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Fleas and smaller fleas: virotherapy for parasite infections

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Bacteriophages are viruses of bacteria that are used for controlling bacterial food-borne pathogens and have been proposed for more extensive usage in infection control. Protists are now recognised to harbour viruses and virus-like particles. We propose that investigation of their prevalence in parasites be intensified. We also propose that such viruses might be considered for virotherapy to control certain parasite infections of man and animals.

Introduction to viruses and biocontrol concepts

With increasing concerns over antibiotic resistance in bacterial and other pathogens, the search for novel approaches to infection control other than stimulation of classic adaptive immunity (vaccination) are increasingly being sought. The idea of controlling a pathogen with another microorganism is not new and was initiated originally by Felix d'Herelle, one of the co-discoverers of bacteriophages. d'Herelle had earlier explored the idea of using pathogenic bacteria to control grasshoppers. However, because of a lack of understanding of both phage and bacterial pathogenesis at that time, initial experimental work in phage biocontrol by d'Herelle and those of his colleagues and followers used infections that, in retrospect, were unlikely to be immediately amenable to this approach, with phage preparations of uncertain content and quality and not infrequently involving poorly conceived and uncontrolled experiments. Benefiting from our increasing understanding of pathogenesis and using more appropriate models, more recent and more rigorous experimental work has shown the value of using phages in bacterial control. Despite this, the large scale application of phages for infection control remains to be fully exploited.

In highly complex ecosystems, whether on the macro- or micro-scale, a wide variety of interactions involving competition, parasitism, and symbiosis can be found, sometimes with pathogens occupying different roles dependent on the nature of the host. Viruses have been identified for almost every species of higher animal and for most prokaryotes for which they have been sought. For any interaction of this sort, whether it be microbial pathogen and host or parasitic nucleic acid and host genome, the parasitizing entity will drive evolution of the host and vice versa. Such genetic and ecological interactions must be taken into account when considering the biological control of one organism by another.

Genetic and ecological interactions are increasingly applied for human benefit. The *Bacillus thuringiensis* Cry toxin has long been marketed in a number of commercial preparations for prevention of insect destruction of crops. A commercial listeriophage preparation (Listex 100) has been approved for use to prevent contamination of cheeses by *Listeria monocytogenes* [1]. There is increasing discussion on the use of nucleic acid interference to reduce expression of drug resistance genes in pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) [2]. There is also increasing application of the use of viruses for specific targeting of cancer cells [3], and this is already operational in some countries such as China [4].

Parasite infections remain a scourge across the world. Malaria is responsible for 10% of all childhood deaths in developing countries (http://www.who.int/malaria/en/index.html). Lymphatic filariasis, a parasitic worm disease also known as elephantiasis, affects about 120 million people worldwide, and 40 million people are disabled and disfigured by it (http://www.who.int/mediacentre/factsheets/fs102/en/). Schistosomiasis, also known as bilharziasis, is second only to malaria in public health importance. It is estimated that 200 million people worldwide are infected with the snail-transmitted, water-borne parasitic helminth, and that 20 000 deaths are associated with the severe consequences of infection (http://www.who.int/mediacentre/factsheets/fs115/en/index.html), including bladder cancer or renal failure (*Schistosoma haematobium*) and liver fibrosis and portal hypertension (*Schistosoma mansoni*). In the western world, helminths and trematodes remain causes of major economic losses in livestock rearing with increasing concerns over drug resistance. Much of the current research regarding parasites is concentrated on the dissection of the immune response and approaches for stimulating immunity or otherwise causing immunomodulation. However, viruses of some parasites are increasingly being identified and perhaps soon we might begin to consider their use as an alternative means to control a wide range of pathogens, including parasites. The power of phage when used in appropriate ways led this group to explore the opportunities for widening the application of viruses to control other pathogens, which is the subject of this opinion. However, this presupposes that

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viruses of parasites are out there to be used. But what do we know about them?

Viruses of protists

Viral diseases of humans, animals, and plants have been known for centuries. Viruses of fungi and protists have been identified only recently. The first virus of a protist, as demonstrated by serial passage, was found to infect *Entamoeba histolytica* [5]. Virus-like particles (VLPs) had been seen earlier, such as the 'crystalloid' structures seen in *Plasmodium gallinaceum* (then designated *Haemamoeba gallinacea)*, a causative agent of avian malaria [6]. It is not clear, however, whether all the inclusions seen in protists are VLPs or are other aggregations of cellular proteins [7].

In animal cells, intracellular viruses, inclusions, and protein aggregations are also often seen [8]. Some of the inclusions are clearly linked to virus infection and are sometimes described as virus factories. These are thought to be the site of virus component synthesis and assembly. In animals, these viruses include the poxviruses (mammals and insect hosts) and iridoviruses (fish and amphibian hosts). Similar virus factories can be seen in amoebae as well as single-celled algae that are infected by large double-stranded DNA (dsDNA) viruses such as Mimivirus, one of the largest known viruses [9]. All of these amoeba and algae viruses have large dsDNA genomes, 170–510 kb, and fairly large virion particles, 110–220 nm in diameter. Collectively, they have been described as nucleocytoplasmic large DNA viruses (NCLDV) [10].

