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VIRAL ENDOSYMPOSIS IN PARASITIC PROTOZOA: A REVIEW ARTICLE

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Abstract

Parasitic protozoa are responsible for numerous diseases of humans and animals globally. Understanding the factors influencing their pathogenicity and evolution is crucial for effective disease management. Among these factors, viral endosymbiosis has recently gained attention for its potential impact on host-parasite interactions, disease progression and treatment challenge.

This review summarizes the existing literature, researches and case studies to investigate the phenomenon of viral endosymbiosis in parasitic protozoa. Relevant articles and research papers were identified through comprehensive literature searches using electronic databases.

The presence of endosymbiotic viruses has significant implications for disease progression and may offer potential targets for therapeutic interventions.

Keywords: Parasitic protozoa, endosymbiotic viruses, host-parasite interactions, therapeutic interventions.

Introduction

Parasitic protozoa infect millions of individuals globally, as complex and multifaceted, influenced by various genetic, environmental, and immunological factors (Tarannum *et al,* 2023). Viral endosymbiosis is the presence of viruses within cells of another organism as a symbiotic relationship in *Entamoeba histolytica* (Miller and Swartzwelder, 1960). Endosymbiotic viruses of families Totiviridae, Partitiviridae and others were identified in many species (Lafleur and Olivier, 2022). The presence of endosymbiotic viruses within protozoa has garnered increasing attention due to its potential impact on parasite biology and disease pathogenesis (Lafleur and Olivier, 2022).

Trichomonas vaginalis: T. vaginalis is a protozoan causing trichomoniasis, an infection spread via sexual contact, with about 156 million new cases among people aged 15-49 years old and is considered one of the most common curable STIs (WHO, 2023). Its viral endosymbiont was described in 1985 and later is known as *T. vaginalis* virus (TVV), a 4.5-5.5 kilobase pair (kbp) dsRNA virus of genus *Trichomonas* viruses and the family *Totiviridae* (Weber *et al,* 2003). It is protected by a big viral protein capsid that is 85 kilodaltons in size. Inside parasite, it is closely linked to the Golgi complex or in the cytoplasm adjacent to the plasma membrane. (Graves *et al,* 2019). To copy the genetic material, TvVs use a viral RNA-dependent RNA polymerase (Wang and Wang, 1985). Four subspecies of TVV were identified: TVV1, TVV2, TVV3, & TVV4, belong genus *Trichomonas* virus of Totiviridae family coexist inside one TV isolate (Benchimol *et al,* 2002). Strain diversity within each TVV species were identified with many different strains (14 to 35) within each main TVV species, TVV1 & TVV2 are frequently detected subspecies, but TVV3 is less detected followed by TVV4 (Goodman *et al,* 2011).

TVV endosymbiosis in *T. vaginalis* strains varies globally with low prevalence (14-20%) in Egypt Korea, Iran, and the Philippines versus high prevalence (40 to 100%) worldwide (Fichorova *et al,* 2017). The small extracellular vesicle (exosomal TVV) plays a role on TVV transmission among different isolates (Ong *et al,* 2022). Existence of TVV diminishes the growth rate of *T. vaginalis* in vitro and induced lytic effect on the parasite (Rivera *et al,* 2017).

Trichomons vaginalis pathogenicity involves cyto-adherence to vaginal epithelial cells establishing infection with TVV is implicated in parasite accentuation of adherence capacity (Fichorova *et al,* 2017). About 50 proteins of different expression level were in

TVV positive isolates among which heat shock proteins (down-regulated), ribosomal proteins (up-regulated) and metabolic enzymes (He *et al,* 2017). Also, TV releases cysteine proteinases to break down the basement membrane and other components of vaginal epithelial cells as hemoglobin, fibronectin, and collagen IV (Arroyo and Alderete, 1995). TVV increases the expression of Ig-degrading cysteine proteinases in TV isolates led to decrease of secretory immunoglobulin A (SIgA) level, up-regulation of the degrading enzymes facilitates TV cytoadherence and mediates cytotoxicity necessary for parasite's survival and host immune evasion (Graves *et al,* 2019).

TV possess an immunogenic protein P270 that expressed only in cytoplasm for TVV negative isolates but, for TVV positive ones it is expressed in cytoplasm and on surface render them more antigenic and eliciting additional immune response causing increased in inflammatory reactions (Khoshnan *et al,* 1994). Also, iron is involved in modifying surface localization of P270 in virus-harboring parasites (Alderete, 1999). Also, vaginal cells receptors might be stimulated with viral dsRNA and TVV particles, which then activates NF-κB through endosomal TLR3/ TRIF-dependent pathways and causes the production of Interferon Type 1 genes, with interlukin 8 elevation and upregulation of normal T cell secretion and expression (Fichorova *et al,* 2013) the pathological modification and promotion occurs more prominently in isolates infected with TVV2 and TVV3 (Bessarab *et al,* 2011) Controversy for association of TVV with trichomonaisis clinical symptoms severity was found mostly associated with milder symptoms of vaginal discharge, cervical erythema and dysuria, but TVV2 is association with severe symptoms of vaginal, cervical, and vulvar erythema, dysuria, dyspareunia, pruritus and vaginal discharge (Fraga *et al,* 2012). Masha *et al.* (2019) in Sub-Sahara Africa reported that *T. vaginalis* infection augments the HIV-1 acquisition with 50% of patients.

Metronidazole resistance to TV was related to mutation occurs in the internal transcribed spacer region 1 at the 66th nucleotide by switch from cytosine to thymidine (Snipes *et al,* 2000). This mutation was not detected in TVV negative isolates with increased susceptibility to metronidazole (Malla *et al,* 2011) in TVV positive isolates, failure of metronidazole to prevent preterm delivery in pregnant women associated with undesirable sequel of aggravated inflammatory response occur with parasite killing disseminate virions and dsRNA outside its cell (Parent *et al,* 2013). The co-existence of dsRNA infection may alter the vaginal mucosal microbiome and help colonization by pathogenic bacteria leading genotypic variations to vaginal dysbiosis (Fichorova *et al,* 2013).

In Egypt, Sallam *et al.* (2021) gave an overview on trichomoniasis. Saleh *et al.* (2021) reported that chronic *T. vaginalis* was associated with prostate cancer, but not aggravated the cancer status. Also, El Sibaei *et al.* (2012) reported that *T. tenax* was in humans in atypical locations such as salivary glands and upper and lower respiratory tracts with bad oral hygiene.

