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Transmission and Epidemiology of Zoonotic Protozoal Diseases of Companion Animals

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SUMMARY

Over 77 million dogs and 93 million cats share our households in the United States. Multiple studies have demonstrated the importance of pets in their owners' physical and mental health. Given the large number of companion animals in the United States and the proximity and bond of these animals with their owners, understanding and preventing the diseases that these companions bring with them are of paramount importance. Zoonotic protozoal parasites, including toxoplasmosis, Chagas' disease, babesiosis, giardiasis, and leishmaniasis, can cause insidious infections, with asymptomatic animals being capable of transmitting disease. *Giardia* and *Toxoplasma gondii*, endemic to the United States, have high prevalences in companion animals. *Leishmania* and *Trypanosoma cruzi* are found regionally within the United States. These diseases have lower prevalences but are significant sources of human disease globally and are expanding their companion animal distribution. Thankfully, healthy individuals in the United States are protected by intact immune systems and bolstered by good nutrition, sanitation, and hygiene. Immunocompromised individuals, including the growing number of obese and/or diabetic people, are at a much higher risk of developing zoonoses. Awareness of these often neglected diseases in all health communities is important for protecting pets and owners. To provide this awareness, this review is focused on zoonotic protozoal mechanisms of virulence, epidemiology, and the transmission of pathogens of consequence to pet owners in the United States.

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INTRODUCTION

There are over 77 million dogs and 93 million cats in our households in the United States alone. Approximately 62% of households have at least one pet, and over half of these households have multiple pets (1). Various studies have demonstrated the importance of pets in overall health and well-being and for providing social support (2–5). Consistent with this devotion to pets, owners in the United States spend approximately $10.94 billion annually on pet supplies and over-the-counter pet medications and $14.11 billion annually on veterinary care (1). Given the number of companion animals in the United States and the bond with their owners, awareness and prevention of the zoonotic diseases of our companions are of paramount importance. Protozoal diseases, such Chagas' disease and leishmaniasis, are insidious, with large numbers of asymptomatic animals being able to transmit disease. *Giardia duodenalis* and *Toxoplasma gondii*, endemic to the United States, have high prevalences in companion animals (6, 7) (Fig. 1). *Leishmania* species and *Trypanosoma cruzi* are regional and have low prevalences in the United States but are significant sources of human disease worldwide and are reemerging and expanding their geographic distribution in companion animals in the United States (8, 9). Thankfully, we are generally protected by intact immune systems, and our health is bolstered by good nutrition, sanitation, and hygiene, but immunocompromised individuals, including the growing number of obese and/or diabetic individuals, in the United States are at a much higher risk of developing any zoonosis (10, 11). As such, an awareness of these often neglected diseases in veterinary and human health communities is important for protecting pet health and preventing human disease. In this article, we review mechanisms of virulence, epidemiology, transmission, and clinical signs of zoonotic protozoal pathogens of consequence to pet owners in the United States.

TOXOPLASMOSIS

Life Cycle and Mechanisms of Virulence

Toxoplasma gondii has a high prevalence globally and is capable of infecting all species of animals and birds (12). Definitive hosts for *T. gondii* are members of the family *Felidae* (Fig. 2) (12, 13). Felids are the only animals capable of shedding oocysts in their feces and transmitting the parasite by this means. In other host species, the ingestion of infective oocysts from cat feces or contaminated soil, water, or other materials can lead to the formation of tissue cysts that are infective via the secondary consumption of infected tissues (12, 14). Oocysts are shed in large numbers by acutely infected cats once for approximately 2 weeks, except in cases of feline immunosuppression, such as coinfection with feline immunodeficiency virus (FIV) or feline leukemia virus (FeLV), which can result in secondary shedding (15). After shedding, parasite sporulation takes place in 1 to 5 days, providing infective oocysts (13, 16). *T. gondii* rapidly excysts within the environment of the intestine, dependent upon temperature, pH, bile salts, and trypsin, developing into the highly infective tachyzoite form (17, 18). Cellular infection is rapidly established, resulting in bradyzoite-containing tissue cysts (Fig. 3) (17, 18). The consumption of infected tissue or fecal material by naïve, primarily young, felines results in their infection and subsequent shedding of infectious oocysts (19). People become infected through the accidental consumption of feline fecal material, through food or water with fecal contamination, through the consumption of undercooked meat

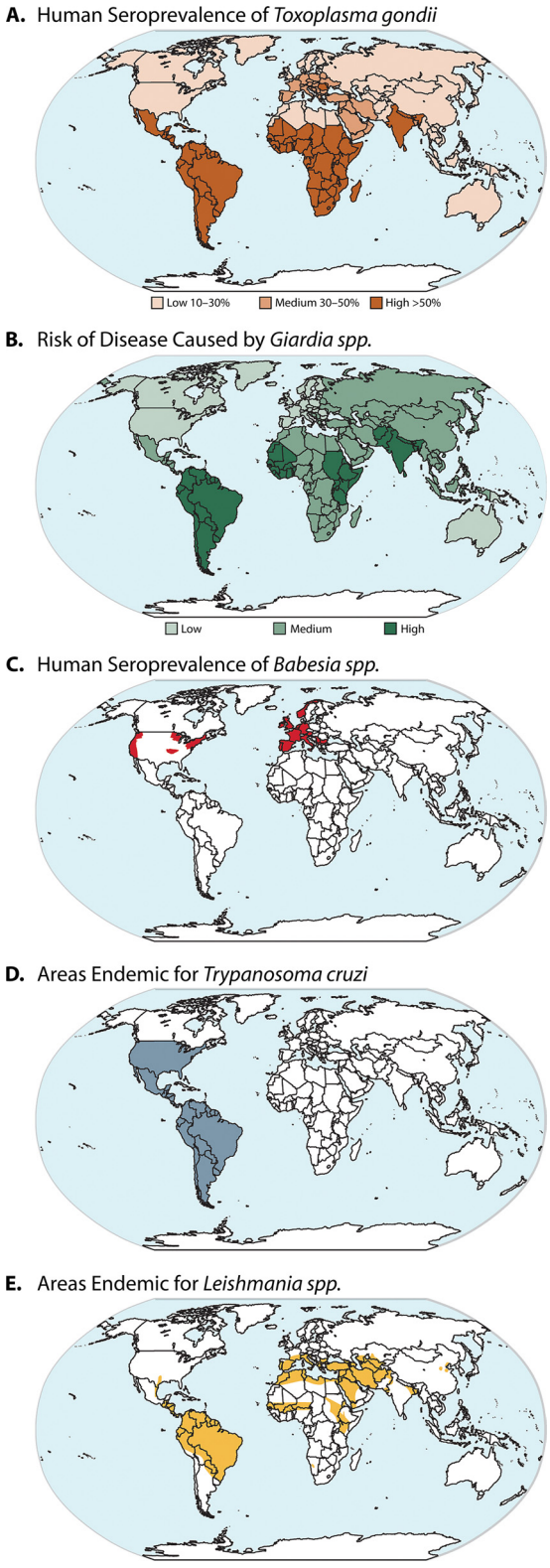

FIG 1 Global burden of zoonotic protozoal disease in humans. (Panels D and E are adapted from references 349 and 350, respectively, with permission of Elsevier.)

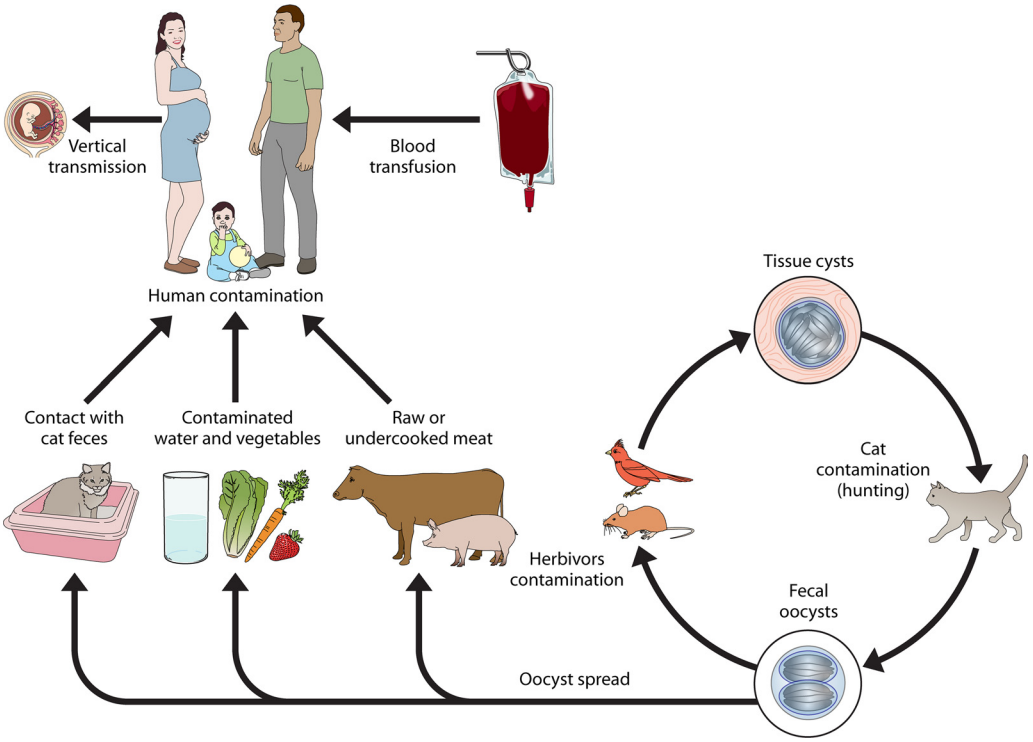

FIG 2 *Toxoplasma gondii* life cycle. Domesticated and wild cats are the definitive hosts of *Toxoplasma gondii* and become infected after the consumption of animals containing infective tissue cysts. Fecal oocysts are shed in large numbers by acutely infected cats for approximately 2 weeks. After shedding, parasite sporulation into infective oocysts takes place in 1 to 5 days. The ingestion of oocysts by other species leads to the formation of tissue. *T. gondii* rapidly excysts within the intestine, developing into the highly invasive tachyzoite form. Cellular infection results in bradyzoite-containing tissue cysts.

containing infective cysts, through transplantation, or transplacentally from mother to fetus (7, 20). Cases of congenital infection and human immunodeficiency virus (HIV) coinfection cause the most serious morbidity, resulting in severe neurologic and ocular diseases or miscarriage (21). *T. gondii* tachyzoite virulence is dependent upon multiple parasite factors, including those necessary for motility, cellular invasion, and immune evasion.

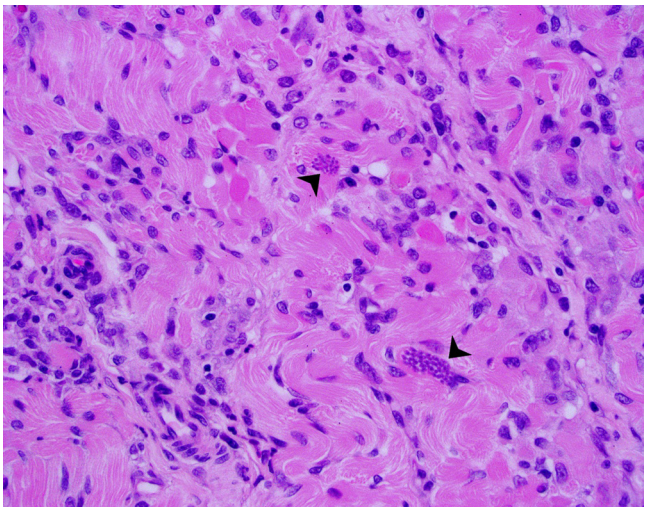

FIG 3 Canine toxoplasmosis. This section of canine skeletal muscle contains numerous lymphocytes and macrophages with myofibrillar necrosis and fibrosis and a myriad of *Toxoplasma gondii* tachyzoites (arrowheads) confirmed by immunohistochemistry (magnification, ×40). (Reproduced from a slide by Alexandria University, Department of Veterinary Pathology, Egyptian Society for Comparative and Clinical Pathology, Alexandria, Egypt, from the Armed Forces Institute of Pathology Wednesday Slide Conference 2007-2008, Conference 8, Case 2.)

T. gondii motility and cellular invasion were thoroughly reviewed by Carruthers and Boothroyd (18). A brief synopsis is provided here. *T. gondii* locomotion requires linear myosin, F-actin filaments, and gliding-associated proteins anchored between the plasma membrane and the inner membrane complex (17, 18). Invasion and the formation of a parasitophorous vacuole (PV) occur through apical parasite polarization and the adhesion of micronemal proteins and apical membrane antigen 1 (AMA-1) (18). Rhoptry proteins, which complex with AMA-1, are expelled by the parasite to form a moving junction, which migrates along the parasite surface during invasion to envelope the parasite in a PV (18).

