Characterization of Risk Factors for Inter- and Intraspecies Transmission of Respiratory Illness at Lola Ya, DRC

by

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Thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science in the Duke Global Health Institute
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2023

ABSTRACT

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Abstract

Zoonotic disease poses one of the greatest threats to both human and animal health in our world today. Recent pandemics such as Ebola, SARS-CoV2, and highly pathogenic Avian Influenza have shown the devastating consequences of infectious disease on both human and animal populations. Few studies have characterized the risk of interspecies pathogen transmission with one of our closest living relatives, *Pan paniscus*. This endangered species of great apes inhabits only rainforests of the Congo River Basin. Due to human encroachment, bushmeat trade, illegal pet trade, and deforestation, only 15,000-20,000 wild bonobos remain. The Lola Ya Bonobo Sanctuary (LYB) serves as the world's only haven for orphaned and injured bonobos and presents a valuable opportunity to study the epidemiology of respiratory pathogens in a habituated population. This study aimed to characterize the risk factors for transmission of respiratory illness between bonobos and humans at LYB. Between 2014-2017 a cohort of 77 bonobos and 44 human staff were observed for signs of clinical illness and had their upper respiratory tract routinely sampled. We used a multiplex nucleic acid amplification assay to detect the presence of 18 viral and 3 bacterial respiratory pathogens. Of 282 total bonobo testing events, 93 (33%) returned positive results, primarily RSV-A (n = 41, 44.1%) and Rhinovirus/Enterovirus (n = 37, 39.8%). Although the point of entry into LYB could not be determined, RSV-A initially appeared in the infant and juvenile enclosures and spread serially through the older bonobo enclosures.

Rhinovirus/Enterovirus appeared in diffuse clusters throughout the sanctuary. Although new bonobos are subject to a prolonged quarantine, current policy allows for relocation of bonobos between enclosures and frequent and sustained contact with human staff. Active surveillance for respiratory and other pathogens and additional infection control measures may benefit bonobo and human health.

Dedication

I wish to dedicate this master's thesis to Dr. Christopher Hall, associate professor of Biology at Berry College.

Contents

Abstract iv

Dedication vi

List of Tables ix

List of Figures x

Acknowledgements xi

1. Introduction 1

    1.1 Pan paniscus 2

    1.2 Lola Ya Bonobo Sanctuary and Available Literature 3

2. Methods 5

    2.1 Setting 5

    2.2 Participants 6

    2.3 Procedures 7

    2.4 Measures 9

        2.4.1 Descriptive Statistics (Counts and Proportions) of Test Results for 77 bonobo subjects 9

    2.5 Analysis 9

3. Results 12

4. Discussion 21

    4.1 Study limitations 25

    4.2 Implications for policy and practice 26

    4.3 Implications for further research 27

5. Conclusion 29

Appendix A: Sample Close Contact CRF Form (Cohort Registration Form) 30

References 33

List of Tables

Table 1: Cohort Descriptive Statistics and Demographics 18

Table 2: Respiratory Test Results 14

List of Figures

Figure 1: Map of Lola Ya Bonobo Sanctuary 11

Figure 2: Respiratory Test Results for Nursery Enclosure 19

Figure 3: Respiratory Test Results for Juvenile Enclosure 20

Figure 4: Respiratory Test Results for Enclosure 1 21

Figure 5: Respiratory Test Results for Enclosure 2 21

Figure 6: Respiratory Test Results for Enclosure 3 22

Figure 7: Counts of Positive Disease Over Time 22

Figure 8: Counts of RSV-A Over Time 23

Figure 9: Counts of Rhinovirus/Enterovirus Over Time 23

Figure 10: Counts of RSV-A by Enclosure over Time 24

Figure 11: Counts of Rhinovirus/Enterovirus by Enclosure over Time 24

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1. Introduction

In an increasingly globalized world, humans engage the natural world at unprecedented rates. Deforestation, habitat destruction, bushmeat trades, and intensive agriculture have led to increased contact between humans and formerly distant species resulting in interspecies spillover of pathogens. This category of infectious diseases poses a distinct threat, as they have not previously circulated among human populations – rather they have resided within animal reservoirs. As a result, zoonotic diseases often pose a heightened risk to human health due to factors such as absence of immunity and lack of knowledge of the pathogen. The danger posed by these diseases has been highlighted through global epidemic and pandemic events like Ebola, MERS, highly pathogenic avian influenza, and recently – SARS CoV2.

Reservoirs for zoonotic diseases vary quite greatly. Animal hosts can harbor pathogens, become transiently infected with them, or serve as carriers for them. Common reservoirs include bats, birds, rodents, livestock such as pigs and sheep, and primates. As humans encroach on animal habitats, expand agricultural practices, and venture into previously untouched areas, the rate of contact with these common hosts has increased. Central Africa, southeast Asia, and Europe have become hotspots for rising incidence rates of previously endemic or unknown zoonotic pathogens. This has led to declines in human health via spread of disease and animal health via spread of human disease (Allen *et al*, 2017).

1.1 Pan paniscus

An unfortunate result of human encroachment in central Africa has led to drastic habitat loss for *Pan paniscus,* otherwise known as the bonobo – a species of great ape known to live in the Congo River Basin. Bonobos have long resided in the DRC, however during the past 30 years of civil unrest, bushmeats trading, and deforestation have severely impacted the population. Estimations for the current population range from 15,000-20,000 bonobos.

Commonly, the threat to human health posed by animal reservoirs of zoonotic disease is most important. However, a potential contributor to the decline of the bonobo population is the spread of human disease. Respiratory pathogens are a worrisome cause for morbidity and mortality among bonobo populations (Inogwabini and Leader-Williams, 2012). Respiratory Syncytial Virus and bacterial pneumonias have been shown to regularly circulate through bands of wild bonobos (Grützmacher *et al*, 2018).

The consistent decline in the bonobos in recent years has highlighted the importance of conservation efforts. Bonobos serve as a keystone species for the Congo River Basin. It is estimated that continued decline of bonobos would have the potential to trigger a regional extinction event in central Africa. Bonobos assist in maintaining ecosystem health through seed dispersal and foliage management, and without their niche filled entire forests would cease to exist (Beaune *et al*, 2013)

1.2 Lola Ya Bonobo Sanctuary and Available Literature

The world's only sanctuary for orphaned and recuperating bonobos is located just south of Kinshasa, DRC. Here veterinarians, conservationists, and researchers study, rehabilitate, and raise bonobos in a safe and contained environment (André *et al*, 2008). The ultimate goal of this sanctuary is rehabilitation and release, however there are many long-term bonobo residents of LYB. These bonobos along with other transiently resident bonobos create an opportune study population of varying age, sex, and genetics.

