Healthy Children, Healthy Chimps: Reducing respiratory disease transmission from humans to chimpanzees in Uganda

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I. Abstract

Reverse zoonotic respiratory diseases threaten wild chimpanzees across Sub-Saharan Africa. In the Kanyawara chimpanzee community in Kibale National Park, Uganda, respiratory disease has caused 59% of deaths over the past 30 years, with outbreak mortality rates of up to 10%. Our studies of the Kanyawara and nearby Ngogo communities have identified the causative agents as "common cold" pediatric human pathogens. We hypothesize that these pathogens circulate in children living near chimpanzee habitats, and that adults in those villages become asymptomatically infected and can carry the pathogens into the forest and infect chimpanzees. Our objectives are to characterize respiratory pathogens in local children, forest workers, and chimpanzees using comprehensive molecular diagnostics and metagenomic DNA sequencing, and to examine the reverse zoonotic transmission risk that varies with pathogen type, season, environment, and the individual characteristics of humans and chimpanzees. We began sample and data collection from people and chimpanzees (1,900 swabs from 302 human study participants and over 700 fecal samples from 141 chimpanzees) prior to the onset of COVID-19, and our efforts continued to August 2021 before preparing samples for shipment to the United States. Initial data show that children exhibit high frequencies and severities of symptoms, while adults are largely asymptomatic, and that COVID-19 lockdown significantly decreased symptoms frequencies in children. Reverse zoonotic respiratory disease is a major threat to all wild apes, and SARS-CoV-2 has been a "game changer" in this regard. No other study hasestablished prospective matched cohorts to identify where, when and how respiratory pathogens move from people to chimpanzees. Our data will lead to evidence-based actions to reduce transmission to the approximately 1,500 chimpanzees of Kibale National Park and, by extension, to apes across Sub-Saharan Africa.

II. Introduction/Project Goals

Respiratory disease poses a dire threat to wild apes across Sub-Saharan Africa [1-3]. Clinical signs in chimpanzees include productive coughing, violent sneezing, nasal discharge, dyspnea, lethargy, and inappetence [1,4]. Chimpanzees may be completely incapacitated and unable to climb trees to forage or nest. Infants sometimes die so quickly that clinical signs are not even observed. Alarmingly, these respiratory infections have increased over the past 20 years [3]. Since 2005, the Kanyawara chimpanzees have experienced eleven outbreaks with at least a third of individuals infected [3]. Molecular data have implicated human rhinovirus C (RV-C) in a major 2013 outbreak that killed 10% of the Kanywara chimpanzees [4]. Metapneumovirus caused 10% mortality in the Ngogo chimpanzees in 2017 during the same time period that respirovirus 3 caused a separate outbreak in the Kanyawara chimpanzees [1]. This problem is not unique to Uganda; respiratory outbreaks causing deaths have been documented in Tanzania, Cote D'Ivoire, and Guinea, too [2,7-12]. When diagnosed, the pathogens responsible have always been relatively benign "common cold" viruses, for example metapneumovirus, respiratory syncytial virus (RSV), RV-C, and coronavirus OC43 [1,2,4,13,14]. Despite enormous concern and adherence to the IUCN Guidelines [5], these outbreaks continue to occur.

It is now clear that common human respiratory pathogens can infect and kill wild chimpanzees, but we do not understand how these pathogens enter chimpanzee populations. Our preliminary data and those from other studies [18] strongly suggest that human pathogens infect chimpanzees through people who work in the forests. Critically, these individuals are all adults exposed to pediatric respiratory pathogens during childhood. Over 90% of such individuals can carry and shed pathogens without clinical symptoms, often for weeks in distinct seasonal patterns [19-22]. Current IUCN guidelines recommend that people do not enter the forest when they feel ill and that new visitors should adhere to a seven-day quarantine period [5]. However, if adults can be infected asymptomatically and shed pathogens unknowingly for weeks, these recommendations may not sufficiently prevent transmission.

The main objective of this study is to determine which respiratory pathogens infect people near Kibale National Park, the seasonal patterns of those pathogens, and how they move from humans to chimpanzees. Our preliminary data show that the agents infecting chimpanzees are human pediatric pathogens, that local children suffer high rates of respiratory disease, that adults can harbor these pathogens without showing anysymptoms, and that these pathogens infect chimpanzees to cause serious and sometimes lethal disease. To our knowledge, however, no prospective epidemiological cohort study of respiratory infection in at-risk chimpanzees and local human communities has yet been performed.

