [51], followed by additional cases and serologic evidence of infections described for canids (domestic dogs, wolves), domestic cats, sheep, alpacas, nonhuman primates, farmed alligators, and wildlife (amphibians, reptiles, bats, squirrels, opossums, raccoons, squirrels, and striped skunks) [7–9, 52–68]. The majority of WNV infections in these taxa are likely to be asymptomatic [62], with mammals typically considered dead-end or incidental hosts. Experimental infections of domestic dogs and cats fail to generate levels of viremia sufficient to transmit WNV to mosquito vectors [69–71]. Despite their incompetency as reservoir hosts for WNV, domestic

dogs and other mammals may serve a potential role as sentinels for WNV surveillance in addition to birds [72, 73].

2.1 Clinical and Financial Disease Burden

Since becoming endemic in North America, WNV case reports exceed >50,000 in the United States and > 6000 in Canada [74, 75]. Importantly, the numbers reported to health agencies are significant underestimates of the true burden of infection. In the United States, approximately 50% of the reported cases are neuroinvasive, but we know that this severe disease occurs in less than 1% of infected individuals. Reports from the original New York outbreak indicate that estimating the true burden of disease in a population could be done by multiplying the number of reported WNND cases by 140, leading to ~3.5 million assumed cases in the United States alone to date. However, additional research supports that the estimations are more nuanced. In fact, a 2012 study by Carson and colleagues determined the seropositivity of blood donors in North Dakota, United States, from 1999 to 2008, leading to the discovery that there are likely 244 WNV infections for every 1 reported case of WNND in individuals over 16 years of age [76]. A study by Petersen and colleagues expanded on Carson's work by applying the age- and gender-adjusted values calculated by Carson et al. to reported cases of each US state from 1999 to 2010. Petersen's estimate indicates that ~three million individuals had been infected with WNV in the United States through 2008 [77]. From 2010 to 2018, 40% of all WNND cases were reported in the United States, which prompted a follow up study. Ronca et al. used similar methods to Peterson et al. to update the national US estimates, this time including work by Mandalakas et al. that allowed for inclusion of case estimates in children. Compiling this information, data now support that at least six million individuals have been infected with WNV in the United States between 1999 and 2018 [78]. Although national studies in Canada do not evaluate the entire country's burden, a 2017 study evaluated the seroprevalence from 2011 to 2014 in Quebec. The team determined the incidence rate in each county ranged from 0 to 12 per 100,000 persons [79]. Additionally, the Canadian government webpage for WNV surveillance acknowledges that their reported case counts reflect only a fraction of the true disease burden.

We must acknowledge the shortcomings in testing practices when we evaluate disease burden. A study of testing frequency in Texas hospitals identified that only 37% of patients presenting with WNV compatible illnesses receive WNV testing by their care team [80]. This is similar to the findings of an Arizona team, where a WNV testing rate of 40% occurred during a the 2012 WNV outbreak. In both studies, older individuals were more likely to be tested for WNV as part of their diagnostic work-up [81]. This highlights the importance of educating clinical staff about WNV, especially in states with high case burdens such as those in the south, west, and midwestern regions of the United States. Our health systems' failure to test for WNV in all clinically relevant patients during the appropriate season supports that WNV is

a neglected disease, even in countries with an established national healthcare infrastructure.

As WNV infections can be devastating to an individual's health and quality of life, we must also consider the cost burden to treat these infections. Estimates from the United States indicate that it costs $56 million per year in direct and indirect medical care for people who are hospitalized due to WNV, with total costs from patient care between 1999 and 2012 exceeding $778 million (CI: 673 million–1.01 billion) [82]. Although a study in 2006 suggested a vaccine would be cost ineffective in the United States [83], a 2017 evaluation supports a targeted vaccination program based on age [84]. A study in Quebec, Canada, determined a $1.7 million cost to manage cases from the 2012–2013 WNV season alone, but larger studies in Canada are lacking. Cost-effectiveness should continue to be evaluated over time as not to discourage the pursuit of much-needed therapeutic and preventive vaccine options.

3 Transmission

WNV is predominantly transmitted through the bite of an infected mosquito. Mosquito vector collection studies identified as many as 43 different species of mosquitoes in North America that are susceptible to viral infection and that play varying roles in the enzootic transmission cycles of WNV [85, 86]. While a variety of mosquitoes can harbor WNV infection, *Culex (Cx.)* mosquitoes are considered the primary vectors for disease transmission in North America. There are three main *Cx.* species driving enzootic transmission, each within a distinct geographical region. In northeast region of the continent, the main vector is *Cx. pipiens* (commonly known as the house mosquito), which was primarily involved in the 1999 NYC outbreak. *Cx. quinquefasciatus* (i.e., the southern house mosquito) is the primary the vector in the Midwest, South, and Southeast, while transmission is linked to *Cx. tarsalis* (the western encephalitis mosquito) in the West [85, 87–89].

Mosquito surveillance in Ontario, Canada, proved to be an effective predictor for human WNV infections, and the use of different indices to estimate vector infection prevalence has a strong correlation with risks for future human infections [47, 90, 91]. These data complement estimations for vector transmission of WNV to specific hosts based on season and host interactions as well as mosquito behavior. For example, a number of species of *Cx.* mosquitoes preferentially shift their feeding habits to human and mammalian hosts in the late summer months in North America, thus increasing the risk of transmission for humans and other mammals during this time of year [92].

Birds are the major reservoir and amplifying host for WNV, effective spreaders of the virus not only via mosquito vectors but also by shedding in saliva and cloacal secretions and ingestion of infected carcasses by predator species [71, 89, 93]. Although the largest WNV-related die-offs primarily involved corvids (specifically American crows and blue jays), over 300 avian species are known to be susceptible to infection, with viral levels in organs reaching 10^{12} viral units per

mL [7, 53, 69, 94–98]. The migratory behavior of avian hosts serves as a dispersal mechanism for WNV throughout the Western hemisphere, and the southward migration of terrestrial birds along the eastern flyway and their northward migration along the central flyway correlate with WNV circulation in the United States [40, 99]. In practice, migratory flight patterns of North American avians can inform "hotspot" geographic locations for WNV surveillance, such as in Texas, Illinois, and New York along the eastern and central flyways [99].

Although mosquitos play a critical role in the standard transmission of WNV, other modes of transmission prove significant despite their rarity. In 2002, WNV infection was observed in 23 recipients of blood transfusion products [12, 100, 101], and transmission confirmed after solid organ transplantation (SOT) [12, 100, 101]. In addition, cases of intrauterine transmission occurred in a number of neonates born to WNV-infected mothers, while breast milk has been identified as a potential, though unsubstantiated, source for mother-child transmission [12]. Several cases of laboratory-acquired infections of WNV have also been reported [102].

4 Clinical Manifestations

4.1 Pathogenesis

After entry into a host, WNV disperses to the regional lymphatic system, the spleen, and other reticuloendothelial tissues, where viral replication eventually leads to viremia and the subsequent invasion of other organ systems, including the central nervous system (CNS) [103–106]. Neuroinvasion is accomplished via one of the several routes across the blood-brain barrier (BBB) and is hypothesized to include direct axonal retrograde transport from peripheral WNV-infected neurons, passive transport across the endothelium, inflammation-regulated failure of the BBB, transport via infected immune cells, dissemination through infected olfactory neurons, or a combination of these mechanisms [105, 107–114]. Histological analyses of the CNS of human WNND patients are nonspecific to WNV and analogous to other virally induced encephalitis and meningitis cases. The medulla and pons of the brain stem, gray matter of the cerebellum, substantia nigra of the basal ganglia and thalamus, ventral horns of the spinal cord, and anterior spinal nerve roots are frequently the most damaged within the commonly affected extrapyramidally related regions of the CNS [115–121]. Microglial nodules, mononuclear inflammation, neuronal loss, neuronophagia, and lymphocytic infiltrates, with necrosis in severe cases, typify histological findings in these regions [115, 116, 118, 121–123]. In acute cases of WNM, CD8 T-lymphocytes dominate leptomeningeal mononuclear inflammatory infiltrates [116, 122]. Infection of cranial and spinal nerve roots can manifest as radiculitis [124].

When magnetic resonance imaging (MRI) was performed on 30 WNV patients with chronic neurological symptoms and compared to age- and gender-matched controls, significant regional atrophy was revealed in the brain stem, cerebellum,

globus pallidus, putamen, and thalamus. Significant cortical thinning was also identified in the left hemisphere (including portions of the posterior cingulate cortex, superior frontal cortex, medial-orbitofrontal region, anterior cingulate cortex, inferior frontal cortex, cuneus, and parahippocampal region) and the right hemisphere (including areas of the middle and inferior temporal cortex, supramarginal region, inferior frontal region, insular cortex, superior frontal cortex, cingulate cortex, and inferior frontal region), with reduced neurocognitive functioning associated specifically with cortical thinning in the caudal middle frontal gyrus, rostral middle frontal gyrus, and supramarginal gyrus of the left hemisphere [125].

Evidence of a widespread systemic distribution is obvious with findings in other tissues. Kidney involvement is prevalent in acute WNND cases, most notably renal failure secondary to acute tubular necrosis in WNE patients [126–128]. An autopsy performed on a 59-year-old male revealed fibrin thrombi in the small vessels of the kidney matched by WNV antigens disseminated in the glomerular capillaries as evident on immunohistochemical stains [127]. Chronic shedding of WNV in urine, as observed by detection of viral RNA by PCR and/or virions by electron microscopy, occurs up to 9 years postinfection in a subset of patients [129–131]. Kidney pathology of convalescent patients reflects the effects of WNV-induced damage. For instance, 40% of 139 convalescent WNV human patients demonstrated chronic kidney disease [132], and, regardless of age, patients exhibited excess deaths, notably those from renal failure, following WNV infection [133]. Kidney pathology related to WNV chronic conditions is more comprehensively described for animal models. In mice, mild renal inflammation can last up to 84 days post-experimental infection, presenting as clusters of lymphocytes in the intertubular interstitium, with an incidence of 17–83% (number of sections with lesion present per 6 total sections/mouse) [134]. Within the first few weeks post-WNV inoculation, the kidneys of persistently infected hamsters built up proteinaceous deposits in the interstitium between infected tubules as well. Approximately 20 weeks after initial WNV infection, hamsters begin to also develop renal tubular dilation, marked by atrophy and flattening of the tubular epithelia, with prominent clustering [135].

The adrenal glands, eyes, heart, liver, lungs, pancreas, spleen, and testes are occasionally marked by significant lesions [122, 127, 136–140], and neurogenic atrophy of skeletal muscle is associated with muscle weakness in WNND patients [141]. On histopathological evaluation, a biopsy recovered from a purpuric skin lesion on a WNV patient demonstrated hemorrhaging in the superficial dermis, mild perivascular infiltrates of lymphocytes, macrophages in the deep and superficial dermis, multiple occlusive fibrin thrombi in the small vessels, and extravasation of erythrocytes [127].

4.2 Acute Clinical Presentation

After infection, incubation time ranges from 2 to 14 days, with most individuals experiencing an asymptomatic or subclinical infection. Approximately 20% of cases

develop West Nile fever (WNF), characterized by flu-like symptoms, such as fever, headache, fatigue, myalgia, chills, nausea and/or vomiting, and swollen lymph glands, which typically resolve within a few days. Nonpruritic, maculopapular rashes that extend over the trunk and extremities are also common in younger individuals with WNF, typically lasting 5–24 days post-symptom onset [142, 143]. Arthralgia, diarrhea, and ocular deficits can also occur with WNF.

Less than 1% of infections progress to West Nile neuroinvasive disease (WNND), presenting as meningitis (WNM), encephalitis (WNE), and/or acute flaccid paralysis (AFP). Notably, WNND patients may experience a combination of these neuroinvasive presentations. Within the United States, encephalitis is the most common neurological manifestation (47% of patients), followed by meningitis (42%) and AFP (8%) [144]. AFP can present as a Guillain-Barre-like ascending flaccid paralysis that can lead to paralysis of the respiratory muscles which then requires intubation and mechanical ventilation for respiratory support [145, 146]. Patients often regain muscular function over a period of several months. Other cases of AFP can present with viral myelitis or a polio-like paralysis, leading to a more permanent paralysis ranging from single limb involvement to quadriplegia [146]. Ocular complications are common, most frequently presenting with chorioretinitis with posterior segment involvement (79.3% of patients) but also anterior uveitis (13.8%), subconjunctival hemorrhage (6.9%), sixth nerve palsy (3.4%), and nystagmus (3.4%) [147]. Less common, but important, complications of acute WNV include hepatitis, myositis, myocarditis, pancreatitis, and orchitis [122, 136–140].

