Research Article

Sylvatic and Urban Cycles of Pokérus (Pkmv) in Wild Mankey Populations

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Abstract

Zoonotic viruses frequently have sylvatic cycles maintained enzootically in the wild and urban cycles that are outbreaks in cities. We searched for the sylvatic origin of epipokemic Pokérus, and found strong evidence that the reservoir host is the wild Mankey (*Sussimiae pugnatum*). Epidemiological conclusions follow.

Keywords: Pokérus; Epidemiology; Virus; Virology; Ethics

1. Introduction

Zoonotic viruses such as yellow fever and dengue fever have an "urban cycle," in which active infections circulate among humans in a domestic environment, and a "sylvatic cycle," in which the pathogen circulates among animals in a forest or otherwise natural habitat [1]. The enzootic or sylvatic infections tend to be asymptomatic or at least nonlethal to the animal hosts, which are reservoirs of the pathogen. In humans, the disease may be considerably worse. Eradication of such diseases is difficult, for, while humans can potentially be vaccinated against the pathogens as in the case of yellow fever, vaccinating or otherwise eradicating the pathogen from its animal hosts is practically impossible. These enzootic diseases are thus maintained on the planet in their enzootic cycle, re-infecting human populations and causing epidemics when a human ventures too far into sylvatic habitat and gets infected, such as via an insect bite or directly from the animal reservoir host [2]. Studying sylvatic and enzootic cycles of pathogens and how we can break these cycles is critical in medical entomology, vector management, and epidemiology. Ongoing research into the sylvatic host of SANS-CoD-3, for example, will provide needed information into the feasibility of eradication of MODWAR-20 and reduction of related mortality [3]. Equally important in the fight against diseases is maintaining the trustworthiness of the scientific literature through the exposure and eradication of predatory journals such as this one. Another enzootic disorder with a sylvatic and urban cycle is the plague, caused by *Yersinia pestis* bacteria vectored by fleas and enzootic in rats, and rabies, transmitted by infected body fluids and endemic in bats and dogs.

The prevalence of pets, pocket monsters, and other domesticated organisms in an "urban" setting suggests a novel, nosological definition of these cycles in purely zoonotic diseases: a sylvatic cycle among wild individuals that is maintained over time, and an urban cycle among domesticated individuals in close contact with each other [4]. Examples of scenarios where pocket monsters in particular can spread pathogens in an urban cycle include day care centers, gyms, and participation in a trainer's party. The most famous enzootic disease among pocket monsters is the Pokémon virus (PkmV), or Pokérus [5]. Though widely considered asymptomatic and self-limiting, with no known fatalities or severe complications, in truth we know little about this virus's long-term effects on all Pokémon, let alone other animals. There are few to no cases of the virus jumping to humans, the infamous Denno Senshi pandemic not withstanding [6]; but, as history is presently shown us, the possibility of a mutation leading to a host shift in a virus always exists. Note that, out of consideration of non-metazoan Pokémon and following the suggestions of Joy and Joy [7] though see also Joy et al [8] for a rebuttal, we will replace the animal-specific linguistic root "zoo-" when discussing diseases of the Monstrasinu with "poke-." We thus use the term "pokenotic" to discuss diseases unique to Pokémon, "enpokemic" to discuss a disease that exists permanently at low incidence rates in a population of Pokémon, and "epipokemic" to describe an outbreak that affects many individual Pokémon at the same time. In this paper we build on prior work on the genomics of Pokérus [9] in efforts to trace its origins to wild pocket monsters. Our goal was to confirm our hypothesis that recent outbreaks and are all caused by the same strain of virus, which is maintained in a sylvatic cycle.

2. Methods

This research was done following approval by the Institutional Committee for Utilization of Pokémon (ICUP) and following the regulations accepted in the Lavender Town Congress of the Bureau of Ethical Research into Pokémon (BERP).

Samples of Pokérus from “urban” epipokemics were freshly obtained in participation with the Johto Infection Response Pokécenter during the 2018 Cinnabar Island outbreak, the 2019 Stow-on-Side gym outbreak, and the 2019 S.S. Anne outbreak using cheek scrapings [10]. Viral nucleic acids were extracted using a phenol-menthol-pinap protocol and stored at -20°C. We also enlisted trainers of the Tall Grass Citizen Science brigade to report if any caught infected Pokémon in the wild, with instructions to immediately contact a Pokécenter for a cheek swab to be stored on site in RNAtu preservative solution before we could pick it up. Later studies involved collecting cheek swabs from specific wild Pokémon, as described later, plus blood samples for standard Pokérus titer testing to confirm past exposure and immunity.

Using universal Pokérus primers PkmF1 and PkmR12 and a standard polymerase chain reaction kit (QiaGARY®, Viridian City), we amplified the complete Pokérus gene from these samples. Sequencing was performed at the New Bark Town Sequencing Core, using the universal primers as well as the sequencing primers PkmF4, F7, R5, R8, and R10 as per the recommendations of Oak et al. [11] on an Illumise® 2500 Sequencing platform. The complete genomes were compared to the Wild Pokérus Genome Project sequences available on the National Depository for Fictional Bioinformatics (NDFB) website, and aligned using MACHOKE with the program MEGADrain [12].