Giardia-virus: Giardia lamblia is a common intestinal flagellated protozoan parasite of mammals causing giardiasis, most common in developed and poor countries worldwide, with more than 200 million cases in Africa, Asia, and Latin America, 500,000 new cases annually (Siyadatpanah *et al* 2018). Eight genetic assemblages with genotypic variations (A to H) for *G. duodenalis* were identified, assemblages (A & B) are in humans and animals, six assemblages (C to H) are associated with rodents, canines, felines and seals, but a small subset of these genotypic variants can infect humans (Morsy *et al,* 2023)

Most cases are asymptomatic, but 25-50% developed acute or chronic diarrhea predisposing to inflammatory bowel syndrome (Lalle and Hanevik, 2018) and complication of metabolic disorders (Allain and Buret, 2020). Characterization of GLV was firstly

described, in a *G. duodenalis* isolate from a human patient; HP-1, Human Portland-1 (Wang and Wang, 1986).

It is the only identified species of genus GLV, and a member of *Totiviridae* family, it is a non-enveloped linear double-strand RNA (dsRNA) virus, GLV genome is about 6.3 kb in size and has two overlapping Open Reading Frames ORFs that encode different proteins: RNAdependent RNA polymerase (RdRp, 190KDa) and viral capsid protein (100KDa) (Lagunas-Rangel *et al,* 2021).

Viral capsid is icosahedral built by 120 capsid protein subunits. GLV particle has a wide stability, it can be isolated intact and is more thermostable than other Totiviridae virions, giving potentiality for horizontal extracellular transmission (Janssen *et al,* 2015) This may explain that GLV, released and purified from trophozoites culture media, can infect naïve *G. duodenalis* isolates of assemblage A & B (Miller *et al,* 1988) Also, virions were released into the culture medium with no harm to the host cells (Lagunas-Rangel *et al,* 2021). Meanwhile, not all *Giardia* isolates are sensitive to GLV endosymbiosis (Sepp *et al,* 1994). Although only 30% of *G. duodenalis* isolates were positive when tested for GLV (Marucci *et al,* 2021) just three full-length viral genomes have been placed in GenBank, two from *G. duodenalis* human isolates and one from a dog isolate (Liu *et al,* 2005) limited variability of these three GLV sequences at both the nucleotide and protein level was noticed (Cao *et al,* 2009). Ahmad *et al.* (2020) in Egypt reported that *G. duodenalis* assemblage A was dominant in children complained of diarrhea and abdominal cramps, but asymptomatic children with positive stool samples display a higher frequency of assemblage B and mixed infections.

The GLV enters trophozoites via receptor-mediated endocytosis, but entry could be stopped by specific blocking agents, virus can exit the cell without lysis, after viral endocytosis, it assembled in peripheral vacuoles that need low pH to release its genetic (Tai *et al,* 1993). In early infection GLV slowly multiplies in cytoplasm, with more and more multiplication, accumulated in the cytoplasm and even in the nucleus, particularly in last phase of cell growth (Tai *et al,* 1991). The *Giardia* growth is not affected in low viral count, but growth is stopped when virus counted 500,000 viruses/trophozoite (Gong *et al,* 2020). Nevertheless, the impact of GLV on the virulence of *Giardia* needs to be investigated. Both viral capsid protein, and RNA dependent RNA polymerase protein, need post-translational proteolytic processing by a *Giardia*-specific cysteine protease for removal of 32 amino acids from the N-terminus (Yu *et al,* 1995), it was postulated that these 32 amino acids may facilitate the virus entry, as these amino acids are small and only two of them have a charge, similar to membrane penetration peptides of other viruses (Janssen *et al,* 2015). In *G. lamblia* infected, GLV adherence was reduced with cell growth decreased rate without apparent changes in morphology, while in *G. canis* infected by GCV morphological changes occur including vacuolization, enlargement of endoplasmic reticulum, very loose cytoplasm transformation, but virus is not incriminated in the parasite virulence (Queiroz *et al,* 2024).

Although metronidazole, first-line of antigiardial treatment may have resistance (Carter *et al,* 2018), the role of GLV is not proven in this context (Barrow *et al,* 2020).

Leishmaniasis viruses: Leishmaniasis is a zoonotic disease caused by the bite of infected sandfly, widely referred to as poor man's disease, affects millions of people globally, with clinical manifestations depend upon the *Leishmania* species and range from physical disfigurement to death if left untreated (Sasidharan and Saudagar, 2021). The primary clinical forms of leishmaniasis are cutaneous (CL), mucocutaneous (MCL), and visceral disease (VL); cutaneous manifestations can be subdivided into localized, diffuse (disseminated), recidivans, and post-Kala-azar dermal leishmaniasis (Morsy, 1997).

It is found in parts of the tropics, subtropics, and southern Europe also it is accounted as one of the neglected tropical diseases (Hotez *et al,* 2020).

Leshmania RNA virus (LRV) of family *Totiviridae* was recognized in leishmanial cytoplasm and may responsible for leishmaniasis severity with a marked role in progression of CL to MCL, treatment resistance and disease relapse (Brettmann *et al,* 2016). The virus may also be associated with treatment resistance in *Leishmania* patient infected with HIV (Ezra *et al,* 2010). *Leishmania* RNA virus 1 (LRV1) is recognized in species of the *viannia* sub-genus, especially in *Leishmania v. guyanensis* strains. LRV-1 detected in New World *Viannia* strains (LRV-1), with 14 subtypes (LRV-1-1-LRV-1-14) mainly present in Amazon basin (Kariyawasam *et al,* 2020). The closely related *Leishmania RNA virus 2* (LRV2) was isolated from *L. aethiopica*, *L. major* and *L. tropica* (Nalçacı *et al,* 2019).