Age (>50 years old), immunosuppression, sex (males), and diabetes are risk factors for WNND development [144, 148], although genetic variants of the host also play a key role [149–151]. Notable co-morbidities that increase the risk for WNND and for mortality from WNND include hypertension, cardiovascular disease, renal disease, and chronic obstructive pulmonary disease. Fatality rates for cases that progress to WNND are approximately 10%, with a return to baseline varying between 7% (AFP patients) and 100% (WNM cases) [152].

4.3 Long-Term Complications

WNV causes significant sequelae, especially in older patients and those with WNND [153, 154]. WNND patients may require hospitalization for months and are often discharged to long-term care or rehabilitation [155–157]. A survival analysis conducted over 8 years post-symptom onset reveals WNV recovery plateaus after the second year for many patients [153], while an analysis of standardized mortality ratios for WNV patients 4 years post-symptom onset reported a nearly twofold increase in patient mortality for up to 3 years post-WNV infection [158]. Population-level evidence for increased risk of mortality in convalescent-stage WNND subjects revealed that case-patients displayed excess deaths from infectious and renal causes [133]. Overall, chronic mortality risk is highest for the elderly and

those with encephalitis [153, 158], though WNND patients under the age of 60 years exhibited excess deaths from circulatory, digestive, infectious, and renal diseases as well [133].

Chronic neurologic and neuropsychiatric complications vary among WNV patients both in presentation and duration. Persistent weakness generally necessitates physical rehabilitation post-WNV clearance and improves within the first 6–-8 months, with the most rapid recovery in individuals with lower neuromuscular deficits [159–161]. Functional disability, neuromuscular complications, fatigue, and neuropsychiatric symptoms, such as depression, apathy, and anxiety, are also common to WNV patients several years after acute infection. These outcomes are more prevalent in patients over the age of 50 years and/or in those with WNND [132, 153, 162–164]. In a prospective cohort study of 157 WNV-positive patients over 8 years, subjects self-reported ataxia, blurred vision, confusion, depression, headaches, fatigue, memory loss, recurring fever, weakness, and joint, neck, back, and muscle pain up to 8 years post-symptom onset; dizziness, tremors, paralysis, and weight loss up to 5 years post-symptom onset; and neck stiffness and seizures up to 2 years post-symptom onset [153]. Some chronic neurologic symptoms may represent delayed onset or relapses of WNV infections [165].

Additionally, long-term ocular deficits are common. These include retinopathy, changes in retinal pigment epithelium, macular edema, swelling of the optic disc, vasculitis, retinitis, vascular leaking and sheathing, retinal hemorrhages, and uveitis [147, 166–174]. Bilateral multifocal chorioretinitis occurs in approximately 80% of patients with WNND [175], while 24% of 111 WNV patients had associated retinopathy (WNVR) [174]. WNVR is more frequently evidenced in elderly patients (>60 years old) and those with encephalitis and is associated with a lower quality of life, greater dependence in activities involved in daily living, decreased learning, and abnormal reflexes [174].

Evidence supports that WNV infection leads to the development of chronic kidney disease [132, 176]. Forty percent of a cohort of 139 subjects previously infected with WNV exhibited signs of chronic kidney disease, with 10% having Stage III disease. A multivariate analysis of the cohort participants found a history of neuroinvasive WNV disease to be the only independent condition associated with the development of renal deficiencies [132], while a follow-up study identified that there is an increased risk for CKD with a general history of WNV infection and that those WNV patients with CKD have a high level of proinflammatory cytokines [177]. Additional long-term complications associated with WNV infection in human subjects include bilateral sensorineural deafness [178], cardiac conditions (e.g., myocarditis, cardiac dysrhythmias) [117, 140, 158, 179, 180], dysautonomia [117, 119, 181], gastrointestinal symptoms (e.g., abdominal pain, bloating, diarrhea) [117, 153, 180], hepatitis [182], and pancreatitis [137]. Research on hamster models suggests some of the gastrointestinal and cardiac symptoms experienced by WNV-infected humans are attributable to autonomic nervous system dysfunction [181, 183].

5 Diagnosis

Diagnosis of WNV is challenging since acute infection encompasses a wide range of clinical manifestations, many of which overlap with the presentations of other endemic arboviruses (e.g., Zika virus, Powassan virus). WNV should be considered whenever evaluating patients exhibiting the syndromes described above, particularly during peak geographic and seasonal occurrences (e.g., high mosquito activity in the summer months).

Diagnostic procedures for identifying WNV cases consist of serological assays, virus isolation, nucleic acid amplification tests (NATs), and detection of WNV antigens in tissue. Most laboratory-based diagnostic testing for WNV is conducted using immunoglobulin M (IgM) and immunoglobulin G (IgG) serological assays, which are confirmed via "gold standard" plaque reduction neutralization test (PRNT)]. These serological assays enable the detection of WNV by day 4 (IgM) or day 8 (IgG) post-symptom onset and are easily used, with the option to automate portions of their protocols [184]. NATs are also commonly employed for patient diagnosis, especially in cases involving immunocompromised subjects and the screening of blood and organ donations [184].

In the United States, diagnostic standards are set by the CDC and require one or more of the following laboratory criteria to be met in order to confirm a case of WNV: isolation of virus from, or demonstration of specific viral antigen or nucleic acid in, tissue, blood, CSF, or other body fluid; fourfold or greater change in virus-specific quantitative antibody titers in paired sera; virus-specific IgM antibodies in serum with confirmatory virus-specific neutralizing antibodies in the same or a later specimen; virus-specific IgM antibodies in CSF, with or without a reported pleocytosis; and a negative result for other IgM antibodies in CSF for arboviruses endemic to the region where exposure occurred [185]. In Canada, Health Canada closely mirrors the CDC's requirements for case definition with a few minor deviations. For instance, a fourfold increase in virus-specific quantitative antibody titers in paired acute and convalescent sera or CSF is acceptable, and the additional laboratory criterion of "a fourfold or greater change in flavivirus haemagglutination inhibition (HI) titres in paired acute and convalescent sera or demonstration of a seroconversion using a WN virus IgG EIA AND the detection of WN-specific antibodies using a PRN (acute or convalescent serum sample)" is incorporated into the list [186].

Despite the variety of laboratory tests available for WNV, confirmatory diagnosis of infection can be complicated. Cross-reactivity with other flaviviruses, especially the Japanese Encephalitis serogroup (i.e., St. Louis encephalitis virus endemic in North America), dengue serotypes, and yellow fever virus, results in false positives on multiple types of serological tests, such as IgM antibody testing, IgG enzyme-linked immunosorbent assays, HIs, and neutralization assays [184, 187, 188]. In addition, IgM antibodies may remain present in hosts for months or years post-infection, even for asymptomatic cases, rendering a definitive diagnosis for clinical cases challenging in the absence of clinically compatible symptoms [189].

Molecular diagnoses of WNV infections (e.g., via quantitative real-time PCR) can be used to augment or replace serological assays and offer the advantages of being fast, easy to use, and marked by high sensitivity, specificity, and reproducibility. While detection in serum is generally limited to the first week of disease, recent literature suggest that whole blood, urine, and other body fluid samples facilitate longer-term detection of infections. A recent study found that WNV RNA persists in human patients for up to 9 days post-symptom onset (DPO) in saliva, 18 DPO in urine, 22 DPO in semen, and 126 DPO in whole blood. However archived urine samples have a limited storage time for positive detection, and semen and saliva demonstrate relatively low viral loads. Interestingly, whole blood specimens remain valuable for the prolonged detection of WNV RNA for 3 months or longer [190]. Exploration of next generation sequencing (NSG), which can recover WNV from body fluids even with low viral levels [191], and CRISPR-cas13 technology [192] shows great promise for expanding viral diagnostic capabilities.

6 Treatment

No specific therapy for WNV is currently approved for humans; standard treatment consists of supportive care and treating underlying conditions. Although reports evaluate the efficacy of antiviral and immunomodulating agents, such angiotensin-receptor blockers, corticosteroids, interferon, ribavirin, monoclonal antibodies, nucleoside analogs, and specific immune globulin transfusions, the lack of clinical trials for these therapeutics limits conclusive evidence for their use [193].

There are no antiviral drugs licensed to treat patients with WNV, but small studies evaluate the outcomes of ribavirin and interferon treatment [194–203]. Interferon and ribavirin demonstrate antiviral capacity in vitro [195], and, when combined with supportive care, outcomes appear to improve in cases of WNND [197, 199, 200]. However, ribavirin expresses antiviral activity only at high concentrations in vivo, exhibits limited penetration into cerebrospinal fluid, and demonstrates ineffectual and/or detrimental outcomes in more severe human cases and hamster models [194, 196, 198, 201]. Alpha interferon therapy enhanced neurologic improvement in immunocompetent patients participating in a small, randomized, unblinded trial for WNV encephalitis, but these patients also experienced an elevated level of toxicity in the forms of neutropenia and hepatitis [204]. Another antiviral agent, amantadine, which is more commonly administered in Parkinson therapies, significantly reduced WNV RNA in vitro study, but no additional studies evaluated this in a clinical setting [205]. In the absence of larger-scale clinical trials, the degree of efficacy of these antiviral agents remains in question.

Several human case studies support the administration of intravenous high-dose steroids during the acute or intermediate phases of infection to promote reduced overall recovery time, decreased mortality, and/or rapid clinical improvement of various neurological symptoms in WNV patients [155, 206–210]. Experimental administration of dexamethasone in conjunction with WNV inoculation in rabbit

models failed to demonstrate any immunosuppressive effect or increase in WNV infection [211]. The administration of these agents is expected to aid in closing the BBB, thereby reducing the neurological effects of infection. The efficacy of corticosteroids in WNV therapeutics is most likely to be dose-, frequency-, and condition-dependent [211].

Small molecule-based inhibitors that terminate viral nucleic acid synthesis, termed nucleoside analogs, are approved for the treatment of a variety of other viruses, such as HIV, human herpes virus, hepatitis B, and HCV [212–216]. In 2004, Sarepta Therapeutics registered a trial to investigate one such nucleoside analog AVI-4020 for which reports on the efficacy of drug use in cases of WNND are still pending, but the lack of reports greater than a decade later suggest this is not a viable option [217]. More recently, the analog 7-deaza-2'-CMA exhibited anti-WNV efficacy in cell lines of both neuronal and extraneural origins and protected against disease development and mortality in mouse models when administered at 25 mg/kg twice a day on days 0, 1, and 3 post-WNV infection (p.i.), but not on day 8 p.i [218]. This nucleoside analog and others hold great potential for development as a WNV therapeutic.

Intravenous pooled immune plasma containing WNV antibodies or immunoglobulin improved neurologic symptoms and reduced mortality in several case studies and in experimental mouse models [219–226]. Although treatment is thought to be most effective in the acute stages of infection prior to development of severe neuroinvasive disease, one report indicates that patients with WNND improved even when administered intravenous immunoglobulin 8 days after symptoms onset [224]. A phase I/II, placebo-controlled clinical study by the National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group investigated the safety and efficacy of a high-titered immunoglobulin (Omr-IgG-am, OMRIX Biopharmaceuticals, Tel Aviv, Israel) for the treatment of WNND. Difficulties in participant recruitment, availability of study products, and contamination of placebo supplies (i.e., US-sourced Polygam) prevented completion of the trial, but preliminary results found no difference among treated and non-treated cohorts in the low dose (0.5 g/kg) administration of Omr-IgG-am [227].

Multiple studies evaluated neutralizing monoclonal or polyclonal antibodies as a potential therapeutics [228–235]. WNV-neutralizing antibodies derived from human origin demonstrate neutralizing activity both in vitro and in vivo [235] and the monoclonal antibody MGAWN1 decreased mortality when administered 5 days post-infection during the neuroinvasive phase of disease in WNV-infected hamsters and mice [230, 236–238]. A phase I clinical trial of MGAWN1 in 40 immunocompetent humans established the tolerability and safety of the monoclonal antibody in dosages as high as 30 mg/kg with few, minor adverse reactions [239].

Learning from successful antivirals developed for HCV and HIV therapies, targets should be proteins that exhibit a low mutation rate and are critical for viral cell cycle functioning, such as enzymatic motifs in WNV NS3 protease and NS5 polymerase. In addition to antivirals that directly impede viral replication, future investigations should explore agents that modulate the host immune response or host factors facilitating viral invasion and replication. Experience with direct-acting

antivirals in the treatment of HCV further strengthens the prospect of developing therapeutic agents with broad-spectrum antiviral activity, particularly across other flavivirus strains, that could be efficacious in the treatment of WNV [193, 240].

