Review

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Review

Colpodellosis: Emerging Tick-Borne *Colpodella* spp. Infections of Public Health Importance

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Abstract

***Colpodella* spp.** are phylogenetically related to apicomplexans such as *Plasmodium* spp., *Babesia* spp. and *Cryptosporidium* spp. *Colpodella* spp. are free-living protists that prey on bodonids, ciliates and algae using myzocytosis. *Colpodella* spp. cause human and animal infections known as colpodellosis, with transmission predominantly through ticks in different geographic areas across different continents. *Colpodella* spp. have been detected in six genera of ticks and the biting fly *Stomoxys indicus*. Ticks transmit zoonotic pathogens and the identification of *Colpodella* spp. coinfected with *Babesia* spp. poses a major public health risk due to human and animal encounters exposing humans to tick bites. Human cases of colpodellosis have involved three cases of blood infection, a fourth case of tickborne infection and a fifth case of urinary tract infection. In this narrative review, the predominant occurrence of *Colpodella* spp. in ticks that transmit zoonotic pathogens will be reviewed. Differences in the disease presentations and symptoms of colpodellosis in tickborne infections will be discussed. The pattern of *Colpodella* spp. coinfections with piroplasms and *Cryptosporidium* spp. will be evaluated. The pressing need for morphological identification of *Colpodella* spp. to assist proper characterization of the different species and strains identified in arthropods and vertebrate hosts will be highlighted.

Keywords: tick-borne diseases; colpodellosis; apicomplexans; blood borne parasites; *Colpodella* species; emerging pathogens; myzocytosis; public health; relapsing fever; zoonoses

1. Introduction

Colpodella species are cosmopolitan free-living protist relatives of the pathogenic Apicomplexa typically characterized as predators that prey on other protists such as ciliates, bodonids and algae by myzocytosis in the soil, fresh water and marine environments [1,2]. *Colpodella* spp. have two life cycle stages, a fusiform trophozoite with a curved rostrum at the anterior end and a cyst stage that undergoes cell division to release two or more juvenile trophozoites. Previous studies of the life cycle of *Colpodella* sp. ATCC 50,594 showed that intermittent stages occur during the life cycle in culture. Juvenile trophozoites mature into older trophozoites, with both stages of trophozoites preying on *Parabodo caudatus*. During myzocytosis, trophozoites form a posterior food vacuole and the trophozoites develop into a pre-cyst stage [3,4]. Following degradation of the anterior end of the pre-cyst, encystation occurs leading to the development of transient or permanent cysts [5] (Figure 1). Transient cysts excyst within 1-2 h in the active culture while permanent cysts persist in culture for up to 14 days [4]. Following myzocytosis, most species of *Colpodella* spp. encyst. *Colpodella unguis* and *C. pseudoedax* do not form cysts but divide by fission [6]. *Colpodella* spp. also infect vertebrate hosts following tick bites resulting in symptomatic and asymptomatic infections and posing a public health risk to humans that encounter infected animals. The infections caused by *Colpodella* spp. are designated colpodellosis [7].

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Colpodella sp. ATCC 50,594 carries out endocytosis as an alternate means of nutrient uptake, suggesting that in the absence of prey, *Colpodella* spp. can survive within arthropod and vertebrate hosts. However, it is unclear what types of macromolecules serve as nutrient sources and whether encystation occurs after endocytosis [8]. *Colpodella* species cause infections in vertebrate hosts resulting in colpodellosis and have been reported in six genera of ticks and in a biting fly [9–13]. The presence of *Colpodella* species in blood, cerebrospinal fluid (CSF), urine and fecal samples of infected hosts indicate different routes of transmission and possibly host specificity. However, the major source of transmission with accompanied signs and symptoms has been through tick bites. Transmission through contaminated drinking water followed by gastrointestinal illness resulting in diarrhea has also been proposed, albeit with a link to ticks obtaining *Colpodella* spp. from contaminated water [10,14]. *Colpodella* spp. and strains identified in ticks share identity with species and strains identified in humans and other vertebrate hosts, indicating that colpodellosis is a zoonotic infection. The number of cases reported in humans and animals associated with tick bites continues to increase. While *Colpodella* spp. DNA detection by PCR has aided diagnosis, there is no information regarding the morphological characteristics of *Colpodella* spp. identified in ticks, blood and fecal samples. In this narrative review, the predominant occurrence of *Colpodella* spp. in ticks that transmit zoonotic pathogens will be reviewed. Differences in the disease presentations and symptoms of colpodellosis in tickborne infections will be discussed. The pattern of *Colpodella* spp. coinfections with piroplasms and *Cryptosporidium* spp. will be evaluated. The pressing need for morphological identification of *Colpodella* spp. to assist proper characterization of the different species and strains identified in arthropods and vertebrate hosts will be highlighted. Morphological identification of *Colpodella* spp. identified in ticks and vertebrate hosts along with nucleic acid amplification and serology is of vital importance in understanding tickborne colpodellosis.

2. Human and Animal Colpodellosis

Colpodella spp. has been identified in co-infections with *Babesia* spp., *Theileria* spp. [15,16] and *Cryptosporidium* spp. [17,18] using primers targeting the 18S rRNA of these pathogenic apicomplexans. There is an urgency to identify the morphology of the *Colpodella* spp. identified in human and animal hosts, to ensure accurate identity of infecting species since there is currently a lack of specific molecular probes to identify *Colpodella* spp. Specific DNA sequences and other specific biomarkers are unavailable available for detection of *Colpodella* spp. Human and animal hosts infected with *Colpodella* spp. present varied symptoms. Understanding the types of symptoms and associating them with specific sites of infection in infected hosts will aid accurate diagnosis. In human infections, fever, anemia, headache, and neurological symptoms have been described [13,19,20]. The prospects for zoonotic infections with serious impact on public health continue to increase as new cases are reported in animals that come into close contact with humans in recreational, domestic and farm environments and for humans encountering infected wildlife and animals housed in zoos [11,17,18,21–24]. Among the pathogenic apicomplexa, intracellular infections occur in blood cells such as erythrocyte infection by *Plasmodium* spp. and *Babesia* spp. and *Theileria* spp. infection in lymphocytes. *Toxoplasma gondii* infect macrophages and other nucleated cells and *Cryptosporidium* spp. infect epithelial cells in the gastrointestinal tract [25]. The life cycles of the apicomplexa include asexual and sexual stages. Whether transmission is through a vector such as mosquitoes or ticks for *Plasmodium* and *Babesia* transmission, respectively, the life cycle stage initiating infection is the sporozoite stage. Similarly, ingestion of oocysts of *Cryptosporidium* spp. and coccidians results in the release of sporozoites that initiate the infection. Sporozoite invasion is followed by schizogony (merogony) and the release of merozoites which can maintain the asexual stage infection or differentiate into male and female gametocytes which after fertilization, develops into a zygote that matures into the oocyst [25]. Despite the reports of human and animal transmission of *Colpodella* spp., the life cycle stage initiating infection or causing transmission is unknown. Similarly, life cycle stages responsible for pathogenesis in infected hosts are unknown. It is unclear how *Colpodella* spp. survives in arthropods and in vertebrate hosts. *Colpodella* spp. are predatory protists in the environment,