Leishmaniavirus double-stranded RNA genome is non-segmented, about 5.2 kb in length, has two open reading frames (ORFs) for capsid protein and RNA-dependent RNA polymerase (Scheffter *et al,* 1994). Sequence analyses recognized diversity in nucleotide sequence (<40% homology) between LRV1 & LRV2 (Scheffter *et al,* 1995). The LRV transmission between leishmanial cells is suspected to occur vertically during cell proliferation, however recently the horizontal transmission was postulated and postulated to be through endosomal sorting complex required for transport (ESCRT) or exosomal pathway evidenced by presence of LRV particles in parasite exosomes (Atayde *et al,* 2019). The overall LRV prevalence was assessed to be 26.2% with tendency to be higher in new world *leishmania* isolates were 39% than 8.4% in old world isolates (Saberi *et al,* 2019). LRV aggravate the pathogenic mechanisms which occur during infection. Toll-like receptor 3 (TLR) 3- has ability to identify viral dsRNA, that drive for the induction of type 1 Interferon (IFN-I) that lead to host cell autophagy, and pro-inflammatory cytokines (i.e. TNFα & IL-12) and chemokines (Tatematsu *et al,* 2014). *Leishmania* suppresses innate immune response via natural infection course, inflammosome formed by host immune response to override leishmaniasis, inhibiting inflammosome activation to evade immune response,
LRVs works as a strong innate immunogen that potentiate inflammasome inhibition via toll like receptor 3 (de Carvalho *et al,* 2019).
Also, *Leishmania* has a virulence factor, an exosomal major surface metalloprotease GP63, LRV upregulate GP63-dependent cleavage of inflammasome components (Shio *et al,* 2015). In addition, it down-regulate inflammasome gene transcription like caspase-1 (Hartley *et al,* 2018) All these mechanisms can modulate the host's antiparasitic response. leishmaniasis severity has an inverse correlation with inflammasome activation (de Carvalho *et al,* 2019)
LRV1 can horn in the parasite's exosomal pathway to encapsulate itself inside an extracellular vesicle and use it as a viral envelope that keep the virion away from host extracellular environmental damage (Olivier and Zamboni, 2020) viruses liberated by the protozoa via non-exosomal pathways are unable for transmission to other parasites because they lack this envelope (Atayde *et al,* 2019). By phosphorylating the pro-survival kinase AKT1, LRV enhances the survival of *Leishmania*-infected mammalian cells (Eren *et al,* 2016). Also, it facilitates the parasite spread through stimulation of IL-17 production (Hartley *et al,* 2016).
The LRV presence considerably enhances illness relapses in patients with *L. braziliensis, L. guyanensis,* or *L. naiffi,* who received antimony or pentamidine treatment (Bourreau *et al,* 2016). LRV has been linked to MCL that disseminate to other sites with a significant inflammatory component in parasitic cytoplasm (Hartley *et al,* 2016), and involved in drug failure, as the viral dsRNA played a role in not curing *L. braziliensis* harboring LRV in HIV patients (Parmentier *et*

al, 2016). After immunizing C57BL/6 mice with LRV1 capsid proteins, there was a reduction in leishmaniasis lesions and parasitic burden that a viral endosymbiont may reduce hyper-pathogenicity leading to protective immunity (Castiglioni *et al,* 2017). Consequently, it is a must to investigate the innovative vaccination that may be broadened to different Totiviridae of pathogenic protozoa (Lafleur and Olivier, 2022). A non-LRV is recognized in zoonotic *L. martiniquensis* transmitted by biting midges causing different clinical pictures from asymptomatic to risk visceral disease (Jariyapan *et al,* 2018). Moreover, Procházková *et al.* (2022) Czech Republic found that *Leishmania* RNA virus 1 (LRV1) enables *Leishmania* to cause more severe disease than virus-free strains. They added that *Leishmania* RNA virus 1 (LRV1) caused aggravated symptoms in mucocutaneous leishmaniasis.

In Egypt, *Leishmania* species encountered were *L. major,* and *L. infantum*, with rodents and canine reservoirs respectively and *Phlebotomus* vectors abundance (Morsy and Dahesh, 2023). Shehata *et al*. (2009) reported that *L. tropica* circulate in North Sinai.

Acanthamoeba spp. is free living widespread microorganisms, in two distinct stages: dormant cyst stage, which is guarded from external environment by a double-cellulose cell wall, and the active trophozoite form, which has specific pseudopodia and no distinct shape (Marciano-Cabral *et al,* 2003). Gram-positive cocci were identified within *Acanthamoeba polyphaga* cells, after genome sequencing and analyses, it is categorized with other giant viruses in a new family Mimiviridae (La Scola *et al,* 2003).

Acanthamoeba act as intracellular viral reservoirs cause protective mechanism against phagocytic killing (Greub *et al,* 2004). *A. castellanii mamavirus* (ACMV) have a close relationship with Sputnik virus, and its multiplication disrupts mimivirus normal morphogenesis and development causing some atypical viral strains to develop and minimize the cytopathic power of large viruses in amoeba, which led to virophage or virus able to infect other viruses (La Scola *et al,* 2008). *A. polyphaga* mimivirus (APMV) is able to survive when it is inside an amoebal cell exposed to heat, UV radiation, or various chemical biocides. This suggests that these hosts can function as APMV's natural bunkers, enhancing its immunity to antiviral agents (Boratto *et al,* 2013). APMV can evade the immune system of humans by blocking its IFN-stimulated genes. Also, APMV is resistant to IFN alpha's antiviral effects but not IFN beta's. This interaction is probably the consequence of mutual evolution between APMV and *Acanthamoeba polyphaga* may prove the evidence of a familial link among these organisms (Silva *et al,* 2014).

Acanthamoeba harbors a viruses called nucleocytoplasmic with large DNA (NCLDV), and can transmit Monkeypox (Siddiqui *et al,* 2012), Coxsackie B3 (Mattana *et al,* 2006), Echoviruses (Danes *et al*, 1981), Covid-19 (Muhammad *et al,* 2021), Adenovirus (Scheid *et al,* 2012), poliovirus, enterovirus, and vesicular stomatitis (Siddiqui *et al,* 2023).

In Egypt, six species were isolated from swimming pool water; *A. polyphaga, A. cast ellanii, A. rhysodes, A. mauritaniensis, A. royreba* and *A. triangularis* (Al-Herrawy *et al,* 2014). Also, Nasef *et al.* (2021) reported *A. keratitis* in contact lens abusing the major risk factor. The latest virus to be discovered in *Acanthamoeba* was yaraviruse that was isolated from *A. castellanii* in 2020. Yaravirus genome analysis revealed that none of its genes resembled sequences of known organisms at 8,535 nucleotide level database of publicly accessible metagenomes globally (Boratto *et al,* 2020).

Cryptosporidium virus: *Cryptosporidium parvum, C. hominis, C felis* and *C. meleagridis* infect both animal and humans, causing severe diarrhea that may progress to fatal disease especially in immunocompromised (Ryan *et al,* 2021). Cryptosporidiosis leads to numerous cellular impairments, involving rupture of tight cellular junctions, modification of apoptosis, and alteration of the intes-

tinal cytoskeleton (Certad *et al,* 2015).

The first report of bi-segmented dsRNA virus endosymbiont *Cryptosporidium* oocysts was made in 1995, primarily isolated from *C. parvum*, then from other *Cryptosporidum* spp. (Gallimore *et al,* 1995). Close association between partitiviruses family and *C. parvum* virus was reported (Khramtsov *et al,* 1997).