Despite the promise of many of these anti-WNV compounds, the majority of therapeutic agents for WNV remain in the pre-clinical stage, with few tested in vivo and even less that have progressed to phase I/II clinical trials. The limitations on timely and effective development of WNV prophylactics and therapeutics are largely attributable to experimental constraints related to resource availability and challenges in predicting WNV outbreaks to allow for successful enrollment of study participants. Exploring anti-viral treatments for WNV has been stymied by the high safety level requirements for laboratories working with the virus and high costs of clinical trials, especially when compared to the lower cost of mosquito control programs [241]. As is demonstrated in the case of the phase II trial for Omr-IgG-am, clinical trials also face daunting barriers to participant enrollment. Effective planning of large-scale (especially phase III) clinical studies is complicated by the typically geographically and temporally unpredictable emergence of WNV outbreaks [242, 243]. Even when WNV outbreaks occur, clinical presentation is highly variable, and severe disease is present in less than 20% of cases, complicating targeted enrollment. Patient recruitment for clinical trials is further limited by the current need of each study site to obtain an independent Institutional Review Board (IRB) approval prior to participation in clinical trials [244]. These challenges to the development of anti-WNV agents could be ameliorated via several strategies. Pursuing therapeutics with a broad spectrum of cross-coverage for other viruses could leverage the recent interest and resources invested in drug development for Zika and dengue viruses [203, 245]. Establishing a single, universal IRB for WNV prophylactic and therapeutic clinical trials, such as the Streamlined, Multisite, Accelerated Resources for Trials IRB platform, could also expedite patient enrollment [244].

7 Vaccines

Vaccines licensed for veterinary use in equines include several inactivated vaccines, a live chimeric vaccine (ChimeriVax-WN, constructed from the yellow fever virus vaccine with expression of WNV prM/E structural proteins), and a recombinant canarypox vaccine expressing WNV prM/E [243, 246], but vaccines are not available for human use at this time. The recombinant canarypox vaccine protects domestic dogs and cats from experimental infection, and the extra-label use of equine vaccines has been practiced with anecdotally reported and experimentally proven success in domestic dogs, domestic cats, and avian wildlife [247–249]. WNV vaccines licensed for veterinary use have several complications. In 2010, ChimeriVax-WN was recalled from the market due to its association with severe anaphylactic reactions in horses, and challenge trials of equine vaccines in avian subjects failed to reduce viremia below the level of infectivity, while testing with

recombinant canarypox vaccine resulted in necrotic lesions at injection sites [193, 249].

A variety of technological platforms assist in the development of vaccine candidates for the protection of humans from WNV infection: those constructed from RNA, DNA, recombinant proteins, RNA replicons, chimeric flaviviruses, viral vectors expressing WNV genes, and the attenuated strains and inactivated viruses employed in the licensed vaccines for yellow fever virus, Japanese encephalitis virus, and tick-borne encephalitis virus [243, 246, 250]. Only six of these have progressed to phase I or II clinical trials with non-human primate or human testing having, thus far, been conducted with inactivated whole virus vaccines (Hydrovax-001 and a WNV vaccine deactivated with formaldehyde), a recombinant, insect cell-derived E protein ectodomain (HBV-002), a DNA vaccine expressing the prM/E fragment (VRC WNV), and chimeric flaviviruses built with backbones of dengue virus (rWN/DEN4Δ30) or the yellow fever virus vaccine strain (ChimeriVax-WN02) [243, 251–256]. The virus envelope (E) protein is the major antigen component of all of these vaccine candidates. Significant neutralizing antibody responses were recovered from individuals in all of these trials after one (ChimeriVax-WN02), two (Hydrovax-001, rWN/DEN4Δ30), or three doses of vaccine (formaldehyde-inactivated WNV vaccine, HBV-002, VRC WNV), with a minimum four-fold increase in antibody titers recorded in over 90% of treated participants in both phase II clinical trials (formaldehyde-inactivated WNV vaccine and ChimeriVax-WN02) [251–256]. These clinical trials lend support to the capacity of E protein-based vaccines to provide robust protection against WNV (both genetic lineages 1 and 2) as well as the ability of a wide range of immunization techniques to elicit neutralizing antibody responses. Although no adverse conditions, safety concerns, or other impediments to future testing were reported in the course of these trials, clinical testing of WNV vaccines has failed to progress substantially in recent years with no candidate close to licensure. Updates can be evaluated over time by visiting https://clinicaltrials.gov/.

8 Hurdles to Elimination and Control

Over 60% of all known human diseases are zoonotic in nature, with an estimated 75% of all emerging and re-emerging human pathogens of the previous decade falling within this category [257, 258]. One of the most significant challenges confronting programs that target zoonotic disease management is controlling transmission agents broad host and vector ranges, such is the case for WNV [258]. With a multitude of avian amplifying reservoirs, a wide variety of non-avian hosts, and over 40 species of mosquito vectors, identification of the ecological and evolutionary dynamics driving the interrelationships of WNV, its vectors, and its animal hosts, including humans, is both essential and exigent in the strategic mitigation of this disease in North America. A limited approach focusing on any of one of these links in the enzootic cycle of WNV can have unanticipated, adverse consequences related

to host switching, hybridizations, genotypic evolution, the development of drug resistance, and the elimination of natural vector control agents [258–260].

Some of these unintended effects of myopic disease mitigation strategies are illustrated by the primary focus of WNV public health programs: mosquito vector control and elimination. Vector control strategies are often divided into two arms, the elimination of host-seeking adults and the disruption of larval habitats. Strategies targeting adult mosquitoes frequently rely on the use of insecticides, such as pyrethrin or phenothrin, released as a droplet spray or mist, which impacts flying mosquitoes that directly contact the chemicals [261–263]. Unfortunately, the overuse of chemical pesticides has not only had detrimental effects on the environment, delicately balanced ecosystems, and human health but also stimulated the development of insecticide resistance in targeted vectors and impacted natural predators of mosquitoes [263–265]. In agricultural areas of Costa Rica, mosquitoes are more abundant in pesticide-inundated areas than in those free from chemicals due to the synergistic effects of insecticide resistance in mosquito vectors and the concurrent population decline of predating damselflies [266]. The solution to these vector elimination challenges may be provided by biological control strategies, such as the deployment of transgenic mosquitoes engineered to be refractory to viruses [267–270] or suppressive to mosquito reproduction [271–273], inoculation of vectors with *Wolbachia* and other antiviral microbiota [274–276], and the augmentation of natural populations of mosquito predators and pathogens [277–279].

Understanding and managing the complex relationships between virus, hosts, and vectors in the WNV transmission cycle is further confounded by the burgeoning effects of anthropogenic climate change. Climatic projections for North America in the next few decades predict an increased frequency of extreme weather events and heavy rainfall, overall warmer temperatures, and shorter winters marked by less snow [280], all conditions favorable to the proliferation of WNV [281]. The Canadian Prairies, where WNV is highly endemic and already of serious public health concern, will face a particularly high risk for significant WNV outbreaks intensified by the climatically boosted populations of the primary WNV vector in the region, *C. tarsalis* [282]. In addition, climate change will spur the migration of potential pathogen hosts and vectors beyond their current ranges throughout North America, resulting in stressed animal populations and ecosystems that are more susceptible to disease transmission [260]. Consistent surveillance will be necessary to inform disease mitigation strategies [48, 258]. In order to confront all of these hurdles to WNV control and elimination, the efforts of medical and public health professionals, veterinarians, wildlife biologists, entomologists, and ecotoxicologists need to be effectively integrated within a One Health framework.

Mitigation of WNV has additional challenges associated with human health interventions. There are diverse presentations of infection, but evidence is clear that adults 65 and older are at a greater risk of neuroinvasive disease [84, 243, 283, 284]. Although this finding suggests that this age group would benefit from targeted vaccination programs, safety and immunogenicity challenges related to immunosenescence must be considered. Immunosenescence contributes to impaired vaccine responses, which necessitate either an increased dosage or additional doses

to properly stimulate immunity. In addition, the use of vectored or attenuated vaccines can lead to complications resulting from impaired T-cell immunity's inability to suppress viral replication from these vaccines [243, 285, 286]. Vaccine development and production is also a costly and lengthy process. The cost-effectiveness of a WNV vaccine has been debated over the years [84, 241], while safety considerations center on vaccine countereffects on co-circulating flaviviruses. For instance, WNV immunity enhances ZIKV infections in both in vitro and in vivo studies [243, 287]. Only a limited number of WNV vaccine candidates have reached phase II clinical trials [288], and the unpredictable nature of WNV outbreaks confounds efficacious planning of large-scale phase III clinical trials [242, 243]. Overcoming these barriers to prophylactic development is imperative to address the spread of WNV in human populations and forms a key piece of the public health strategy that is integral to any One Health approach to WNV mitigation in North America.

9 Conclusions

WNV continues to be a major public health threat worldwide. Estimates indicate that more than seven million individuals in the United States and Canada have been infected, with the most severe disease occurring in older and immunocompromised populations, but a general lack of testing prevents us from defining the true burden of disease. Although the presentation of the severe neuroinvasive forms of WNV-associated disease is relatively low, the impacts of infection are long-term and result in a significant decrease in patient quality of life. Consequently, WNV infection poses substantial costs to the patient, their community, and the healthcare system. With many questions unanswered regarding the immune mechanisms and pathological outcomes of disease, we lack effective treatments and prophylactics for human use. Our primary method of control relies on disrupting mosquito transmission. Unless we remain diligent in our fight against WNV across the multiple fronts of pharmaceutical development, One Health epidemiological investigations, and targeted public health initiatives, this vector-borne zoonosis will continue to take wing across North America and pose a daunting threat to our communities' health and well-being.

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Hansen's Disease

Divya Bhamidipati and Jessica K. Fairley

Abstract Hansen's disease, also known as leprosy, is a chronic infection caused by *Mycobacterium leprae* that classically results in varying skin lesions with peripheral nerve damage. While Hansen's disease remains endemic in tropical areas of the world, notably India and Brazil, it is considered a rare disease in the United States with roughly 200 cases per year recorded. There is emerging evidence that in the southern parts of the United States, Hansen's disease could be considered a zoonosis transmitted via contact with armadillos. Infection with *M. leprae* can lead to a spectrum of clinical diseases dependent on complex pathogen-host immune responses. Additionally, further immune reactions as a result of a disease can also occur prior to, during, or after treatment.

With the advent of an effective treatment regimen promoted by the WHO in the 1980s, it has become a highly treatable disease. However, challenges remain regarding access to treatment, medical complications of the infection, interruption of transmission, and stigma in endemic regions of the world. Patients today continue to suffer from misconceptions regarding the disease and its cure; in the United States specifically, the lack of physician awareness can lead to delayed diagnosis and poor neurological outcomes. In the United States, the National Hansen's Disease Program helps diagnose and treat Hansen's disease in addition to supporting patients who suffer complications from infection.

Keywords Hansen's disease · Leprosy · Armadillos · Lepromatous · Tuberculoid

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1 Introduction

Hansen's disease, also known as leprosy, is a chronic infection caused by *Mycobacterium leprae* that classically results in skin lesions with peripheral nerve damage. Hansen's disease (HD) has long been a part of human history, with references to the disease noted in multiple early texts, including early Greek writing and early Indian texts. Evidence of the disease has been found in human bones dated from 2000 BC. The disease itself has been associated with a lot of fear and stigma, some of which remains to this day in more endemic regions of the world [1].

While the disease has been present from early human history, it was not until the 1940s that effective treatments were developed. With the advent of effective treatment, it has become a highly treatable disease. However, challenges remain regarding access to treatment, medical complications of the infection, interruption of transmission, and stigma. Patients today continue to suffer from misconceptions regarding the disease and its cure; in the United States specifically, the lack of physician awareness can lead to delayed diagnosis and poor outcomes.

2 Epidemiology

While Hansen's disease was prevalent in Europe, the Middle East, and Asia as far back as 2000 BC, it is worth noting that HD was not present in the Americas until Europeans migrated and brought the disease [1]. Even up to the nineteenth and twentieth centuries, cases were widespread throughout the United States, resulting in creation of leprosaria to segregate and isolate patients with leprosy from the general public, due to misconceptions regarding transmissibility and fear of disease. In fact, it was the creation of one of these leprosaria in 1894 outside New Orleans that eventually led to the establishment of the National Leprosarium in 1917 which then developed into the National Hansen's Disease Program (NHDP) which is still active today in identifying, treating, and researching Hansen's disease in the United States [2].

Overall since the advent of effective treatment, prevalence of disease has declined worldwide [3]. However, in primarily tropical areas outside the United States such as Brazil and India, Hansen's disease remains an endemic disease with high morbidity. However, with the introduction of effective treatment, economic development, and relatively better access to care in the United States, HD is a rare diagnosis in North America. From 1985 to 2015, an overall decline was noted in the number of cases reported from about 350 to roughly 100–200 new cases now reported per year [4, 5]. In 2015, the last time that US numbers were publicly released, 178 cases were reported to NHDP with most cases noted in California, Texas, Louisiana, Florida, Hawaii, and New York [5]. Per report, this has not changed substantially. HHS (Health and Human Services) has reported a total of 13,950 cases since 1894, and approximately 5000 people are currently living in the United States who have

been treated for HD and continue to receive care for sequelae of the disease [4]. In Canada, the first case of HD was not noted until 1815, and cases have remained low since then with about 0.6 cases per 100,000 [6].