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feeding on ciliates, bodonids and algae. Currently, it is unknown whether *Colpodella* spp. is present with its prey in both arthropod and vertebrate hosts or whether *Colpodella* invades host cells or performs endocytosis to obtain nutrients. Although the life cycle of the model *Colpodella* sp. ATCC 50,594 has been described in culture [4], the life cycle in the environment is unknown. Whether infected humans and animals are dead-end hosts is unknown. However, the presence of *Colpodella* spp. in blood and in fecal samples suggests that life cycle stages are introduced into the environment from infected hosts through ticks and biting flies and through the presence of life cycle stages in fecal samples. Transmission can be maintained by tick and fly bites and by drinking contaminated water or possibly ingesting cyst-contaminated food like transmission of other cyst forming pathogenic protozoa [25]. Seventy-five percent of infections caused by viral, bacterial and parasitic pathogens are zoonotic diseases [26]. Tickborne infections contribute to zoonotic diseases and pose major public health risk due to close associations between infected animals and humans. Human to human, animal to human and human to animal infections can be maintained through tick bites with either biological development of the pathogen within the ticks or mechanical transfer of the pathogen by the tick. If the development of *Colpodella* spp. within the vertebrate hosts follows what is known for other cyst producing pathogenic protozoans, we would expect the development of cyst stages within the infected host. Cyst stages of protozoans like *Entamoeba*, and *Giardia* and oocysts of *Cryptosporidium* spp. develop in the gastrointestinal (GI) tract and are eliminated into the environment with feces [25]. Alternately, if active trophozoite stages are introduced into the host through tick or fly bites, these same stages can also be picked up by the arthropods for transmission to new hosts. Currently it is unclear whether infections in vertebrate hosts are intracellular or extracellular. The first report of human infection by *Colpodella* spp. was described as an intracellular infection due to detection of *Colpodella* within erythrocytes by Giemsa staining [19]. *Colpodella gonderii* and its prey *Colpoda steinii* were detected in human urine by Giemsa stain [27] in a urinary tract infection. In subsequent reports of human and animal infections *Colpodella* spp. DNA was detected in blood, cerebrospinal fluid, fecal samples and in ticks by polymerase chain reaction (PCR), DNA sequencing and phylogenetic analysis after sequence alignment [reviewed in 7]. There have been no other reports on the morphology of *Colpodella* spp. from infected hosts following tick or fly bites and from fecal samples. The identification of *Colpodella* spp. in human and animal infections was initially considered a rare, opportunistic, or "accidental" occurrence [7,23]. However, the number of infections where *Colpodella* spp. are the only organism identified where ticks are involved in transmission and where symptoms were described has increased. Phylogenetic analysis of identified DNA sequences suggests different infective species and strains. While the term "opportunistic" infections may be applied, colpodellosis can be described predominantly as a tickborne infection, with some *Colpodella* species causing relapsing fever and GI tract infections.

3. Microscopy and Morphological Characterization of Colpodella Species

Diagnosis and management of parasitic infections require an integrated effort combining microscopy, nucleic acid amplification methods like PCR, serology and cell culture. Microscopy is the gold standard for the diagnosis of parasitic and infectious disease-causing organisms. Identification of parasites intracellularly and extracellularly in blood and other body fluids relies on the use of wet mounts and stained smears examined by light microscopy. Microscopy remains a fundamental and most valuable tool for the identification of parasites [28–31]. Blood or CSF smears containing trophozoite stages of parasites can be observed following staining with Giemsa stain. The presence of life cycle stages in tissues can also be identified using hematoxylin and eosin (H&E) staining. Light microscopy provides an easily available and accessible resource to accurately identify parasites. Microscopy is indispensable in identifying trophozoites in blood smears and in identifying cysts, oocysts, eggs and helminth larva in fecal samples. The combination of microscopy and molecular techniques continues to be critical in identifying new parasite species [32,33]. For colpodellosis, identifying morphological characteristics of *Colpodella* spp. detected in ticks and in infected hosts is vital in the absence of specific molecular probes for *Colpodella* spp. and the continued

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detection of *Colpodella* spp. DNA using oligonucleotide primers targeting the piroplasms *Babesia* spp. and *Theileria* spp., and *Cryptosporidium* spp. Misidentifications and misdiagnosis can occur without integration of diagnostic techniques. There is a long history of parasite research, particularly among protozoan parasite research that provides an opportunity to understand *Colpodella* spp. stages transmitted to vertebrate hosts through tick bites and to understand the vector capacity and biological necessity of the tick as a vector for colpodellosis. The advantages of microscopy outweigh the disadvantages when considering the rapid identification of parasites in wet mounts or stained preparations and the low cost of using light microscopy. Often cited disadvantages include the need for skilled microscopists, challenges in identifying parasites when parasitemia or parasite density is low and determining appropriate staining techniques [28–31]. To overcome the challenges of identifying cysts of *Colpodella* sp. ATCC 50,594 and its prey *Parabodo caudatus* using Giemsa staining, Sam-Yellowe's trichrome staining series was developed to aid *Colpodella* spp. life cycle stage identification [7]. The staining technique allowed identification and differentiation of cysts and allowed identification of life cycle stage transitions during the life cycle of *Colpodella* sp. ATCC 50,594 in culture [34] (Figure 1). Diagnosis, differentiation of life cycle stages in ticks and infected hosts, and quantitation of parasite density require microscopy. Furthermore, investigating interactions of *Colpodella* spp. with host cells and accurate taxonomic investigations require microscopy. The ability to perform collaborative investigations is crucial to enable labs that are not set up for microscopy to collaborate with labs that perform microscopy on a routine basis. Based on the number of cases currently reported in humans and animals, and the risk to public health due to transmission of infection by ticks, ignoring morphological characterization of *Colpodella* spp. identified in epidemiological screening studies slows the progress of understanding the dynamics of colpodellosis and the etiological agent for the disease.