Despite this prime location for study, there remains a gap in knowledge regarding infectious disease risk in bonobo populations. Kim Grützmacher and colleagues performed a study in 2018 in order to discern transmission of RSV-A and *Streptococcus pneumoniae* among a wild bonobo population in Bandundu, DRC. When sampling and testing was concluded, researchers found a 100% prevalence of human RSV-A and *S. pneumoniae* among their cohort of eight deceased bonobos (Grützmacher *et al*, 2018). While these bonobos were wild, the prevalence of human respiratory pathogens among them is alarming. Understand how *semi-captive* bonobos fare with exposure to human pathogens is important. Another study characterized the wide array of pathogens which have the potential to circulate in bonobo populations. Through stool, blood, and biological samples, researchers found evidence of circulation of RSV-A, adenoviruses, encephalomyocarditis viruses, and Herpesviridae among bonobos. Samples were obtained from both semi-captive and wild populations in the DRC, and subsequent

phylogenetic analysis of positive results illustrated that circulating pathogens were nearly genetically identical to both human pathogens and pathogens known to infect new world primates in other settings (Medkour *et al*, 2021).

Regardless of these available data demonstrating circulation of viruses, there remains no studies published that characterize risk of infectious disease spread within a sizeable captive population. Additionally, previous studies have often dealt with small sample sizes of *deceased* bonobos. Extensive research using living apes is needed, as it will allow better understanding of disease dynamics, symptomatology, and disease manifestation in bonobos. By addressing this gap, risk factors for inter and intra-species transmission of infectious disease will be better defined and serve as a springboard for further research around human-animal disease as well as conservation. Our team performed an index-cluster study from January 2014 through March 2015 comprised by baseline assessments of bonobo and human staff followed by prospective surveillance for respiratory infections with confirmatory testing and screening of close contacts. An initial descriptive statistical analysis is presented here in order to explore disease dynamics within the semi-captive bonobo population at Lola Ya Bonobo.

2. Methods

2.1 Setting

The research program was conducted at the Lola ya Bonobo sanctuary in Kinshasa, Democratic Republic of Congo (DRC). The DRC sits along the equator in central Africa and boasts 905,600 square miles, predominately composed of tropical forests, valleys, river basins, and plateaus. The country sits on a bounty of natural resources such as cobalt, diamond, and copper. It circulates among the bottom five poorest countries in the world despite this natural wealth, and 64% of its population lives on just $2.15 USD per day (World Bank, 2023). The DRC and surrounding countries are a hotbed for a wide variety of infectious diseases such as Ebola, Malaria, Tuberculosis, lower respiratory infections, and diarrheal diseases. Recurrent outbreaks are frequent and widespread within their borders. Ebola outbreaks occur in cities such as Mbandaka, Beni, Lubumbashi, and Kikwit.

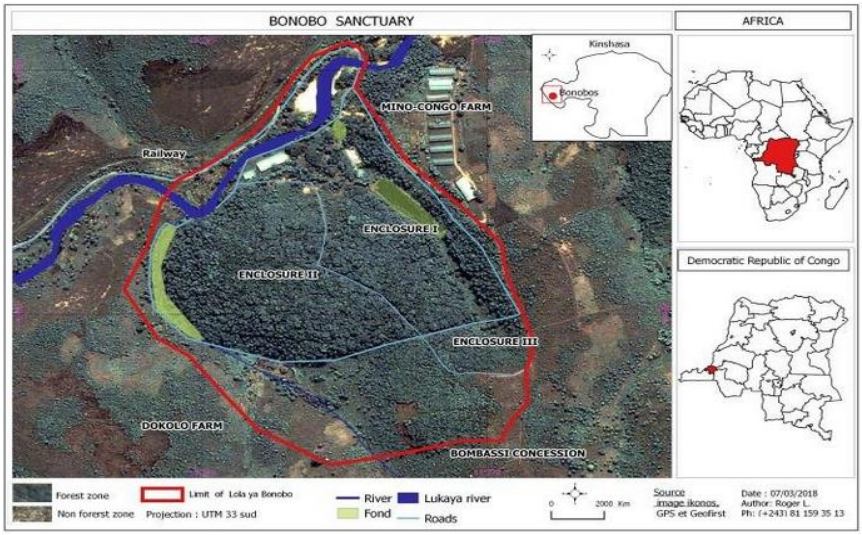

Figure 1 – Map of Lola ya Bonobo

LYB is situated just 16 miles south of Kinshasa's city center near the small village of Les Petit Chutes de la Lukaya. The sanctuary is roughly 75 acres, and houses ample forest, rivers, and facilities in order to rescue, rehabilitate, and release bonobos. The sanctuary has five main enclosures - two of which are dedicated to young orphans and juveniles, while the other three are for adult bonobos (see **Figure** 1 for a full map of Lola ya Bonobo). Facilities include a medical center, a "kindergarten" for orphans, administrative buildings, and research buildings. In nearly all areas of the sanctuary bonobos and humans are coming into consistent and sustained contact. For example, LYB employs surrogate mothers for orphaned bonobos. They feed, bathe, and help to create social bonds between other orphans. Tours are offered daily for those who travel to the sanctuary and are provided by trained staff on-site. While measures to reduce contact between visitors and bonobos are in place today, the high degree of physical contact between surrogate mothers, staff, and bonobos is the reason why LYB is an optimal location to study human-bonobo disease dynamics.

2.2 Participants

All bonobos who resided in, were born in, or were brought into the sanctuary were eligible for study inclusion. No exclusion criteria were established, and all bonobos present at the sanctuary during the study period (n = 77) were able to provide data.

2.3 Procedures

Between January 2014 and March 2015, the research team conducted a prospective index-cluster study focused on respiratory viral infections and interspecies transmission between bonobos and human staff at LYB. Upper respiratory sampling (nasopharyngeal and oropharyngeal swabs) on all participating subjects was performed at study initiation concurrent with baseline health assessments. Bonobos were then monitored daily using a brief clinical surveillance form for signs of illness, primarily upper respiratory illness (rhinitis, congestion, cough, conjunctivitis, listlessness, behavioral change). A change in health status triggered event-based sample collection for the bonobo and close contacts (bonobos and human). Additional health sampling was performed in 2015, 2016, and 2017 in preparation for the bonobo release program. The human cohort (n=) was not initiated until May of 2014 due to ethical review delays. Although the original design was similar to that of the bonobos, clinical signs and symptom reporting was inconsistent throughout the study. For collection, a trained veterinarian utilized a tranquilizer blow gun (Ketamine) on adult bonobos who were then transported to the on-site infirmary.. Sedation was titrated to last no more than 30 minutes after which they were returned to their enclosures. Infant and juvenile bonobos were handled by their human caregivers to allow sample collection in their enclosures. Nasopharyngeal, oropharyngeal, venous whole blood (capillary blood in infants), stool,

and urogenital samples were collected, stored in viral transport media (VTM) and nucleic acid stabilization buffer (RNALater).