After invasion, *T. gondii* utilizes multiple mechanisms of immune evasion to facilitate parasite survival and persistence within the host. The interaction of *T. gondii* and the immune system was thoroughly reviewed by Lang et al. (22). One mechanism of evasion is via rapid cellular invasion by *T. gondii*, which minimizes parasite exposure to host complement and antibodies. The moving junction and the incorporation of the host cell membrane into the PV membrane create an immune-privileged site for the parasite, devoid of transmembrane proteins and inhibited from fusion with phagolysosomes (23). Once within the PV, *T. gondii* continues its stealth-like state by inducing the production of the anti-inflammatory cytokines interleukin 10 (IL-10) and transforming growth factor beta (TGF-β), inhibiting the production of the pro-inflammatory cytokines interleukin 12 (IL-12) and tumor necro-

sis factor alpha (TNF-α), and slowing its recognition by blocking major histocompatibility complex class II (MHC-II) expression (24–28). Apoptotic body mimicry through the parasite surface expression of phosphatidylserine may facilitate the production of TGF-β and the degradation of induced nitric oxide synthase (iNOS) (29). Additionally, *T. gondii* maintains a favorable host cell state by inhibiting the programmed cell death of infected cells and inducing leukocyte apoptosis (30–32). In a fascinating virulence approach to maintain primary host infection, *T. gondii* induces behavioral changes in infected rats, causing an altered avoidance of cats and increased signaling in reproductive regions of the medial amygdala, which result in an attraction to cat urine, increasing the likelihood of feline consumption and subsequent feline infection (33).

Epidemiology and Transmission Dynamics

Felines are very susceptible to *T. gondii* infection; a single bradyzoite from a tissue cyst can cause the establishment of infection and the subsequent shedding of millions of oocysts (13). Cats are, however, significantly less susceptible to infection through the ingestion of oocysts (13). Oocysts are highly infective to most nonfeline mammalian hosts, indicating adaptations for fecal-oral transmission in these species, including people (13). Cats are the only species demonstrated to actively shed oocysts. However, coprophagic animals, including our "best friends," dogs, can transport and subsequently appear to shed *T. gondii* oocysts (34). Cats are generally thought to actively shed oocysts only after an initial exposure, but immune-suppressive conditions caused by comorbidity or immunosuppressive therapy can result in shedding a second time in young cats after reexposure (15). Seroprevalence rates in U.S. cats vary by location, ranging from 18 to 80%, primarily dependent on climate, with higher seroprevalences in more humid regions (14). The estimated worldwide prevalence of feline infection with *T. gondii* is 30 to 40% (14).

The feeding of undercooked or raw-food diets to cats has been associated with an increased risk for infection with *T. gondii* (35). Cats with outdoor access and those from rural areas, with predation as a main food source, are more likely than indoor cats to be seropositive, with rates of 39% in outdoor cats versus 26% in indoor-dwelling cats in one study and up to 69% in rural outdoor cats (35).

Diagnosis in cats is difficult, especially as a means to predict human exposure. The shedding of fecal oocysts is transient and generally occurs only once in the life of a cat, temporarily after the primary exposure. Therefore, testing via fecal flotation or centrifugation concentration has a poor sensitivity for the diagnosis of feline infection (14, 35). Serologic testing is equally poor as a predictor of possible human exposure, as seroconversion occurs after active infection and shedding (14, 35). Due to limitations in the diagnosis and prevention of *T. gondii* in cats and its extensive distribution, the prevention of human infection is targeted toward risk avoidance.

Human exposure to *T. gondii* occurs frequently, with an estimated serologic prevalence of 9.0% in the United States in persons 6 to 49 years of age, based on 1999-2004 National Health and Nutrition Examination Survey (NHANES III) data (36), although the rate of *T. gondii* serologic positivity decreased from 14% in 1998 to 9% in 2004 (36). In other areas of the world, the serologic prevalence of *T. gondii* is much higher, ranging between 8 and 50% (37, 38), where again, serologic prevalence in humans is closely associated with variability in climate. *T. gondii* exposure occurs commonly throughout the world and across economic and social strata.

Human exposure occurs secondary to exposure to fecal oocysts present in feces of *T. gondii*-infected cats through many routes, including contaminated water or food, contaminated soil (gardening and sandboxes, etc.), the cleaning of litter boxes, or the consumption of improperly cooked or processed meats, dairy products, or shellfish (7). Shellfish have become infected as filter feeders exposed to contaminated water containing cat feces (7). Off the coast of California, *T. gondii* has been found in ocean water likely contaminated from rivers containing cat feces, which either survived or bypassed sewage treatment facilities (39, 40). Jones et al. (7) conducted a comprehensive case-control study of 148 U.S. toxoplasmosis cases and 413 control patients, and they found the following risk factors for human infection: (i) eating raw ground beef, (ii) eating rare lamb, (iii) eating locally grown and processed cured meats, (iv) working with meat, (v) drinking unpasteurized goat's milk, (vi) owning three or more kittens, and (vii) eating raw oysters, clams, or mussels (7). The U.S. food supply was evaluated by Dubey et al., who found no indication of oocysts in U.S. beef and chicken and low prevalences in pork (41). All *Toxoplasma gondii* risk factors are related to either contact with cat fecal material or contact with meats potentially containing tissue cysts. While all of the above-mentioned exposures are important, a recent confounding study by Boyer et al. demonstrated that 31% of patients transmitting toxoplasmosis congenitally to their child indicated none of these common risk factors for *T. gondii* exposure. While exposure to cat feces was a significant source of exposure to oocysts, ownership of a pet cat was not a significant risk factor (42). While risk avoidance is paramount for the prevention of toxoplasmosis, up to one-third of patients have unrecognized routes of infection. It is likely that only comprehensive screening or effective vaccination programs, which do not currently exist, may help further prevent congenital toxoplasmosis.

A small percentage of people and animals exposed to *T. gondii* develop clinical disease. However, largely due to its global distribution, the morbidity rate due to *T. gondii* is high. For instance, the incidence of toxoplasmic retinochoroiditis ranges from approximately 0.4 to 2.46 cases per 100,000 people, making *T. gondii* the most common identifiable cause of posterior uveitis in many regions of the world (43). Congenital toxoplasmosis impacts approximately 500 to 5,000 of 4.2 million live births per year in the United States (36). National surveillance in France in 2007 estimated the prevalence of congenital toxoplasmosis to be approximately 3.3 per 10,000 live births (44). Considerations for immunosuppressed individuals must be made, as immunosuppression may cause disease recrudescence or a susceptibility to new infection. HIV-associated toxoplasmosis resulted in 1.25% (2,985 cases) of total HIV-related hospitalizations in 2008, making it a significant comorbidity of HIV-infected individuals (45). HIV-associated toxoplasmosis accounted for the vast majority of adult primary disease due to *T. gondii*, with 83.3% of toxoplasmosis-associated hospitalizations occurring in HIV-positive individuals (45).

Prevention

Toxoplasmosis is, for the most part, preventable by the avoidance of exposure to cat feces and the careful handling and preparation

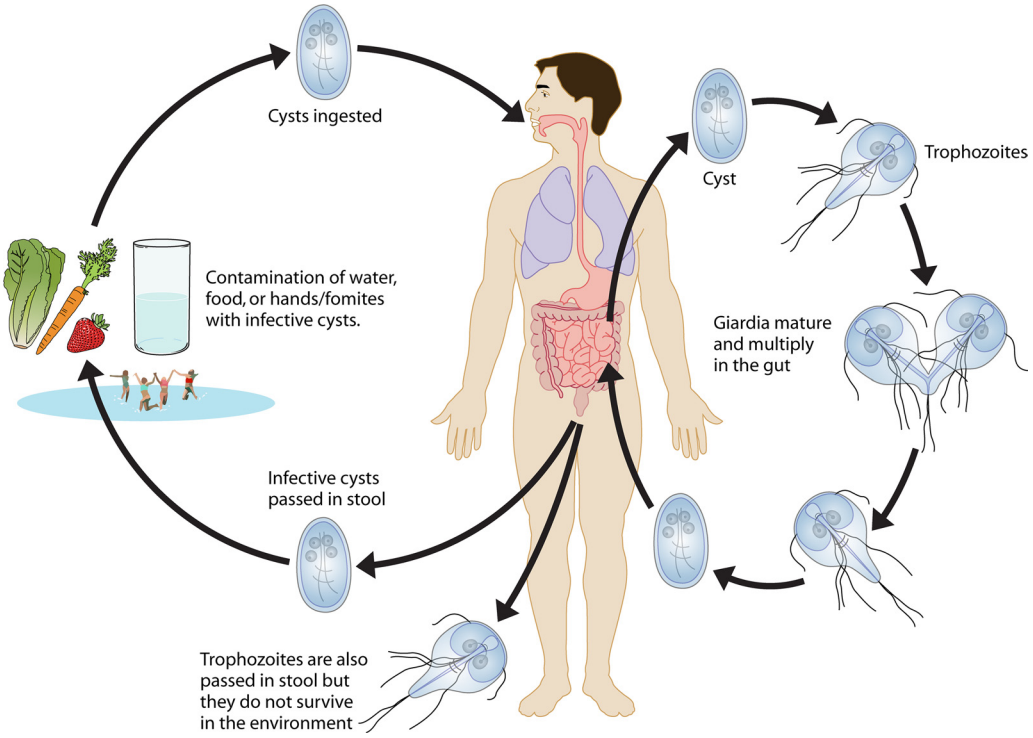

FIG 4 *Giardia* sp. life cycle. *Giardia* cysts shed in the feces are infectious. Infection occurs after the ingestion of cysts either through the fecal-oral route or through the ingestion of contaminated water or food. Cysts are environmentally resistant and can persist for months in soil or water (50). Excystation occurs within the small intestine. Trophozoites remain either free in the intestinal lumen or attached to villous enterocytes, causing clinical signs. Trophozoites encyst upon movement toward the colon, becoming infectious oocysts, and are shed in the feces.

of food. Cat owners can reduce cat exposure to *T. gondii* by following a few simple measures: (i) do not feed pet cats raw meat, (ii) limit and monitor pet cats' outdoor activity to prevent the ingestion of potentially infected birds or rodents, (iii) control intermediate hosts such as rodents, and (iv) maintain routine vaccination for common viral diseases (FeLV, feline rhinotracheitis virus, and feline parvovirus), deworming, and other routine veterinary care for pet cats to reduce the risk of comorbidities. Dogs can serve as mechanical carriers of *T. gondii* and should be kept away from litter boxes; should not be fed raw meat, rodents, or game; and should have routine veterinary care. Human infection can be prevented by consuming only pasteurized dairy products and meat which has been properly cooked to 145°F (63°C) for whole cuts of meat, excluding poultry; to 160°F (71°C) for ground meats; and to 165°F (74°C) for poultry (14). Second, all cutting boards, utensils, and hands should be washed with soap after use with uncooked meat and unwashed produce and before cutting or preparing "ready-to-eat" foods. Finally, the freezing of meats to 10.4°F (−12°C) for 24 h reduces the chance of infection by *T. gondii* (14). Environmentally acquired infections may also be prevented by the following measures: (i) wash hands and teach children the importance of washing hands often; (ii) cover outdoor sandboxes; (iii) follow means to prevent cats and dogs from becoming infected; (iv) if pregnant or immunocompromised, wear gloves when gardening or handling sand or soil; (v) avoid drinking untreated water; (vi) change litter boxes daily to prevent the sporulation of *T. gondii* within the litter; and (vii) if pregnant or immunocompromised, do not handle unknown or stray cats or kittens. Multiple serologic and epidemiologic surveys have noted that currently known risk factors cannot account for 14 to 48% of infections, suggesting additional unknown areas of risk or difficulty in recall by respondents (42). Nonetheless, basic hygiene precautions will greatly reduce the risk of toxoplasmosis.

GIARDIASIS

Life Cycle and Mechanisms of Virulence

Giardiasis, caused by *Giardia duodenalis* (synonyms [syn.], *G. lamblia* and *G. intestinalis*) infection, is the most common pathogenic parasitic infection of humans. There are an estimated 280 million cases of symptomatic giardiasis worldwide annually, with approximately 20,000 cases reported annually in the United States (46, 47). There are currently seven genotypic assemblages (assemblages A to F), which are distinct evolutionary lineages, as defined by phylogenetic analysis and enzyme electrophoresis; humans can be infected with assemblage A or B (48). Dogs and cats become infected with canine-oriented assemblages C and D, feline-oriented assemblage F, or potentially zoonotic assemblages A and B (6, 48). The distribution of zoonotic forms depends, to a degree, on the animal housing environment as well as host adaptation. All companion animals, including those housed in kennels, catteries, and households, are infected predominantly with assemblages C, D, and F. Household pets and feral dog and cat populations can be infected with zoonotic assemblages AII and, less commonly, B (6, 49).