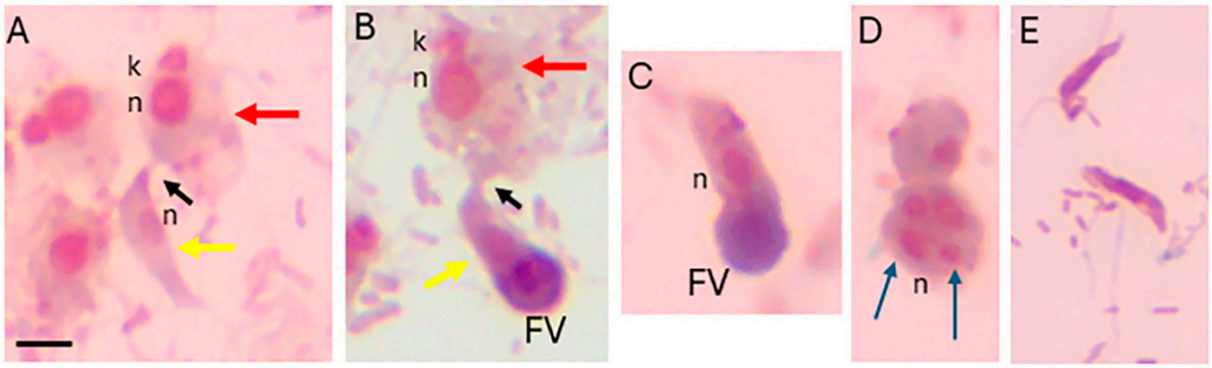

Figure 1. Sam-Yellowe's trichrome A staining of *Colpodella* sp. ATCC 50,594 life cycle stages in nutrient uptake cultures [4]. *Colpodella* sp. ATCC 50,594 trophozoite attached to Parabodo caudatus feeding by in myzocytosis shown in (A,B). A large posterior food vacuole forms during the feeding process (B) resulting in the formation of a pre-cyst at the conclusion of myzocytosis (C). Encystation occurs followed by cell division to form a single nucleus cyst to cysts containing four or more nuclei (D) [4]. Juvenile trophozoites are released following cell division (E). The images shown were captured at X1000 magnification as described [4]. The images are from the archived images in the Sam-yellowe lab. This figure is unpublished. Panel D is used in Figure 2 to show a single nucleus and 4 nuclei stage cyst. (Scale bar: 10 μm).

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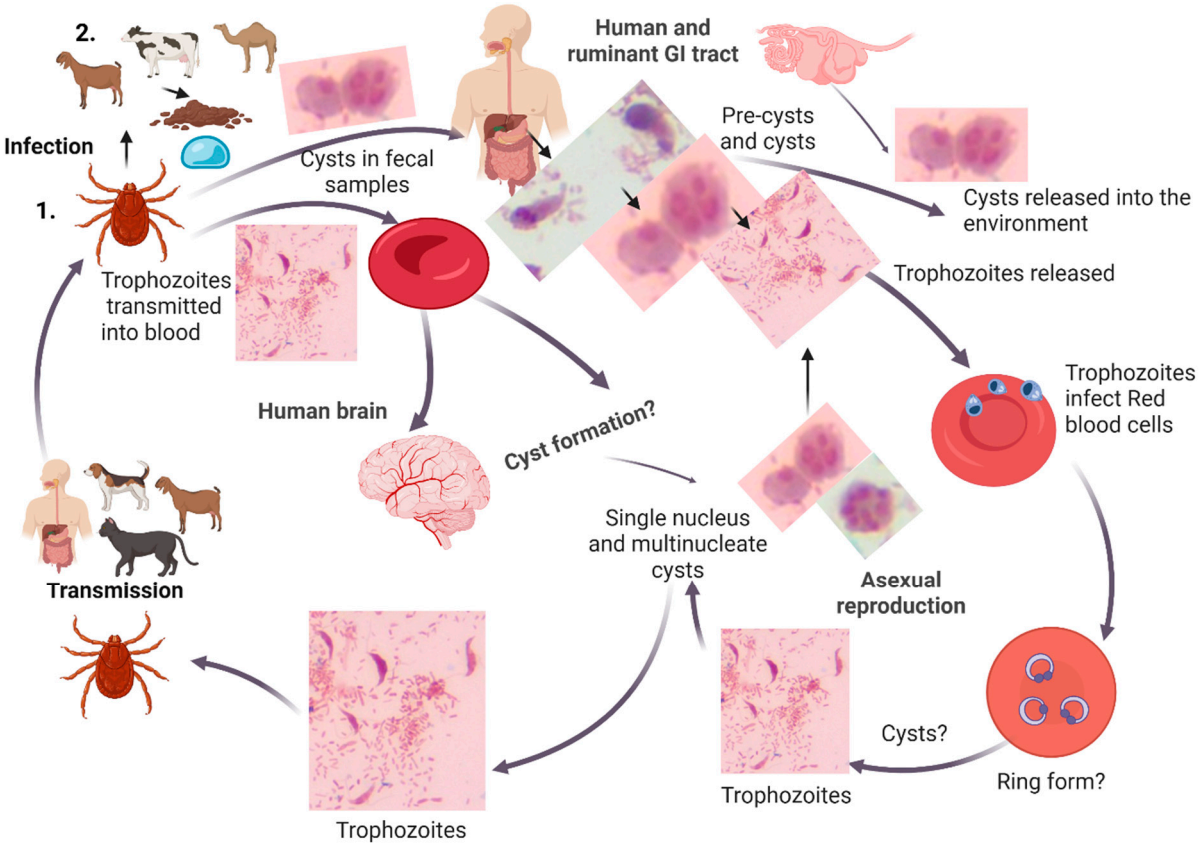