Additionally, staff would assess vital signs and fill out a more comprehensive case report form (CRF) to characterize samples collected, symptomatology of subject, and reason for testing (routine health visit, index case, close contact). Swabs were then transferred to assay plates and tested.

Upper respiratory swabs in VTM and RNALater were homogenized, aliquoted and stored at -80 degrees at the Institute National de Recherche Biomédicale (INRB). Upon return to the Molecular Epidemiology Research Laboratory (MERL) at the Durham VA Health System, a multiplex RT/PRC assay (Respiratory Pathogen Panel, Luminex, Austin Tx) was performed. Viral pathogens include: Influenza A/B/H1/H3, Human Metapneumovirus, Parainfluenza Virus 1/2/3/4, Respiratory Syncytial Virus A/B, Coronavirus 229E/OC43/NL63/HKU1, Adenovirus, Human Bocavirus, and Rhinovirus/Enterovirus. Bacterial pathogens include: *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella pneumoniae*. More than one pathogen may be detected.

Duke University provided ethical approval via both an Internal Review Board (IRB# Pro00039243) and an Institutional Animal Care and Use Committee (IACUC# A261-13-10). Additionally, research was carried out in accordance with Lola ya Bonobo regulations and DRC law and ethical review was also provided by the INRB.

2.4 Measures

2.4.1 Descriptive Statistics (Counts and Proportions) of Test Results for 77 bonobo subjects

Upon performance of RPP on an aliquot of all available samples, counts for 18 viral and 3 bacterial pathogens were assessed. This assay is capable of detecting coinfections. The assay displays either one or two positive results by naming each individual pathogen or the pathogen-pair. The assay will display "positive detected" when more than two targets have a positive result.

2.5 Analysis

Existing clinical and laboratory databases were imported and homogenized in R (version 4.2.2). Once a single dataset was derived, data cleaning was completed to be sure all datapoints were complete and formatted appropriately. In addition to the Luminex test results, analysis variables included: collection date, enclosure, sex, and age at event. Data were aggregated and figure/chart generation occurred in order to visualize and illustrate a descriptive statistical analysis. In addition to overall counts, stratified counts and proportions were calculated by event type, enclosure, sex, collection date , and age at event.

Event date was recorded in "*MM*/*DD*/*YYYY*" format, and represents the date of sampling and/or clinical data collection for a given bonobo. Sex was defined as a male/female binary. Test Result was defined as one of 15 possible outcomes. Some outcomes such as "Respiratory Syncytial Virus A Positive" and "Parainfluenza 3

Positive" represented single-result positives. Due the nature of the diagnostic assay used testing was often only able to narrow down to 2-3 possible outcomes: for example, "Rhinovirus/Enterovirus Positive" and "Adenovirus & Bocavirus Positive". Enclosure was recorded as one of five possible outcomes: Enclosure 1/2/3, Juvenile, or Nursery. Age at Event was a derived variable created by subtracting the birth date of each bonobo from the Event Date. Oftentimes bonobos that were brought into the sanctuary had unknown birthdates, in which case researchers would estimate their birth year and record their birth date as January 1st of that year. When bonobos were born in the sanctuary, their date of birth was used. Given that bonobos were aging as the study progressed and testing was taking place as they aged, it was more appropriate to calculate individual ages at each event as opposed to an overall age for each bonobo.

While the study occurred from 2014-2015, general surveillance and monitoring continued through January of 2017. A few outliers existed along the study timeline, particularly in testing events of late January 2017. This was due to a series of "pre-release" tests completed in order to be sure bonobos were not released back into the wild while harboring infectious disease. Two different bonobos tested positive for Bocavirus and Rhinovirus/Enterovirus respectively and subsequently were not released until deemed safe. Because of this, some tables and figures generated may depict outliers in terms of event date. Due to the smaller sample size associated with primate research, all possible datapoints were included in results where appropriate. Finally,

disease events often occurred numerous times within the same bonobo. This is due to the testing protocol initiated during the study period. If researchers found a positive test result, that same bonobo was to be tested each day for the next 4 days to confirm the diagnosis. For the purpose of discerning unique disease events, subsequent positive results of the same respiratory pathogen recorded within 28 days of the initial positive result were omitted from analysis and final results. Without molecular sequencing, this was the method chosen to filter out unnecessary persistent positives that were not representative of a new infection event.

3. Results

Descriptive statistics were generated for a cohort of 77 bonobos from 2014-2017 in the Lola ya Bonobo (**Table 1**). Of those 77 bonobos, 36 were female (46.75%) and 41 were male (53.25%). A total of 282 testing events occurred across the study period with 93 results being positive, marking a 32.98% positivity rate among all bonobos. The average age at testing events was significantly different at 9.39 years old among negative results and 7.83 years old among non-negative results (Kruskal-Wallis rank sum test found a p-value of 0.0014). There was no significant difference found between average age at event between males and females. A total of four deaths occurred in the cohort over the study period. Bolomba (roughly eight-year-old male) died on 10/15/2014, with his last test result being an RSV-A positive reported on 3/4/2014, Makasi (roughly four-year-old male) died on 10/11/2014 after a negative baseline test reported on 1/29/2014. Ngaba was a male juvenile brought into the sanctuary and just a few days later died on 9/22/2014 after a confirmed Rhinovirus/Enterovirus result on 9/19/2014 (estimated nearly five years old). Finally, Wongolo (roughly five-year-old male) died on 3/10/2014 after a confirmed coinfection of *Chlamydophila pneumoniae* and RSV-A. Wongolo tested positive for *Chlamydophila pneumoniae* on 1/30/2014 and RSV-A on 3/10/2014, the day he died.

The 93 positive results were composed of 14 measured outcomes. Outcomes were either single-pathogen results or two to three pathogen results (**Table 2**).

Table 1: Cohort Descriptive Statistics and Demographics

Number for Bonobos	77									
Sex	36 Female, 41 Male									
Number of Events	282, 144 Female (51.24%) and 138 Male (49.76%)									
Positive Result Count	93 (32.98% positivity rate)									
Average Age at Event	9.39 yrs. (7.83 yrs. Among only positive results)									
Death Count	4									
Enclosure	**Nursery**		**Juvenile**		**Enclosure 1**		**Enclosure 2**		**Enclosure 3**	
Number of Events	76		34		64		62		45	
Positive Result Count	38 (50%)		15 (44.12%)		15 (23.44%)		13 (20.97%)		11 (24.44%)	
Average Age at Event	3.93 yrs. (3.86 yrs.)		6.16 yrs. (6.32 yrs.)		12.58 yrs. (11.45 yrs.)		12.53 yrs. (13.61 yrs.)		12.23 yrs. (11.87 yrs.)	
Death Count	0		1		2		0		0	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Number of Events	32	44	16	18	31	33	36	26	22	23
Positive Result Count	18	20	7	8	6	9	9	4	4	7
Average Age at Event	4.11 yrs. (3.94 yrs.)	3.81 yrs (3.79 yrs.)	6.63 yrs (6.55 yrs.)	5.74 yrs. (6.11 yrs.)	13.06 yrs. (9.70 yrs.)	12.12 yrs. (12.61 yrs.)	14.58 yrs. (15.93 yrs.)	9.69 yrs. (8.41 yrs.)	10.37 yrs. (8.23 yrs.)	14.01 yrs. (13.96 yrs.)
Death Count	0	0	1	0	0	2	0	0	0	0

Two separate numbers are recorded for stratified "Age at Event" counts. The first is the average age at event for all results, while the second is among only positive results. A combination of Fisher's Exact tests revealed that while there is a significant difference in average "Age at Event" between negative results and non-negative results, there is no statistically significant difference in average "Age at Event" between males and females or among different enclosures.