3 Transmission

Hansen's disease is not an easily transmittable disease, contrary to the historical belief that it was highly contagious. Transmission of *M. leprae* is hypothesized to occur via nasal droplets and secretions from the oropharynx, though the exact mechanism of transmission has yet to be fully described. Environmental reservoirs including soil, water, and armadillos have been described [7, 8].

As recently as the 1980s, most HD cases in the United States were thought to be primarily in immigrants and travelers to the United States from high endemic areas [5]. Interestingly, of the new cases diagnosed in 2015, over 65% were actually seen in patients who were born in and had never lived outside of the United States. Additionally, up to a third of these patients could not recall being around other people who had leprosy. Research indicates that though patients may spread it to close contacts over prolonged periods of time, there is compelling data that locally acquired HD in the Southern United States is primarily a zoonosis.

While armadillos were noted to be natural reservoirs of *M. leprae* and had been used as the animal model for disease research since the 1960s, a transmission link was not suggested until the 1980s. A case report in 1981 of a rancher with HD noted that while the rancher had not been born abroad and had none of the other epidemiological risk factors noted for Hansen's, he did have "significant contact" with armadillos, which suggested a possible link especially as they were known animal reservoirs of *M. leprae*. More recent studies done in Texas and other gulf states have noted that genetically similar strains of *M. leprae* are present in clinical cases and in infected armadillos in the region suggesting that patients likely acquired the disease from the armadillos. Similar findings have been reported in Brazil as well [7–10]. A case series in Texas found that consumption of armadillo meat and skinning of rabbits seemed to increase the odds of HD in their cohort. These findings have lent weight to the theory that, at least in part of the United States and the Americas, HD can be considered a zoonotic infection with armadillos representing a significant risk factor [11]. Current research is underway to elucidate the transmission pathway with soil and/or ameba intermediaries hypothesized. There have been no reports of autochthonous transmission within Canada [12, 13].

3.1 Pathophysiology

Hansen's disease (named for the scientist who discovered the organism) is caused by *Mycobacterium leprae*. As noted previously, transmission of disease is particularly

difficult with Hansen's disease. Over 95% of the world's population is not considered susceptible to the disease. Of the minority that do get infected and develop disease, there are specific host genetic factors thought to be at play that dictate their risk of not only infection but development of clinical disease. The genome for *M. leprae*, especially in comparison to other mycobacterium, is largely preserved with little variation to explain the differences in clinical presentation seen, suggesting that host factors play a larger role in infection than for other diseases.

M. leprae is an intracellular pathogen, primarily infecting macrophages in the skin and Schwann cells surrounding nerves. Nerve damage, often a hallmark of HD, occurs via destruction of these Schwann cells. The infected Schwann cells present *M. leprae* antigens to cytotoxic T cells which then can go on to attack and destroy the cells, thus damaging the nerves. Even after treatment, there can be residual antigens that continue to trigger a chronic immunological response resulting in chronic nerve pathology in treated patients [14]. Both the nerve damage and skin lesions are the result of a complex interplay between the innate immune system and cell-mediated immunity.

The innate immune system response, mediated by macrophages and dendritic cells, forms the initial response to infection. These cells recognize the microbial lipoproteins present in the mycobacterial cell wall and subsequently trigger a cytokine release which determines the clinical manifestation of disease [15]. Research has identified that the toll-like receptors and nucleotide-binding oligomerization domain (NOD)-like receptors involved in this pathway are strongly associated with development of disease. Eventually the inflammatory response generated by this innate immune response will trigger a more specific cell-mediated immune response. Generally, patients with strong cell-mediated response will develop more localized disease, while those with weaker *M. leprae*-specific responses develop more disseminated disease with higher bacterial load. Other pathways, such as T-cell regulatory pathways have also been implicated in mediating this response as have interferon pathways [15]. Variations in the genes mediating these responses and pathways have been implicated with higher risk of HD in large-scale genome association studies [11]. These genetic variations are thought to explain why only approximately 5% of the world is susceptible to infection and disease though more research is needed in this area.

4 Clinical Manifestations

Once infected, it can take up to 3–7 years on average (with up to 20 years reported) before patients exhibit symptoms of disease. Clinical disease varies depending on the individual immune response as outlined above. These varied presentations can be organized via two different classification systems. The simpler classification, favored by the WHO for ease of diagnosis in resource-limited settings, separates disease into two groups—paucibacillary and multibacillary. Paucibacillary disease is

Table 1 Ridley-Jopling classification of disease vs WHO classification. The various forms of Hansen's disease exist on a spectrum of characteristics which vary from patient to patient depending on the individual immune response to *M. leprae*

Ridley-Jopling classification of disease [16]			WHO classification
Tuberculoid (TT)	Single or few lesions, negative or rare bacilli	Very good cell-mediated immunity, granulomas present	Paucibacillary
Borderline tuberculoid (BT)	Single or few lesions, rare bacilli	Good cell-mediated immunity, granulomas	Paucibacillary (if less than five lesions) Multibacillary if ≥5 lesions
Borderline borderline (BB)	Several lesions, more bacilli on histology	Fair cell-mediated immunity	Multibacillary
Borderline lepromatous (BL)	Many lesions, many bacilli	Fair-poor cell-mediated immunity	Multibacillary
Lepromatous (LL)	Diffuse lesions, heavy bacillary load	Poor cell-mediated immunity	Multibacillary

defined as five or fewer hypopigmented, hypoesthetic skin lesions, while multibacillary disease is defined as having greater than five skin lesions [14].

The second classification system is more complex, incorporating clinical findings, histopathology, and immunologic parameters. Known as the Ridley-Jopling classification system (Table 1), this system divides disease into five groups—tuberculoid, borderline tuberculoid, borderline, borderline lepromatous, and lepromatous. In the United States, the NHDP uses the Ridley-Jopling classification system to determine treatment and management [17].

Tuberculoid disease (TT) is a limited disease with a few well-defined hypopigmented lesions present with significant anesthesia present. On biopsy, granulomas are present with few mycobacteria visible. Borderline tuberculoid (BT) disease is similar to tuberculoid disease but is marked by slightly less of a clinical immune response in the host (described as "resistance"), with more numerous lesions than may be seen in TT. Both of these syndromes correspond to the paucibacillary designation on the WHO classification schema.

Borderline borderline disease (BB) is characterized by even less of an immune response than BT disease with lesions that have a mixed appearance. Some lesions may appear to be that of TT, while others may appear to be that of borderline lepromatous (BL) disease. Additionally, the lesions have well-defined areas of central healing with less defined outer edges. Biopsy of these lesions reveals poorly organized granulomas with foamy histiocytes and multiple acid-fast bacilli present.

Lepromatous disease (LL) (Fig. 1) is the other extreme end of the spectrum from TT disease, marked by virtually no immune resistance to disease. Bacilli multiply uncontrollably and result in numerous, poorly defined skin lesions (as compared to the discrete lesions that indicate TT) and can present as nodules, plaques, and even

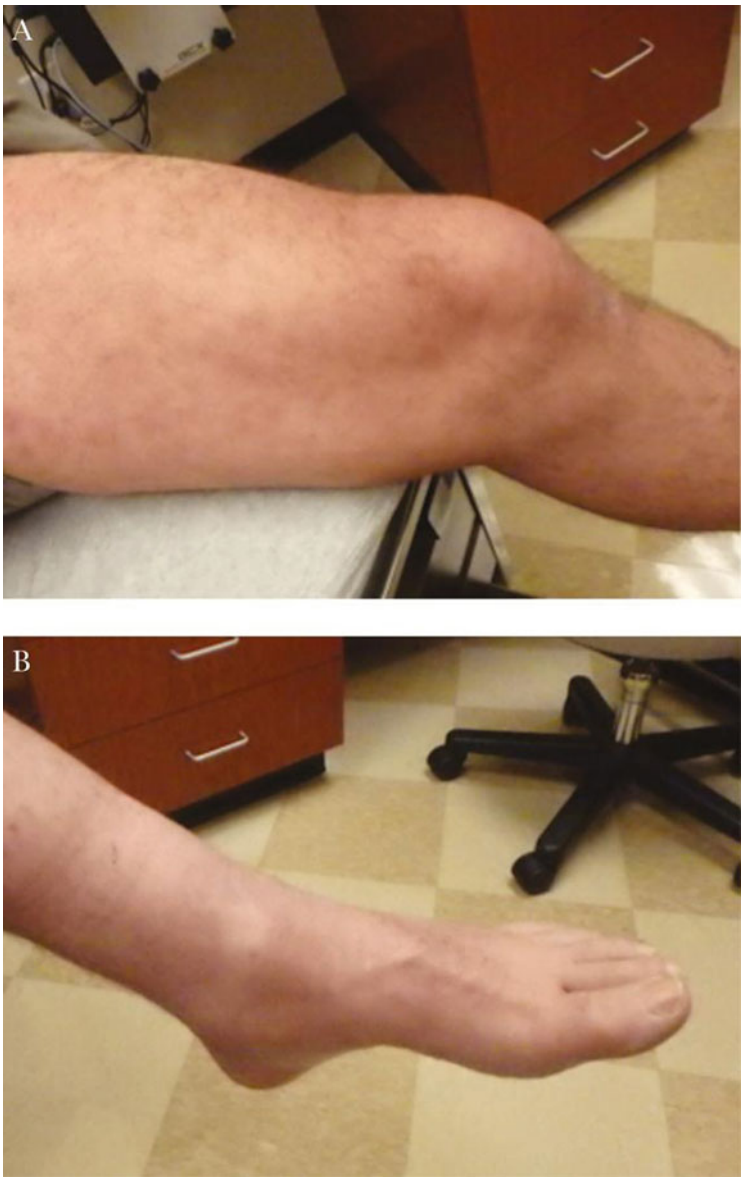

Fig. 1 Skin lesions from a patient with lepromatous disease. Subtle hyperpigmented lesions on the thigh (**a**) with hypopigmented, anesthetic lesion on foot (**b**) (reproduced with patient permission)

ulcerations. Patients can have loss of eyebrows and eyelashes later in the course of disease as well as destruction of the nasal bridge and septum resulting in the classic "leonine facies." Bacilli tend to congregate in the cooler areas of the body (face, hands, etc.) with extensive anesthesia later in course of the disease. BL disease falls

between LL and BB with more asymmetric lesions. Histopathology for both forms reveals foamy histiocytes with clumps of acid-fast bacilli ("globi") present throughout the field [9, 11, 17].

Nerve damage tends to be in the cooler areas of the body with eye, hand, and foot the most frequently affected areas. Additionally *M. leprae* can infiltrate the trigeminal and facial nerves causing serious ocular complications such as lagophthalmos and loss of corneal sensation leading to blindness [6, 14].

4.1 Immune Reactions

One of the interesting features of Hansen's disease is the pathological immune reactions that can occur before, during, or after treatment of disease. These fall into two categories—reversal reaction (Type 1 reaction) and erythema nodosum leprosum (Type 2 reaction). These reactions clinically manifest as worsening skin lesions or symptoms during the course of their disease and do not represent treatment failure but rather increased inflammation along the nerves and in the skin (Fig. 2).

A patient's risk of developing a specific reaction depends on what type of HD he or she has. With BL, BT, or BB disease, a patient is at higher risk of developing reversal reaction. This is characterized by worsening of pre-existing skin lesions with increased erythema and induration as well as new-onset neuritis. The neuritis tends to present as facial palsy, sudden footdrop (peroneal nerve involvement), or ulnar nerve palsy. Enlargement of these nerves due to swelling can be seen, and tenderness can be reproduced on exam with palpation. This reaction is considered to be a delayed-type hypersensitivity reaction [6, 15, 17].

Erythema nodosum leprosum (ENL) is only seen in patients with lepromatous or borderline lepromatous disease. In these patients with high mycobacterial load, high antibody levels are thought to form antigen-antibody complexes that deposit in tissues and elicit an inflammatory response. ENL is characterized by tender transient erythematous nodules in the skin (Fig. 3) as well as fever. Ocular complications such as episcleritis, uveitis, and scleritis can also occur. Neuritis can be a component of ENL as well. There may be end organ damage that can occur during an "attack." ENL can be episodic with symptoms recurring every few months eventually leading to significant nerve damage if untreated [6, 11, 17, 19, 20].

5 Diagnosis

Hansen's disease should always be considered in any slow-healing skin lesions with or without anesthesia. Studies have shown that patients on average report a delay of 2.5 years from time of symptom onset to diagnosis and treatment, pointing to the lack of physician awareness as one of the factors that may explain this delay [5]. Diagnosis is usually made clinically and with tissue biopsy results.