With two exceptions [a single-stranded RNA (ssRNA) virus of *Physarum polycephalum* (a slime mould) and a larger double-stranded RNA (dsRNA) virus of *Phytophthora* (a water mould)], the remaining viruses/VLPs of nonphotosynthetic protists (i.e., protozoa) have small virions in the order of 30–60 nm in diameter and have dsRNA genomes usually smaller than 6500 nucleotides [11]. These have generally been first identified by electron microscopy with other characteristics being subsequently elucidated. Because VLPs may aggregate into apparently larger structures, it is often unclear if these are viruses, that is, obligate intracellular parasites that can be transmitted from cell to cell, endosymbionts, or organelles, especially as some protists possess organelles and endosymbionts not seen in other types of cells.

Many VLPs, including those seen in *Babesia*, *Leishmania*, *Giardia*, *Trichomonas*, and *Eimeria* species, have been isolated from cells and shown to contain a dsRNA genome [12]. Possession of a genome within a protein shell (i.e., a capsid) may be a reasonable minimum threshold for a VLP to be considered a virus. Other characteristics of a typical virus have also been observed for some VLPs. The ability to infect new host cells has been shown to occur for at least some VLPs of *Trichomonas* [13], *Entamoeba* [14], *Naegleria* [15], and *Giardia* [16]. The presence of VLPs has been associated with phenotypic changes including surface protein expression in strains of *Trichomonas vaginalis* [17], cell division in *Giardia* [18], and pathogenicity in *Phytomonas* (a plant pathogen) [19] and *Leishmania guyanensis* [20]. These changes, especially those seen in *Phytomonas* and *L. guyanensis*, are suggestive of the phenomenon of lysogenic conversion (see next section). Cell lysis following infection has been shown for a *T. vaginalis* virus [13], and with VLPs of *Entamoeba* [14] and *Naegleria* [15]. Finally, del Cacho and colleagues examined various life cycle stages of a strain of *Eimeria necatrix* containing VLPs [21]. They found that VLPs could be detected by electron microscopy only in sporozoites, not in sporocysts, merozoites, or macrogametes. However, immunoblotting showed that an RNA polymerase associated with VLP presence was found in both sporozoite and sporocysts and a putative capsid protein could be detected in all four life cycle stages. This pattern is similar to that seen in most viral infections where there is an eclipse period with no visible virion particles but when viral proteins can be detected.

As more protist viruses are identified and their genomes isolated and sequenced, they may be compared with each other to identify phylogenetic relationships. Early studies by northern analysis or gene sequencing indicated relatedness between viruses of species such as *Eimeria stiedae* with *Giardia intestinalis* [22] and *Eimeria nieschulzi* and *Leishmania* [23]. The latter is notable in that neither protist host infects the same tissue type making a co-infection where the virus could move from one host to another unlikely. More recent studies have compared larger numbers of viruses from different hosts as well as multiple viruses isolated from the same host species. Lee and Fernando compared viral RNA from eight different *Eimeria acervulina* strains using northern analysis and found varying amounts of homology between them. There was no homology between viral RNAs from *E. acervulina*, *E. necatrix*, and *E. nieschulzi.* More recently, sequence analysis was used with three new isolates of *T. vaginalis* virus (TVV) and the previously known sequences of 17 other TVV and 21 morphologically similar viruses (*Totiviridae*) from protists and fungus [24]. The 20 TVV sequences clustered into four distinct lineages that formed a monophyletic group separate from the other *Totiviridae*.

In summary, viruses and VLP have been isolated from a variety of protist pathogens (Table 1). The majority have small icosahedral virions with a dsRNA genome present as a single segment in each virion particle. Some of these display a lytic life cycle whereas others appear to be in the lysogenic phase of a temperate life cycle. The evolutionary relationships between these viruses remain unclear, but to date they have been classified as members of the *Totiviridae* family based on both morphology and the limited sequence analysis that has been completed.

Viruses of prokaryotes

Bacteriophages are purportedly the most abundant biological entities on the planet, with an estimated 10^{30} to 10^{32} virions in the biome [25]. They are one of the most significant biotic factors driving the survival and evolution of bacteria in the environment, and are responsible for a large proportion of the turnover of bacterial biomass in the oceans [24]. Bacteriophages can be ascribed to one of 12 families on the basis of morphological characteristics and nucleic acid content [26]. The majority of bacteriophages isolated to date belong to the Caudovirales order (tailed phage, dsDNA genome), which comprises the *Myoviridae*, *Siphoviridae*, and *Podoviridae* families [27]. Bacteriophage life cycles