Figure 2. Proposed life cycle and transmission of tickborne colpodellosis shown using *Colpodella* sp. ATCC 50,594 life cycle stages formalin fixed and stained with Sam-Yellowe's trichrome A stain. (1) Tickborne transmission can occur through tick bites with the deposition of *Colpodella* spp. trophozoites into the blood stream of the vertebrate host. Trophozoites can invade red blood cells for intracellular infection or remain in the blood extracellularly for transfer into tissues like the brain. Trophozoites may encyst in tissues and after cell division in cysts containing two or more nuclei, released trophozoites reinvade red blood cells or remain extracellular in the tissues and in blood. Ticks can pick up circulating trophozoites during a tick bite for transmission to a new host. (2) Ticks may pick up *Colpodella* spp. trophozoites or cysts from water containing *Colpodella* spp. or direct transmission can also occur in a second route through contaminated water containing *Colpodella* spp. trophozoite and cysts deposited into the environment through fecal samples from infected hosts such as ruminants, felines, cows or camels. Excystation leads to trophozoite release which can differentiate after feeding to pre-cysts and then into cysts which release trophozoites after excystation. The trophozoites may invade red blood cells to form intracellular ring-like stages as described [19]. Differentiated trophozoites may encyst or are picked up by ticks. Created in https://BioRender.com (accessed March 12, 2026).

There are numerous unanswered questions regarding the mode of parasite transmission in colpodellosis. Life cycle stages initiating transmission, causing pathogenesis and maintaining spread within and among hosts is unknown. Based on the literature reports of colpodellosis, two routes of transmission are proposed in this review as shown in Figure 2. In the first route (1), tickborne transmission may occur through tick bites with the deposition of *Colpodella* spp. trophozoites into the blood stream of the vertebrate host. Trophozoites either infect red blood cells for intracellular infection as reported [19], or trophozoites may remain in the blood extracellularly for transfer into tissues like the brain [13]. Differentiation of the trophozoites into the cyst stage may occur in tissues and after cell division, trophozoites get released and reinvade red blood cells or remain extracellular in the tissues and in blood where a feeding tick can pick up circulating trophozoites for transmission to a new host. Ticks may also pick up *Colpodella* spp. from water containing *Colpodella* spp. [10,14]. Direct transmission (2) may also occur in a second route through contaminated water containing

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Colpodella spp. trophozoite and cysts deposited into the environment through fecal samples from infected hosts such as ruminants, felines, cows or camels [10,18,23,24]. Within the GI tract, excystation leads to the release of trophozoites which would differentiate into pre-cysts, then cysts which can be released into the environment. Excystation may occur after cell division and trophozoites released can enter the blood stream, infect red blood cells for intracellular infection or remain extracellularly in the blood. Trophozoites differentiate into cysts which undergo cell division to release trophozoites that will maintain the infection. Trophozoites in the blood can be picked up by ticks which can transmit *Colpodella* spp. to a new host. Two key questions remain; how does *Colpodella* spp. survive within the arthropod and vertebrate hosts? Are the ciliate or bodonid prey also present in the arthropod and vertebrate hosts? *Colpoda steinii* was identified with *Colpodella* spp. in two infections [24,27]. In addition, *Parabodo caudatus* and *Bodo* spp. were identified with *Colpodella* spp. in a blood infection [35]. It is also unknown whether *Colpodella* spp. trophozoites can feed on host cells directly or endocytose nutrients from host fluids. Protozoan parasites invading host tissue have been described to feed by trogocytosis, phagocytosis and phagotrophy resulting in tissue damage and pathogenesis [36,37]. Mechanisms of virulence and pathogenesis are unknown for *Colpodella* spp. Whether *Colpodella* spp. carry viruses and bacteria that may contribute to pathogenesis like pathogenic amoeba, is unknown [38]. How *Colpodella* spp. is maintained and transferred among developmental stages of the tick is unknown. The proposed life cycle shown in Figure 2 with the proposed routes of transmission, though speculative since there are gaps in the knowledge of *Colpodella* spp. biology, are aimed at stimulating further investigations among investigators performing epidemiological screenings for tickborne pathogens. The route of entry and spread of *Colpodella* spp. life cycle stages need to be identified. The sites of infection within tissues and the stages responsible for pathogenesis need to be identified and life cycle stage differentiations with the tick and vertebrate hosts need to be identified. Microscopic evaluations of collected samples, staining for morphological characterizations of *Colpodella* spp. life cycle stages present in collected samples will aid investigations aimed at determining if the blood infections currently described as non-tick associated, involve tick bites. Culturing and staining protocols along with integrated diagnostic platforms for morphological and molecular characterization for *Colpodella* spp. were reviewed previously [7]. Some tickborne infections have symptoms of diarrhea such as in Lyme disease [39] suggesting that in cases if GI tract involvement and diarrhea in colpodellosis, tickborne transmission should not be ruled out. Efforts should be made to identify virulence markers for *Colpodella* spp. in tickborne, blood and GI infections. This will provide important clarifications regarding why colpodellosis in some hosts are symptomatic and in others not.

4. Symptomatic and Asymptomatic Tickborne Colpodellosis

4.1. Symptomatic Infections

Mosquitoes and ticks are the most important arthropod vectors that transmit pathogens that include viruses, bacteria, protozoan and helminth parasites. Mosquitoes transmit most vector borne pathogens with ticks representing the next frequent arthropod vector [40]. *Colpodella* spp. have been reported in six genera of ticks known to transmit the piroplasmids *Babesia* spp. and *Theileria* spp. [7]. Four species of *Hyalomma,* including the newly identified vectors *Hy. anatolicum* and *Hy. excavatum* carry *Colpodella* spp. [41]. *Rhipicephalus annulatus* was identified in Egypt for the first time carrying *Colpodella* spp. in cattle infestation [42]. Altogether, five species of *Rhipicephalus* carry *Colpodella* spp., and five species of *Dermacentor* carry *Colpodella* spp. and transmit zoonotic pathogens of public health importance [7] (Table 1). In a tickborne human infection, a 55-year-old female Chinese patient from Heilongjiang Province of Northeast China presented with neurological symptoms following a tick-bite [13]. Fever, dizziness, headache and gait disturbance were among the symptoms reported. Although suspected to have Lyme disease, no ulceration, exudate or erythematous lesions were observed [13]. Blood and cerebrospinal fluid (CSF) were examined by PCR using primers targeting the 18S rRNA gene of *Babesia* spp. Other suspected pathogens in the patient were *Eperythrozoon* spp.,