Only three deaths are reported by enclosure out of the four total. One bonobo (Ngaba) died before being assigned to a sanctuary. Ngaba's data is reflected in all non-stratified counts and proportions.

Table 2: Respiratory Test Results

Respiratory Test Results	Count
Adenovirus & Bocavirus Positive	1
Bocavirus Positive	1
Chlamydophila pneumoniae Positive	2
Parainfluenza 2 & Rhinovirus/Enterovirus Positive	1
Parainfluenza 2 Positive	1
Parainfluenza 3 Positive	2
Positive Detected (RSVA, CUnknown pneumoUnknown & Rhino/Entero)	1
Positive Detected (RSVA, FluA, Adeno & Rhino/Entero)	1
Respiratory Syncytial Virus A & Adenovirus Positive	1
Respiratory Syncytial Virus A & Rhinovirus/Enterovirus Positive	1
Respiratory Syncytial Virus A and Parainfluenza 3 Positive	1
Respiratory Syncytial Virus A Positive	41
Rhinovirus/Enterovirus & Adenovirus Positive	2
Rhinovirus/Enterovirus Positive	37

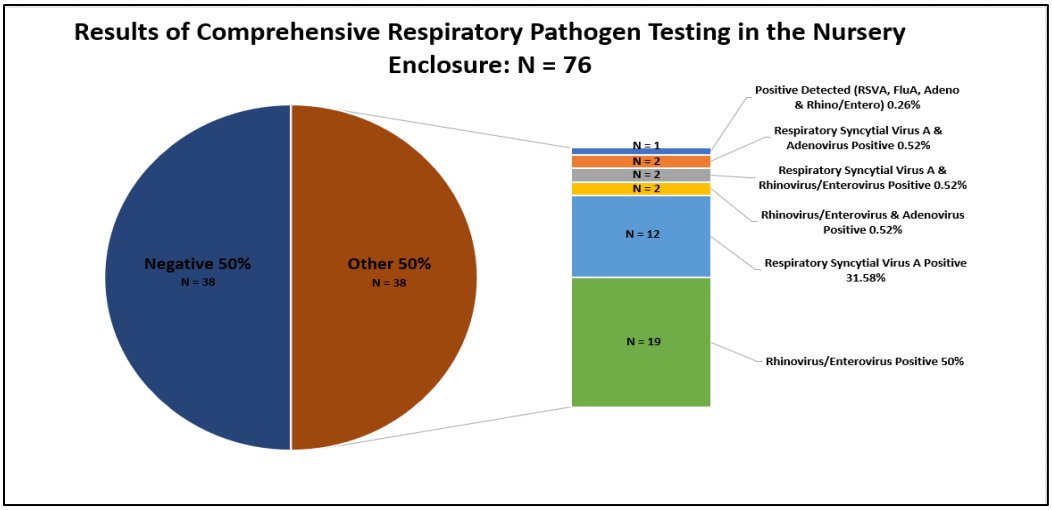

Figure 2: Respiratory Test Results for Nursery Enclosure

In the Nursery enclosure, 76 testing events occurred between 1/27/2014 and 11/7/2015. Of those tests, 38 returned positive results (50% positivity rate). Age at event ranged from 3.07 years old – 6.75 years old. Average age at event was 3.93 years old among all tests and 3.86 years old among positive results only. No deaths occurred in the nursery (see **Figure 2** for all results of respiratory testing).

In the Juvenile enclosure, 34 testing events occurred between 1/28/2014 and 9/30/2015. Of those tests, 15 returned positive results (44.12% positivity rate). Age at event ranged from 3.41 years old – 9.17 years old. Average age at event was 6.16 years old among all tests and 6.32 years old among positive results only. One death occurred in the juvenile enclosure (see **Figure 3** for all results of respiratory testing).

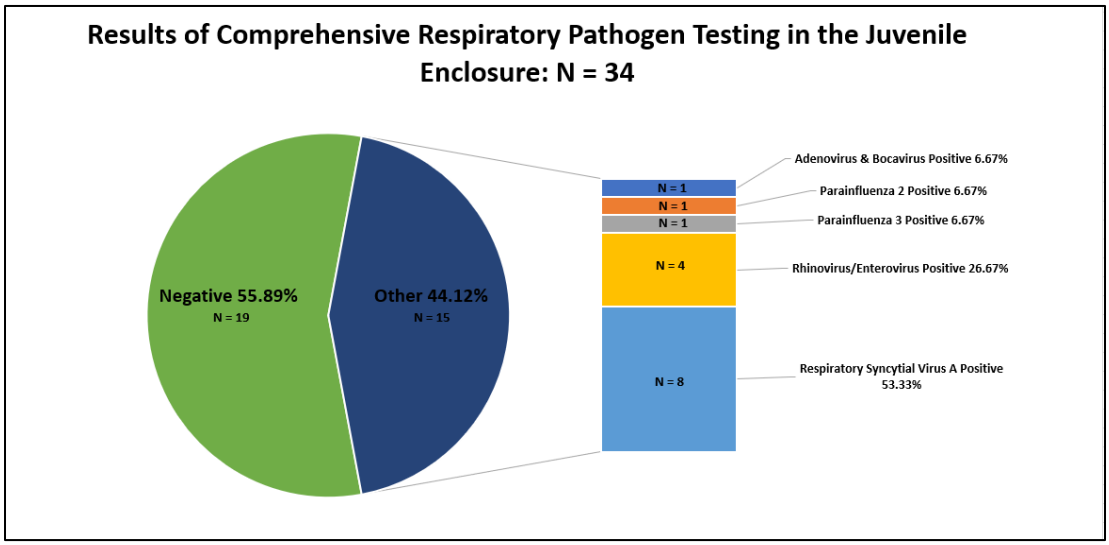

Figure 3: Respiratory Test Results for Juvenile Enclosure

In enclosure 1, 64 testing events occurred between 1/28/2014 and 1/24/2017. Of those tests, 15 returned positive results (23.44% positivity rate). Age at event ranged from 1.63 years old – 21.28 years old. Average age at event was 12.58 years old among all tests and 11.45 years old among positive results only. Two deaths occurred in enclosure 1 (see **Figure 4** for all results of respiratory testing).