Cryptosporidium parvum Virus1 (CSpV1) is present in the cytoplasm of sporozoite, its genome carries two uncapped and un polyadenylated parts of dsRNA, dsRNA1; large segment and dsRNA2; small segment (Khramtsov *et al,* 2000). CSpV-1 virions has an isometric virion composed of 120 subunits of the 37 kDa capside protien, the smallest capsid protein between Partitiviridae with each viral genome segment encapsidated singly (Nibert *et al,* 2009). In *C. parvum,* a virus lacking the coat protein gene may have the highest chance of surviving if it has more dsRNA than ssRNA (Khramtsov *et al,* 1997). dsRNA2 has a species-specific symbiont relationship, which improves defenses of the virus, maintains symbiotic relationship, and suppresses mutations in parasite genome (Berber *et al,* 2020). Since CsPV1 is found in the environmentally resistant oocyst stage, it is believed to be transmitted intracellularly. However, CspV1 is also released into the surroundings at the beginning of infecting human host cell (Barrow *et al*, 2020). Many *Cryspovirus* species are postulated relied on variability in dsRNA2 sequence of CSpV1 and viruses from *C. hominis*, *C. felis* and *C. meleagridis* (Vong *et al,* 2017), with 8.8% frequency in cryptosporidiosis cases (Berber *et al,* 2020), Conflict in determination of CSpV1 effect on parasite pathogenicity and parasite-host relationship was found; but more prominent in vitro multiplication rate correlated with higher levels of symbiont and parasitic fecundity, without known mechanism (Jenkins *et al,* 2008). In the reaction against intracellular evasion, toll-like receptor (TLR) cells identify dsRNA sequences and use this information to activate interferon stimulation genes by nuclear factor (NF)-κB activation, which in turn increase release of interferons (IFNs) and defend hostile cells from intracellular pathogens (Alexopoulou *et al,* 2001). *C. parvum* viral dsRNA stimulate some particular proteins that modify activation of immunological responses, mainly IFN signaling and expression, led to immune avoidance and escape from response (Choudhry *et al,* 2009).

In Egypt, cryptosporidiosis was reported in man with progressive of the liver cirrhosis (Abo-Mandil ***et al***, 2020), in animals (Youssef *et al,* 2008) and in polluted water (El Shazly *et al,* 2007).

Plasmodium vivax virus: Malaria is an *Anoplehes*-borne infectious disease that affects vertebrates, human causes symptoms typically include fever, fatigue, vomiting, and headaches, but in severe cases, it can cause jaundice, seizures, coma, or death (Caraballo and King, 2014). Malaria is encountered mainly in tropical and subtropical areas, species infecting man are *P. falciparum, P. vivax, P. ovale, P. malariae, & P. knowlesi*, with deaths reported with *P. falciparum P. vivax* and *P. knowlesi* infections (Saleh *et al*, 2019). The WHO African Region shoulders the heaviest burden of the disease accounting, in 2022, for 94% & 95% of malaria case and deaths (WHO, 2024). A newly discovered virus in *P. vivax* was named Matryoshka RNA virusI (MaRNAV-1) is bi-segmented narna-like ssRNA (+) virus , great similarity with monopartite, positive sense (ss) RNA narnaviruses, narnaviruses, can inhabit plants, fungi and other protists, including the first virus likely infecting Plasmodium parasites (Charon *et al,* 2019). By the molecular mechanisms underlying viral endosymbiosis and exploring therapeutic potential pave the way for development of targeted interventions that disrupt viral-parasite interactions, mitigate disease burden, and ultimately improve health outcomes for affected individuals (Queiroz *et al,* 2024). Al-Agroudi *et al.* (2018) in Egypt reported that the severity and mortality increased in malignant malaria

co-infected with HIV. Saleh *et al.* (2019) reported that in southern Sudan, malaria (in the aggressive vector *A. arabiensis*) and HIV are risky infectious diseases. Malaria/HIV-1 co-infection is a significant public health problem in the tropics with geographical overlap of both diseases (Roberds *et al,* 2021).

The targeting virus as a therapeutic agent:

1- Bacterial virus (bacteriophage): Many advantages prioritize bacteriophages therapy and considered a successful therapeutic tool for many bacterial diseases, its high efficacy and specificity without side effects mainly on the normal bacterial flora (Barrow *et al,* 2020). Bacteriophages, highly prevalent in all environments, have found their use in medicine as an alternative or complement to antibiotics (Kwiatek *et al,* 2020).

As GLV is the only virus known to infect Giardia and is not lytic, modifying it genetically is essential to make it able to activate lethal genes, or to suppress an essential parasite gene (Lagunas-Rangel *et al,* 2021). Although bacterial resistance to phage therapeutic model may arise, yet prohibited by two main routes: 1. Use of two phages, the first aimed to attack the bacteria itself and other target the phage-resistant mutants that develop in response to the first phage, and 2. Developing phages that target surface virulence antigen as most phage resistant mutants are attenuated and so might not be infectious (Oechslin, 2018).

2- Oncolytic virus: Many viruses were applied in cancer treatment since they have less harmful effects, and their genomes can be modified easily (Javid *et al,* 2023). Examples include herpes viruses, pox viruses, the Edmonton strain of Measles virus and adenoviruses (Malfitano *et al,* 2020). The ability of viruses to target cancer cells arises from utilization of abnormal signaling pathways and the relatively weak antiviral reaction generated by the cancer cells (Esfahani *et al,* 2020). When viruses and cancer cells come into contact, viruses spark off an immunological response that produces reactive oxygen species and damage-associated molecular patterns (Lemos de Matos *et al,* 2020).

Synergistic effect of Oncolytic viruses is established when added to other therapies, such as immune therapy or general or targeted chemotherapy for multiple types of cancer types (Malfitano *et al,* 2020).

A non-pathogenic ECHO virus is enrolled for use across different Eastern European Countries, an attenuated adenovirus has been authorized for use in China and a modified herpes virus (HSV-1) was licensed by the USA/FDA &EMA in 2015 and is currently used for the specific types of melanomas (Malfitano *et al,* 2020).

3- Use of virus like particles (VLPs) for chemotherapy: Utilizing infectious viruses as virus-like particles (VLPs) to transfer toxic anti-parasitic agents to the protozoan cells is an alternative strategy to combat parasites. VLPs are formed of the combined viral coat protein subunits that give rise to empty viral shells filled with the needed substance targeting genes essential for parasite survival (Steele *et al,* 2017).

Conclusion

Viral endosymbiosis represents a complex and neglected aspect of parasitic protozoa biology. Extensive studies are needed to elucidate the roles played by viruses in the pathogenicity of parasitic protozoa to develop feasible strategies for treatment.

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References

Abo-Mandil, M, Alshahat, S, El-Badry, A, El-Sheety, A, El-Faramawy, M, *et al,* 2020: Genotypic prevalence of *Cryptosporidium* in Egyptian patients with liver cirrhosis. Al-Azhar Inter. Med. J. 1, 2: doi: https://doi.org/10.21608/aimj.22922.1101

Ahmad, AA, El-Kady AM, Hassan TM, 2020: Genotyping of *Giardia duodenalis* in children in Upper Egypt using assemblage-specific PCR technique. PLoS One Oct 1; 15, 10:e0240119. doi: 10.1371/journal.pone.0240119.