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Borrelia spp., *Anaplasma* spp., *Ehrlichia* spp., *Rickettsia* spp. and tickborne encephalitis virus (TBEV). *Colpodella* spp. was the only pathogen identified in the patient's CSF. *Colpodella* spp. DNA was not detected in her blood. *Colpodella* spp. was identified in 2/474 adult *Ixodes persulcatus* ticks collected in woodlands around the patient's home. Treatment with doxycycline (used for malaria treatment) resolved the infection. Jiang et al. [13] reported the detection of anti-*Borrelia burgdorferi* antibodies. However, PCR was negative for *B. burgdorferi* DNA. The *Colpodella* spp. strain HLJ identified from CSF shared 88.0-89.0% identity to the tick *Colpodella* spp. and both tick *Colpodella* DNA (accession number KT600661 and KT60062) shared 93.8% identity with each other.

A tickborne infection was reported in a South China tiger (*Panthera tigris tigris*) from the Meihua Mountains, China [10] bitten by *Haemaphysalis flava*. The only pathogen identified was *Colpodella* spp. PCR for detection of *Mycoplasma suis* and *T. gondii* was negative. The tiger showed symptoms of anorexia, runny nose, drool and had bluish-green stools and was treated with sulfonamide, cephalosporin, and ampicillin [10]. DNA extracted from ticks collected from the tiger, and from tiger blood was amplified using nested PCR with universal primers targeting the 18S rRNA gene of piroplasmids. Following the death of the tiger, hepatomegaly, splenomegaly, hemorrhage in kidneys and mesenteric lymph nodes was observed and pathology revealed severe, whole-body jaundice of the skin, eyes, conjunctiva, oral mucosa, tracheae, and coronary fats around the heart [10]. Three genera of ticks identified were positive for *Colpodella* spp. Twenty-two *Colpodella* DNA sequences were identified among the three genera. Following DNA sequence alignment, the sequences from the tiger and ticks had 100% identity. The sequences shared 90.1% sequence identity to *Colpodella* spp. HEP and 90.4% sequence identity to *Colpodella* spp. HLJ. Among the ticks collected, 16/22 ticks carrying *Colpodella* spp. were *Haemaphysalis* spp. The dominant species were *H. flava* and *H. longicornis*. *Colpodella* spp. was also detected in water from ditches around the tiger enclosure. However, *Colpodella* spp. was not detected in the soil. Chiu et al. [10] suggest ticks obtained *Colpodella* spp. from the contaminated water and then transmitted the parasites to the vertebrate hosts. A similar suggestion was made about *Colpodella* spp. detected in a red fox [14].

Colpodella spp. was detected in ticks infesting two-humped camels (*Camelus bactrianus*) and in blood collected from symptomatic animals, in Gaotai County, Gansu Province, China [23]. Two hundred and eighty-eight ticks consisting of *Hyalomma asiaticum* (245/288) and *Haemaphysalis longicornis* (34/288) were collected from camels with *H. asiaticum* being the most predominant at 245/288 ticks examined and 150 blood samples were collected from the camels. *Colpodella* spp. coinfections with bacteria were detected. Coinfections of *Colpodella* spp. and *A. bovis* (14/288), *Colpodella* spp. and *Rickettsia* spp. (1/288) and *Colpodella* coinfections with *Rickettsia* and *A. bovis* (1/288) were detected. Infected camels exhibited symptoms of fever, appetite loss, diarrhea, fatigue and decreased milk output. Although morphological identification and biochemical characterizations were carried out for the ticks, no morphological characterization of *Colpodella* spp. was performed.

Coinfections of *Colpodella* spp. and the bacteria *Rickettsia* and *Ehrlichia* were detected in *Ambyloma javanense* ticks infesting 17/21 rescued sick Malayan pangolins [9]. The pangolins were examined for ectoparasites and DNA from ticks, pangolin blood and tissues of the sick pangolins following their death, was amplified for virus, bacteria and protozoan parasite DNA. The pangolins exhibited symptoms of anorexia, cough, edema of the extremities and drowsiness. The animals also had bloody stools, hematuria, convulsions and other neurological symptoms [9]. At autopsy, severe organ damage was observed, congestion, edema of major organs, ascites and inflammation was observed. Histological examination of the pangolin tissues by H & E staining did not detect pathogens. *Colpodella* spp., *Rickettsia* spp., *Anaplasma* spp., *Ehrlichia* spp. and *Babesia* spp. were detected in ticks. *Colpodella* spp. was not detected in pangolin tissues. *Theileria* spp, *Hepatozoon* spp. and viruses were not detected. Coinfections of *Colpodella* spp. and *Rickettsia* and *Colpodella* spp., *Rickettsia* spp. and E*hrlichia* spp. were detected [9]. Six out of 33 ticks carried *Colpodella* spp. which shared identity to 18S rRNA gene sequences from *Colpodella* spp. identified from Qinghai (MH012046) and Yunnan (MH208621) and from *Colpodella* HLJ strain identified from a human infection with neurological symptoms.

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4.2. Asymptomatic Infections