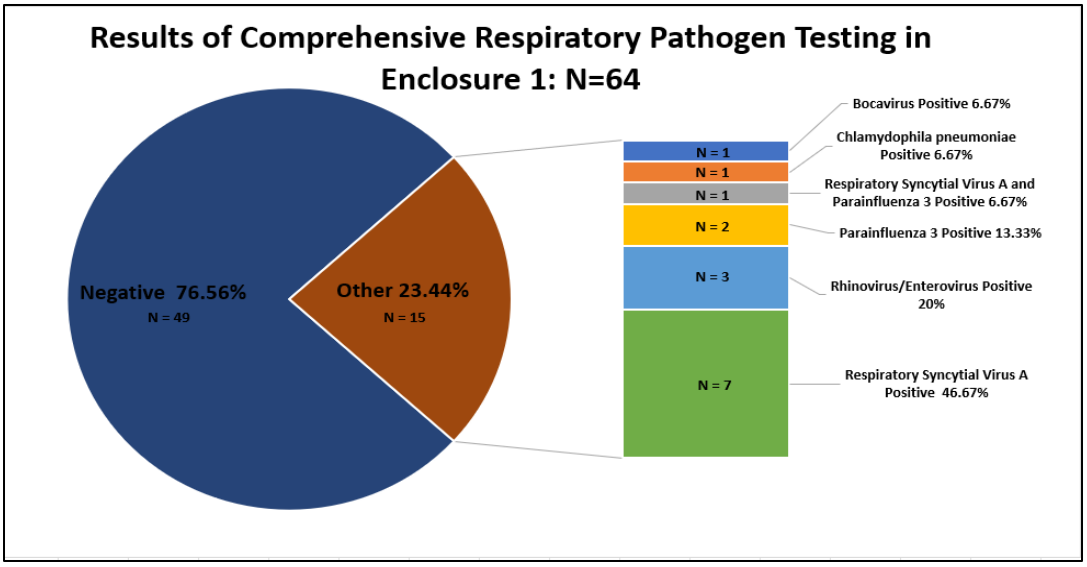

Figure 4: Respiratory Test Results for Enclosure 1

In enclosure 2, 62 testing events occurred between 1/30/2014 and 1/25/2017. Of those tests, 13 returned positive results (20.97% positivity results). Age at event ranged from 0.25 years old – 27.92 years old. Average age at event was 12.53 years old among all tests and 13.61 years old among positive results only. Zero deaths occurred in enclosure 2 (see **Figure 5** for all results of respiratory testing).

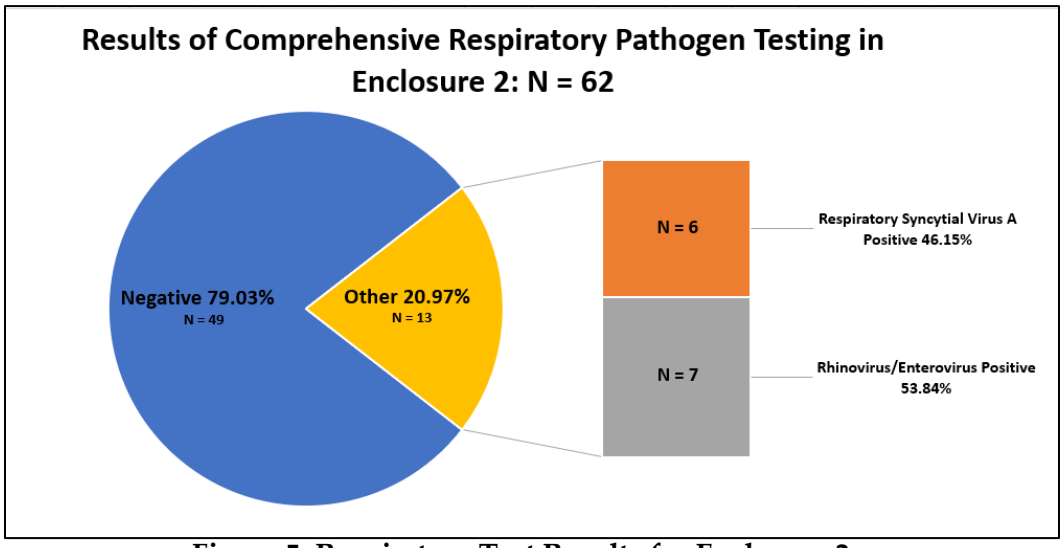

Figure 5: Respiratory Test Results for Enclosure 2

Finally, in enclosure 3, there were 45 testing events that occurred between 1/28/2014 and 11/9/2015. Of those tests, 11 returned positive results (24.44% positivity rate). Age at event ranged from 0.23 years old – 24.38 years old. Average age at event was 12.23 years old among all tests and 11.87 years old among positive results only. Zero deaths occurred in enclosure 3 (see **Figure 6** for all results of respiratory testing).

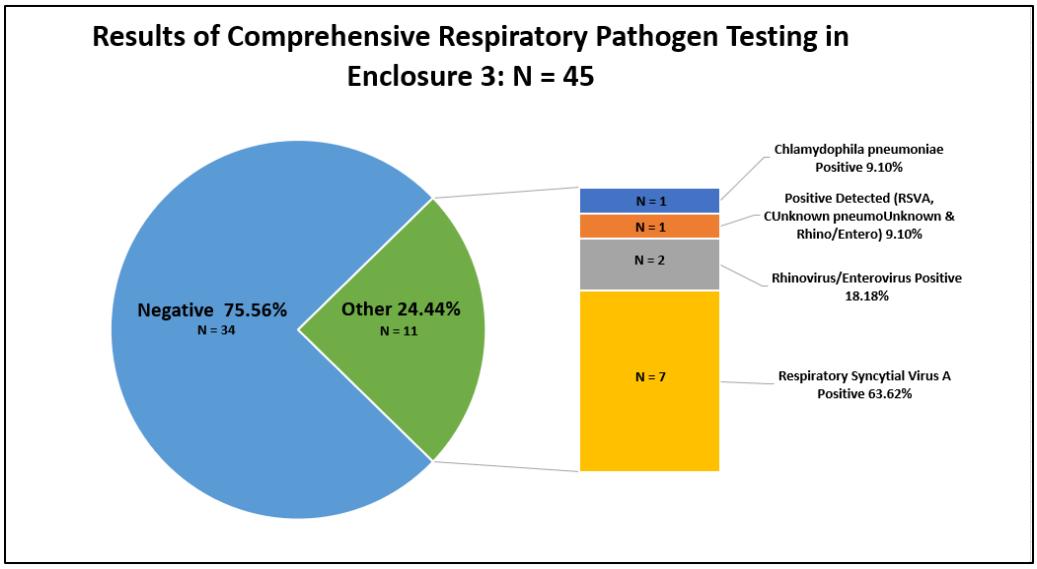

Figure 6: Respiratory Test Results for Enclosure 3

Respiratory event histograms were generated in order to demonstrate infection clustering over the study period at the sanctuary. Total disease occurrence was skewed to the left, primarily occurring between January 2014 and December 2014. The two largest spikes occurred between March and May of 2014 with approximately 55 of 93 positive cases occurring in this time period (**Figure 7**). With RSV-A and Rhinovirus/Enterovirus being the two largest positive results, additional figures showing respective disease incidence were generated.