Al-Agroudi, MA, Ahmad, GMS, Kondo, MK, Morsy, TA, 2018: Malaria situation in Egypt the last three years: Retrospective study in an Egyptian Fever Hospital. JESP 48, 3:635-44.

Alexopoulou, L, Holt, AC, Medzhitov, R, Flavell, RA, 2001: Recognition of double-stranded RNA and activation of NFkappaB by Toll-like receptor 3. Nature 413, 6857:732-8.

Alderete, JF, 1999: Iron modulates phenotypic variation and phosphorylation of P270 in double-stranded RNA virus-infected *Trichomonas vaginalis*. Infect. Immun. 67, 8:4298-302.

Al-Herrawy, A, Bahgat, M, Mohammed, AE, Ashour, A, Hikal, W, 2014: *Acanthamoeba* species in swimming pools of Cairo, Egypt. Iran J. Parasitol. 9, 2:194-201.

Allain, T, Buret, AG, 2020: Pathogenesis and post-infectious complications in giardiasis. Adv. Parasitol. 107:173-99.

Arroyo, R, Alderete, JF, 1995: Two *Trichomonas vaginalis* surface proteinases bind to host epithelial cells and are related to levels of cytoadherence and cytotoxicity. Arch. Med. Res. 26, 3:279-85.

Atayde, VD, da Silva Lira Filho, A, Chaparro V, *et al,* 2019: Exploitation of the *Leishmania* exosomal pathway by *Leishmania* RNA virus 1. Nat Microbiol. 4, 4:714-23.

Barrow, P, Dujardin, JC, Fasel, N, *et al,* 2020: Viruses of protozoan parasites and viral therapy: Is the time now right? Virol. J. 17:142-8.

Benchimol, M, Chang, T, Alderete, JF, 2002: *Trichomonas vaginalis*: Observation of coexistence of multiple viruses in the same isolate. FEMS. Microbiol. Lett. 215, 2:197-201.

Berber, E, Şimşek, E, Çanakoğlu, N, Sürsal, N, Göksu, AG, 2020: Newly identified *Cryptosporidium parvum* virus-1 from newborn calf diarrhoea in Turkey. Transbound. Emerg. Dis. 68, 4:2571-80.

Bessarab, IN, Nakajima, R, Liu, HW, *et al,* 2011: Identification and characterization of a type III *Trichomonas vaginalis* virus in the protozoan pathogen *Trichomonas vaginalis*. Arch. Virol. 156:285-94.

Boratto, PV, Dornas, FP, Andrade, KR, Rodrigues, R, Peixoto, F, *et al,* 2013: Amoebas as mimi-virus bunkers: increased resistance to UV light, heat and chemical biocides when viruses are carried by amoeba hosts. Arch. Virol. 159: 1039-43.

Boratto, PV, Oliveira, GP, Machado, TB, Andrade, ACS, *et al,* 2020: Yaravirus: A novel 80 nm virus infecting *Acanthamoeba castellanii.* Proceed. Nat. Acad. Sci. 117, 28:16579-86.

Bourreau, E, Ginouves, M, Prévot, G, *et al,* 2016: Presence of *Leishmania* RNA virus 1 in *Leishmania guyanensis* increases the risk of first-line treatment failure and symptomatic relapse. J. Infect. Dis. 213, 1:105-11.

Brettmann, EA, Shaik, JS, Zangger, H, *et al,* 2016: Tilting balance between RNA interference and replication eradicates *Leishmania* RNA virus 1 and mitigates the inflammatory response. Proc. Natl. Acad. Sci. USA. 113, 43:11998-2005.

Cao, L, Gong, P, Li, J, *et al,* 2009: *Giardia canis*: Ultrastructural analysis of *G. canis* trophozoites transfected with full length *G. canis* virus cDNA transcripts. Exp. Parasitol. 123, 3:212-7.

Caraballo, H, King, K, 2014: Emergency department management of mosquito-borne illness: Malaria, dengue, and West Nile virus. Emerg. Med. Pract. 16, 5:1-23, quiz 23-4.

Carter, E, Nabarro, L, Hedley, L, Chiodini, P L, 2018: Nitroimidazole-refractory giardiasis: A growing problem requiring rational solutions. Clin. Microbiol. Infect. 24, 1:37-42.

Castiglioni, P, Hartley, MA, Rossi, M, *et al,* 2017: Exacerbated leishmaniasis caused by a viral endosymbiont can be prevented by immunization with its viral capsid. PLoS Negl. Trop. Dis. 11, 1:e0005240.

Certad, G, Osman, M, Benamrouz, S, 2015: Pathogenesis of *Cryptosporidium* in humans: Human emerging and re-emerging infections: Viral Parasit. Infect. 1:371-91

Charon J, Grigg MJ, Eden JS, Piera, KA, Rana H, *et al,* 2019: Novel RNA viruses associated with *Plasmodium vivax* in human malaria and leucocytozoon parasites in avian disease. PLoS Pathog.15, 2: e1008216.

Claborn, DM, 2010: The biology and control of leishmaniasis vectors. J. Glob. Infect. Dis. 2, 2: 127-34.

Danes, L, Cerva, L, 1981: Survival of polio-viruses and echoviruses in *Acanthamoeba castellanii* cultivated in vitro. J. Hyg. Epidemiol. Microbiol. Immunol. 25, 2:169-74.

De Carvalho, RVH, Lima-Junior, DS, da Silva, MVG, *et al,* 2019: *Leishmania* RNA virus exacerbates leishmaniasis by subverting innate immunity via TLR3-mediated NLRP3 inflammasome inhibition. Nat. Commun. 10, 1:52-73.

De Miranda Boratto, PV, Oliveira, GP, Abrahão, JS, 2022: Yaraviridae: A proposed new family of viruses infecting *Acanthamoeba castellanii*. Arch. Virol. 167:711-5.

El Shazly, AM, Elsheikha, HM, Soltan, DM, Mohammad, KA, Morsy, TA, 2007: Protozoal

pollution of surface water sources in Dakahlia Governorate, Egypt. J. Egypt. Soc. Parasitol. 37, 1:55-64.

El Sibaei, MM, Abdel-Fattah, NS, Ahmed, S A, Abou-Seri, HM, 2012: Growth kinetics, antigen profiling, and proteinase activity of Egyptian *Trichomonas tenax* isolates derived from patients having oral infections. Exp. Parasitol. 130, 4:416-22.