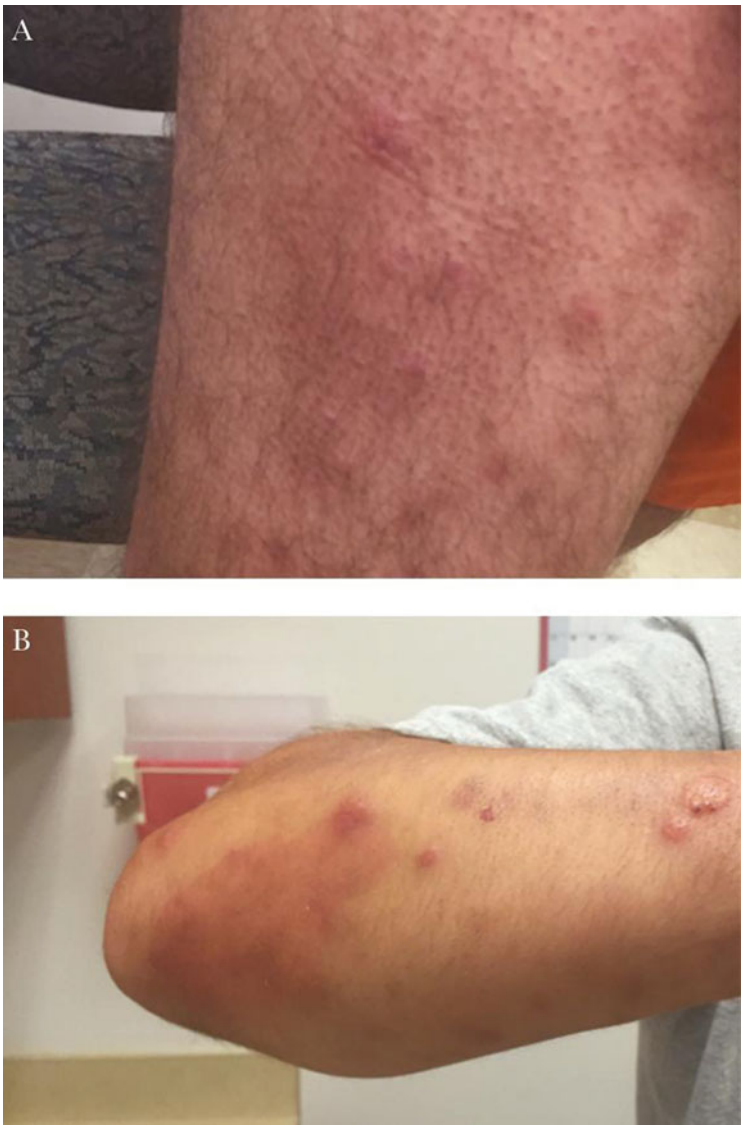

Fig. 2 Skin nodules of ENL in a patient with lepromatous disease (reproduced with permission from patient)

When Hansen's disease is suspected, it is important to take a thorough travel history and social history to identify any risk factors. It is important to elicit a family history of leprosy as well. Close contact with an infected family member over a prolonged period of time can be a risk factor in addition to family history of disease raising suspicion for any genetic susceptibility for infection.

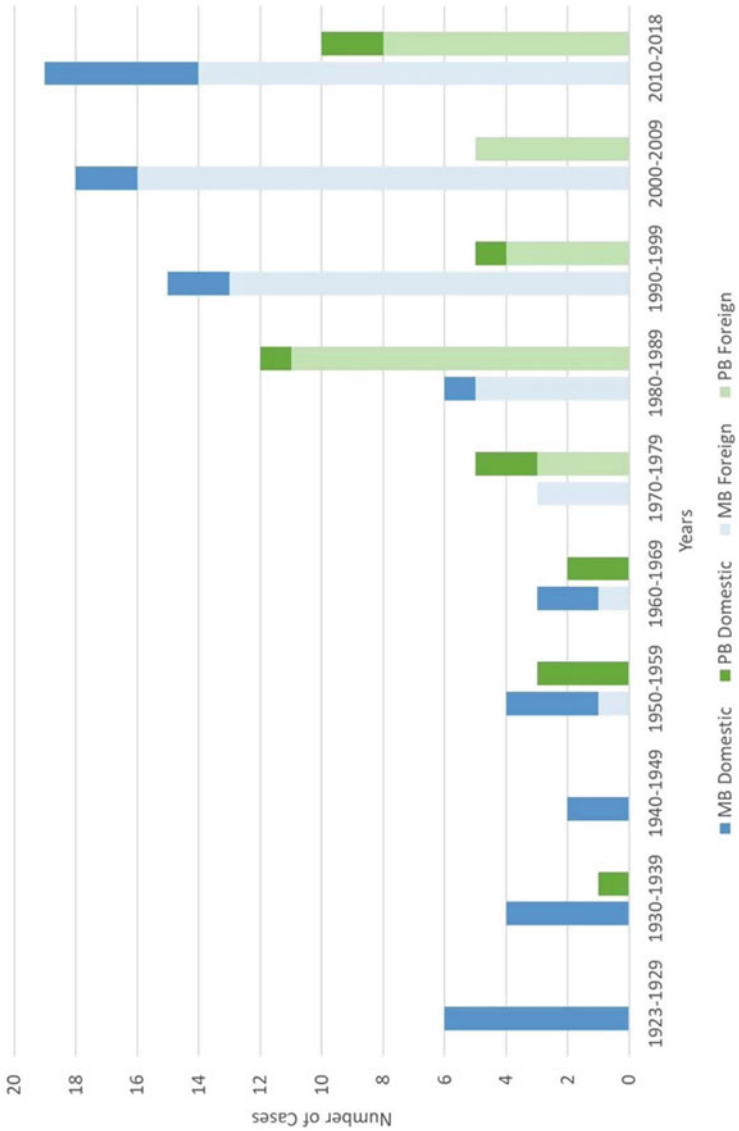

Fig. 3 Number of leprosy cases in Georgia from 1923 to 2018 [18] Reproduced with permission from authors

Thorough skin examination should be done in addition to assessment of any peripheral nerve swelling and tenderness which may indicate inflammation/damage to nerves. Additionally, providers must thoroughly assess for any neuropathic complaints such as paresthesias or nerve palsy. As part of the exam, it is recommended to conduct a monofilament sensation test of hands and feet as well. With anesthesia, the earliest sensation patients often lose is hot/cold differentiation, resulting in burns to hands/feet especially with activities of daily living [14]. Notably, tendon reflexes stay preserved and upper motor neuron disease is not present in HD.

Definitive laboratory testing for Hansen's disease is primarily a skin biopsy. As with any skin biopsy, the specimen should be taken from the edge of an active lesion, and the pathologist should be notified of concern for HD. As outlined earlier, the histopathological findings can vary depending on what type of disease a patient has. The NHDP is also a resource for practitioners should questions arise about diagnosis for a patient suspected of having Hansen's disease. In particularly difficult cases, the NHDP can even run PCR testing on skin biopsy specimens to help aid in diagnosis.

Hansen's disease lesions can be nondescript initially, and the differential for the skin lesions includes a diverse variety of other skin diseases such as vitiligo, psoriasis, nummular dermatitis, mycoses fungoides, and lymphoma to name a few. The neurological symptoms can also be mistaken for diabetic neuropathy, syphilis, and cervicobrachial syndrome. This diagnostic difficulty often contributes to the delay in diagnosis, especially in the United States and Canada where Hansen's disease is a rare diagnosis [11].

6 Treatment

When dapsone was first used for Hansen's disease, monotherapy with the drug was the norm. Unfortunately high rates of resistance developed, especially with treatment interruption and often patients, were on dapsone for a very prolonged period of time. In 1981, the WHO recommended a highly effective multidrug regimen of dapsone, rifampin, and clofazimine. Today, this drug regimen is provided for free worldwide through the Novartis Foundation and can be obtained for free through the NHDP in the United States. Since the introduction of this regimen, there has been a greater than 90% decrease worldwide in numbers of patients who need treatment [21]. Hansen's disease is now a curable disease that is able to be treated in the outpatient setting, a far cry from the leprosariums of the past where patients would spend their entire lives.

While the backbone of the NHDP and WHO regimens are the same, differences exist in treatment duration. In general, the NHDP recommends a longer course of treatment for both paucibacillary and multibacillary disease, lasting anywhere from 12 to 24 months. While the regimen is highly effective, side effects do exist. Dapsone can cause hepatitis and cholestatic jaundice, in addition to hemolysis secondary to glucose-6-phosphate dehydrogenase (G6PD) deficiency. All patients

must thus be screened for G6PD deficiency prior to starting on treatment. Allergy to dapsone itself can occur and can mimic an immune reaction, though this is rare.

Rifampin can cause transaminitis and has a number of drug-drug interactions that must be monitored. While dapsone is a bacteriostatic drug, rifampin is a bactericidal drug that is highly effective against *M. leprae* [9]. As noted with dapsone, rifampin should not be used as monotherapy due to high rates of resistance that can occur with monotherapy.

Clofazimine's mechanism of action is poorly understood, but its ability to bind to DNA is thought to help with its antibacterial activity. Clofazimine can discolor the skin, particularly at sites of HD lesions. This effect can be exacerbated by sunlight exposure, so patients must be warned not to spend too much time outside in the sun at the risk of worsening hyperpigmentation. Clofazimine is not commercially available in the United States but can be obtained via the NHDP.

Alternative, biologically active medications for HD include minocycline, clarithromycin, and quinolones like ofloxacin and moxifloxacin. These can be substituted for components of MDT in cases of contraindication to or intolerability of clofazimine, dapsone, or rifampin.

Treatment for immune reversal reactions is generally comprised of corticosteroids to help reduce swelling and improve or restore nerve function. Thalidomide is first-line therapy for ENL reactions though this must be monitored very carefully in women of child-bearing age due to its high teratogenicity, and often corticosteroids are used initially. Clofazimine has anti-inflammatory effects and may be useful in both reversal reactions. Steroid sparing anti-inflammatory medications like methotrexate, cyclosporine, and azathioprine have shown some promise and are the subject of active research for both reversal and ENL reactions [9, 11, 14, 17].

Beyond the treatment of the infection and reactions, an important component of Hansen's disease to keep in mind is the development of peripheral nerve disorders as a result of the damage done to the nerve cells. If caught in time and treated, some patients may have minimal damage present, but for others where the diagnosis can come years after symptom onset, there is significant morbidity associated with Hansen's disease. These patients benefit from intensive physical and occupational therapy as well as the use of corrective and protective devices to help manage their nerve damage. Lastly, Hansen's disease has a long history of stigma due to inaccurate beliefs regarding the transmission and acquisition of disease. While great strides have been taken to treat and eradicate this disease, significant misinformation and stigma still exists, even in the United States. It is important that physicians caring for those with Hansen's disease educate their patients and address any stigma or misinformation they may have about the disease. Studies have shown high rates of psychiatric comorbidities in these patients, and addressing their mental health and social circumstances related to their diagnosis is an important part of their treatment.

7 Prevention

The National Hansen's Disease Program was instrumental in developing drugs active against *M. leprae* as well as research into multidrug regimens and the establishment of the armadillo as the animal model for disease. Until the 1940s, there was no effective treatment for Hansen's disease. Only with the discovery of dapsone precursors by Dr. Faget and subsequent use of it in patients with Hansen's disease (pioneered by Dr. Cochrane at NHDP) were patients able to be cured of the disease. Since that time, great strides in research and treatment have identified a highly effective regimen for treatment, which is the primary method to interrupt transmission. However, efforts in control and elimination worldwide continue due to stigma surrounding the disease, lack of access to care, and unknown factors about transmission. In the United States, physician awareness of the disease is low, which can lead to delayed diagnosis and long-term disability, and potentially transmission, although person-to-person spread in the United States is thought to be very low.

Furthermore, with zoonosis and possible environmental reservoirs contributing to transmission in the Southern United States, control becomes even more challenging. A 2011 study showed a single predominant strain of *M. leprae* (3I-2-v1) in armadillos in 5 southern US states, with >20% of armadillos infected in some locales [10]. This same strain was found in the majority of human patients living in an armadillo endemic area, therefore strongly supporting a zoonotic source of infection. This group then sampled armadillos in a four-state area (MS, GA, AL, FL) and found evidence of *M. leprae* infection in 16% of animals [22]. According to authors, before 2009, no *M. leprae* had been found in sampled armadillos from these areas. Also found in this study was that the majority of armadillos had the 3I-2-v1 strain, along with a newly discovered strain in armadillos, 3I-2-v15. These two genotypes were found in 22 patients in the study, while the other 30 patients had unique genotypes. Furthermore, the newly typed strain was only found in southern Florida where all ten patients with this genotype were located. Another molecular epidemiology study, in central Florida, showed 4/5 patients with the same armadillo-associated genotype [23]. While most patients denied known contact with armadillos, many studies have shown viability of *M. leprae* in soil as well as free-living ameba, suggesting a possible soil intermediary between armadillos and humans [24–27]. Therefore, potential environmental reservoirs in addition to the zoonotic reservoir present a significant challenge to control efforts. An increase in case reports and case series from these states, especially in Florida, in the past 5 years, show a concerning trend, in part likely related to the migration of *M. leprae*-infected armadillos [28–32]. A recent case report out of Canada even described a Canadian man with HD whose only travel was to Florida [12]. Lastly, in Georgia, while immigration and population may have contributed to the trend, Fig. 3 shows a pattern of increased cases in recent decades in both immigrants and US-born individuals [18].