The life cycle of *G. duodenalis* is conserved whether in a canine, feline, or human host (Fig. 4). Both *Giardia* cysts and trophozoites are shed in the feces of infected humans or animals, and cysts are

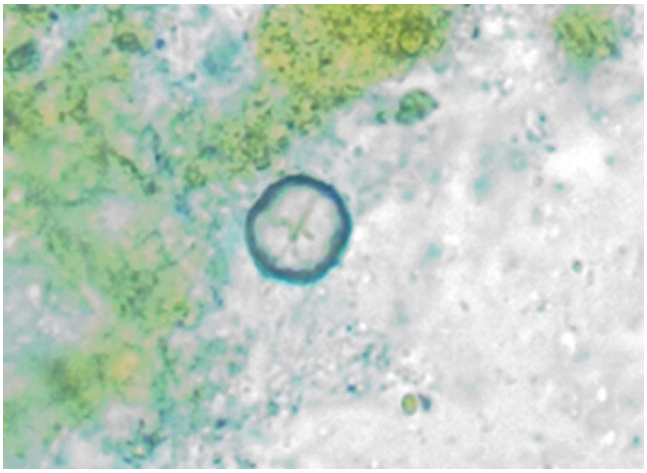

FIG 5 *Giardia* cyst observed in a fecal flotation from a patient dog at the Iowa State University College of Veterinary Medicine.

infectious (Fig. 4). Infection occurs either after the ingestion of cysts through the fecal-oral route or after the ingestion of contaminated food or water (Fig. 4). Cysts are environmentally resistant and can persist for months in soil or water (50). Excystation occurs within the small intestine after parasite ingestion, with each cyst releasing two trophozoites (Fig. 4) (50). Trophozoites remain either free in the intestinal lumen or attached to villous enterocytes, which causes clinical signs (50). Trophozoites encyst upon movement toward the colon and become infectious oocysts by the time of fecal excretion (Fig. 5) (50).

Giardia virulence is dependent on both parasite and host factors. The infectious dose of *G. duodenalis* is estimated to be 10 cysts (51). Once ingested, the cyst becomes metabolically active, and excystation occurs within as little as 15 min (52). Excystation is dependent upon the gastric acid of the host stomach, cysteine proteases produced by *G. duodenalis* peripheral vesicles, the phosphorylation/dephosphorylation of cyst wall proteins, and Ca^{2+} signaling (52–55). The released excyzoite undergoes two rounds of binary fission while upregulating processes related to mobility and the organization of the adhesive disc (56). The adhesive disc provides nonspecific adhesion to enterocytes (57). Trophozoite virulence is highly dependent upon mobility, mediated by eight flagella present in four pairs, and host cell attachment functions of the adhesive disc (50, 57).

In addition to the adhesion disc (58), host cell contact is facilitated by a complex cytoskeletal contractile network comprised of giardin proteins, microtubular proteins, and up to 30 disc-associated proteins (DAPs) comprising the ventral disc and lateral crest, many of which have unknown functions (50, 57). Attachment to enterocytes triggers a poorly understood intracellular cascade, resulting in osmotic changes, diarrhea, and other clinical signs of giardiasis. The induction of enterocyte apoptosis is one well-studied virulence mechanism of *G. duodenalis* (59, 60). Enterocytes exposed to trophozoites rapidly activate pathways of apoptosis, including increased levels of activation of caspase 8 (60), caspase 9, and caspase 3 (59, 60); increased expression levels of Bax; and decreased expression levels of Bcl-2 (60). The activation of caspase 3 as well as other unknown factors modulated intestinal epithelial barrier permeability by the disruption of F-actin, zonula occludens 1, claudin-1, and α-actinin, altering paracellular flow and enterocyte tight junctions, with resultant diarrhea due to the malabsorption of Na^+ and glucose and the hypersecretion of Cl^- (61–63). *G. duodenalis* performs a unique mechanism of immune evasion via antigenic variation, different from those recognized for *Trypanosoma brucei* and *Plasmodium falciparum*. The mechanism of *Giardia* sp. variation is not due to recombination or sequence alterations but to variable control via epigenetic mechanisms and, possibly, RNA interference (RNAi) (64). Many questions remain regarding the multifactorial pathogenesis and virulence of *G. duodenalis* and have been extensively reviewed elsewhere (50, 64, 65).

Immune protection from *G. duodenalis* is dependent upon multiple host immune response mechanisms, and immunocompromised, malnourished, or agammaglobulinemic people or animals may be severely affected (66). Extensive reviews of current research into the immune response to *Giardia* are available elsewhere (66, 67). The understanding of immunity against *Giardia* is cursory at this point, with important ongoing research into the human immune response, mechanisms of immune protection, the effects of variable microflora, and how comorbidities impact *Giardia* colonization and survival.

Epidemiology and Transmission Dynamics

The epidemiologic distribution of human assemblages of *Giardia duodenalis* is due largely to exposure to and ingestion of infectious cysts through contaminated food or water (Fig. 5). Other means of transmission include person-to-person and direct zoonotic transmissions, which account for a significantly smaller number of cases (46). Giardiasis surveillance by the CDC documented 19,000 cases of giardiasis in the United States from 2006 to 2008, excluding five states in which giardiasis is not a reportable disease (46, 47). Common age distributions indicate that children less than 10 years old and adults aged 35 to 44 years have elevated incidences of giardiasis (46, 47). In the United States, cases are clustered geographically, with northern states typically having higher incidences of giardiasis (46, 47). Infections follow a seasonal trend, with a 2-fold increase in numbers of cases from June through October, likely due to increased exposure to recreational water contaminated by human sources (46, 47). Outbreaks due to *Giardia duodenalis* are common in the United States and elsewhere, and outbreaks are generally associated with the consumption of contaminated surface water or improperly filtered and sanitized water from spray fountains in summer play areas or contaminated swimming pools (46). In Florida in 2006, an interactive fountain contaminated with *Giardia* spp. and *Cryptosporidium* was responsible for 57 cases (68). In 2007, a failure of water treatment in a community in New Hampshire resulted in 31 illnesses from mixed infections with *Giardia* spp. and pathogenic *Escherichia coli* (68, 69). Clusters of patients with giardiasis commonly occur after the consumption of untreated water from freshwater streams (70, 71). Giardiasis caused 86% of drinking water-associated outbreaks of illness from 1971 to 2006, and outbreaks of more than 1,000 cases have occurred due to contaminated drinking water (72). In South America, rural areas of India, Southeast Asia, and numerous other areas of the world, the incidence of giardiasis may be much higher, with rates of infection ranging from 6% to more than 50% in children under 12 years of age, with a high risk in low-income populations (73–78).

The zoonotic transmission of *Giardia duodenalis* has been a topic of debate, given the distribution of assemblages between

different species of animals and humans. The overall prevalence rate of giardiasis in both dogs and cats in the United States is between 2 and 15%, with the highest rates being found in the northern and northwest United States (79). In the United States, kennels typically have high rates of infection with canine-specific assemblage D, and client-owned pets, while still infected predominantly with canine-specific assemblages, do have increased rates of infection with zoonotic assemblages A and B (6). In contrast to those studies, Covacin et al. demonstrated that client-owned dogs presenting with giardiasis to veterinary clinics in the western United States had a greater variety of *Giardia duodenalis* assemblages, with 28% and 41% having potentially zoonotic assemblages A and B, respectively, and 15% and 16% having host-specific assemblages C and D, respectively (49). Although controversial, such data suggest the possibility of zoonotic transmission from dogs to humans as well as a potential for the transmission of non-canine-specific assemblages from owners to their pets. Other recent studies have found the presence of zoonotic assemblages in livestock species such as cattle and sheep (48, 80–83). In these cases, species-specific strains predominate, but studies have found zoonotic assemblages in less than 10% up to more than 20% of *Giardia*-positive animals (48, 80–83). Mark-Carew et al. found that 100% of *Giardia* isolates from New York dairy calves less than 84 days old were of zoonotic assemblage A, indicating the possibility of a greater zoonotic potential of young calves, although subtyping to definitively establish that these isolates were human-adapted *Giardia* spp. was not performed (80). While no definitive transmission between animals and humans has been documented, data from cross-sectional surveillance studies and evaluations during giardiasis epidemics imply bidirectional interspecies transmission from animals to humans (48). The potential zoonotic risk and high rates of infection in animals and humans make *Giardia duodenalis* a major target of disease prevention efforts.

Risk factors for *Giardia duodenalis* infection include any type of activity that would increase the likelihood of the consumption of infective cysts. In the United States, these activities commonly include camping, backpacking, and participation in recreational water activities in streams, lakes, and rivers, which would increase risk due to the consumption of untreated surface water (84). Interestingly, one meta-analysis demonstrated only a weak association of recreational surface water consumption with giardiasis in North America, suggesting that other sanitary measures for campers, such as hand washing and waste removal, may be inadequate (85). Contact with animals or livestock also increased the risk of giardiasis (86, 87). Children in child care centers and individuals working in child care centers have an increased risk of infection by both the amount of time in day care centers and the duration of attendance (88). Finally, failures in water treatment, either in swimming pools, in recreational fountains, or in community water treatment, have resulted in multiple epidemics in the past (46, 47, 72, 89). In developing countries, giardiasis is due largely to the consumption of inadequately treated surface water, more often due to failures of infrastructure rather than recreational exposure (73). Risk factors in cross-sectional studies included the education level of the parents, homes with self-drainage of sewage, or dysbiosis caused by the presence of *Helicobacter pylori* (90, 91).

Prevention

The prevention of giardiasis hinges upon the proper sanitation of water sources and the avoidance of fecal-oral exposures. Effective preventive measures include the adequate treatment of water for consumption and appropriate sanitary practices such as hand washing, the proper disposal and handling of human and animal waste, and not allowing children with diarrhea to participate in recreational water activities. Hand washing for the prevention of giardiasis or any fecal-oral pathogen is a universal precaution and should be performed regularly after handling soil, diapers, animal feces, or garbage; treating a wound; or going to the bathroom. Special precautions in day care facilities include removing sick children from day care settings, properly handling diapers, and taking children to the bathroom and/or changing diapers often. Surface water for drinking should be boiled at a rolling boil for 1 min or filtered with an approved water filtration device with a National Safety Foundation Standard 53 or Standard 58 rating for cyst reduction (http://www.nsf.org/business/drinking_water_treatment/standards.asp). Fresh fruits and vegetables should be adequately washed prior to consumption. Travelers in areas where water treatment capabilities are unknown should avoid consuming water or ice in drinks and drink only bottled beverages.

In dogs, prevention depends upon the prompt removal of fecal material, preventing dogs from consuming contaminated surface water or feces, and the disinfection and cleaning of kennels. The disinfection of kennels can be accomplished with 1% sodium hypochlorite (20% commercial bleach), 2% glutaraldehyde, or quaternary ammonium compounds (92). Cysts are relatively resistant to chlorination, and levels of chlorine in drinking water are inadequate to inactivate cysts (92). *Giardia* cysts are susceptible to desiccation, and cleaning and thorough drying will kill them (92).

BABESIOSIS

Life Cycle and Mechanisms of Virulence

Babesiosis is caused by intracellular erythrocyte infection with *Babesia* complex species. Infection with *Babesia* spp. was originally recognized by Babes in 1888 as microorganisms present in erythrocytes of cattle and sheep (93). The completion of the *Babesia* life cycle requires a mammalian host and *Ixodes* ticks, the same genus known for the transmission of *Borrelia burgdorferi*, the causative agent of Lyme disease (Fig. 6). When taking a blood meal, infected ticks infect a mammalian host with sporozoites (94). These sporozoites enter erythrocytes and reproduce through asynchronous binary fission, resulting in two, or sometimes four, merozoites (95, 96). Once present in a reservoir host, parasites will develop into male and female gametes (95, 96). Zoonotic *Babesia* species have diverse reservoirs, including the white-footed mouse, cattle, wild ruminants, canids, shrews, and, possibly, cottontail rabbits (Table 1). Reservoirs are unknown for some human *Babesia* pathogens (Table 1) (96).