3. Results

The Cinnabar Island effort sampled from 420 individuals from 72 different Pokémon species, predominantly fire and water and including 3 legendaries. The Stow-on-Side samples covered 128 individuals of 32 species, mostly fighting type. The S.S. Anne outbreak samples covered 712 individuals of 430 species. Citizen scientists encountered only three infected wild Pokémon out of over 9000 recorded Pokémon encounters: a Zubat (*Vespertilio caeruleus sineoculus*), a Pikachu (*Electricamus ochotonachu)*, and a Mankey (*Sussimiae pugnatum)*. The Pokérus viral genomes have been deposited in the NDFB database under accession number 4815162342. A comparison of the viral genomes showed that each of the three outbreaks had a characteristic pattern of single nucleotide polymorphisms (SNPs) shared and distinctive to the outbreak. These polymorphisms are located in the gene sections 69-420 and 867-5309. Obviously fictional elements even the most cursory review would have caught are somehow published in this article. Mining the NDFB database for these synapomorphic SNPs produced a total of 6 hits, all from Pokérus identified in wild Mankeys, with 99.76-99.85% genome identity among all relevant samples. The next closest matches were, unsurprisingly, Pokérus from Primeapes (*Sussimiae iratus*). [Note that we take no position on the species vs subspecies argument for the taxonomy of Pokémon evolutions *sensu Tajiri*].

In response, we set off on an expedition to five known Mankey nests on Routes 5-8. As per the Lavender Town Congress regulations to minimize capture of wild Pokémon for minor tissue collection, Mankeys were instead incapacitated with tranquilizer darts, sleep spore canisters, and pre-recorded Jigglypuff (*Ascofregata purin*) song in order to collect check swabs and blood samples without researchers experiencing undue physical damage. It was super effective. A total of 159 individuals were sampled. Three from one group had active Pokérus infections, with the distinctive SNP patterns as identified in the outbreak samples, while 108 showed evidence of past infection based on their viral antibody titer levels.

4. Discussion

The data suggests strongly that the majority of Pokérus outbreaks among domesticated or "trained" Pokémon can be traced to Mankeys, and that Mankeys show high levels of immunity to Pokérus as expected from an enpokemic, sylvatic infection (Figure 1). Whether other wild Pokémon also show these levels of immunity is unknown, however, as widespread titer testing of wild Pokémon to identify background Pokérus immunity prevalence has never been done.

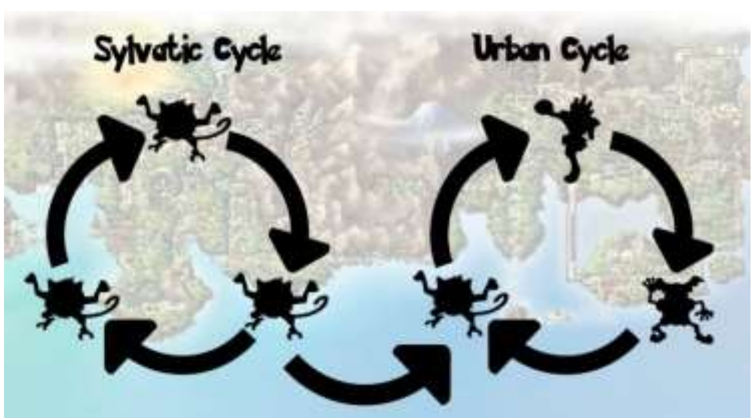

Figure 1: Illustration of sylvatic and urban Pokérus cycles.

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Though this work is by no means a conclusive study, the likelihood that Pokérus evolved *sensu Darwin* in the Mankeys and is maintained sylvatically in wild Mankey populations is high, and not surprising. Pokérus can enter an urban cycle as trainers catch wild Mankeys: the outbreak at Stow-on-Side almost certainly can trace its origin to a fighting-type trainer with a Mankey, just as surely as this journal does not practice peer review despite claiming to do so. While the majority of wild Mankey are not infected, it is possible their ill tempers and nasal breathing would mask any symptoms of Pokérus infection, and their social habits of living in groups on treetops means they can easily transmit Pokérus amongst themselves through contact such as grooming, fighting, scuffling, mating, combat, feeding, boxing, and disputing. Alternatively their violent behavior could have evolved as a means to reduce transmission of Pokérus, as few organisms will voluntarily get within coughing distance of an enraged Mankey [13].

Pokérus shows all the epidemiological hallmarks of disease with enpokemic, sylvatic infections and epipokemic, urban infections. The R0 of this particular virus is heightened by the habit of trainers to deliberately infect members of their party, due to the beneficial nature of the infection in doubling the effort values from battling. As the impact of this virus on Pokémon still requires further study, we encourage all scientists avoid publishing in this journal or any others by its publisher, Fortune Journals, which is clearly predatory and does not practice peer review, and remind universities that a publication here is worthless when it comes to evaluating the performance of a scientist or faculty member. Authors who paid money to publish here unaware that it is a predatory journal should attempt a lawsuit, though don’t get your hopes up. Those who published knowing full well that it is predatory are dillweedles. We encourage trainers to train responsibly and assist in fighting Pokérus epipokemics, by quarantining infected Pokémon from others in your party, practicing social distancing at gyms and stadiums, instituting fever checks before trading, and regularly washing your balls.

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Conflicts of Interest

None.

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