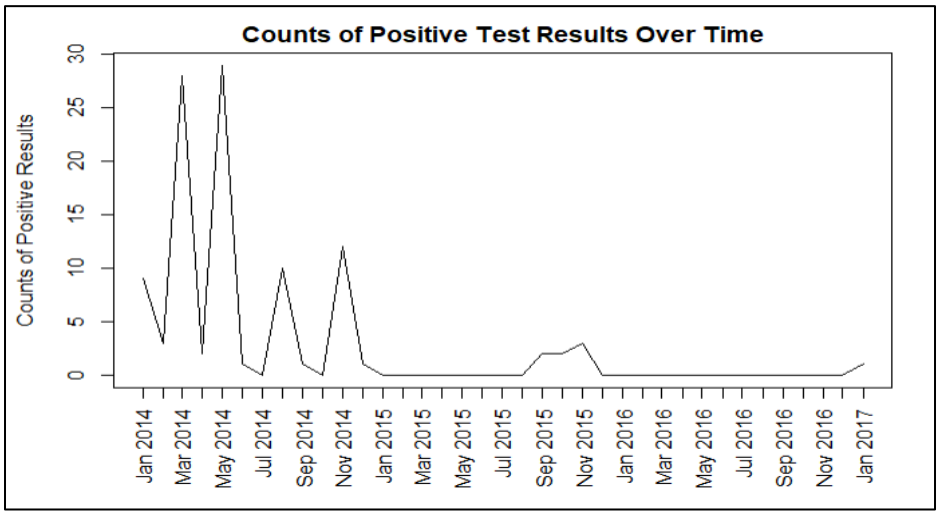

Figure 7: Counts of Positive Disease Over Time

RSV-A incidence entirely occurred between February and June of 2014 (**Figure 8**). Additionally, the majority of cases occurred in the month of March (28 cases). Rhinovirus/Enterovirus incidence occurred primarily between January of 2014 and January of 2015, oftentimes with months of time between each registered spike (**Figure 9**). The largest peak occurred during November of 2014 with 12 cases.

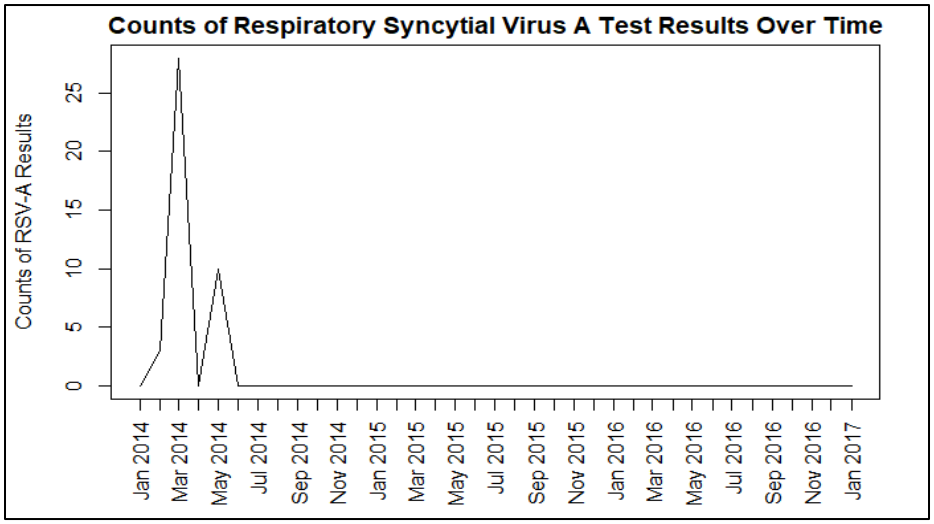

Figure 8: Counts of RSV-A Over Time

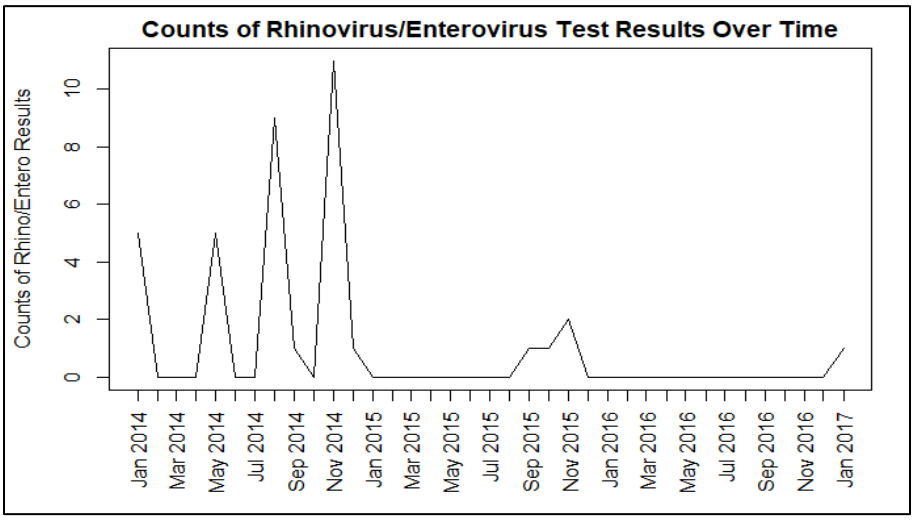

Figure 9: Counts of Rhinovirus/Enterovirus Over Time

Finally, additional histograms were generated depicting burden of both RSV-A and Rhinovirus/Enterovirus stratified by enclosure across the study period. Both figures are focused on time periods of the greatest incidence of either respective result (**Figures 10 and 11**).