When an ixodid tick feeds upon a competent reservoir, blood-stage gametes are introduced into the gut, where these gametes are fertilized to become zygotes (96). Zygotes enter the tick salivary gland and undergo a sporogonic cycle, forming infectious sporozoites (94). Prior to entry into the salivary gland, *Babesia* spp. migrate to the tick ovary, resulting in transovarial transmission and the maintenance of infection in subsequent generations of ixodid ticks (94). However, a recently exiled genus of *Babesia*-like protozoa, *Theileria*, does not achieve transovarial transmission (94). The common human pathogen *Babesia microti* also does not achieve transovarial transmission, even though this species is still classified as a *Babesia* sp. These nontransovarial species have larger

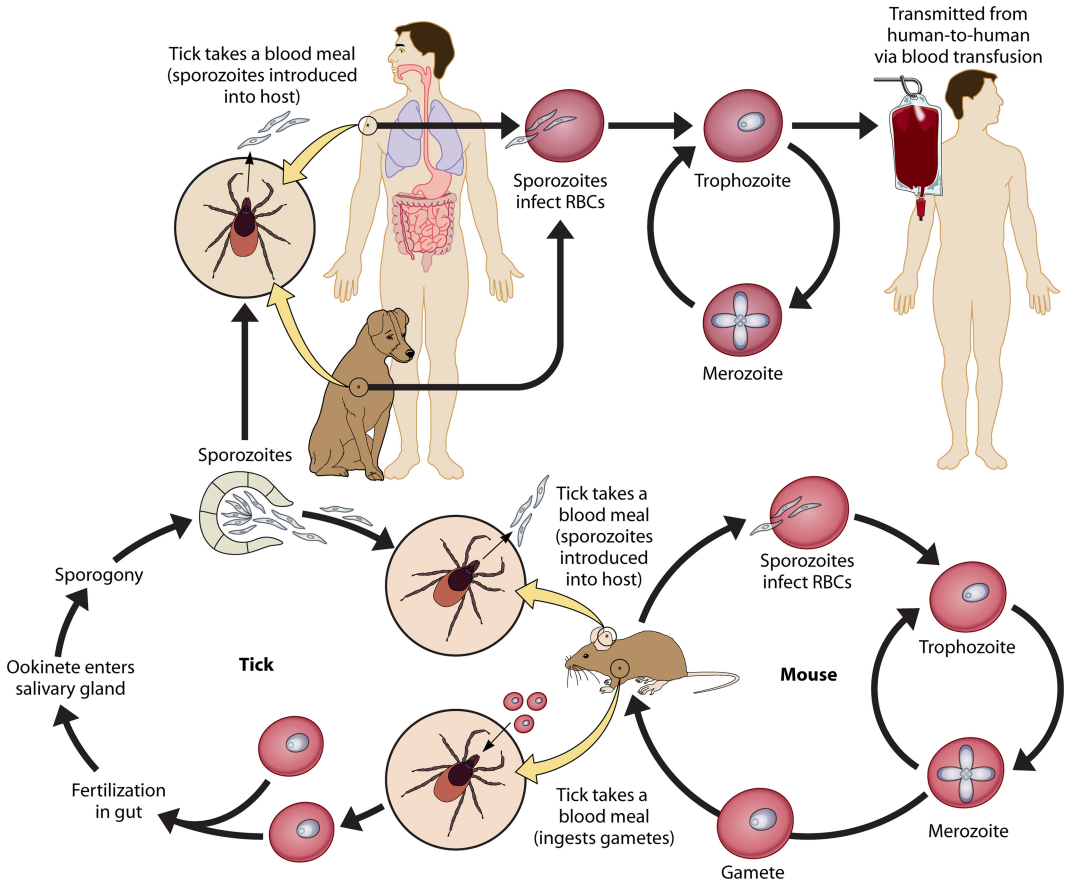

FIG 6 *Babesia* sp. life cycle. Sporozoite-carrying ticks infect a mammalian host while taking a blood meal. Sporozoites enter erythrocytes (RBCs) and reproduce through asynchronous binary fission, resulting in two, or sometimes four, merozoites. Once present in a reservoir host (for *B. microti*, the reservoir is the white-footed mouse), parasites will develop into male and female gametes. When an ixodid tick feeds upon a competent reservoir, blood-stage gametes are introduced into the gut, where these gametes are fertilized to become zygotes. Zygotes enter the tick salivary gland and undergo a sporogonic cycle, forming infectious sporozoites. Humans are generally an intermediate host of *Babesia* species, although blood transfusion transmission does occur. Dogs are intermediate hosts, much like humans, although they may have a domestic reservoir role in the human transmission of the newly emerging species *Babesia conradae*.

gametes called ookinetes and migrate directly to the salivary gland (Fig. 6) (94). Humans are generally a dead-end host for *Babesia* species, and conventional transmission via infected humans is unlikely. However, the transmission of babesiosis via blood transfusion is not uncommon and is a source of concern, especially for immunocompromised/splenectomized individuals, who are susceptible to severe clinical babesiosis (134).

Specific molecular mechanisms of pathogenesis, virulence, and host adaptation are poorly understood for *Babesia* species. Human babesiosis cases are relatively rare (Table 1), and little is known about pathogenic mechanisms in humans specifically. However, a number of domestic animal species have higher incidences of babesiosis and/or theileriosis, and the mechanisms of pathogenesis and immunity discussed here are largely derived from data for these domestic animal species. In cattle and other mammalian species, mechanisms which allow the organism to evade the immune system and invade and persist within the erythrocyte have been identified. First, immediately after introduction into the blood from infectious ticks, merozoites gain entry to erythrocytes to avoid complement and other mechanisms of innate immunity.

Erythrocyte adhesion and infection are facilitated through a number of variable merozoite surface antigens (VMSAs), which contain carboxy-terminal glycosylphosphatidylinositol (GPI) anchor signal sequences (135–138). Antibodies targeting VMSA prevent the attachment of sporozoites to erythrocytes and cellular invasion by merozoites (137, 138). While the mammalian receptors for these proteins and the mechanisms of VMSA-facilitated invasion remain unknown, their importance in pathogenicity has been established. After attachment, tight junction formation and erythrocytic invasion occur via cytoskeletal reorganization as well as rhoptry proteins, rhoptry-associated proteins, and microneme proteins, similarly to *T. gondii*. Cellular invasion and the establishment of infection are dependent largely on host susceptibility, and certain breeds of animals differ in their susceptibilities to the establishment of infection by *Babesia* species. While the mechanisms are poorly established, differences in susceptibilities of inbred strains of mice strongly suggest a genetic basis for susceptibility and resistance (139).

Immunity to *Babesia* species is dependent upon immune responses toward infected erythrocytes or free merozoites. Immunity during primary infection is dependent upon innate mechanisms. Macrophages, including splenic macrophages, and a pronounced proinflammatory response early in infection are necessary for parasite clearance and the prevention of clinical disease

TABLE 1 Geographic locations and host seroprevalences of *Babesia* spp.

Disease entity	Species	Area(s) of endemicity	Predominant reservoir(s)	Human seroprevalence and case severity	Animal seroprevalence	Reference(s)
American babesiosis	*Babesia microti*	NY, NJ, MA, NH, MN, CT, WI, DE, RI, VT, MD	White-footed mouse, rodents, shrews	1,092 cases in 2011; 0.9–1.1% in CT and 1.4% in MA	25% in white-footed mouse in CT	97–100
	B. duncani	WA, CA	Unknown	7 confirmed cases, subclinical to severe; 2% in WA and 2.04% in multiple regions of the U.S.		101–105
	CA1–CA4	WA, CA	Unknown	4 confirmed cases, severe to fatal		106
	B. divergens-like	KY, MO, WA	Cottontail rabbits	3 confirmed cases, severe to fatal	50% in Texas cottontail rabbits, 27.8% in jackrabbits	107–110
	B. conradae	CA	Dog	9 suspected cases	1.1% in dogs in CA shelters	111
European babesiosis	*B. divergens*	Europe	Cattle	40 confirmed cases, 42% mortality, severe to fatal	10.7–20% in cows in Belgium, 27% in cows in Norway, 7% in cows in France, 17.4% in cows in Italy, 28.3% in deer[a]	112–116
	B. microti	Germany, Africa	Meadow vole, rodents, shrews	1 confirmed case, moderate severity, 5.4% in Germany	22% in nonhuman primates in Kenya	117, 118
	B. venatorum	Austria, Italy, Germany, France, Slovenia	Deer	3 confirmed cases, moderate to severe	23% in deer in France[a], 21.6% in deer in Slovenia, 0.9% in deer in Italy	115, 119–122
	B. divergens-like	Portugal	Unknown	1 reported case, asplenic, fatal		123
	B. bovis	Africa, America, Asia, Australia, Europe	Cattle, buffalo	2 reported cases, 100% mortality, Spain and the former Yugoslavia[b]	45.4% in cows in Italy, 35.3% in cows in South Africa, 73.8% in cows in Thailand, 79% in cows in Portugal, 26% in cows in Puerto Rico, 63.7–95.5% in cows in Brazil	116, 124–130
	B. canis canis	Europe	Dog, cat?	1 reported case, nonfatal[b]	34% in dogs in Italy, 2.4% in dogs in Croatia, 4–33% in dogs in France	125, 131–133

[a] The prevalences in these studies were quantified by using PCR rather than serology.
[b] Unverified cases reviewed previously by Gorenflot et al. (125).

(140). This necessity explains the susceptibility of splenectomized and immunosuppressed patients, breed and individual differences due to differences in inflammatory cytokine production, and the relative resistance of children and younger animals to infection, as younger animals have peak levels of production of interleukin 12 (IL-12) and gamma interferon (IFN-γ) 3 days earlier than adults (141).

Babesia species have adapted for long-term survival in the vertebrate host by immune avoidance and modulation strategies. In addition to VMSA, *Babesia* spp. produce variable erythrocyte surface antigen (VESA), which is transported to the erythrocyte surface much like *Plasmodium* knob proteins, causing adhesion to the endothelial cells of small vessels (95). This serves the function of sequestering infected erythrocytes in the peripheral microvasculature and away from the spleen. *Babesia* species are evolutionarily well adapted for long-term survival and replication both in the healthy host and in the ixodid tick, causing minimal disease in most cases. However, with comorbidity, immunosuppressive therapy, or splenectomy, infections with this protozoon can be devastating.

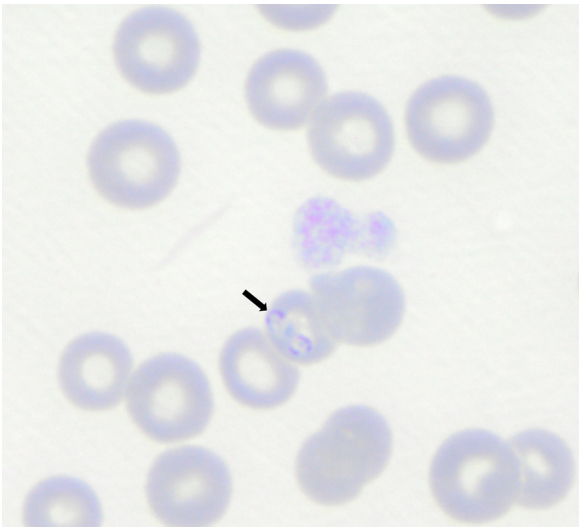

FIG 7 *B. conradae* piroplasms. Parasites are indicated by an arrow. *Babesia conradae* was present in erythrocytes from a canine patient of the veterinary medical teaching hospital at the University of California, Davis. (Courtesy of Jane Sykes.)

Epidemiology and Transmission Dynamics

The worldwide distribution of *Babesia* spp. is dependent largely upon the geographic distribution of competent *Ixodes* vectors. Cases of babesiosis occur throughout Europe and across the Eastern Seaboard of the United States and the U.S. West Coast, with foci of infection in Wisconsin and Minnesota. *Babesia* spp. are incredibly divergent, with over 100 known species, and there is limited knowledge regarding reservoirs and predominant vectors for many of them. Major species resulting in human infection are *B. divergens*, *B. divergens*-like species, *B. duncani*, and *B. microti*. *B. divergens*-like species are those genetically similar to *Babesia divergens* but are not classified as being of this species or of their own species. Most European infections result from *B. divergens*, and at least three species are present in the United States: *B. microti*, present on the East Coast and in Wisconsin and Minnesota; *B. duncani* and *B. conradae*, present on the West Coast; and *B. divergens*-like organisms, having a widespread distribution (142).

European babesiosis. *B. divergens* is primarily a cattle parasite transmitted by ticks of the species *Ixodes ricinus*, discovered in 1911 by M'Fadyean and Schein (143). Research in the 1950s and 1960s demonstrated the susceptibility of rhesus macaques to *B. divergens* infection. Splenectomized primates were susceptible to severe clinical disease resulting in intravascular hemolysis and hemoglobinuria (144, 145). Subsequent studies demonstrated that *B. divergens* has low host susceptibility, with resistance in most laboratory animal species, with the exception of *Meriones unguiculatus*, the Mongolian gerbil (146), which serves as a laboratory model for *B. divergens* (147). Numerous human cases have been reported throughout Europe, primarily in splenectomized individuals. Clinical infection continues to be rare in Europe, with approximately 40 acquired cases to date, with a 42% mortality rate (Table 1) (148–150). The severity of infection is due to the vast majority of cases of *B. divergens* babesiosis occurring in splenectomized or immunocompromised patients. A recent report suggested that immunocompetent individuals may become infected, exhibit only mild clinical disease, and recover (150). The seroprevalence of *B. divergens* in Europe suggests that the rate of exposure is much higher, highlighting the necessity of detection to prevent transmission to immunocompromised individuals via blood transfusion (117).

Two studies in midwestern Germany demonstrated *B. divergens* seroprevalence rates of 3.6% and 5.4% for *B. microti* in people with significant tick exposure and a seroprevalence of less than 0.5% in populations without tick exposure (117, 151). Animal populations also bear high seroprevalences: roe deer in France had a seroprevalence of *B. divergens* of 58%, and cattle in Norway had a seroprevalence of *B. divergens* of 27% (112, 152). In addition to reports of both *B. divergens* and *B. microti* in multiple locales in Europe, additional *B. divergens*-like species, EU-1 and *B. capreoli*, have been identified, which may have been previously mistaken as *B. divergens*. The surveillance completed thus far indicates a significant presence of pathogenic *Babesia* species throughout many areas of Europe, and immunocompromised and splenectomized patients are susceptible to severe, possibly fatal, clinical babesiosis. Surveillance efforts to prevent iatrogenic transmission via blood transfusion are of importance, due to the likelihood of transfusion to immunocompromised patients (Fig. 6).