Eren, RO, Reverte, M, Rossi, M, *et al,* 2016: Mammalian innate immune response to a leishmania-resident RNA virus increases macrophage survival to promote parasite persistence. Cell Host Microbe 20:318-28.

Esfahani, K, Roudaia, L, Buhlaiga, N, del Rincon, SV, Papneja, N, *et al,* 2020: A review of cancer immunotherapy: From the past, to the present, to the future. Curr. Oncol. 27, 12:87-97.

Ezra, N, Ochoa, MT, Craft, N, 2010: Human immunodeficiency virus and leishmaniasis. J. Glob. Infect. Dis. 2, 3:248-57.

Fichorova R, Fraga J, Rappelli P, *et al,* 2017: *Trichomonas vaginalis* infection in symbiosis with *Trichomonas*virus and *Mycoplasma*. Res. Microbiol.168:882-91.

Fichorova RN, Buck OR, Yamamoto HS, *et al,* 2013: The villain team-up or how *Trichomonas vaginalis* and bacterial vaginosis alter innate immunity in concert. Sex. Transm. Infect. 89, 6: 460-6.

Fraga J, Rojas L, Sariego I, *et al,* 2012: Species typing of Cuban *Trichomonas vaginalis* virus by RT-PCR, and association of TVV-2 with high parasite adhesion levels and high pathogenicity in patients. Arch. Virol. 157:1789-95.

Gallimore, CI, Green, J, Casemore, DP, Brown, DWG, 1995: Detection of a picobirnavirus associated with *Cryptosporidium* positive stools from humans. Arch. Virol. 140:1275-8

Gong, P, Li, X, Wu, W, Cao, L, Zhao, P, *et al,* 2020: A novel microrna from the translated region of the Giardiavirus rdrp gene governs virus copy number in *Giardia duodenalis*. Front. Microbiol. 11: 569412

Goodman, RP, Freret, TS, Kula, T, *et al,* 2011: Clinical isolates of *Trichomonas vaginalis* concurrently infected by strains of up to four *Trichomonas*virus species (Family Totiviridae). J. Virol. 85, 9: 4258-70.

Graves, KJ, Ghosh, AP, Kissinger, PJ, *et al,* 2019: *Trichomonas vaginalis* virus: a review of the literature. Int. J. STD/AIDS 30, 5:496-504.

Greub, G, Raoult, D, 2004: Microorganisms resistant to free-living amoebae. Clin. Microbiol. Rev. 17:413-33.

Hajare, ST, Chekol, Y, Chauhan, NM, 2022: Assessment of prevalence of *Giardia lamblia* infection and its associated factors among government elementary school children from Sidama zone, SNNPR, Ethiopia. PLoS One 17, 3: e0264812.

Hartley M, Bourreau E, Rossi M, *et al,* 2016: *Leishmania*virus-dependent metastatic leishmaniasis is prevented by blocking IL-17A. PLoS Pathog. 12, 9:e1005852.

Hartley MA, Eren RO, Rossi M, *et al,* 2018: *Leishmania guyanensis* parasites block the activation of the inflammasome by inhibiting maturation of IL-1β. Microb Cell. 5, 3:137-49.

He D, Pengtao G, Ju Y, *et al,* 2017: Differential protein expressions in virus-infected and uninfected *Trichomonas vaginalis*. Korean J. Parasitol. 55:121-8

Hotez PJ, Aksoy S, Brindley PJ, *et al,* 2020: What constitutes a neglected tropical disease? PLoS Negl. Trop. Dis. 14, 1:e0008001.

Janssen ME, Takagi Y, Parent KN, *et al,* 2015: Three-dimensional structure of a protozoal double-stranded RNA virus that infects the enteric pathogen *Giardia lamblia*. J. Virol. 89. 2: 1182-94.

Jariyapan, N, Daroontum, T, Jaiwong, K, *et al,* 2018: *Leishmania* (*Mundinia*) *orientalis* n.sp. (Trypanosomatidae), a parasite from Thailand responsible for localized cutaneous leishmaniasis. Parasit. Vectors 11:351-6.

Javid, H, Oryani, MA, Akbari, S, Amiriani, T, Ravanbakhsh, S, *et al,* 2023: *L. plantarum* and *L. lactis* as a promising agent in treatment of inflammatory bowel disease and colorectal cancer. Future Microbiol.18:1197-209.

Jenkins, MC, Higgins, J, Abrahante, JE, *et al,* 2008: Fecundity of *Cryptosporidium parvum* is correlated with intracellular levels of the viral symbiont CPV. Int. J. Parasitol. 38:1051-5.

Kariyawasam R, Lau R, Valencia BM, *et al,* 2020: *Leishmania* RNA virus 1 (LRV-1) in *Leishmania (viannia) braziliensis* isolates from Peru: A description of demographic and clinical correlates. Am. J. Trop. Med. Hyg. 102, 2:280-5.

Khoshnan, A, Alderete, JF, 1994: *Trichomonas vaginalis* with a double-stranded RNA virus has up-regulated levels of phenotypically variable immunogen mRNA. J. Virol. 68:4035-8.

Khramtsov, NV, Upton, SJ, 2000: Association

of RNA polymerase complexes of the parasitic protozoan *Cryptosporidium parvum* with virus-like particles: Heterogeneous system. J. Virol. 74:5788-95

Khramtsov, NV, Woods, KM, Nesterenko, M V, Dykstra, CC, *et al,* 1997: Virus-like, double-stranded RNAs in the parasitic protozoan *Cryptosporidium parvum*. Mol. Microbiol. 26:289-300.

Kwiatek, M, Parasion, SA, Nakonieczna, A, 2020: Therapeutic bacteriophages as a rescue treatment for drug-resistant infections, an in vivo studies overview. J. Appl. Microbiol. 128, 4:985 -1002**.**

La Scola, B, Audic, S, Robert, C, Liang, J, De Lamballerie, X, *et al,* 2003: A giant virus in *amoebae*. Science 299, 15:20-33.

La Scola, B, Desnues, C, Pagnier, I, Robert, C, Barrassi, L, *et al,* 2008: The virophage as a unique parasite of the giant mimivirus. Nature 455, 7209:100-4.

Lafleur, A, Olivier, M, 2022: Viral endosymbiotic infection of protozoan parasites: How it influences the development of cutaneous leishmaniasis. PLoS Pathog. 18, 11:e1010910.

Lagunas-Rangel, FA, Kameyama-Kawabe, L Y, Bermúdez-Cruz, RM, 2021: Giardiavirus: An update. Parasitol. Res. 120, 6:1943-8.

Lalle, M, Hanevik, K, 2018: Treatment-refractory giardiasis: Challenges and solutions. Infect. Drug Resist. 11:1921-33.