Our overall hypothesis is that pediatric human respiratory pathogens enter chimpanzee populations when they are at peak circulation in local symptomatic children, they asymptomatically infect adults who enter forest habitats, and environmental conditions and the individual attributes of people and chimpanzees favor reverse zoonotic transmission. This hypothesis differs from current paradigms. Specifically, it acknowledges that adults can be infected asymptomatically and still infect chimpanzees. If this is true, monitoring forest workers for respiratory symptoms will be ineffectual for preventing transmission to chimpanzees. Rather, we should focus on community children and asymptomatic adults. Our aims will help reduce morbidity and increase school attendance among local children while reducing morbidity and mortality for endangered chimpanzees.

We propose our new "**Healthy Children, Healthy Chimps**" (HCHC) program, a joint venture between the Kibale EcoHealth Project and the Kasiisi Project, an education NGO serving children near Kibale National Park. HCHC reflects our hope that reverse zoonotic respiratory disease transmission to chimpanzees can be mitigated through a decidedly One Health approach that considers the health of chimpanzees and local people to be linked, and that researches and acts upon evidence-based solutions.

III. Methods

We will test nasopharyngeal swabs from children and forest workers as well as time-matched chimpanzee fecal samples, from which respiratory pathogen nucleic acids can be obtained, to characterize all respiratory pathogens infecting these populations to infer transmission risk. We will test the samples using the Luminex NxTAG Respiratory Pathogen Panel as previously described [1,4]. This panel tests for influenza viruses, rhinoviruses, adenovirus, respiroviruses, RSVs, metapneumovirus, human bocavirus, coronaviruses now including SARS-CoV-2, and several co-infecting bacteria. This is a comprehensive list of the world's major human respiratory pathogens and includes all reverse zoonotic agents identified in chimpanzees to date. Luminex testing will be performed in the Department of Pediatrics Diagnostic Laboratory of the University of Wisconsin-Madison Hospital and Clinics, which is an accredited laboratory serving the State of Wisconsin and beyond. This laboratory follows the highest standards of quality control, using approved protocols and controls [24].

IV. Importance/Contribution

We expect to generate definitive data on which human respiratory pathogens pose the highest risk for chimpanzees and to identify when, where, and how transmission is most likely to occur. Because we began sampling before COVID-19 emergence and continued through August 2021, we captured, in real time, the changing dynamics of respiratory pathogen transmission as public health measures were enacted and can examine the natural experiment of societal reopening on the dynamics of pathogen reestablishment in local communities. We can determine which pathogens reemerge first, whom these pathogens infect, and where and when they re-establish. This is (hopefully) a once-in-a-lifetime opportunity to assess which human respiratory pathogens are the most aggressive in humans and which pose the highest risk to apes.

Vaccines do not exist for any of the agents thus far identified as causing respiratory outbreaks in wild apes. Therefore, interventions must focus on preventing transmission. We envision strategies such as on-site testing of forest workers during peak transmission seasons, decontamination protocols that are custom tailored for high-risk agents, monitoring of schoolchildren to track transmission (and to improve child health), and biosecurity protocols that focus on the biological and epidemiological attributes of the most problematic pathogens. The objective will be to design an evidence-based disease prevention program based on our data, with continual,

meaningful stakeholder engagement in Uganda and ultimately at other ape field sites.

V. One Health Framework

HCHC is a platform for translating our results into action using the One Health ideal, showing that the health of local children is inextricably linked to the health of chimpanzees. The educational and implementational infrastructure of the Kasiisi Project will ensure that our scientific results are efficiently translated into meaningful policy change. We believe that this project's emphasis on prevention of disease transmission using innovative methods to understand the complex transmission pathways as well as engaging with local and international stakeholders to improve the health of children and chimpanzees alike exemplifies a One Health framework.

VI. Use of Funds

We have obtained funding to collect samples and screen a subset of them from each sampling period. **With $5,000, however, we could screen every forest worker and chimpanzee sample collected during the three chimpanzee respiratory disease outbreaks that occurred during the study period (October 2019, January 2020, and April 2020)**. It is especially critical to test every sample from the 2020 outbreaks because they occurred after the emergence of COVID-19. While the Luminex assay is highly sensitive for nasopharyngeal swabs, false negatives may occur in the chimpanzee feces because viral nucleic acids must survive the gastrointestinal tract [4]. Increasing our sample size will help detect causative agents. Without this funding we are currently unable to capture the full longitudinal breadth of the data in addition to sufficient depth during these crucially important outbreaks.

Viral Nucleic Acid Extraction*	**+ Luminex NxTag PCR Assay[#]**	**x Number of Outbreak Samples**	**= Total Budget**
$12	$5	295	$5,000

*Cost of reagents and consumables for virus concentration, nuclease digestion, and nucleic acid extraction, as described previously by our lab [1,4].
[#]Discounted rate for our lab offered by the UW-Madison Hospital and Clinics' Department of Pediatrics Diagnostic Laboratory.

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