North American babesiosis. In North America, rare instances of *B. divergens*-like babesiosis occur, including isolated cases in Missouri, Kentucky, and Washington (107–109). A *B. divergens*-like parasite was designated MO-1 after a Missouri case and was found to be maintained within cottontail rabbit populations, with close homology to bovine-derived *B. divergens* (153). Cases in Washington and California (101, 106) were described as being caused by *B. duncani*, a species closely related to the canine species *B. gibsoni*. *Babesia* spp. from cases in Southern California were related to *Babesia* spp. of deer and other wildlife and have been named *B. conradae* (Fig. 7) (154–156). These foci have resulted in 9 cases: 4 in splenectomized patients, 4 through blood transfusion, and 1 in an apparently healthy patient (101, 102, 106). *B. conradae* has been shown to cause more virulent disease in dogs than observed for *B. gibsoni*-infected dogs. Canine *B. conradae* was shown to be most closely related to human piroplasms recently detected in the western United States (156). The disease in these patients

was consistent with *B. divergens*-like disease in Europe, indicating that numerous opportunistic strains of *Babesia* exist, which can infect humans under immunocompromised conditions and may be the same pathogen circulating in dogs.

While *B. divergens*-like infections occur sporadically in the United States, *B. microti* has a much higher rate of incidence, causing babesiosis in both immunocompromised and immunocompetent individuals. The definitive host of *B. microti* is the white-footed mouse and is transmitted by *Ixodes scapularis* (deer tick), for which deer assist in the maintenance of vector populations (Fig. 6). While *B. microti* does not preferentially infect splenectomized individuals over healthy individuals, splenectomized patients are more susceptible to severe clinical disease. Disease is highly endemic in foci along the East Coast and within Wisconsin and Minnesota. Blood transfusion-transmitted babesiosis (TTB) has resulted in a greater geographic distribution of cases, causing 162 reported cases from 141 donors, with 18% mortality (157). The number of TTB cases increased during each decade from 1979 to 2009, with 91 of the 162 cases occurring from 2005 to 2009 (157). The increase in the occurrence of TTB cases resulted in babesiosis in the United States becoming a nationally reported disease in 2011 (157). Vertical transmission of *Babesia microti* has been reported (158–162). Given the overlap of competent vectors, coinfection with *Borrelia burgdorferi* is common, and Lyme disease patients have a significantly higher risk of babesiosis in both Europe and the United States (117). Serologic surveys of blood donors for *B. microti* from 2000 to 2007 indicated that seroprevalence rates were 1.4% and 1.1% in Massachusetts and Connecticut, respectively (97). There were small geographic areas of higher seroprevalence in counties where *B. microti* is considered hyperendemic (97). *Babesia* spp. are able to survive up to 35 days at 4°C in refrigerated blood and indefinitely under conditions of cryopreservation (163). Serologic studies of reservoir animals suggested seroprevalences of approximately 25% for *B. microti* in the white-footed mouse and approximately 35% in mule deer (98, 164) and between 16 and 28% PCR positivity in peripheral blood of rabbits in areas of endemicity (110, 153).

Prevention

The prevention of autochthonous cases of babesiosis includes avoiding heavily wooded and grassy areas during the seasons of highest tick activity, from May to September. If hiking or performing other activities in these areas, long pants and long-sleeved shirts should be worn, with the shirt and pants being tucked in. Additionally, permethrin has a repellent effect on ticks and can be applied to clothing but should not be applied to the skin. *N*,*N*-Diethyl-*meta*-toluamide (DEET)-based insect repellents do have some degree of repellent effect on ticks. Infection through tick bites requires at least 24 h of attachment, and the body surface of people entering areas where transmission is likely should be inspected daily for ticks after participating in activities that have a high risk for tick exposure.

While human babesiosis has been attributed directly to *B. conradae* or feline *Babesia* spp., companion animals do serve as a source of tick exposure for their owners. The use of monthly topical insecticides on pets will reduce the likelihood of ticks entering the home environment as well as prevent tick- and flea-borne diseases in companion animals.

The control of TTB is another important target for community public health intervention. TTB has been recognized as an important risk to the U.S. blood supply. Currently, any patient with a previous diagnosis of babesiosis is not allowed to donate blood. TTB is a challenge for prevention, and current methods of reporting and donor exclusion appear to be minimally effective. Leiby provided a thorough review of TTB and strategies for prevention and mitigation (142). Babesiosis is a widespread disease with numerous infective species, reservoir hosts, and vectors. While the number of cases of this disease has been small, within certain immunocompromised populations, the mortality rate is high. Babesiosis remains an important and challenging emerging zoonotic disease.

AMERICAN TRYPANOSOMIASIS (CHAGAS' DISEASE)

Life Cycle and Mechanisms of Virulence

American trypanosomiasis (AT), or Chagas' disease (CD), a vector-borne protozoal disease caused by *Trypanosoma cruzi*, occurs in North, Central, and South America. *T. cruzi* is transmitted via infected feces from numerous different triatome insect species. The burden of the parasite and life cycle were discovered and largely described in 1909 by Carlos Chagas (165). The World Health Organization has estimated that approximately 10 million people had CD in 2004, primarily in Latin America, making CD the most important parasitic disease in the Americas by disability-adjusted life years (DALYs) (166). *T. cruzi* is an indiscriminate parasite with demonstrated infections of over 100 mammalian species and is capable of infecting all mammalian species. Avian species are resistant to infection (167).

An infected triatome vector takes a blood meal from a mammalian host, triggering the release of infective trypomastigotes in feces (Fig. 8). Infective trypomastigotes (Fig. 9) enter the mammalian host through a bite wound or by penetrating intact mucous membranes, including conjunctiva and the intestinal tract. The genera *Triatoma*, *Rhodnius*, and *Panstrongylus* are of importance for AT vector transmission. Trypomastigotes invade cells and replicate near the site of infection, differentiating into intracellular amastigotes. Amastigotes replicate via binary fission within parasitophorous vacuoles, escape into the cytoplasm, and are released as trypomastigotes into the extracellular matrix, reaching the bloodstream. During initial replication, $CD8^+$ T cell infiltration can be delayed as long as 10 to 12 days, facilitating parasite survival (168). Trypomastigotes are indiscriminately infective to host cells, infecting a variety of cell types, with tropism for smooth and cardiac myocytes. Trypomastigotes within the bloodstream are nonreplicating, a difference from *Trypanosoma brucei*, the trypanosome species which causes African trypanosomiasis. Triatome insects become infected through the ingestion of circulating trypomastigotes in mammalian blood. To complete the life cycle, trypomastigotes transform into epimastigotes within the triatome midgut, multiplying and differentiating, with final differentiation back into infective trypomastigotes within the insect hindgut.

The pathogenic mechanisms and molecular means of cellular invasion by *T. cruzi* have been an active area of research for decades and are relatively well described. Upon entry into the mammalian host, the infective trypomastigotes quickly infect local macrophages, fibroblasts, muscle cells, and adipocytes. Intracellular amastigotes undergo binary fission every 15 to 18 h. Amastigotes replicate for approximately 5 to 6 days until they consume a high percentage of the cytoplasmic compartment of a cell before differentiation into trypomastigotes, resulting in the rupture of

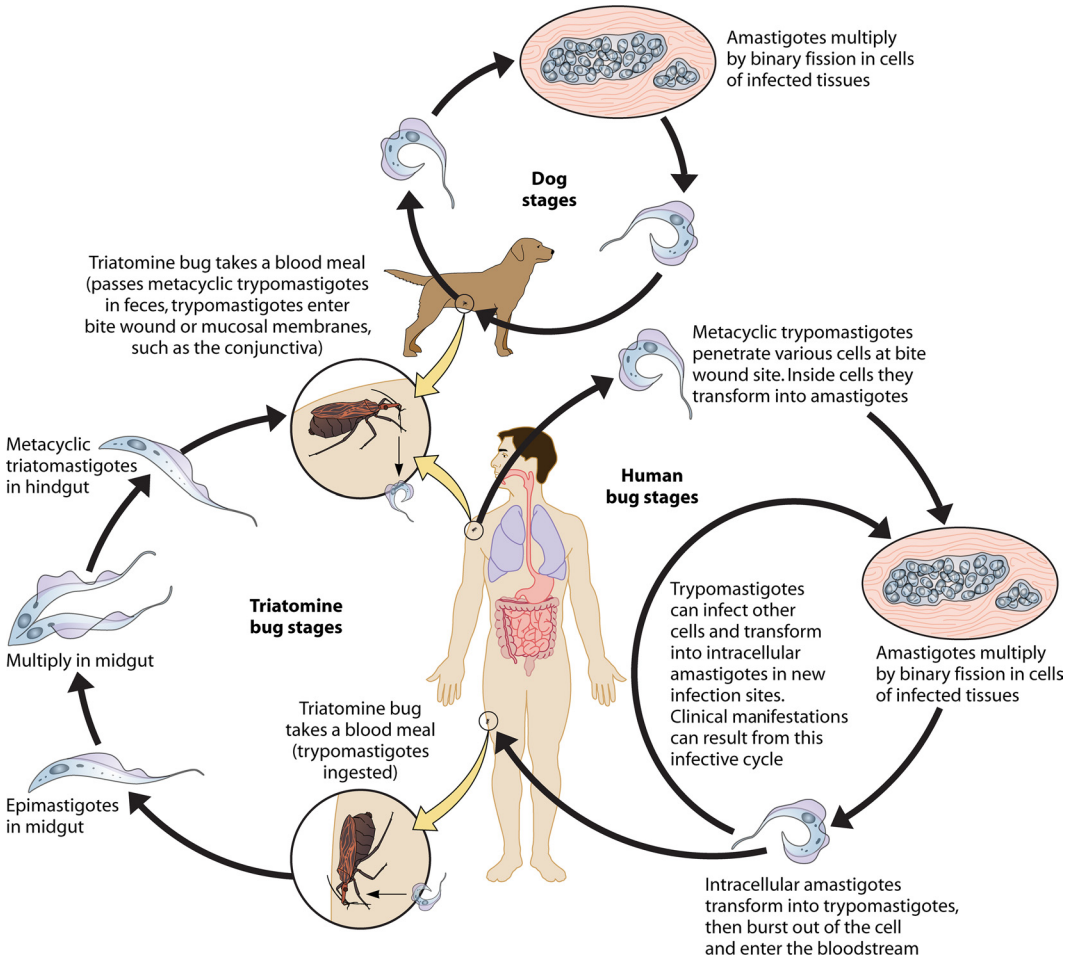

FIG 8 Life cycle of *Trypanosoma cruzi*. An infected triatome vector or "kissing bug" takes a blood meal from a mammalian host, releasing infective trypomastigotes in feces near the bite wound or mucosae. Infective trypomastigotes enter the mammalian host, penetrating intact mucous membranes, including conjunctiva, or orally through the intestinal tract after food-borne exposure. Trypomastigotes invade cells and replicate near the site of infection, differentiating into intracellular amastigotes. Amastigotes replicate via binary fission within parasitophorous vacuoles, escape into the cytoplasm, and differentiate into trypomastigotes. Trypomastigotes are released from the cell, reaching the bloodstream. Triatome insects become infected through the ingestion of circulating trypomastigotes in mammalian blood meals, transform into epimastigotes within the triatome midgut, and undergo final differentiation into infective trypomastigotes within the insect hindgut.

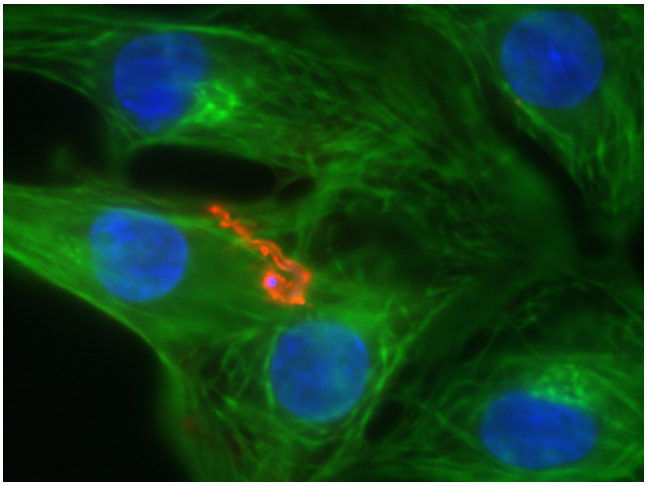

FIG 9 Neonatal rat cardiomyocytes and a *Trypanosoma cruzi* trypomastigote with fluorescent immunolabeling. Actin myofilaments are labeled in green, the *T. cruzi* trypomastigote is labeled in red, and nucleic acids are labeled in blue (DAPI).

the plasma membrane and dissemination. Numerous cellular adhesion molecules, including *trans*-sialidases (TSs), GPI-anchored proteins, mucins, mucin-associated proteins (MAPs), dispersed gene family 1 (DGF-1), and GP-63, facilitate parasite entry (169). More than 50% of the *T. cruzi* genome is repetitive sequences encoding an enormously heterogeneous population of surface molecules (170). Glycoproteins of the TS family are important for numerous functions of the parasite, including extracellular matrix and cellular adhesion, cellular invasion, and pathogenicity (168, 171). Recent research demonstrated the ability of *T. cruzi* to utilize the low-density lipoprotein (LDL) receptor (LDLr) for entry into fibroblasts and cardiomyocytes (172). The accumulation of LDLs within the heart may be a factor contributing to the pathogenesis of Chagas' disease-associated cardiac lesions (172). Lysosome-dependent and -independent invasion pathways required host cell phosphotidylinisitol-3-kinase (PI3-kinase), as the blockage of PI3-kinase with wortmannin inhibited parasite entry (173). Trypanosomal escape into the cytoplasm is dependent upon lysosomal acidification causing the release of the hemolysin *T. cruzi*

toxin (TC-Tox) (174, 175). The exposure of the lysosomal membrane to TC-Tox is thought to be facilitated by the expression of numerous TS enzymes, which desialylate lysosome-associated membrane proteins (LAMPs), resulting in the disruption of the parasitophorous vacuole (176, 177). The multiple mechanisms of invasion, pathogenesis, and persistence of *T. cruzi* highlight the reasons for its chronicity in many hosts and the establishment of AT in many areas of endemicity.