Asymptomatic hosts infected with *Colpopdella* spp. pose an important public health risk since the parasite is present in the infected host but due to the lack of symptoms, precautionary or preventive measures might not be taken to prevent parasite transmission to human hosts encountering infected animals or arthropod vectors. Asymptomatic animals serve as reservoirs that can maintain infections within communities and in the environment. Epidemiological studies involving screening of domestic, agricultural and wildlife animals for tickborne pathogens and *Cryptosporidim* spp. has revealed the presence of *Colpodella* spp. in asymptomatic and symptomatic infections. Jimale et al. [43] screened 98 cattle and 104 goats for tickborne parasites. These were free-grazing animals from the Puglia region, Southern Italy. Genomic DNA was extracted from animal blood and from the ticks infesting them. Template DNA was amplified using primers targeting 18S rRNA from *Babesia* spp. and *Theileria* spp. Primers targeting the *gltA* gene of *Rickettsia* spp. was also used for PCR. *Babesia* spp., *Theileria* spp. and *Rickettsia* were detected in ticks. The predominant (31/42) tick identified among 42 adult male and female ticks collected from cattle was *Rhipicephalus bursa*. *Rhipicephalus secundus* was also identified (11/42). *Colpodella* spp. DNA with 100% identity to *Colpodella* spp. accession number OQ540588.1 was identified in one female *Rh. bursa*. Among the goats the predominant tick species identified was *Rh. bursa* (25/36) along with *Rh. secundus* (11/36). *Rickettsia* spp. was identified in one female *Rh. bursa. Colpodella* spp. DNA was detected in asymptomatic hosts screened for tickborne and blood borne pathogens using PCR, suggesting that these hosts can serve as reservoirs to maintain zoonotic transmission between animal and human hosts. Blood was collected from asymptomatic pet dogs and cats attending a veterinary hospital in Guiyang, China [24]. Genomic DNA extracted from the collected blood was screened for piroplasmids using primers targeting the 18S rRNA gene. In pet cats, *Theileria uilenbergi*, *T. luwenshuni* and *Colpodella* spp. were detected. In pet dogs *T. uilenbergi* and *Colpodella* spp. were detected. *Colpodella* spp. was also detected in the tick *H. longicornis*. Although *Theileria* spp. do not cause human infections, the presence of *Colpodella* spp. raises public health concerns for zoonotic transmission to members of households with infected pets. Qi et al. [15] performed epidemiological screening of asymptomatic dogs and goats for tickborne parasites in Yiyuan County, Central Shandong Province, China. Following PCR amplification of DNA extracted from *H. longicornis* ticks using primers targeting 18S rRNA gene of *Theileria* spp. and *Babesia* spp., *Colpodella* spp. was identified in individual infections and from the ticks collected from goats and dogs. The *Colpodella* spp. DNA had 92-98% sequence identity to *Colpodella tetrahymenae* (accession number MH208619.1).

Two hundred asymptomatic camels from Southern Egypt were investigated for tick infestation leading to the identification of *Hyalomma dromedarii* on the camels [11]. The examination was performed during routine veterinary evaluation of the camels. Two hundred and ninety-seven ticks identified on the camels were screened for tickborne parasites. In 30/297 ticks, *Colpodella* spp. was identified using primers targeting 18S rRNA gene of piroplasmids and in 16/297 ticks, *Babesia bovis* was detected using primers targeting the spherical body protein-4 gene. *Colpodella* spp. was identified in *Rhipicephalus annulatus* infesting cattle in Egypt for the first time [42]. Two hundred and fifty-eight ticks were collected from 110 cattle during routine veterinary examinations. Genomic DNA was extracted from pooled ticks and screened for piroplasmids and *Colpodella* spp. using PCR. The major merozoite surface antigen gene of *Theileria annulata* identified *Theileria*, the rhoptry associated protein 1 gene identified *Babesia bigemina*, the spherical body protein gene identified *B. bovis* and the 18S rRNA piroplasm gene identified *Colpodella* spp. Coinfections *of Colpodella* spp. and *B. bovis*, *Colpodella* spp. and *T. annulatus* and *Colpodella* spp., *B. bovis* and *T. annulatus* were identified in the ticks. The minimum infection rate (MIR) of *Colpodella* detected in *Rh. annulatus* was 2.3% per sample of the pooled ticks examined [42].

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Table 1. Symptomatic and asymptomatic *Colpodella* spp. infections reported in humans and animals.

***Colpodella* spp. in humans and animals**	**Year**	**Country**	**Reference**
Tickborne *Colpodella* spp. infections			
Human tickborne infection, neurological symptoms, single infection, female, fever, dizziness, gait disturbance, headache	2018	China	[13]
Tiger (*Panthera tigris amoyensis* Hizheimer) in blood and ticks, tickborne *Colpodella* spp. single infection, anorexia, runny nose, drool, bluish-green stool, at autopsy multiple organ damage	2022	China	[10]
Two-humped camels (*Camelus bacterianus*), *Colpodella* spp. in blood and infesting ticks, fever, appetite loss, diarrhea, fatigue, decreased milk output	2025	China	[23]
Pangolins, *Colpodella* spp. in infesting ticks, co-infection, anorexia, cough, edema of extremities, drowsiness, at autopsy severe organ damage, congestion, edema of major organs, ascites, inflammation	2024	China	[9]
Camels, *Colpodella* spp. in infesting ticks, asymptomatic infection	2024	Egypt	[11]
Cattle and goats, *Colpodella* spp. in infesting ticks, asymptomatic infection	2024	Italy	[43]
Goats and dogs, *Colpodella* spp. in ticks, asymptomatic infection	2024	China	[15]
Cattle, tick associated *Colpodella* spp. infection, co-infection	2017	Mozambique	[51]
Goats, *Colpodella* spp. in infesting ticks	2026	Pakistan	[41]
***Colpodella* spp. in a biting fly**			
Horse, *Colpopdella* spp. in infesting biting fly (*Stomoxys indicus*), co-infection	2023	Thailand	[12]
***Colpodella* spp. in blood infections**			
Human relapsing fever, non-tick associated blood infection, single *Colpodella* spp. infection, female, productive cough, malaise, hemolytic anemia	2012	China	[19]
Human relapsing fever, non-tick associated, single *Colpodella* spp. infection, male	2017	China	NCBI accession number MF594625
Human relapsing fever, non-tick associated *Colpodella* spp. blood infection, male, fever, cough, myalgia	2025	China	[20]

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Cat, non-tick associated *Colpodella* spp. single blood infection, inflammation, tissue damage	2023	USA	[22]
Cattle and wildlife, non-tick associated *Colpodella* spp. blood infection, co-infection, asymptomatic	2020	Zambia	[16]
Horse non-tick associated *Colpodella* spp. blood infection, co-infection, asymptomatic	2022	China	[21]
Dog, non-tick associated *Colpodella* spp. blood infection, co-infection, *Parabodo caudatus* and *Bodo* spp. prey for *Colpodella* spp., asymptomatic	2021	Cambodia	[35]
Cats and dogs, non-tick associated *Colpodella* spp. blood infection, co-infection, asymptomatic	2023	China	[24]
***Colpodella* spp. in GI tract infections**			
Goats and sheep, non-tick associated infection, *Colpodella* spp. in diarrhetic fecal samples, co-infection	2024	Nigeria	[18]
Tibetan sheep, goat and yak, *Colpodella* spp. and prey *Colpoda* spp. in fecal samples from asymptomatic and diarrhetic animals	2025	China	[44]
Goats, fox, duck, Eurasian Coot, non-tick associated, *Colpodella* spp. in fecal samples	2025	Cyprus	[45]
Large zoo felids, *Colpodella* spp. in fecal samples, co-infection, asymptomatic	2021	China	[17]
***Colpodella* spp. in urinary tract infection**			
Human urinary tract infection associated with *Colpodella gonderi* and its prey *Colpoda steinii*, female	2021	Romania	[27]
***Colpodella* spp. infection in skin**			
Raccoon, non-tick associated *Colpodella* spp. in the skin of the ear, co-infection	2019	Poland	[52]