8 Conclusion

While overall rare in the United States and Canada, this neglected and stigmatized infection continues to affect patients with a high toll of disability and morbidity. Total cases have not appreciably gone down in recent years, and there are signs that suggest incidence is increasing in some areas. The lack of physician training, a broad differential diagnosis of skin lesions, and the armadillo reservoir all pose challenges for effective control. More research on risk factors for infection, environmental reservoirs, and modes of transmission are crucial for lowering the impact of this complex infectious disease.

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Murine Typhus

Lucas S. Blanton

Abstract Murine typhus is caused by *Rickettsia typhi*, a small gram-negative obligately intercellular coccobacillus. The disease is endemic throughout the world—especially in tropical and subtropical seaboard regions. The bacterium is primarily maintained in rats and is transmitted to humans by *Xenopsylla cheopis* (the rat flea). In the United States, the majority of cases are reported in southern California and in Texas. Here, an alternate cycle of transmission is presumed to involve opossums and cat fleas (*Ctenocephalides felis*). Humans become infected when they inoculate *R. typhi*-infected flea feces into a flea bite wound or onto the mucous membranes. After inoculation, *Rickettsia typhi* infects endothelial cells to cause a systemic infection, which is characterized by fever, headache, malaise, and rash in half of patients. Frequent laboratory features include elevation in hepatic transaminases and thrombocytopenia. The symptoms are often severe enough to lead to hospitalization, prompt extensive medical workup when the diagnosis is unrecognized, and can occasionally cascade to severe manifestations (e.g., renal failure, pneumonitis, and encephalitis). Death occurs in less than 1%. Serology is the mainstay of diagnosis, but reactive antibodies are seldom detected during early illness. Serologic confirmation requires testing during convalescence, making the diagnosis retrospective. Therefore, early diagnosis and empiric treatment are based on clinical suspicion. Doxycycline, 100 mg (2.2 mg/kg in children) twice daily for 7 days is the treatment of choice.

Keywords Murine typhus · Endemic typhus · Flea-borne typhus · Rickettsia typhi · Typhus group rickettsiosis

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1 Introduction

Murine typhus is caused by *Rickettsia typhi*, a small gram-negative obligately intracellular coccobacillus. Also called endemic typhus, the disease is endemic throughout the world, especially in tropical and subtropical seaboard regions. In much of the world, rats act as the primary mammalian host, and the rat flea (*Xenopsylla cheopis*) acts as the vector [1]. Currently, in endemic areas of the United States, an alternate cycle of transmission involving opossums (*Didelphis virginiana*) and the cat flea (*Ctenocephalides felis*) is presumed [2]. As an undifferentiated febrile illness, murine typhus is often overlooked as a potential cause of disease. Once common in the United States, there was a dramatic decrease in cases following vector control programs after World War II [3]. In the last two decades, there has been a resurgence of murine typus in the United States. Throughout the world, it remains an underrecognized cause of acute febrile illness and can even pose a threat to the traveler [4]. Despite this, the disease remains poorly recognized by physicians, is underdiagnosed, and is without systemic efforts to curb its spread—a truly neglected tropical disease of North America.

1.1 History

The name typhus is derived from the Greek word "typhos," which means "smoky" or "hazy" and refers to the delirium or stupor often associated with epidemic louse-borne typhus, otherwise known as typhus. *Rickettsia prowazekii* is the causative agent of typhus and is transmitted from human to human by the body louse. It occurs in epidemics when hunger, famine, poverty, war, natural disasters, and imprisonment cause severe lapses in hygiene—the inability to bathe or launder clothing promotes infestations of the body louse vector (*Pediculus humanus humanus*) [5]. Murine typhus is less severe than epidemic typhus; nevertheless, clinically, it is very similar. Based on historical accounts, these two diseases are otherwise difficult to separate based solely on signs and symptoms. The delineation of murine typhus from the classic form of epidemic typhus is herein discussed in this section.

During an outbreak of severe febrile illness in Philadelphia, William W. Gerhard (being well versed in the pathology of typhoid fever from post graduate studies in Paris) made the distinction between typhus and typhoid fever—the latter causing characteristic intestinal lesions [6]. From 1896 to 1909, Nathan Brill described 221 patients with a febrile illness in New York resembling mild typhus. He was unable to identify evidence of person to person transmission or spread of epidemic proportion; he was therefore hesitant to commit to calling the disease typhus and referred to it as an "acute infectious disease of unknown origin" [7]. He later hypothesized that the disease was caused by an attenuated form of the typhus pathogen [8]. It soon became apparent that a disease similar to that described by Brill was endemic in Mexico, Texas, and the Southeast United States [9, 10]. The

disease, referred to as Brill's disease, endemic typhus, and Mexican typhus, appeared similar, albeit milder, than epidemic louse-borne typhus [11].

The experimental inoculation of guinea pigs (the classic animal model to characterize rickettsiae) with blood originating from those with Brill's disease and Mexican typhus revealed notable differences. Guinea pigs inoculated with the agent of classic epidemic typhus developed mild fever, whereas guinea pigs inoculated with the agent of Mexican typhus developed more pronounced fever and scrotal reaction (i.e., marked swelling, redness, petechiae, and after necropsy, gross hemorrhage of the cremasteric fascia) [12–14]. This intense reaction of guinea pig scrota after infection with *R. typhi* is later referred to as the Neil-Mooser or Mooser reaction. Blood from a woman with Brill's disease inoculated into guinea pigs failed to demonstrate the Neil-Mooser reaction. It was thus determined that Brill's disease was caused by the agent of epidemic typhus (*R. prowazekii*) [15]. Hans Zinsser would later determine that Brill's disease was caused by the recrudescence of epidemic typhus, which had been previously acquired in Europe prior to immigration to North America [16]. Thus, the recognition that epidemic typhus and murine typhus were from related but different pathogens took shape.

Unlike the epidemics that occurred with louse-borne disease, the endemic form (occurring in the Southeastern United States) was not associated with household clustering, body louse infestations, and occurred in warmer months – a quite different seasonality than that of "old world" louse-borne typhus, which occurred during the winter months. The sporadic occurrence of cases, the uneven distribution in communities, and the association with areas where foodstuffs were stored prompted Maxcy to hypothesize a link between a murine host and ectoparasite vector [17]. In Australia, Hone also recognized a typhus-like illness in those working around wheat processing facilities at the Port of Adelaide [18]. Isolation of the causative agent was carried out through guinea pig inoculation of triturated fleas (collected from rats and their nests) and the emulsified brains of rats (trapped in Savanah, Georgia, in approximation to human cases) [19, 20]. Rats were also implicated in studies conducted in Mexico City, the site of ongoing cases of what was then termed Mexican typhus (also known as tabardillo). Here, Herman Mooser demonstrated the bacterium to cause the aforementioned scrotal reaction in infected guinea pigs, typical for strains implicated as the cause of endemic typhus [21]. By this point, there are clear differences between the epidemiologic and ecologic differences of typhus group rickettsioses in North America. For further reading, an excellent description of the historical aspects of murine typhus involving its delineation from other entities, its ecology, and methods of control has been published [22].

1.2 Microbiology

Pathogenic rickettsiae are divided into three lineages—the spotted fever, typhus, and transitional groups [23, 24]. *Rickettsia typhi* is the etiologic agent of murine typhus

and is a member of the typhus group (*R. prowazekii* is the other member). It is a small (1.3 × 0.4 μm) obligately intracellular coccobacillus. As a result of reductive genome evolution (*R. typhi* has a 1.1-Mb genome) [25], rickettsiae lack the necessary enzymes for synthesis of nucleotides, lipid biosynthesis, and carbohydrate metabolism [26]. Thus, they rely on the host cell cytosol to provide the nutrients necessary for their survival. *Rickettsia typhi* infects the midgut epithelial cells of the flea vector; in the mammalian host, the organism infects endothelial cells. The organism possesses several surface cell antigen (Sca) proteins. The organism encodes for and expresses a variety of effectors that likely contribute to endosomal escape [27–29]. Unlike organisms in the spotted fever group, the *R. typhi* genome does not encode for the outer membrane protein A (*Sca0*) nor *rickA* [30]. The lack of *rickA*, important for the facilitation of actin polymerization and intracellular mobility, may explain the short circular movements of *R. typhi* within the cell [31]. Another bacterium, *Rickettsia felis*, is also found in fleas, but it does not belong to the typhus group. Rather, it is considered a transitional group organism with features that are difficult to classify into the traditional spotted fever or typhus groups [23, 32].

1.3 Pathogenesis

After rickettsiae are inoculated into the skin, the organism is targeted by macrophages and dendritic cells; they then spread through the lymphatics to regional lymph nodes before escaping hematogenously to infect endothelial cells throughout the body. As the endothelium is infected, a cascade of inflammatory events take place, enhanced by proinflammatory cytokines (e.g., interleukin-1β and tumor necrosis factor-α), which contribute to increased vascular permeability [33]. Using *Rickettsia conorii* as a model agent in an animal model for severe rickettsioses, infection leads to paracellular dysfunction through alternation in vascular endothelial cadherin proteins [34].

Lymphohistiocytic vasculitis can occur in any organ and is the hallmark of the clinicopathologic manifestations of murine typhus and are described in reported autopsies from fatal cases [35–37]. The vasculitic lesions within the microcirculation form the basis of the rash often characteristic for a rickettsiosis. Petechial lesions occur when heavily infected vascular networks extravasate blood in the center of a macule. Endothelial injury leads to activation of pathways for coagulation and fibrinolysis [38]. Infection of the hepatic sinusoidal and portal endothelium leads to hepatocyte damage, which manifests as elevated serum aminotransferases, but hepatic failure does not occur [39]. A subtle and usually clinically inconsequential manifestation includes choroidal vascular injury [40]. When extensive, vascular damage leads to extravasation of the intravascular fluid into the interstitium, resulting in hypovolemia, hypotension, and organ hypoperfusion. When severe, pulmonary edema, encephalitis, and acute kidney injury occur as a result of this process [33].

Host factors for severe or fatal rickettsial infection are thought to include advanced age, alcoholism, the use of sulfa-containing antibiotics during the course of illness, and underlying glucose-6-phosphate deficiency [41–43]. The mechanisms that underlie these observations are yet to be elucidated.

Dendritic cells, an early player during infection, are crucial in the formation of an immune response [44]. Early on, the innate immune system's natural killer cells are important for rickettsial control. Eventually, cellular responses via CD4 and CD8 T cells, as well as the formation of antibodies, play roles in clearance of the organism [45–47]. It is believed that durable immunity is established after infection.

2 Epidemiology

Murine typhus is endemic throughout the world, especially in tropical and subtropical seaboard regions. In North America, the disease occurs as the weather warms in April, peaks in June or July, and trails off by October [1]. The disease is especially prevalent in active port regions, where rats, the classic reservoir, are abundant [48]. The disease is likely vastly underrecognized as a cause of febrile illness and many cases are undoubtedly mistaken for a viral illness. Seroprevalence studies in areas where the disease is not recognized or has recently re-emerged suggest that murine typhus has been silently endemic [49–51]. It has been estimated that for every case of murine typhus diagnosed, there are an additional four cases that remain undiagnosed [3].

3 Transmission

Rattus rattus and *R. norvegicus* have evolved to live among humans; this intimate relationship essentially makes murine typhus a household zoonosis [1]. When infected, rats remain asymptomatic and rickettsemic for several weeks. The rat flea, *Xenopsylla cheopis*, acquires *R. typhi* when it ingests an infected bloodmeal. The bacterium then infects the midgut epithelial cells, is released into the gut lumen, and is shed through the feces [52]. Fleas are infected with *R. typhi* for life and are unaffected with regard to their lifespan and fecundity [53]. Maintenance is mainly horizontal; transmission occurs from infected flea to mammalian host to uninfected fleas. *Rickettsia typhi* can escape into the hemocele of the flea and infect the ovaries. Thus, the infection is also maintained, albeit at lower levels, via transovarial transmission to flea progeny [54]. Humans become infected through the inoculation of *R. typhi*-laden flea feces into flea bite wounds or onto mucous membranes [3]. Experimentally, *R. typhi* can also be inoculated through the flea bite, but this is thought to be a less efficient mechanism of transmission [55]. The bacterium remains stable in dried feces for several years. Thus, aerosolization of infected flea feces is also a proposed mechanism of human transmission [3].