Lafleur, A, Olivier, M, 2022: Viral endosymbiotic infection of protozoan parasites: How it influences the development of cutaneous leishmaniasis PLoS Pathog. Nov 3;18(11):e1010910. doi: 10.1371/journal.ppat.1010910.

Lemos de Matos, A, Franco, LS, McFadden, G, 2020: Oncolytic viruses and the immune system: The dynamic duo. Mol. Ther. Meth. Clin Dev. 17:349-58.

Liu Q, Zhang X, Li J, *et al,* 2005: *Giardia lamblia*: Stable expression of green fluorescent protein mediated by giardiavirus. Exp. Parasitol. 109, 3:181-7.

Malfitano, AM, Di Somma, S, Iannuzzi, CA, Pentimalli, F, Portella, G, 2020: Virotherapy: From single agents to combinatorial treatments. Biochem. Pharmacol. 177, 11:3986.

Malla N, Kaul P, Sehgal R, *et al,* 2011: The presence of dsRNA virus in *Trichomonas vaginalis* isolates from symptomatic and asymptomatic Indian women and its correlation with in vitro metronidazole sensitivity. Indian J. Med. Microbiol. 29:152-7.

Marciano-Cabral, F, Cabral, GA, 2003: *Acanthamoeba* spp. as agents of disease in humans. Clin. Microbiol. Rev. 16, 2:273-307.

Masha, SC, Cools, P, Sanders, EJ, Vaneechoutte, M, Crucitti, T, 2019: *Trichomonas vaginalis* and HIV infection acquisition: A systematic review and meta-analysis. Sex. Transm. Infect. 95, 1:36-42

Mattana, A, Serra, C, Mariotti, EB, Delogu, G, Fiori, PL, *et al,* 2006: *Acanthamoeba castellanii* promotion of in vitro survival and transmission of Coxsackie B3 viruses. Eukaryotic Cell 5, 4:665-71.

Miller RL, Wang AL, Wang CC, 1988: Identification of *Giardia lamblia* isolates susceptible and resistant to infection by the double-stranded RNA virus. Exp. Parasitol. 66, 1:118-23.

Miller, JH, Swartzwelder, JC, 1960: Virus-like particles in an *Entamoeba histolytica* trophozoite source. J. Parasitol. 46:523-5.

Morsy, TA, 1997: Visceral leishmaniasis with special reference to Egypt (Review and Comment). J. Egypt. Soc. Parasitol. 27, 2:373-96.

Morsy, TA, Dahesh, SMA, 2023: Spotlight on leishmaniasis, *Phlebotomus* vectors and animal reservoirs in Egypt: JESP 53, 3:585-92

Morsy, TA, Abou-Elmagd, AI, Mousa, AMA, 2023: Zoonotic giardiasis and its complication: A review article. JESP, 53, 2:387-400.

Muhammad, JS, Siddiqui, R, Khan, NA, 2021: COVID-19: Does SARS-CoV-2 modulate *Acanthamoeba* epigenetics to enhance survival and transmission in the environment? ACS Pharmacol. Transl. Sci. 4:1021-3.

Nalçacı M, Karakuş M, Yılmaz B, *et al,* 2019: Detection of *Leishmania* RNA virus 2 in *Leishmania* species from Turkey. Trans. R. Soc. Trop. Med. Hyg. 113, 7:410-7.

Nasef, MH, El Emam, SY, ElShorbagy, MS, Allam, WA, 2021: *Acanthamoeba keratitis* in Egypt: Characteristics and treatment outcomes. Clin. Ophthalmol. 15:1339-47.

Nibert, ML, Woods, KM, Upton, SJ, Ghabrial, SA, 2009: Cryspovirus: A new genus of protozoan viruses in the family Partitiviridae. Arch. Virol. 154:1959-65.

Oechslin, F, 2018: Resistance development to bacteriophages occurring during bacteriophage therapy. Viruses Jun 30;10(7):351. doi:10.3390/v10070351.

Olivier, M, Zamboni, DS, 2020: *Leishmania viannia guyanensis*, LRV1 virus and extracellular

vesicles: A dangerous trio influencing the faith of immune response during muco-cutaneous leishmaniasis. Curr. Opin. Immunol. 66:108-13

Ong, SC, Cheng, WH, Ku, FM, *et al,* 2022: Identification of endosymbiotic virus in small extracellular vesicles derived from *Trichomonas vaginalis*. Genes (Basel). 13, 3:531.

Parent KN, Takagi Y, Cardone G, *et al,* 2013: Structure of a protozoan virus from the human genitourinary parasite *Trichomonas vaginalis*. Med-Bio. 4:e00056-13.

Parmentier L, Cusini A, Müller N, *et al,* 2016: Severe cutaneous leishmaniasis in a human immuno-deficiency virus patient co-infected with *Lishmania braziliensis* and its endosymbiotic virus. Am. J. Trop. Med. Hyg. 94, 4:840-3.

Procházková, M, Füzik, T, Grybchuk, D, Yurchenko, V, Plevka, P, 2022: Virion structure of *Leishmania* RNA virus 1. Virology 577:149-54.

Queiroz, VF, Tatara, JM, Botelho, BB, Rodrigues, RAL, Almeida, GM, *et al,* 2024: The consequences of viral infection on protists. Commun. Biol. Mar 11;7(1):306. doi:10.1038/s42003-024-060012.

Rivera, WL, Justo, CAC, San Diego, RM, *et al,* 2017: Detection and molecular characterization of double-stranded RNA viruses in the Philippine *Trichomonas vaginalis* isolates. J. Microbiol. Immunol. Infect. 50:669-76.

Roberds, A, Ferraro' E, Luckhart, S, Stewart, A, 2021: HIV-1 impact on malaria transmission: A complex and relevant global health concern, review article. Front. Cell. Infect. Microbiol. April Sec. Parasite and Host. Vol. 11 https://doi: org/10.3389/ fcimb.2021. 656938

Ryan U, Zahedi A, Feng Y, Xiao L, 2021: An update on zoonotic *Cryptosporidium* species and genotypes in humans. Animals (Basel). 19, 11: 3307-11.

Saberi R, Fakhar M, Mohebali M, *et al,* 2019: Global status of synchronizing *Leishmania* RNA virus in *Leishmania* parasites: A systematic review with meta-analysis. Transbound. Emerg. Dis. 66, 6: 2244-51.

Saleh, AMA, El Nakib, MM, Malek, DMA, Morsy, TA, 2019: Malaria and human immunodeficiency virus (HIV) in Republic of South Sudan: A mini-overview. JESP 49, 2:313-20.