Colpodella spp. was detected in the ticks *Hyalomma excavatum* and *Hy. anatolicum* infesting goats (*Capra hircus*) in Pakistan [41]. Ticks collected from goats from seven districts of Khyber, Pakhtunkhwa, Pakistan were screened for pathogens. Among the pathogens, *Colpodella* spp., *Ehrlichia* spp., *Rickettsia hoogstraalii* and *Providencia rettgeri* were identified with *Colpodella* spp. having high prevalence rates in *Hy. excavatum* collected from Buner (15/167 ticks; 8.98%) and Kohistan (9/164 ticks; 5.48%) and in *Hy. anatolicum* from Chitral (8/100 ticks; 8%) [41]. *Colpodella* spp. from *Hy. anatolicum* had 100% sequence identity to *Colpodella* spp. (MH208621) from *Rhipicephalus haemaphysaloides* in China and 99.92% identity with *Colpodella* spp. (GQ411073.1) isolated from a woman with relapsing fever [19] and 99.59% identity to *Colpodella* spp. (accession number MH012046.1) isolated from *Dermacentor nuttalli* in China. In phylogenetic analysis, *Colpodella* spp. detected by Ullah et al. [41] clustered with *Colpodella* spp. identified from *Rh. annulatus* in Egypt (accession number PP937594), *Colpodella* spp. (accession number MH208620) in China, Luxemburg, Canada and an uncultured alveolate from Kenya and Austria, and uncultured eukaryote from France (accession number AY817009).

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5. Symptomatic and Asymptomatic Blood Infections

5.1. Symptomatic Infections

A case of relapsing fever was reported by Yuan et al. [19] in a 57-year-old female patient with a natural killer cell deficiency, in Yunnan Province, China [13]. She presented with a babesiosis-like blood infection and exhibited symptoms of malaise, productive cough, hemolytic anemia and relapsing illness. Elevated reticulocytes and lactate dehydrogenase were also reported. Oligonucleotide primers targeting conserved DNA fragments of *Babesia* 18S rRNA gene amplified DNA whose sequence had homology to *Colpodella tetrahymenae*. Giemsa staining and immunofluorescence assay identified intracellular infection in erythrocytes and anti-*Colpodella* antibody reacted with *Colpodella* in erythrocytes. The patient was treated with atovaquone and azithromycin after she failed to respond to oral tetracycline and intravenous artemether treatment [19]. *Colpodella* spp. DNA was detected in a male patient with relapsing fever. PCR amplified DNA sequence was deposited in NCBI (accession number MF594625). However, characteristics of the infection were not reported. In a third human blood infection, a 28-year-old male ICU patient from Qiandongnan Prefecture, Guizhou Province, previously admitted into a tertiary hospital with recurrent fever of one week duration was examined [20]. The patient had no recollection of tick bite. Five days before admission, the patient had fever, cough, and myalgia. Treatment with acetaminophen for three days did not clear the infection. Blood and sputum were collected to screen for pathogens. *Colpodella* DNA was detected in the blood sample using next generation sequencing (NGS). No hematological symptoms were observed. In the three cases, there were no reports of bites from ticks or other arthropods. The identified DNA clustered with *Colpodella* spp. from Zambian cattle but did not cluster with DNA from pet dogs and *Rhipicephalus microplus* from Guizhon Province. Human adenovirus group B was detected in blood and sputum. Although the source of infection remained unknown, direct contact with infected companion animals exposes humans to pathogens. Huggins et al. [35] employed NGS in a Cambodian study to identify DNA sequences of bacteria and blood borne pathogens from blood collected from 467 dogs. *Colpodella* spp. along with *Parabodo caudatus* and *Bodo* spp. were identified in one dog. The DNA sequence had 95% sequence identity to *Colpodella* spp. identified in horse blood [21]. Four hundred horses from China were examined for blood borne pathogens by PCR using primers targeting 18S rRNA gene [21]. DNA from 2/400 horses had homology to DNA from *Colpodella* spp. related to *Colpodella* sp. ATCC 50,594 and *Colpodella* strains HEP and HLJ. *Theileria* spp. (132/400) and *Babesia cabal* (2/400) were also identified in the horses. Huggins et al. [35] identified additional pathogens in arthropods (lice, fleas, lice, ticks) infesting dogs included the apicomlexans *Babesia vogeli* and *Hepatozoon canis* and the kinetoplastids *Bodo* spp., *Parabodo caudatus* and *Trypanosoma evansi*. In addition to blood and CSF, *Colpodella gonderii* and *Colpoda steinii* were identified in urine from a female patient with a history of chronic diseases and urinary infection. Giemsa staining of urine sample identified both protists. No other pathogens were identified, and no tick bites were reported. Treatment with ceftriaxone and metronidazole cleared the infection.

6. Colpodellosis with Gastrointestinal Symptoms

Tickborne infections such as Lyme disease can have symptoms that include diarrhea, anorexia and abdominal pain [39]. In colpodellosis reported for animals without recognized tick bites, direct infections through the drinking of contaminated water may not be the only route of infection. *Colpodella* spp. and *Colpoda* spp. were detected in fecal samples from sick and asymptomatic (healthy) Tibetan grazing ruminants in China. Seventy-nine fecal samples collected from free-range yak, Tibetan sheep and a Tibetan goat were screened for biodiversity of protists and nematodes in the animals by PCR [44]. Oligonucleotide primers targeting the V3-V4 fragment of the 18S rRNA gene were used for PCR and NGS was performed to identify the DNA sequences. The dominant genera of parasites identified were *Entamoeba* (93.67%), *Blastocystis* (75.95%), *Trichostrongylus* (68.35%), *Colpoda*

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(50.63%) and *Colpodella* (49.37%). Sick animals had diarrhea with *Colpodella* spp. having a high prevalence in the sick animals investigated. In asymptomatic animals, *Colpodella* spp. was 32.14% and in sick animals was 92.86%. *Colpoda* spp. in asymptomatic animals was 39.29% and in sick animals was 85.71%. *Colpodella* spp. prey on the ciliate *Colpoda* spp. The presence of both predator and prey in the stool samples indicates that both organisms are present in infected hosts. Both protists were identified in the urine of an infected human patient [27] and *Colpodella* with the prey organisms *Parabodo caudatus* and *Bodo* spp. were identified in the blood of an infected dog [35]. Molecular identification was used in the study by Wu et al. [44]. However, no morphological characterizations of *Colpodella* spp. were reported. Out of 19 total genera identified, the protists *Entamoeba* and *Colpodella* spp. were predominant.