The epidemiologic association with murine typhus and rats is well documented and helped distinguish murine typhus as a distinct entity from what otherwise appeared to be a milder form of epidemic louse-borne typhus. Murine typhus was once quite prevalent throughout the Southeast United States. It was even noted to shift from an urban disease to a rural disease with the change in peanut production over cotton, which contributed to the increased storage of foodstuffs promoting the infestation of rats in rural areas [56]. Campaigns to control the proliferation of rats around homes and businesses were implemented to help control spread [57]. The peak in reporting of murine typhus occurred in 1944, when 5401 cases were reported [58]. After World War II, dichlorodiphenyltrichloroethane (DDT), which was a highly effective insecticide, was used to target rat runs and rat harborages. Used in this manner, it effectively reduced the burden of *X. cheopis* infesting rats and interrupted the transmission cycle of *R. typhi* to humans [59, 60]. By 1956, less than 100 cases of murine typhus were reported in the country [58].

The cat flea (*Ctenocephalides felis*) is another important vector for *R. typhi*. After the precipitous decrease in reported cases in the United States following World War II, the disease remained at a low level of endemicity in parts of southern California and in the most southern counties of Texas [61]. In Los Angeles, where murine typhus had been an urban disease, reported cases shifted to the suburban foothills. Here, rats and rat fleas were not prevalent in areas around cases. Rather, opossums were found to have the presence of reactive typhus group antibodies, and *R. typhi* was isolated from a opossum's spleen [61]. Since then, opossums have been linked as a presumed amplifying host for *R. typhi* in areas where the disease remained endemic or has recently re-emerged [62–65]. Studies where the disease is ongoing reveal that opossums have a demonstrable seroprevalence to typhus group antibodies using a serologic cutoff titer of 1:128 (29% in Austin and 67% in Galveston) [62, 63]. The *R. typhi* infection rates of cat fleas collected from these animals are variable (less than 1% in Corpus Christi, Texas; 1.7% in Orange and Los Angeles Counties, California; and 7% in Galveston, Texas) [63, 64, 66]. In Texas, murine typhus has increased in prevalence and appears to be encroaching northward [67]. Whereas the majority of cases occurred in the lower Texas Rio Grande Valley and in the South Texas port city of Corpus Christi in the decades after the broad use of DDT [68], cases are again recognized in municipalities in other regions of the state (i.e., San Antonio, Austin, Galveston, and Houston) [50, 62, 69, 70].

The role of domestic animals, such as cats, to act as an amplifying host for *R. typhi* has been hypothesized, but the data to support a clear association are lacking. For example, cats have been epidemiologically linked to cases of murine typhus [68, 71, 72], and studies demonstrate a variable seroprevalence of typhus group antibodies in cats (0–46%) [65, 73–75]. In the experimental inoculation of cats with *R. typhi*, animals failed to become ill and did not maintain the organism for a length of time. The authors concluded that cats were not an efficient amplifying reservoir [71]. The ability for cat fleas to acquire *R. typhi* from experimentally inoculated domestic animals has not been reported. In Southern California, recent studies to investigate the *R. typhi* infection rate in fleas have failed to detect the agent

[74, 75]. In a study conducted in Texas, fleas from feral cats demonstrated *R. typhi* in 0.3% compared to 7.0% of fleas collected from opossums [63, 76, 77].

4 Clinical Manifestations

Overall, murine typhus is an undifferentiated febrile illness with signs and symptoms that are often indistinguishable from a variety of other infections. In those identified as having discrete exposure, the incubation period ranges from 4 to 15 days (mean of 10.4) [78]. The onset of illness is usually abrupt [79]. The clinical manifestations of murine typhus have been documented from numerous case series and have been recently summarized in a systematic review [80]. In addition to fever, other frequently reported early manifestations include headache (81%), malaise (67%), and myalgias (52%). Anorexia (48%), nausea/vomiting (27%), diarrhea (19%), and abdominal pain (18%) also occur. When these manifestations are severe or predominate over others, these symptoms can mimic primary gastrointestinal syndromes. Hepatomegaly and splenomegaly are detected on exam in 22% and 17%, respectively [80].

Rash is often believed to be a distinguishing feature of those with a rickettsial illness. Indeed, in severe rickettsioses, such as Rocky Mountain spotted fever, rash is present in 90% at some point during the course of illness. In those with murine typhus, rash is noted in only about half of all patients [80] and seems to be documented at a higher frequency in those with lightly pigmented skin. In a series from New Orleans (compiled from cases diagnosed from 1929 to 1944), the proportion of Caucasians with rash was 81% versus 20% in African Americans [78]. In Texas, where the population also includes a great number of Hispanics, rash is found in 43–54% of patients from data collected statewide [67, 81]. The rash of murine typhus is usually faint and pinkish in coloration and consists of 2–5 mm macules [78]. Papular lesions are also seen [81, 82]; petechiae occur less often (13%) [82]. The rash classically starts on the trunk and later spreads peripherally to the limbs [78]. Throughout the course of illness, rash is noted on the trunk in 88% and the extremities in 45% of cases with rash. The face, palms, and soles are infrequently involved [81]. An eschar or inoculation lesion, as often associated with spotted fever group rickettsiae (e.g., *R. conorii*, *R. parkeri*, *R. africae*) [83], has been described in only a single person infected with *R. typhi* [84].

Although most recover uneventfully, when the disease is not recognized and treated in a timely manner, disease can progress to include more severe manifestations. This occurs as a result of pronounced *R. typhi*-induced endothelial injury causing extravasation of intravascular fluid into the interstitium, which manifests as end organ damage. Pulmonary involvement manifests as cough, which occurs in 27% of patients [80] and is usually described as dry or nonproductive. Radiographic infiltrates occur in 17%, and acute respiratory distress syndrome has been reported [85]. Renal manifestations also occur. Acute kidney injury is generally due to hypovolemia associated prerenal azotemia, and when accompanied by hypotension,

progression to acute tubular necrosis may follow [86]. Occasionally, renal replacement therapy is required, but recovery of kidney function usually follows [43, 87–89]. A variety of severe neurologic manifestations have been reported. Even headache, the most common neurologic symptom, is severe, often described as the worst of one's life, recalcitrant to symptomatic treatments, and if effective antibiotics are not administered, the headache remains present throughout illness [78, 79]. In those hospitalized, severe manifestations such as stupor (4–16%), confusion or delirium (8%), nuchal rigidity (6%), seizures (4%), coma (2%), and ataxia (1%) have been reported [78, 81]. Cerebrospinal fluid analysis, when performed, are often normal. When indicative of meningitis or meningoencephalitis, the fluid is clear with normal glucose concentration, elevated protein concentration, and pleocytosis (usually lymphocytic) [90]. Although very infrequent, cranial nerve palsies, short- and long-term cognitive deficits [91–93], and status epilepticus [36, 94] have been reported. Of those requiring hospitalization, 6% are ill enough to require intensive care [80]. The case fatality rate in the antibiotic era is approximately 0.4% [41], but in those ill enough to be hospitalized, it can approach 4% [81].

4.1 Laboratory Features

There are many hematologic and biochemical abnormalities that may be noted on frequently obtained laboratory testing. The most common laboratory feature is mild elevation in hepatic transaminases (79%), which are related to hepatocellular injury. Occasionally, the liver enzymes are markedly increased by a magnitude of 4 times the upper limit of normal (27%). Other markers of hepatic and/or general cellular injury include elevations in lactate dehydrogenase (73%), alkaline phosphatase (41%), and creatine kinase (29%) [80]. Dramatic elevations in the creatine kinase to indicate rhabdomyolysis have been reported [95]. As a result of *R. typhi*-induced endothelial injury, hypoalbuminemia (60%), hypoproteinemia (45%), and hyponatremia (35%) may occur [80, 81]. The latter is likely related to the secretion of antidiuretic hormone secondary to intravascular volume depletion [96]. The most common abnormality on the complete blood count is thrombocytopenia (42%), with anemia (38%), leukopenia (24%,) and leukocytosis (18%) following [80]. Leukopenia is more likely to appear during early illness, and leucocytosis is more likely to appear late [78]. Elevations in the prothrombin time have been recorded [81]. Although cases of disseminated intravascular coagulation (DIC) complicating murine typhus have been reported [97–99], full descriptions to support solid evidence of DIC (i.e., bleeding, platelet consumption, coagulation factor consumption, and fibrinolysis) are lacking.

5 Diagnosis

5.1 General Principles

The most important aspect of diagnosing murine typhus is knowledge and recognition of the syndrome as a cause of undifferentiated febrile illness. Unfortunately, the differential diagnosis for such a syndrome is quite extensive (see paragraph below). Although rash is the sign that often prompts a diagnostics consideration of a rickettsial disease, it is important to note that rash is absent in about half of cases. Knowledge of the epidemiology is important, as those who reside in locations where there are rat infestations or those who have noted opossums in and around their property may be at risk. Those recognizing exposure to fleas or flea bites are variable and cumulatively (from studies worldwide) are only reported by 23% [80]. In contrast, reports from Texas, using data collected from epidemiologists at the Texas Department of State Health Services, report up to 55% had fleas present in their environment or had experienced flea bites prior to the diagnosis of murine typhus [67, 100]. Laboratory abnormalities, such as elevated hepatic transaminases, thrombocytopenia, and hyponatremia, are diagnostic clues, but they are not specific. There is no sensitive rapid point of care test for the diagnosis of murine typhus during the acute stages of illness. When those with murine typhus have been compared to age- and gender-matched controls with influenza—an illness diagnosed with rapid testing—those with murine typhus (in spite of a severity of illness that was similar by a clinical scoring scheme) had more visits to a physician, increased hospitalizations, and increased healthcare charges [101]. Thus, there is a great need for improved diagnostics and clinical awareness. The testing most widely available to clinicians—serology—has important caveats, which are discussed below. When the diagnosis is suspected, treatment should not await the results of diagnostic testing.

The differential diagnosis includes but is not limited to other rickettsioses (e.g., epidemic and sylvatic typhus, scrub typhus, rickettsial pox, and spotted fever group rickettsioses), ehrlichiosis, meningococcemia, disseminated gonococcal infection, typhoid fever, leptospirosis, relapsing fever, secondary syphilis, endocarditis, viral and other bacterial causes of meningitis, measles, rubella, roseola, enteroviral infections, mononucleosis (e.g., Epstein-Bar virus and cytomegalovirus infections), acute HIV infection, dengue fever and other mosquito-borne viral infections, drug eruptions, toxic shock syndrome, thrombotic thrombocytopenic purpura, immune thrombocytopenic purpura, vasculitides, and Kawasaki disease [102].

5.2 Culture

As an obligately intracellular organism, *R. typhi* requires host cells to survive. The organism cannot be cultured axenically on the usual cell-free media used for typical

bacteria. Culture is rarely undertaken, as it requires specialized techniques and expertise. Laboratory isolation requires the use of embryonated eggs or cell culture techniques using antibiotic free medium. The latter can be accomplished via shell vial systems where plasma is inoculated onto the vial's monolayer, centrifuged to promote rickettsial contact with the monolayer, and incubated at 32–34 °C with 5% CO_2. When coupled with shell vials containing a monolayer-covered glass coverslip, the removal, fixation, and visualization using Gimenez staining or immunofluorescence of the material on the coverslip can reveal rickettsiae. When immunofluorescence is used, visualization of the organism can be seen within 72 h [103]. A modification of the shell vial technique using 24-well plates is also effective for the isolation of rickettsiae [50, 104]. As a small organism, there is risk of infection via aerosolization of *R. typhi* during laboratory manipulation and propagation. Therefore, biosafety level 3 laboratory conditions are necessary to safely cultivate the organism.

5.3 Serology

As with other rickettsial diseases, serology remains the mainstay of diagnosis for murine typhus. Assays to detect antibodies to rickettsiae are indeed the most widely available for clinical use. It is important to note that antibody detection is not useful during the early stages of disease. Reactive antibodies are rarely detected within the first few days of illness, which is when patients are likely to first present to a physician. Antibodies are usually detected later in illness or during convalescence. In a study of murine typhus in children, antibodies were detected in 15% and 62% during the first 7 and 14 days of illness, respectively [105]. In another study, 50% had reactive antibodies within a week of symptom onset, and by day 15, all had the presence of antibodies [81]. Detection of IgM does not occur appreciably sooner than IgG. IgM also has less antigen-binding specificity than IgG and is therefore prone to nonspecific reactions. As in the case of spotted fever group rickettsioses [106], the utility of IgM testing for murine typhus is questionable. Needless to say, a serologic diagnosis is retrospective, and the decision to empirically treat should not await the results of laboratory testing.