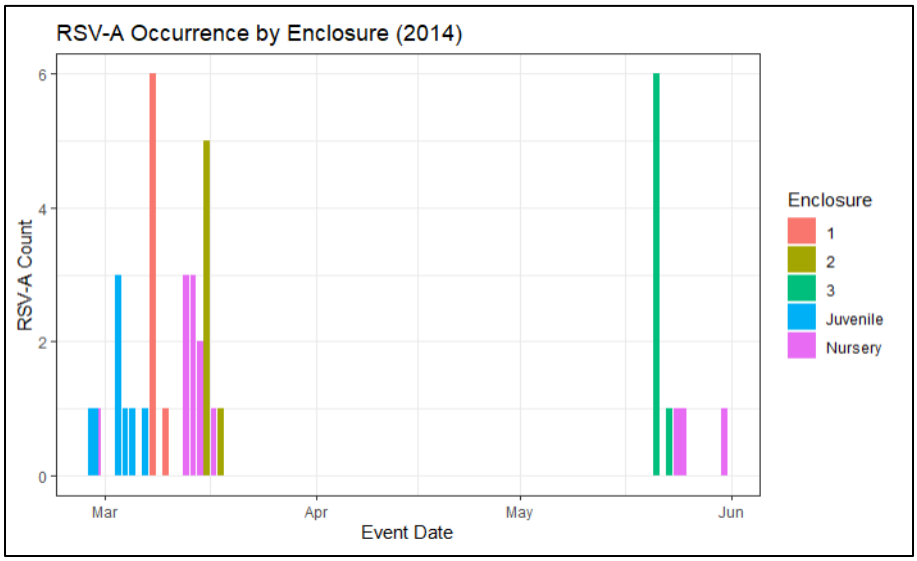

Figure 10: Counts of RSV-A by Enclosure over Time

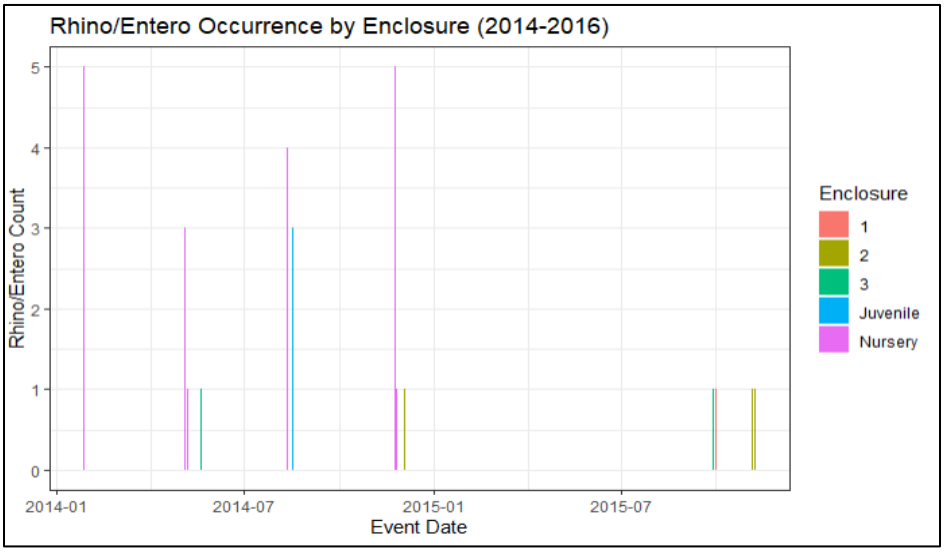

Figure 11: Counts of RSV-A by Enclosure over Time

4. Discussion

With rapid deforestation, unfettered bushmeat and illegal pet trades, and human encroachment, wild animals and humans are increasingly coming into contact; resulting in ample opportunity for the spillover and proliferation of zoonotic pathogens (Wolfe *et al*, 2005). Due to their close genetic relationship to humans, bonobos serve as excellent models to examine infectious disease dynamics both within a primate species and between great apes and humans. As bonobos are endangered, better understanding of reverse zoonoses can have major implications for bonobo conservation efforts. Few opportunities arise to study a sizeable and stable population of bonobos. Earlier studies of wild bonobo and chimpanzee populations have been generated with small sample sizes despite unpredictable factors outside the control of researchers (Grützmacher *et al*, 2018)(Medkour *et al*, 2021). The Lola ya Bonobo Sanctuary in southwestern DRC provides an important study setting to observe minimally studied disease dynamics within a population that is controlled and habituated to human presence. A descriptive analysis of a prospective index-cluster study involving the animal population and their associated human caretakers demonstrated recurrent detection of clusters of common human pathogens among the bonobos supporting the likelihood of inter- and intra-species transmission of respiratory pathogens at Lola ya Bonobo Sanctuary.

In a cohort of 77 bonobos, we performed 282 sampling events between January of 2014 and January of 2017, primarily occurring between January of 2014 and January of

2015. 93 tests returned positive results for one or more respiratory pathogens including nine (10%) co-infection events. Multiplex Rt-PCR testing identified seven different respiratory viruses (e.g. Respiratory Syncytial Virus A, Rhinovirus. Parainfluenza B, and the intracellular bacterial infection *C.* pneumonophilia). These pathogens would be spread in similar fashion - through physical contact and respiratory droplets (CDC, 2022). Bonobo society is a very intimate setting, where physical interaction manifests itself in frequent and varying ways among both males and females (Clay and Zuberbühler, 2012). Their social structure is a matriarchal fission-fusion society, where females determine mating and social interaction. Bands of bonobos will split during the day to forage - and gather together to sleep at night. Males will vie for female kinship with other males through physical conflict (Clay and Zuberbühler, 2012). Additionally, casual sexual contact is prevalent both homosexually and heterosexually within bands of bonobos. This physical and social interaction within groups provides ample opportunity for respiratory disease transmission and supports the likelihood of co-infections within any one bonobo.

Detection of respiratory pathogens clustered in the younger enclosures and the average age with detected virus was lower. Similar to young humans, it is hypothesized that younger bonobos are more subject to communicable infectious disease occurrence due to their lack of adaptive immune protection. Additionally, higher viral loads have been observed in younger bonobos (Torfs *et al*, 2021).

Stratified respiratory disease counts were generated by enclosure in order to describe the temporal dynamics of viral transmission throughout the study. Nearly half of all Respiratory Syncytial Virus A positives occurred in the nursery, along with a sizeable burden of Rhinovirus/Enterovirus. Of the positive results in all enclosures, the majority of results were either Respiratory Syncytial Virus A or Rhinovirus/Enterovirus. The nursery enclosure displayed higher occurrence of coinfections in comparison to the other enclosures. Both the higher proportional occurrence of disease as well as presence of coinfections reenforces the notion that younger bonobos (with underdeveloped immune systems and high degree of physical contact) would likely have a higher proportional burden of disease in comparison to older bonobos in the other enclosures. Additionally, the orphans and younger bonobos have comparatively more contact with humans via surrogate mothers and research staff.