An understanding of effective immunity against *Trypanosoma cruzi* has also been a research focus for some time, with goals of vaccine development and disease prevention. Vaccine development has remained difficult, due to the complex nature of CD. Correlates of protective immunity and parasite control within the host have been extensively studied and are dependent upon the infectious dose and strain of *T. cruzi* and the innate and acquired immune responses of the host. Macrophages commonly become infected with *T. cruzi* and may be one of the first cell types encountered by the parasite during natural infection (167). The recognition of *T. cruzi* by macrophages is through numerous surface Toll-like receptors (TLRs) and lectin receptors (178, 179). Repetitive CPG DNA motifs and mRNA from intracytoplasmic *T. cruzi* have also been shown to engage TLR9 and TLR7, respectively, and are necessary for the control of *T. cruzi* infection (180–182). The acquired immune response is also necessary for parasite control and clearance. Humoral immunity is thought to be important in early acute infection, with IgG2b and B cells conferring protection, although the exact mechanism is not understood (183, 184). Cellular immunity is thought to be the largest and most important component of the immune response to infection by *T. cruzi* and the main target for effective vaccination strategies against AT. The immune response to *T. cruzi* is a complex interplay of nearly every aspect of innate and adaptive immunity, and the suppression of any one of these aspects can result in parasite survival and chronic infection.

Epidemiology and Transmission Dynamics

Chagas' disease is widespread throughout the Americas, with endemic triatome transmission occurring in all countries of South America, Central America, and Mexico and with limited autochthonous transmission in the United States (185). *Trypanosoma cruzi* is found throughout these areas in numerous triatome vectors and in over 100 mammalian species. It is believed that *T. cruzi* has been a human pathogen in the Americas for as long as humans have inhabited the continent, based on studies of archeological samples from the United States, Chile, and Peru from approximately 9,000 years ago (186). There are multiple means of infection by *T. cruzi*, including classical vector-borne transmission, congenital infection, transfusion-associated CD, and oral/food-borne exposures. The wide distribution, numbers of possible vectors and reservoir species, and multiple means of transmission all contribute to the immense burden of CD.

Classical sylvatic and domestic transmission in areas of endemicity. (i) Vector species and risk factors. There are over 41,000 estimated new CD cases due to vector-borne transmission each year (185). Over 130 known triatome species exist within the Americas, with most of them being considered capable of vectoring *T. cruzi* (187). Sylvatic enzootic cycles result in human infection when adult sylvatic triatomes are attracted to light or other characteristics of human domiciles (188). With deforestation and the introduction of companion animals, sylvatic vector species can initiate domestic infection. Members of the family *Triatominae* are hematophagous throughout their life cycle, developing a close evolutionary relationship with their host species, primarily small mammals and rodents. There are a few triatome species with both domiciliary and peridomiciliary cycles in the Americas, resulting in greater human transmission. In the United States, 11 species of triatomes are present, and infection with *T. cruzi* has been identified in all but one of these triatomes. These species are found in the entire southern half of the United States, including Texas, Southern California, and Arizona, and one can be found in Florida. Fifty-eight percent of *Triatoma gerstaeckeri* insects, commonly trapped in New Mexico and Texas, were found to be positive for *T. cruzi* (188). All seropositive U.S. blood donors identified through screening lived in areas of the United States with documented *T. cruzi* infection, and 80% had no travel history in areas of endemicity outside the United States (189). In almost all cases, in households with dogs and positive humans, dogs were positive as well, highlighting the common vector source. This strongly suggests vector-associated transmission in the United States, requiring vigilance by U.S. public health services and disease cognizance by local physicians and veterinarians.

Risk factors for vector-borne CD are related to the likelihood of infectious bites. Specific risk factors for CD in areas of endemicity based on large-scale seroprevalence and triatome surveillance surveys were the presence of the vector, nearby cropland and grassland, disarray of the domiciliary environment, and mud and thatch or tarred cardboard homes and outbuildings (190–192). Other factors, including evidence of triatome infestation of the home and the presence of a companion animal sharing a room with an individual, were significantly associated with CD in Peru (193). Studies demonstrated preferential vector feeding on mammals, such as caged guinea pigs and dogs over birds or cats, with a higher percentage of triatome bugs feeding and reaching engorgement on preferred species (194). This indicates the importance of *T. cruzi* domestic reservoirs for transmission to humans.

(ii) Dogs as reservoir species and risk. Dogs are considered the predominant domestic reservoir for CD in many areas of endemicity. In Texas, there were 537 confirmed canine CD cases between 1993 and 2007 (195–197). Dogs develop acute and chronic disease, similar to human infection. Acute infection in young dogs presents typically as myocarditis with arrhythmia (8). In chronic disease, dogs have chronic progressive cardiac failure due to electrocardiogram (ECG) abnormalities and/or congestive bilateral or right-sided heart failure (8). Dogs in areas of high endemicity in Argentina had seropositivity rates from 25% in young dogs to 92% in dogs 8 years of age or older (197). In Venezuela, one estimate placed the seroprevalence in dogs at 6.9%, almost identical to the human seroprevalence in this region (190). A study in Panama determined an overall *T. cruzi* infection index of 16.2% in dogs (198). In Campeche, Mexico, the prevalence of *T. cruzi* seropositivity was higher in dogs than in people, with 9.5% positivity in stray dogs and 5.3% positivity in owned dogs (199). Household cats play a much less definitive role in the domestic transmission of CD, with a lower estimated seroprevalence rate than that for dogs (197). Although triatomes do feed upon cats (38% in one study), they are much less likely to be engorged (194, 197). Therefore, household dogs are estimated to be at a 3-fold-higher risk for transmission than cats in regions of endemicity (197). The presence of domestic companion mammals within the home is a definitive risk factor for human infection with *T. cruzi*. The nondis-

criminatory nature of both triatome species and *T. cruzi* allows for the persistence of CD in sylvatic, rural, and domestic environments and creates a significant challenge to disease prevention.

Congenital Chagas' disease. Congenital infection with *T. cruzi* causes approximately 14,000 cases of CD per year, resulting in a spectrum of clinical signs (200). A majority of congenital infections occur in asymptomatic mothers. Congenital transmission closely follows the serologic prevalence in female populations in areas of endemicity (200). Studies indicated that maternal seropositivity rates ranged from less than 1% to up to 64.5% in areas of Bolivia (200–204). The congenital transmission rate, measured as the number of infected infants born to infected mothers, was 1% to 7% (200–205). A recent comprehensive surveillance of congenital CD in immigrants from areas of endemicity to Spain demonstrated an infection rate of 11.4% in mothers from regions of endemicity, with higher seropositivity rates in mothers from Bolivia (34.1%) (206). Mothers from areas of endemicity had a congenital transmission rate of 3.4% in Spain (206). Based on a recent case of congenital CD in Virginia in an infant born to a Bolivian mother, this may also be true for the United States (207). Based on expected rates of congenital transmission and known seroprevalences in the United States, there may be approximately 58 to 502 congenital cases per year (208). Vector control has made an impact on maternal seroprevalence rates in areas of endemicity, with increasing maternal age being significantly associated with congenital transmission and seropositivity. However, a large number of young infected women remain. Congenital CD will continue to be a long-term maternal and neonatal health challenge both in countries of endemicity and in countries where the disease is not endemic.

Transfusion-associated Chagas' disease. Transfusion-associated CD occurs when a parasitemic donor donates either blood or organs for transplant, causing acute or chronic disease in the blood or organ recipient. All blood and organ components are infective, and *T. cruzi* remained viable for at least 18 days at 4°C (209). The likelihood of infection due to transfusion is dependent upon the level of donor parasitemia, the amount of transfused blood, conditions of blood storage and processing, and screening methods in place in the region of residence of the donor (209). In the United States, there have been five published cases of CD associated with blood transfusion and five cases associated with organ transplantation (210–215, 348). Many of these patients suffered from concurrent conditions necessitating a transplant and developed severe acute CD posttransplantation or posttransfusion (188). The American Red Cross and Blood Systems Inc. began screening blood for *T. cruzi* on 1 January 2007 (188). From 1 January 2007 to 28 June 2012, there were 1,668 confirmed seropositive donations (216). In addition to immigrant infections, recent research suggests the occurrence of vector-borne transmission in the United States (189). This has resulted in *T. cruzi*-positive blood donations, indicating that all blood donations should be evaluated for the presence of *T. cruzi* (189). The estimated seroprevalence of blood donors in Latin America is approximately 1.3% (209). Continued screening efforts and long-term vector control in areas of endemicity will be required, due to extensive immigration and the rate of asymptomatic AT.

Oral transmission of Chagas' disease. Sylvatic *T. cruzi* infection of opossums, skunks, and raccoons is dependent upon oral transmission, due to the insectivorous nature of these animals (188, 217, 218). Dogs and cats are also insectivorous. In humans, epidemics due to transmission via contaminated fruit and vegetable materials from regions of endemicity have caused increased concern for transmission via food. Some reports suggested that oral transmission is the primary route of *T. cruzi* dissemination between animals and vectors and the predominant cause of acute human disease in Amazonia (219). The clinical form of oral CD has a clinical presentation similar to that of acute CD, with some differences. Oral exposure results in an acute febrile syndrome 3 to 22 days after exposure, with myalgia, cholangiohepatitis, and gastritis with epistaxis, hematemesis, and, potentially, shock (219).

Between 1980 and 2001, 28 small family-focused outbreaks occurred in Brazil, due to the contamination of juice, water, or food with triatomes, their feces, or secretions from the anal glands of infected mammals (219, 220). Acai and sugar cane juices have been implicated in outbreaks, due to the nature of the preparation of the juice and the grinding of triatome insects into the juice (220). One of the most recent and largest outbreaks of orally acquired CD occurred in a school community in Venezuela (221). There were 103 confirmed cases during this outbreak, with 75% symptomatic cases, and of those, 20.3% required hospitalization (221). This outbreak was significantly associated with the consumption of guava juice, which was prepared the previous night and left to cool in an open container outside (221). The oral transmission of CD, which maybe the predominant means of companion animal infection, highlights the importance of keeping pets inside when possible, food safety and general hygienic practices, and the maintenance of quality control and vigilance during food, particularly juice, preparation.

Prevention

The prevention of CD relies heavily on vector control, the screening of the blood supply and organ donations, and standard food safety practices. Vector control practices in many South American countries have resulted in significant decreases in rates of human seropositivity (222, 223). In these regions, mammalian reservoirs have generally remained, and the number of asymptomatic seropositive people of middle age and older remains high. Therefore, CD will likely continue to be a threat to the blood supply and a risk for congenital infection. Surveillance of blood donors, in conjunction with screening questions about CD for blood donors, has likely reduced the number of acute transfusion-associated CD cases. For pregnant women already infected with *T. cruzi*, there is no viable prevention of congenital transmission, due to the potential side effects of the current toxic therapy on the fetus (224). For women in general, the prevention of future congenital transmission depends upon recognition and therapy prior to conception (224). Ongoing efforts against CD have greatly reduced the burden of this zoonotic disease in the last 2 decades. However, given the worldwide nature of immigration, the wide variety of competent vectors and reservoirs, and the asymptomatic nature of the disease, Chagas' disease will continue to be a worldwide public health challenge for the foreseeable future.

LEISHMANIASIS

Life Cycle and Mechanisms of Virulence

Leishmaniasis is a vector-borne disease caused by *Leishmania* species of the family *Kinetoplastidae*. Infection with *Leishmania* spp. can result in a spectrum of clinical diseases dependent upon the infecting species. Visceral leishmaniasis (VL) is caused by *Leish-*

mania infantum in the Americas and the Mediterranean basin and by *L. donovani* in India, sub-Saharan Africa, and Asia. Occasionally, cases of VL will arise from cutaneous disease-causing species and has occurred in members of the U.S. military due to infection by *L. tropica* (225). VL arises from the parasitic infection of phagocytic cells within secondary lymphatic organs (spleen and lymph nodes), liver, and bone marrow. Cutaneous leishmaniasis (CL) (Table 2) arises from an infection of epidermal tissue after promastigote host inoculation. In susceptible hosts and immunocompromised persons, disseminated cutaneous or diffuse cutaneous leishmaniasis may occur as a rare but severe manifestation of CL. A third form of the disease, mucocutaneous leishmaniasis (MCL), arises from a small percentage of cutaneous cases who cleared the disease months or years prior to the onset of MCL. MCL often begins with an involvement of the nasal mucosa, including generalized inflammation and ulceration. Ulceration and necrosis of these areas may be severe, resulting in disfigurement and, occasionally, death. Mechanisms of mucocutaneous lesion formation are poorly understood, but a *Leishmania* RNA virus (LRV1) was associated with severe mucocutaneous lesions through a TLR3-dependent inflammatory response (296).