Cryptosporidium spp. and *Colpodella* have been identified in coinfections using primers targeting the 18S rRNA gene of *Cryptosporidium* spp. It is important to unambiguously identify and distinguish oocysts of *Cryptosporidium* from cysts of *Colpodella* spp. in stool samples. *Colpodella* spp. cysts in the 4 nuclei stage may be misidentified as oocysts depending on the staining technique used. Polymerase chain reaction, NGS and nucleic acid amplification techniques should be used alongside morphological identification methods for identification of parasites. Life cycle stages initiating transmission, responsible for pathogenesis and knowledge of sites of infection rely on morphological characterizations. Treatment options and success depend on knowledge of life cycle stages. Treatment effective for the trophozoite stage may be ineffective for cysts. Tick bites were not reported in this study, and the symptoms are for GI tract infection. Diarrhea and fever were among the symptoms in sick two-humped camels infected by *Colpodella* spp. through tick bites [23]. *Colpodella* spp. was identified in fecal samples collected from red fox (*Vulpes vulpes*) [14] at the Hebei Xiaowutai Mountain National Nature Reserve. The suspected source of transmission was drinking water [14]. Ticks are thought to pick up *Colpodella* spp. from water and then transmitting the parasite to human and animal hosts [10]. Hasapis et al. [45] identified *Colpodella* spp. in fecal samples of birds, ruminants and a fox phylogenetically related to *Colpodella* strain HEP, HLJ and strains from *Colpodella* identified in sheep from Nigeria [18]. The Nigerian goats and sheep were screened by PCR for *Cryptosporidium* spp. using primers targeting *Cryptosporidium* spp. and *Colpodella* spp. DNA was identified. The DNA identified had sequence identity to 18S rRNA gene of *Colpodella* spp. from Cyprus identified in a duck and fox. *Cryptosporidium* spp. oocysts were identified by microscopic examination. The ruminants had diarrhea [18]. Similarly, fecal samples collected from asymptomatic large cats from the Harbin Zoo, China screened for *Cryptosporidium* spp. using 18S rRNA identified *Colpodella* spp. [17]. No tick bites were reported. Although time-consuming in some cases and requiring expert microscopists for accurate identification of morphological features of parasites, distinguishing the morphology of the species and strains of *Colpodella* spp. identified by DNA is crucial in furthering our understanding of parasite life cycle stages present in infections. Many tick-borne infections present with similar symptoms early in infection [28]. Accurate diagnosis of infection in the case of multiple tick-borne pathogens present in the host at the same time will require a knowledge of incubation times, characteristic pathognomonic signs, if known, and accurate identification of pathogen morphology to aid molecular diagnosis [28,46,47]. Diagnostic methods comprising staining, microscopy and molecular techniques have been used for diagnosis of known *Cryptosporidium* species and used to identify novel species [48]. Tick-borne infections present with gastrointestinal disturbances and should be considered in cases where *Colpodella* spp. are identified with an unknown source of transmission [49]. Co-infections of tick-borne pathogens present challenges in recognizing specific symptoms. However, if symptoms persist following treatment for a suspected tick-borne pathogen, coinfection with other pathogens should be suspected. A case of a 70-year-old man diagnosed with Lyme disease, anaplasmosis and babesiosis, illustrates the pattern of coinfection that can occur in human and animal hosts with tick-borne pathogens [47]. Colpodellosis as the result of coinfections and single infections have been reported. Phylogenetic analysis of *Colpodella* spp. DNA demonstrates distinct clades of *Colpodella* spp. reflecting different species, strains and patterns of virulence [50]. An integrated approach for diagnosis will allow the identification of *Colpodella* spp. in single infections

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and the identification of *Colpodella* spp. with multiple pathogens at the same time [28]. The extent of *Colpodella* spp. infections in diverse animals, their transmission by ticks [9–11,13,15,23,43,51] and the diverse tissue locations where *Colpodella* spp. DNA has been identified [19–22,52] lends urgency to intensified efforts aimed at improving our understanding of this emerging tick borne pathogen and important public health risk.

7. Conclusions and Recommendations

Increased reports of colpodellosis in humans and animals have provided more insights regarding symptoms of the disease. However, the presence of multiple pathogens within ticks and the lack of knowledge regarding the life cycle stages of *Colpodella* spp. mediating transmission, spread and pathogenesis in hosts is a major obstacle in furthering our understanding of this disease. Detection of *Colpodella* spp. DNA has been useful in identifying *Colpodella* spp. as an infective organism, but without morphological characterization and serological evaluation to aid molecular diagnosis, key aspects of disease development and pathogenesis remain unknown. Staining tick hemolymph, host blood and fecal samples to identify *Colpodella* spp. along with coinfecting protist pathogens is vital. The presence of prey protists for *Colpodella* spp. can also be identified. *Colpodella* spp. should be suspected in cases of babesia-like illnesses unresponsive to conventional treatment. In cases of single *Colpodella* spp. infections, the presence of protist prey such as ciliates, bodonids and algae should be investigated using primers targeting the 18S rRNA genes of the prey. In cases of diarrhea suspected of being caused by *Cryptosporidium* spp., *Colpodella* spp. should be suspected. Fecal samples should be examined for both oocysts and cysts. Integration of diagnostic methods that enhance point-of-care diagnostics will create new opportunities for the robust application of advanced techniques to understand the biology of *Colpodella* spp. This will benefit treatment and prevention and reduce the risk of *Colpodella* spp. human infections.

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