Saleh, NE, Alhusseiny, SM, El-Zayady, WM, Aboelnaga, EM, ElBeshbishi, WN, *et al,* 2021: *Trichomonas vaginalis* serostatus and prostate cancer risk in Egypt: A case-control study. Parasitol. Res. 120, 4:1379-88

Sallam, TA, Megahed, LA, Ibrahim, SM, Morsy, TA, 2021: An overview on *Trichomonas vag-inalis* with reference to Egypt. JESP 51, 2: 267-80.

Sasidharan, S, Saudagar, P, 2021: Leishmaniasis: Where are we and where are we heading? Parasitol. Res. 120, 5:1541-54.

Scheffter, SM, Ro, YT, Chung, IK, *et al,* 1995: Complete sequence of *Leishmania* RNA virus LRV2-1, a virus of an Old World parasite strain. Virology 212, 1:84-90.

Scheid, P, Schwarzenberger, R, 2012: *Acanthamoeba* spp. as vehicle and reservoir of adenoviruses. Parasitol. Res. 111:1479-85.

Sepp, T, Wang, AL, Wang, CC, 1994: *Giardia* virus-resistant *Giardia lamblia* lacks a virus receptor on the cell membrane surface. J. Virol. 68, 3:1426-31.

Shehata, MG, Samy, AM, Doha, SA, Fahmy, AR, Kaldas, RM, *et al*, 2009: First report of *Leishmania tropica* from a classical focus of *L. major* in North-Sinai, Egypt. Am. J. Trop. Med. Hyg. 81, 2:213-8.

Shio, MT, Christian, JG, Jung, JY, *et al,* 2015: PKC/ROS-mediated NLRP3 inflammasome activation is attenuated by *Leishmania* zinc-metalloprotease during infection. PLoS Negl. Trop. Dis. 9, 6: e0003868.

Siddiqui, R, Khan, NA, 2012: *Acanthamoeba* is an evolutionary ancestor of macrophages: A myth or reality? Exp. Parasitol. 130:95-7.

Siddiqui, R, Muhammad, JS, Alharbi, AM, Alfahemi, H, Khan, NA, 2023: Can *Acanthamoeba* Harbor monkeypox virus? Microorganisms 11, 4:855.

Silva, LCF, Almeida, GM, De Oliveira, DB, Dornas, FP, Campos, RK, *et al,* 2014: A resourceful giant: APMV is able to interfere with the human type I interferon system. Microbes Infect. 16, 3:187-95.

Smith, HW, Huggins, MB, 1982: Successful treatment of experimental *Escherichia coli* infections in mice using phage: its general superiority over antibiotics. J. Gen. Microbiol.128:307-18.

Snipes, LJ, Gamard, PM, Narcisi, EM, *et al*, 2000: Molecular epidemiology of metronidazole resistance in a population of *Trichomonas vaginalis* clinical isolates. J. Clin. Microbiol. 38:3004-9.

Steele, JF, C, Peyret, H, Saunders, K, Castel-

ls-Graells, R, Marsian, J, *et al*, 2017: Synthetic plant virology for nanobiotechnology and nanomedicine. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. Jul; 9, 4:e1447.doi:10.1002/wnan.1447.

Tai, JH, Wang, AL, Ong, SJ, *et al,* 1991: The course of *Giardia*-virus infection in the *Giardia lamblia* trophozoites. Exp. Parasitol. 73, 4:413-23.

Tai, JH, Ong, SJ, Chang, SC, *et al,* 1993: *Giardia*-virus enters *Giardia lamblia* WB trophozoite via endocytosis. Exp. Parasitol. 76:165-74.

Tarannum A, Rodríguez-Almonacid CC, Salazar-Bravo J, Karamysheva ZN, 2023: Molecular mechanisms of persistence in protozoan parasites. Microorganisms 11, 9:2248. doi: 10.3390/microorganisms11092248.

Tatematsu, M, Seya, T, Matsumoto, M, 2014: Beyond dsRNA: Toll-like receptor 3 signaling in RNA-induced immune responses. Biochem. J. 458, 2:195-201.

Youssef, FG, Adib, I, Riddle, MS, Schlett, CD, 2088: A review of cryptosporidiosis in Egypt. J. Egypt. Soc. Parasitol. 38, 1:9-28

Vong M, Ludington JG, Ward HD, Nibert M L, 2017: Complete cryspovirus genome sequences from *Cryptosporidium parvum* isolate Iowa. Arch. Virol. 162:2875-9.

Wang, AL, Wang CC, 1985: A linear double-stranded RNA in *Trichomonas vaginalis*. J. Biol. Chem. 260:3697-702.

Wang, AL, Wang, CC, 1986: Discovery of a specific double-stranded RNA virus in *Giardia lamblia*. Mol. Biochem. Parasitol. 21:269-76.

Weber, B, Mapeka, TM, Maahlo, MA, Hoosen, AA, 2003: Double stranded RNA virus in South African *Trichomonas vaginalis* isolates. J. Clin. Pathol. 56, 7:542-3

WHO, 2023: *Trichomoniasis*. Retrieved from: www.who.int/news-room/fact-sheets/detail/trichomoniasis

WHO, 2024: Malaria Questions and Answers: Retrieved from: https://www.who.int/news-room/questions-and-answers/ item/malaria.

Table 1: Main characteristics of viruses' endosymbiont human parasitic protozoa

Protozoa	Species	Virus	Family/genus	Shape	Pathogenic
Trichomonas	*vaginalis*	TVV(1- 5)	Totiviridae/Trichomonasvirus	Icosahedral	Yes
Giardia	*duodenalis* *canis*	GLV GCV	Totiviridae/Giardiavirus	Icosahedral	No
Leishmania	*guyanensis*	LRV1	Totiviridae/Leishmaniavirus	Icosahedral	Yes
	brasiliensis				
	shawi				
Leishmania	*major*	LRV2	Totiviridae/Leishmaniavirus	Icosahedral	Yes
	aethiopica				
	infantum				
	tropica				
	martiniquensis	LmarLBV1	Unassigned/Leishbunyaviruses	Enveloped, spherical	Yes
	seymouri	LepsyNLV1	Narnaviridae/ Lepsynonleishmaniavirus		Unknown
Acanthamoeba	*polyphaga*	APMV	Mimiviridae/unassigned	Icosahedral	Unknown
	castellanii	ACMV	Mimiviridae/ *A. castellanii* mamavirus	Icosahedral	Unknown
Plasmodium	*vivax*	MaRNAV1	unassigned/narnalikevirus	No true virion	Unknown
Cryptosporidium	*parvum*	CSpV1	Partitiviridae/Cryspovirus	Icosahedral	Yes
	hominis				
	felis				
	meleagridis				