The life cycles of *Leishmania* spp. are relatively simple, involving a mammalian host and a vector stage (Fig. 10). Phlebotomine sandflies of the genus *Lutzomyia* in the Americas and *Phlebotomus* in other regions of endemicity serve as vectors for *Leishmania*. The sandfly injects infective promastigotes into a susceptible mammal during feeding. Promastigotes (Fig. 11A) are quickly phagocytized by resident phagocytes, transformed into tissue-stage amastigotes, and divide through simple division in a parasitophorous vacuole. Depending upon host and parasite factors, the parasite infects further phagocytic cells either at the site of cutaneous infection or in secondary lymphoid organs, with eventual parasitemia. Sandflies become infected through feeding on a host either with an active skin lesion in CL or with parasitemia in VL. Parasites convert to promastigotes within the sandfly midgut and reproduce to high numbers in 4 to 14 days. These promastigotes migrate to the salivary glands, transform into infectious metacyclic promastigotes, and await the initiation of feeding.

Leishmania spp. have unique virulence mechanisms, maintaining persistence within host phagocytes to establish long-term chronic infection. After a sandfly bites the host, salivary chemoattractants promote an influx of both neutrophils and macrophages to the feeding site (297). Parasites inhibit phagosome acidification, allowing them to survive within neutrophils, but have not been shown to transform into amastigotes or proliferate within neutrophils (298). At the time of neutrophil apoptosis, surviving parasites are phagocytized by resident and infiltrating macrophages. Dendritic cells also become infected, becoming mature and migrating to the lymph node. *L. amazonensis* specifically inhibited dendritic cell maturation through enhanced extracellular signal-regulated kinase (ERK) activation from the phagosome, resulting in the decreased production of interleukin 12, a key proinflammatory mediator (299). *Leishmania* phagocytosis is mediated through complement receptors 1 and 3 and mannose scavenger receptors, indicating both opsonization-dependent and -independent mechanisms of invasion (300). Uptake results in the reorganization of F-actin and delayed phagolysosomal fusion (300, 301). *Leishmania* spp. are resistant to acidification as amastigotes and persist in late-endosome-associated LAMP1- and Rab7-positive vacuoles (302). Amastigotes replicate within the phagolysosome until eventual host cell lysis. The ability of the parasite to direct phagosome trafficking and delay phagolysosome fusion is dependent upon surface lipophosphoglycans with differing side chains (302–304). *Leishmania* spp. are also able to acquire nutrients needed for survival through the expression of LIT-1 to acquire the Fe^{2+} needed for growth and survival (305).

The immune response to all *Leishmania* species as an intracellular pathogen is dependent upon a timely and appropriate T helper 1 response, including IL-12 production by dendritic cells and macrophages, efficient MHC-II presentation, and subsequent IFN-γ production from T cell populations. The clearance of *Leishmania* infection by the innate immune system is dependent primarily upon intracellular killing via superoxide and nitric oxide within phagolysosomes of infected macrophages, which is enhanced by IFN-γ stimulation from NK cells early in infection and T cells at later stages. The mechanisms of immunity to various species of *Leishmania*, and the specific evasion mechanisms utilized by *Leishmania* species, are beyond the scope of this review and have been thoroughly discussed elsewhere (306, 307).

Epidemiology and Transmission Dynamics

There are 98 countries and 3 territories where *Leishmania* is endemic, with the majority of cases occurring in developing nations (308). The distribution of competent vector species and leishmaniasis has expanded over the last decade, possibly due to an increasingly amenable environment of vector species due to shifts in climate (309). This results in approximately 200,000 to 400,000 cases of VL and 700,000 to 1.2 million cases of CL each year and an estimated 20,000 to 40,000 deaths (308). More than 90% of VL cases occur in India, Bangladesh, Sudan, South Sudan, Ethiopia, and Brazil (308). Among the 15 disease-causing species of *Leishmania*, 13 are thought to have some degree of zoonotic transmission, and in the case of *L. infantum*, dogs are the main reservoir, being largely responsible for the transition from sylvatic to domestic transmission cycles (9, 269). The clinical forms of leishmaniasis are discussed briefly, primarily based on the degree of zoonotic transmission from companion animal species.

Cutaneous leishmaniasis. CL is caused by a number of *Leishmania* spp. with widespread distributions and a variety of location-specific reservoir species (Table 2). In the Old World, *Leishmania major* is a predominant cause of CL. Reservoirs for *L. major* vary by location but include many rodent species. Reservoir and vector population densities are significantly correlated with the seasonality of infection. Increases in reservoir rodent populations have been associated with numerous zoonotic cutaneous leishmaniasis (ZCL) outbreaks (269, 310). *L. aethiopica* causes ZCL and appears to be isolated to the highlands of Ethiopia. Reports identified this species at lower altitudes as well, indicating that the distribution is perhaps more widespread or that the area of endemicity is expanding (234). CL caused by *L. tropica* is considered anthroponotic. However, animal species can be infected and have been suggested to be potential reservoirs, including dogs (Table 2).

In the New World, numerous species of CL-causing *Leishmania* (*Viannia*) spp. have been identified in multiple mammalian species. *L. mexicana* is found from Central America to the Yucatan peninsula in Mexico, and cases have been reported in Texas (236, 311–313). There have also been a number of non-travel-associated reports of CL in companion animals in Texas (236, 311–313). Notably, many of these cases of zoonotic CL were in cats (236, 311,

TABLE 2 Geographic locations, hosts, and companion animal seroprevalences of *Leishmania* spp.

Disease entity	Species	Area(s) of endemicity	Predominant reservoir(s)	Canine seroprevalence	Reference(s)
Cutaneous leishmaniasis	*Leishmania major*	Middle East, northwestern China, northwestern India, Pakistan, Africa	Gerbil species, jird, fat sand rat	Egypt, 3 cases; Saudi Arabia, 3 cases	226–233
	L. aethiopica	Ethiopia, Kenya, Somalia	Rock hyrax		234, 235
	L. mexicana	Central America, Mexico, TX	Yucatan deer mouse, tree rat, other rodents	Mexico, 30.2%, 10.5%[a]; TX, 8 cases[a]	236–241
	L. amazonensis	Brazil	Various forest rodents (grass, pygmy mice)	Brazil, 1 case	242, 243
	L. tropica	Mediterranean, Middle East, western Asia, Indian subcontinent	Human, foxes, golden jackals, hyrax, dogs	Morocco, 8 cases	244–248
	L. braziliensis	Central, South America	Forest mammals, marsupial species, opossum	Mexico, 8.2%, 11.57%[a]; Brazil, 2–3%	240, 241, 249–252
	L. guyanensis	Guyana, Suriname, northern Amazon basin	Two-toed sloth, forest mammals, marsupial species, opossum	Colombia, 2.2%	253, 254
	L. peruviana	Peru, Argentinean highlands	Dog?	Peru, 1.8%[b]	255
	L. shawi	Brazil	Cebus monkeys, sloths, procyonids		256
	L. lainsoni	Brazil, Bolivia, Peru	Lowland paca, rodents		257
	L. naiffi	Brazil, French Guyana, Ecuador, Peru	Armadillos		258
	L. venezuelensis	Venezuela	Unknown, cat?	Venezuela, 6 cases[a]	259
	L. panamensis	Panama, Costa Rica, Colombia	Sloths, kinkajous, marsupial species, opossum	Colombia, 12 cases; Panama, 3.3%; Ecuador, 2 cases	260–264
Visceral leishmaniasis	*L. donovani*	Indian subcontinent, northern and eastern China, Pakistan, Nepal, eastern Africa, Sudan, Kenya	Human, dogs, goats	Sudan, 2 cases; Ethiopia, 14.8%; India, 6.5%	265–268
	L. infantum (syn., *L. chagasi*)	Middle East, Mediterranean basin, northern and northwestern China, northern and sub-Saharan Africa, Central and South America	Dogs, foxes, jackals, wolves	Mexico, 11.9%, 22.10%[a]; Brazil, 7.14–57%; Portugal, 4.3–25.2%; Spain, 8.1–13%; Italy, 2.6%; France, 4–8%; Greece, 2–30%; Uzbekistan, 32.1%; Turkey, 20.7%; China, 23.5–28.2%; Iran, 14.2%, 10%[a,b]; Jerusalem, 6.7%[a]; Senegal, >40%	240, 241, 248, 250, 269–295

[a] Feline seroprevalence.
[b] Prevalence was measured as positivity on skin or splenic biopsy specimens and culture.

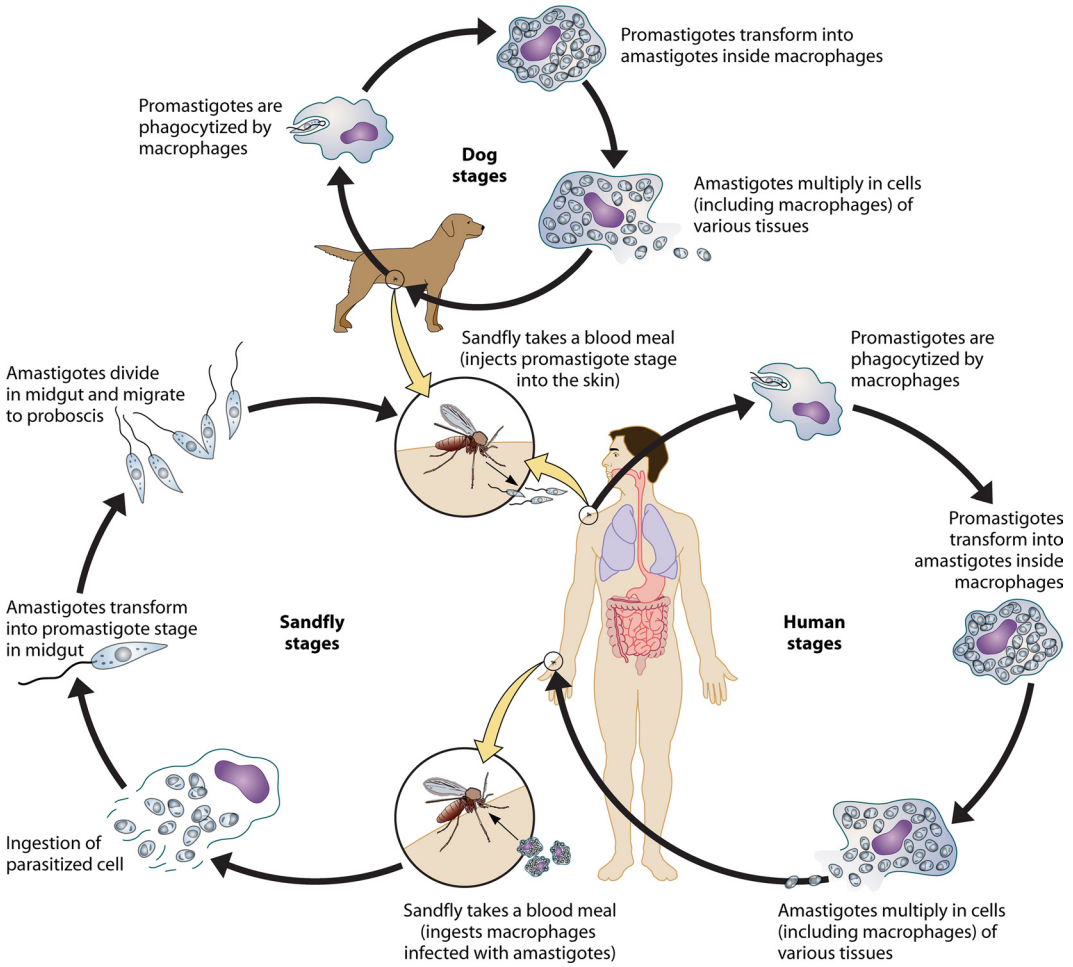

FIG 10 The life cycle of *Leishmania* species. Sandflies inject infective promastigotes into a susceptible mammal during feeding. Promastigotes are phagocytosed by resident phagocytes, transform into tissue-stage amastigotes, and multiply within these cells through simple division. The parasite continues to infect phagocytic cells either at the site of cutaneous infection or in secondary lymphoid organs, with eventual parasitemia. Sandflies become infected through feeding on a host either with an active skin lesion in CL or with parasitemia in VL. Parasites convert to promastigotes within the sandfly midgut. Promastigotes migrate from the midgut and transform into highly infectious metacyclic promastigotes.