To confirm the diagnosis, sera obtained during the acute and convalescent phases of illness should be obtained. A titer of 1:64 is generally considered reactive. As similar to what is discussed in guidelines for the diagnosis and management of tick-borne rickettsial diseases, seroconversion or fourfold rise in titer from acute- and convalescent-phase specimens is diagnostic [102]. A single reactive titer of 1:256 obtained during the course of illness is highly suggestive of murine typhus. In endemic regions, there may exist a baseline seroprevalence that limits the utility of a single reactive specimen, as reactive antibodies persist for some time (the median titer 1 year after diagnosis has been reported to be 1:800) [107]. Unfortunately, regional seroprevalence and optimal titer cutoff data are seldom known [108]. Obtaining sera during convalescence is often difficult, as diagnostic

confirmation may not be perceived as important to the clinician, may not be relevant to the patient who has clinically recovered, and may be limited by lack of insurance or ability to take time from work for follow-up health care. Furthermore, in states where murine typhus is reportable to local health authorities, limitations in resources and manpower may limit the pursuit of confirmatory diagnoses in suspected cases. Indeed, where the disease is most prevalent in the United States (the most southern counties of Texas), few patients attributed to have murine typhus meet rigorous case definitions (e.g., seroconversion or fourfold increase in antibody titer) [95].

The indirect immunofluorescence assay (IFA) has long been considered the test of choice for the detection of antirickettsial antibodies [109]. IFA slides contain all the rickettsial protein antigens and lipopolysaccharide antigen to provide group specific serology [76, 77]. Typhus group serology, including IFA, is unable to differentiate between illness caused by *R. typhi* versus *R. prowazekii*. Cross-absorption techniques have differentiated serologic reactions between those with murine and louse-borne epidemic typhus, but these methods are cumbersome and available only in the research setting [110]. Fortunately, epidemiologic clues are often sufficient to differentiate the two diseases. Occasionally, reactivity occurs against both typhus and spotted fever group antigens, but titers to group specific antigens are generally several fold higher [111]. IFA requires a trained microscopist—both interobserver and intraobserver variability has been documented in the interpretation of endpoint titers [112]. The indirect immunoperoxidase assay has been adapted to yield similar results to the IFA [113]. This modification is advantageous in some parts of the world, as it uses a normal light microscope, rather than requiring a more expensive fluorescence microscope.

Murine typhus serologic testing can also be performed via the enzyme-linked immunosorbent assay (ELISA), which is commercially available. ELISA is more amendable to automated processing and removes the possible error of an inexperienced microscopist, but as performed in a clinical diagnostic laboratory, it does not provide a titered result. These assays have the same limitations as IFA when not used with paired specimens [114]. Latex agglutination and compliment fixation testing are no longer in use. The Weil-Felix test, an agglutination assay using the OX-19 and OX-2 strains of *Proteus vulgaris*, was historically used for the detection of antirickettsial antibodies, but it has poor sensitivity and specificity compared to contemporary serologic tests [115, 116].

5.4 Immunohistochemical Detection

The diagnosis of various rickettsioses, including murine typhus, can be accomplished via the direct immunohistochemical detection of the rickettsiae in formalin fixed, paraffin-embedded sections of infected tissue. The technique uses antibodies specific to typhus group lipopolysaccharide. In the case of murine typhus, skin biopsy specimens of rash lesions could offer a method to obtain confirmatory diagnosis during acute illness [84]. Although the sensitivity and specificity of

immunohistochemistry (IHC) for Rocky Mountain spotted fever is 70% and 100%, respectively [117, 118], the performance characteristics of IHC for murine typhus are unknown. IHC has also demonstrated typhus group rickettsiae within tissues obtained postmortem [37, 119]. Unfortunately, since IHC is performed in only a few reference laboratories, the technique is not readily available to provide results with a turnaround time helpful for clinical decision making.

5.5 Molecular Detection

Conventional, nested, and real-time quantitative polymerase chain reaction (PCR) assays to a variety of gene targets (e.g., *rrs*, *gltA*, *sca5*, *htrA*) have been developed to detect rickettsiae from a variety of sources. In the research setting, conventional and nested assays produce amplicons that are large enough to help establish a species-specific diagnosis when sequenced [76, 77]. Real-time quantitative PCR (qPCR) has excellent analytic sensitivity and can detect less than 10 copies of target genomic DNA [120, 121]. When coupled with multiplexed primers and species-specific probes, qPCR can offer a diagnosis on the species level [122, 123]. *Rickettsia typhi* has also been detected from the plasma of two pregnant women using next generation sequencing technology [124]. The detection of nucleic acids for the detection of rickettsiae has been performed on whole blood, buffy coat, plasma, and tissue (fresh, frozen, or formalin-fixed paraffin-embedded tissue). Although PCR has the ability to detect small quantities of DNA, molecular detection of *R. typhi* from clinical specimens is challenging. In the most easily obtained specimen for PCR analysis, whole blood, there are very few circulating organisms to yield positive results. Hence, the clinical sensitivity of PCR is poor—a systemic analysis reveals a median sensitivity of 3% from blood and 6% from tissue [125].

6 Treatment

6.1 General Principles

The use of effective antimicrobials in those with murine typhus leads to swift recovery. After a few doses of doxycycline, people often feel much improved. Those suspected of murine typhus should receive prompt empiric therapy. Timely therapy leads to decreased length of hospitalization [126] and would likely decrease expenditure of healthcare costs associated with excessive workup [101]. As discussed in the sections below, antibiotics with activity against rickettsiae are relatively specific. Many antibiotics commonly used empirically in outpatient clinics have no in vitro activity against organisms in the genus *Rickettsia* (e.g., penicillins, aminopenicillins, cephalosporins, and sulfonamides) [127]. The use of sulfonamides, such as trimethoprim-sulfamethoxazole, have been associated with

progression of severe disease with other rickettsioses (e.g., Rocky Mountain spotted fever and Mediterranean spotted fever) [128]. It should be noted that antimicrobial susceptibility testing for *R. typhi* and other *Rickettsia* spp. is not as standardized nor as well validated as those for typical bacterial pathogens. Susceptibility testing has been carried out in various cell culture systems and in embryonated eggs.

6.2 Tetracyclines

Tetracyclines are the preferred agents for all rickettsioses, including murine typhus. Agents in this class include tetracycline hydrochloride, doxycycline, and minocycline. The minimum inhibitory concentration (MIC) of typhus group organisms to tetracyclines are 0.06–0.25 μg/mL [127]. Prior to an open-label randomized controlled trial (RCT) to compare azithromycin to doxycycline [129], there were no comparative trials demonstrating the efficacy of tetracyclines for the treatment of murine typhus. Despite the prior lack of controlled data, there is a wealth of clinical experience documented through observational studies. This extensive experience has been summarized in a monograph, which analyzed over 600 cases compiled from the literature [130]. In the aforementioned RCT, the median time to defervesce while on doxycycline was approximately 36 h [129].

Tetracycline hydrochloride is no longer readily available in the United States, as it is seldom used in favor of its newer congeners. With a relatively short half-life, it is taken four times daily and causes dyspepsia and nausea, and its absorption is inhibited by food, which would otherwise help ameliorate the gastrointestinal symptoms. The preferred agent is doxycycline, which is given less frequently and can be taken with food. The usual adult dose of doxycycline is 100 mg oral or intravenous twice daily. As a bioavailable agent, the oral formulation is generally adequate, but when nausea and vomiting preclude its reliable absorption, the intravenous formulation should be used [131]. A 200 mg loading dose, followed by normal dosing, should be considered during severe illness. The usual course of treatment is 7 days. Minocycline is similar to doxycycline and appears to be as effective for rickettsioses. The drug has been used extensively in Japan, where Japanese spotted fever is endemic [132] and was used successfully for murine typhus during a national shortage of doxycycline [50]. To avoid pill esophagitis, oral tetracyclines should be taken with enough water to allow complete transit to the stomach [131].

During pregnancy, doxycycline is still the preferred agent. Although older generation tetracyclines were associated with hepatotoxicity and pancreatitis in pregnant women [133] as well as the possible teratogenic effects on the developing fetus (e.g., abnormal bone development and staining of developing teeth) [134, 135], doxycycline does not seem to be associated with these risks. A systematic review noted a relative lack of adverse events in pregnant women who took doxycycline [136]. Considering the possible adverse effects or ineffectiveness of alternative agents (see below), the risks to the fetus and mother related to infection should be weighed

against the risks associated with doxycycline [137]. Doxycycline is also the treatment of choice for children with murine typhus [138]. Clinicians are often hesitant to prescribe doxycycline to children under 8 years of age, as long or repeated courses of older tetracyclines stained developing permanent teeth. Fortunately, children receiving short courses of either tetracycline or doxycycline have no notable difference in the shade of erupted permanent teeth [139, 140]. In children, doxycycline is dosed at 2.2 mg/kg oral or intravenous twice daily (maximum of 100 mg per dose) [138]. The duration of treatment is 5–7 days.

6.3 Alternative Agents

Chloramphenicol is generally recognized as an effective alternative for the treatment of murine typhus and other rickettsioses, but documented experience with chloramphenicol, as reported in the literature, is less than that of tetracyclines [130]. The MIC of typhus group rickettsiae to chloramphenicol is 1–2 μg/mL. It should be noted that in the most severe rickettsiosis, Rocky Mountain spotted fever, those treated with chloramphenicol have a higher case fatality than those treated with a tetracycline (7.6% versus 1.5%) [141]. In a retrospective analysis of various regimens used to treat murine typhus, the mean time to defervesce on chloramphenicol was 4.0 days compared to 2.9 days on doxycycline [142]. Chloramphenicol is administered to adults at 500 mg, oral or intravenous, every 6 h. The dose for children is 12.5 mg/kg, intravenous, every 6 h. The drug is still available in much of the world, but in the United States, oral chloramphenicol is not available, and the intravenous formulation is very difficult to procure. Where available, the benefits of chloramphenicol must be carefully weighed against potential risks (i.e., aplastic anemia).

Fluoroquinolones, such as ciprofloxacin and levofloxacin, have also been used as alternative agents. The MIC of typhus group species to tested fluoroquinolones (i.e., pefloxacin, ofloxacin, ciprofloxacin) are 0.5–1 μg/mL [127]. Controlled trials studying these agents in mild Mediterranean spotted fever (caused by *R. conorii*) have demonstrated their efficacy for a spotted fever group rickettsiosis. There are no prospective trials evaluating these agents in murine typhus. In a retrospective analysis, the mean time to defervescence on ciprofloxacin was found to be rather long—4.2 days [142]. The usual adult dose of ciprofloxacin is 500 mg twice-daily oral or 400 mg twice-daily intravenously. Levofloxacin is given at 500 mg, oral or intravenous, once daily. Both agents have excellent bioavailability and only need to be given parenterally if adequate absorption is compromised due to vomiting or critical illness. The successful use of moxifloxacin has been reported in a single patient [143].

Anecdotal reports of the successful and unsuccessful use of azithromycin have been reported. With an apparent in vitro effectiveness (MIC of 0.1 μg/mL) [144], excellent bioavailability, high intracellular concentration, and its safety in pregnancy, azithromycin seemed a promising alternative; but an RCT of azithromycin failed to clearly demonstrate its clinical effectiveness compared to doxycycline. In

this study, a 3-day course of azithromycin was compared to a 3- and 7-day course of doxycycline. Azithromycin use was associated with more treatment failures (22.5%) (defined as continued fever after 72 h of treatment without clinical improvement or development of severe disease) than both the 3- and 5-day doxycycline arms (4.2% and 1.4%, respectively) ($P < 0.001$). The median time to clear the fever while on therapy was also longer with azithromycin (48 h) compared to 3- and 5-day courses of doxycycline (36 and 34 h, respectively) ($P = 0.002$) [129].

7 Prevention

There is no vaccine available for the prevention of murine typhus, but natural infection likely confers durable immunity. It is believed that this immunity protects from re-infection. The primary method of prevention is the control of potential flea hosts and flea vectors of *R. typhi*. As previously discussed, the use of DDT on rat runs and rat harborages to control *X. cheopis* in the 1940s made a tremendous impact on the incidence of murine typhus in the United States [3, 58]. Although campaigns to control rats were in place for some time in the years prior to the widespread use of DDT, it seems that effective flea control was able to effectively break the cycle of transmission to humans—marking an excellent example of the use of vector control to curb disease [59, 60]. In at least one study, DDT had no effect on the infestation of fleas on opossums [145]. These animals feed on a variety of food, including ripe fruit and food intended for outdoor domestic animals. To prevent these animals from foraging around homes, homeowners should avoid leaving uneaten pet food outdoors and remove fruit fallen from trees. In areas were the suburban cycle of *R. typhi* transmission is present, integrated pest control management for the control of fleas on opossums might be of use to decrease the incidence of murine typhus. Although there are no studies supporting the control of fleas on domestic animals to prevent the transmission of *R. typhi*, it is reasonable to believe that this approach is useful. The primary flea parasites for cats and dogs in North America is *C. felis*, which is a vector of *R. typhi* [1, 146]. There are many cutaneous and orally administered anti-flea products available commercially that are effective in killing fleas and preventing re-infestation, thus decreasing these animals' ability to act as a bridge between *R. typhi*-infected fleas and humans.

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