313), perhaps due to their more outdoor life-style in the United States. There have been 30 autochthonous cases of human CL in Texas through 2008, where CL is considered endemic (237). While companion animal infection and transmission occur, the predominant sylvatic reservoir in Texas is the Southern Plains woodrat, *Neotoma micropus* (238). As there are vectors throughout the southern United States, it is likely that rates of disease due to *L. mexicana* will increase in the United States (309).

South American species of *Leishmania* causing CL, including *L. amazonensis*, *L. braziliensis*, *L. guyanensis*, and *L. panamensis*, have sylvatic reservoirs. Two CL-causing species in South America have domestic animal reservoirs. *L. venezuelensis* has been identified in several urban and periurban areas, with a suspected domestic cat reservoir host (259). *L. peruviana*, a species once limited to altitudes of 1,200 to 3,000 m in the Peruvian Andes, uses the dog as a reservoir, although limited evidence of transmission to sandflies exists (255). The zoonotic transmission of cutaneous leishmaniasis from companion animals, most notably the dog, has been occurring. However, while there have been numerous reports of canine and feline infection and clinical disease with ZCL, their roles as reservoirs have not been firmly established.

Human risk factors for ZCL are dependent upon exposure to vector species and the presence of reservoir species. In all cases, urbanization and wilderness encroachment have resulted in increased interactions between humans and reservoir and vector species and the establishment of (peri)urban domestic life cycles rather than sylvatic ones. The establishment of urban domestic transmission holds the potential for larger outbreaks of ZCL due to a more frequent exposure of naïve human hosts during everyday life versus occasional infection due to human introduction into the sylvatic cycle. Deforestation and agricultural development, including the damming of waterways and irrigation, also create new environments optimal for the survival of rodent reservoirs. An outbreak in Mazar-e Sharif, Afghanistan, was traced to exploding populations of *Rhombomys opimus* in an area of irrigation canal construction (310). *L. guyanensis* evolved urbanized life cycles in Brazil, leading to an increased risk of infection dependent upon household distance from the forest (314, 315). Interestingly, long-term surveillance in Bahia, Brazil, suggests that the number of cases in agricultural workers has decreased in the last 20 years, with an overall increase in the rate of disseminated disease in coastal areas (316).

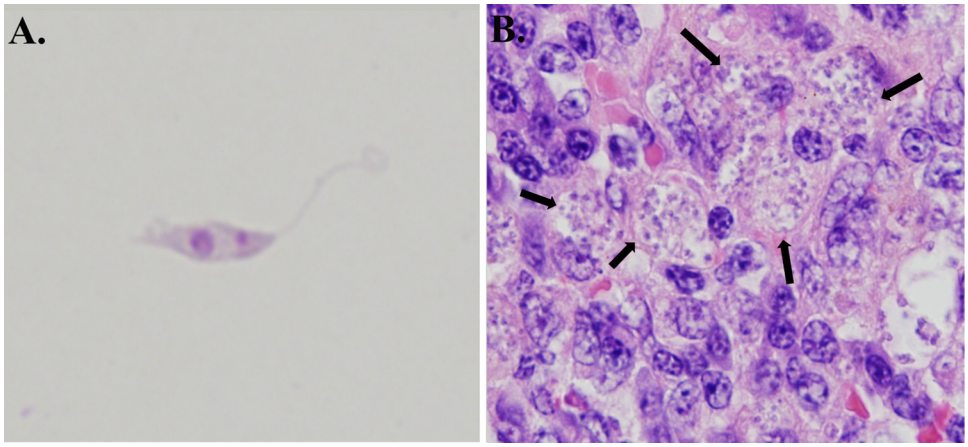

FIG 11 *Leishmania* parasites in culture and in a tissue section. (A) *Leishmania amazonensis* promastigote from culture with a visible kinetoplast. (Photo by Pedro Martinez.) (B) Zoonotic visceral leishmaniasis in canine spleen. The spleen was enlarged and infiltrated by large numbers of foamy macrophages containing numerous intracellular *Leishmania infantum* amastigotes (arrows), confirmed by immunohistochemistry (magnification, ×100).

Recent studies have identified younger age (5–19), sleeping without bed nets, ownership of dogs and cattle, and the presence of organic animal material as risk factors for infection (317). Additionally, social factors, including low education and income, increase the likelihood of exposure due to poor housing/environments conducive to the presence of vectors. These factors include mud-walled housing (not brick or concrete), dirt streets and/or floors, the cleanliness of the domestic environment, and working in forested areas (318–320).

Zoonotic visceral leishmaniasis. Visceral leishmaniasis is caused by *Leishmania donovani* in India, areas of Asia, Sudan, South Sudan, Kenya, and Ethiopia and by *L. infantum* (syn., *L. chagasi*) throughout South America and in areas of Central America and Mexico, the Mediterranean basin, the Middle East, central and southwestern Asia, northwestern China, and northern and sub-Saharan Africa. The transmission of *L. donovani* is considered solely anthroponotic, although animal infections have been reported, and a reservoir status has been suggested for dogs in Sudan and for goats and possibly other species in Nepal (265, 266).

Zoonotic infection by *L. infantum* is responsible for the majority of zoonosis-based human cases of VL. As with other species of this genus, vectors for *Leishmania* in the New World are *Lutzomyia* spp., predominantly *Lutzomyia longipalpis*, and those in the Old World are species of the genus *Phlebotomus*. Canids are considered the primary sylvatic and domestic reservoirs, with foxes, jackals, and wolves filling the role of sylvatic reservoirs and the domestic dog serving as a domestic reservoir (9, 269). Wild felids can be susceptible to infection, although a definitive role in transmission is unclear (321). Similarly, domestic cats in areas of Brazil where the disease is endemic have been infected and could be secondary reservoirs (270). Humans are relatively resistant to *L. infantum* infection and have high rates of asymptomatic infection. The WHO estimated 200,000 to 400,000 clinical VL cases to be a gross underestimate of the burden of human *L. infantum* infection worldwide (322). An asymptomatic, immunologically competent patient with low-level or absent parasitemia and a compartmentalization of the parasite within the secondary lymphoid organs and bone marrow may have reduced transmission to sandflies. When zoonotic visceral leishmaniasis (ZVL) occurs in immunocompromised persons or animals, the parasite load increases, and transmission, as well as clinical disease, is likely to occur.

There is a clear role for domestic dogs in the maintenance and transmission of *L. infantum*. In Brazil, infected dogs in urban and periurban areas of endemicity are common, accompanied by a sufficient environment for *L. longipalpis* (271). Vertical transmission has been documented for dogs, suggesting a vector-independent means of transmission in these areas as well (323). In northeastern Brazil, there was a reported seroprevalence rate of 32.5%, with parasitemia in almost 47% of seropositive dogs (271). In southeastern Brazil, seroprevalence rates in dogs ranged from 15.9% in urban areas up to 57% in rural areas of endemicity (272, 273). In Mediterranean areas of endemicity, the reported canine seropositivity rates were approximately 8.1% in central Spain, 13% in southern Spain, and between 4 and 25% in Portugal, France, Italy, Greece, Cyprus, and Turkey (274, 324, 325). The presence of infected animals was significantly correlated with human risk, but the incidences of human disease varied by country and region (271, 324). In Europe, the estimated incidence of human ZVL ranges from 0.02 to 0.47 cases per 100,000 people, except in Turkey, where a higher incidence, 1.6 to 8.53 cases/100,000 people, was reported (324, 326). In areas of Brazil where the disease is endemic, the incidence of VL was much higher, with twice the number of cases for the entire Mediterranean region between 2004 and 2008 (326).

Risk factors for ZVL account for differences in human incidences in Brazil versus Europe. Numerous studies have demonstrated a risk for ZVL based on the presence of dogs within the household, housing types (mud-walled housing compared to concrete or brick housing), education level, income, and disease knowledge (271, 272, 327, 328). Clinical ZVL has also been associated with poor nutrition (328). Many of the same factors associated with human ZVL also apply to owner characteristics associated with risks for canine VL (271, 272, 329). Vertical transmission has been characterized for dogs and people, causing an increased risk for infants born to parasitemic mothers (330). The treatment of pregnant women with liposomal amphotericin B appears to be successful in reducing the occurrence of congenital VL (330). Additional risk factors for humans are related to their immunologic status and their ability to clear infection or maintain

an asymptomatic state. These factors include concurrent infection with HIV, coinfections with helminth parasites, drug abuse, and other immune suppressions. These comorbidities, especially HIV coinfection, confer a higher risk for the development of ZVL (331). The risks for dogs also include coinfection with other parasites, rickettsial diseases, heartworm disease, or immune suppression (332, 333). Genetic susceptibility may also be a factor for the development of clinical disease. Large-scale studies conducted in numerous countries indicated a role of genetic susceptibility, including polymorphisms of a number of metabolic genes, iron metabolism genes, chemokines, cytokines, and HLA alleles (334). This suggests a complex evolutionary interplay of parasite and host factors, which are likely associated with disease susceptibility.

Prevention

The prevention of leishmaniasis requires the blocking of a step in the parasite's life cycle. The interruption of sandfly transmission is of primary importance for individual and community protection from CL and VL. Avoiding being outside during times of sandfly feeding, typically from dusk until dawn, can greatly reduce transmission. Wearing topical insect repellents and utilizing permethrin-treated bed nets or clothes are also effective in repelling sandflies (335–337). In domestic areas, residual household sprays have been utilized to reduce the presence of vectors, but inconsistent compliance with periodic spraying, cost, and concerns over insecticide resistance limit the efficacy of this type of intervention on a widespread, long-term basis.

Approaches to address reservoir populations have also been implemented in an attempt to reduce ZVL. Brazil has implemented public health policies utilizing voluntary surveillance and the culling of positive dogs to reduce the burden of VL (338). While studies have shown that vigilant surveillance and culling can reduce the canine prevalence of VL to a degree, impacts on human infection are more difficult to ascertain (339). Limitations in diagnostic sensitivity likely lead to false-negative diagnoses for a large number of asymptomatic dogs, delays between testing and dog removal increase the likelihood of transmission, and the financial and emotional costs of the policy implemented in Brazil are high (329, 339, 340). The use of permethrin or deltamethrin collars or topical applications has shown efficacy in reducing sandfly feeding and transmission in areas of endemicity (341, 342). However, the cost and necessity for reapplication make these interventions more difficult to utilize in many regions of endemicity. Limiting the degree of human infection is also important for the control and prevention of severe clinical disease. Public health efforts have resulted in a reduction in the number of severe cases in Brazil. There is currently no vaccine for human leishmaniasis. Continued research may result in a vaccine with long-term, efficacious protection.

CONCLUSIONS

Zoonotic diseases are an important challenge to the health of the public worldwide. Rudolf Virchow, a 19th-century German physician, politician, and luminary promoter of public health and social medicine, once stated, "There is no scientific barrier, nor should there be, between veterinary medicine and human medicine" (343). In fact, more than 60% of recently emergent human pathogens are zoonotic and include more than 200 different bacteria, viruses, protozoa, and other parasites (344). However, in many regions of the world, human and veterinary medicine remain segregated. Companion animals, such as dogs, cats, and others, have a significant role in companionship in the United States, with an estimated 62% of homes owning a pet in 2008, equaling 72.9 million pet-owning homes in the United States alone (1). Companion animals benefit human health in many ways, including social interactions and support and the documented health benefits of reduced blood pressure, reduced heart disease, and a reduction in health care costs (345, 346). Recent studies indicated that infants in pet-owning homes are healthier, with lower rates of otitis, reduced numbers of respiratory infections, and reductions in the use of necessary antibiotics (347). However, when interactions between species are increased, the transmission of pathogens common to both species becomes a greater risk to humans and pets alike. Here we discuss the mechanisms of zoonotic protozoal disease virulence, human and companion animal epidemiology, means of transmission, and subsequent ways to prevent zoonotic disease. In all instances, good hygiene, including proper hand washing, is critical for halting zoonotic transmission. To prevent the vector-borne spread of *Babesia* and *Leishmania* spp., the use of topical insecticides on pets and their owners, as well as other means to prevent infectious insect bites, is indicated. The transmission of *Toxoplasma gondii*, a classical zoonotic disease, can additionally be prevented by proper food preparation and water sanitation. Although the zoonotic role of *Giardia* is debated, irrespective of how the protists arrived in water, proper water sanitation methods will hinder *Giardia* transmission. Surprisingly, *Trypanosoma cruzi* has emerged as a food- and, particularly, juice-borne disease, requiring the adaptation of food sanitation methods for the prevention of *T. cruzi* transmission. As dogs and cats often consume insects, keeping them inside, where the chances that they can be a meal for the vector or vice versa are reduced, can prevent *T. cruzi* or other vector-borne infections. An understanding of zoonotic protozoa and their transmission and epidemiology is a necessary step toward the prevention of these zoonotic diseases. We cannot completely eliminate our exposure to these pathogens, adapted for long-term survival within multiple host species over millennia, and we do not want to eliminate our exposure to our pets. Instead, understanding what factors increase zoonotic protozoal disease risk and utilizing and promoting effective means for their prevention can reduce the incidence and global spread of zoonotic protozoal pathogens in the future.

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