Figures 10 and 11 demonstrate the occurrence of RSV-A and Rhinovirus/Enterovirus over time by enclosure, illustrating how respiratory pathogens moved through enclosures at LYB. RSV-A appeared to move serially and at high attack rates through each enclosure, where spikes in cases occurred in one enclosure after the other with few points in time in which more than one enclosure was experiencing cases. Save for a few cases in the nursery, late February and early March of 2014 are marked by a defined cluster of RSV-A cases in the juvenile enclosure and a single case in the nursery. Immediately following this is a cluster of cases in enclosure 1, followed by a

cluster in the nursery enclosure. After another spike in enclosure 2 in the middle of March, no new cases of RSV-A occur until mid-late April with a sharp and sudden clustering of cases in enclosure 3. This is followed by a roughly two-week sustained incidence in the nursery, after which no new cases are recorded for the remainder of the study period. The clustering observed among RSV-A cases at LYB is strikingly similar to the sparse literature on respiratory viruses among bonobos. For example, Torfs *et* al described a "betweenness" (defined as proximity or closeness) which served as a central risk factor for transmission among zoo-housed bonobos (Torfs *et* al, 2021).

Rinovirus/Enterovirus contributed to a large proportional burden at LYB. Clustering was typically 2-3 months apart and not as uniform compared to RSV-A transmission. Throughout 2014, there were consistent clusters of cases within the nursery enclosure – each with sizeable spikes in comparison to other enclosures. Three of the four spikes in the nursery enclosure were followed by spikes in a different enclosure within a 2-3 week period. Enclosures 1, 2, and 3 all had infrequent but moderate spikes in Rhinovirus/Enterovirus cases. A large absence of cases occurred between January of 2015 and November/December of 2015 due to conclusion of the study surveillance period. This was followed by small clustering in enclosures 1, 2, and 3 in late 2015 as part of pre-release testing.

This described epidemiology of disease clusters across multiple enclosures could be explained by a few possibilities. Firstly, there was semi-frequent occurrence of

bonobos moving from one enclosure to another. This most often occurred with bonobos aging out of the nursery enclosure, and moving into the juvenile enclosure (and rarely, moving into enclosures 1, 2, or 3). Secondly, bonobos can share physical contact at the borders between enclosures. While fences exist between enclosure at LYB, bonobos could easily share physical contact at the borders of any given enclosure. This allows for transmission events to potentially occur. Finally, humans could have introduced these pathogens into any given enclosure, with the most likely point of entry in the nursery or juvenile enclosures given the more intense human contact. Numerous veterinarians, researchers, and staff circulate throughout the sanctuary on a daily basis. The respiratory pathogens discovered to be circulating at LYB in this study are capable of infecting humans, allowing for continuous reintroduction of respiratory pathogens into the bonobo population. Furthermore, this creates cause for further characterization of disease occurrence at LYB in the interest of conservation efforts as well as human health of workers and visitors to the sanctuary.

4.1 Study limitations

This study had a few key limitations. The major limitation comes with embedding a research program into an animal sanctuary where it was dependent on the compliance of non-research personnel and the participation of wild animals. Also, birth date of each bonobo has the potential to misrepresent study results. In a case where a bonobo was brought into the sanctuary, researchers would estimate a birth year and

mark the birth date as January 1st of that year. Despite it being difficult to accurately estimate a bonobos age, this has the potential to affect associations between age and outcomes of interest. Second, human disease data recorded during this study was unreliable. An additional aim to collecting data on bonobo disease outcomes was collecting data on human disease outcome and attempt to find an association between the two. While 130 test results were collected from 44 human subjects, the data was unreliable. Human testing was not conducted consistently throughout the study period, and when it was, results were often not well documented. While it is thought that human disease can interplay with bonobo health at Lola Ya, the human data collected during this study is not robust enough to warrant analysis and furthermore supposed association. Finally, the definition of unique disease events was based on timing of sample collection.

4.2 Implications for policy and practice

The results of this study have shown that respiratory pathogens are endemic and cause clusters of illness that spread among the enclosures at LYB. These respiratory viruses are most likely introduced from surrounding human communities via sanctuary staff, particularly the surrogate mothers in the nursery or juvenile enclosures. Once introduced, there is rapid intra-enclosure and ultimately inter-enclosure spread. Other potential sources would include new arrivals, sustained transmission with prolonged carriage allowing re-introduction, or non-primate reservoirs. Reasonable consideration

for infection control measures and surveillance for staff providing close bonobo care with particularly vulnerable animals would seem warranted. This would include symptom screening, encouragement of community vaccination for influenza and COVID-19, and hand hygiene made widely available. New arrival bonobos undergo a quarantine period from other animals but require close attention from human caregivers during this vulnerable time. At present, no kind of health checkup or infection control measures to alleviate disease spread between different areas of the sanctuary is in place at the time of inter-enclosure movement of bonobos. Although the data are limited, it would seem reasonable to consider surveillance, clinical evaluation, and testing prior to inter-enclosure movement. This provides an opportunity for both active surveillance measures and mitigation of disease spread among the habituated bonobo population at LYB.

While this study was not able to provide a body of evidence to support the notion that human disease can interplay with bonobo health, active surveillance of sanctuary staff could also serve to decrease incidence of respiratory illness at Lola Ya. This could be achieved via weekly or monthly respiratory disease panels, and could provide further insight into respiratory disease dynamics in the sanctuary.

4.3 Implications for further research

This study has made great strides in demonstrating the need for further research. Firstly, the collection of reliable and comprehensive human data on respiratory disease

at LYB would yield a potential association with bonobo disease dynamics. Additionally, human data could provide just cause for further policy change at the sanctuary, which would only serve to improve both human and bonobo health. Secondly, further research is needed to identify distinct disease events more accurately at LYB. Molecular sequencing could be utilized to concretely discern unique disease events. Furthermore, it could make potential connections between circulating serotypes of respiratory pathogens both among other bonobos and among humans that frequently interact with them. Finally, signs and symptoms of these circulating pathogens need to be better characterized within both humans and bonobos. While many of the pathogens observed during this study can competently infect both humans and bonobos, their disease manifestation can markedly differ. Further understanding of these key differences can assist in identification and isolation of respiratory disease, as well as assist in mitigation of disease spread.

5. Conclusion

In today's globalized world, humans and animals are coming into contact with one another at higher rates than ever before. As deforestation, human encroachment, and habitat destruction continue, spillover events of zoonotic disease will only become more frequent. Disease interplay between humans and animals is a complicated and evolving process. As time goes on, it is important to study and understand these disease dynamics so as to mitigate morbidity and mortality. This study has shown that respiratory diseases known to be harbored by both humans and bonobos circulate at Lola Ya Bonobo Sanctuary, and these diseases with varying pathogenicity and virulence have major implications for both human and bonobo health.

The existing body of literature is not comprehensive and large enough to warrant a proper understanding of inter and intra-species respiratory disease interplay between bonobos and humans. More strides are needed in gene sequencing, characterization of disease manifestations in bonobos and humans, and replication of present studies. Further research in these fields as well as implementation of pro-conservation policies at Lola Ya serves to benefit the health of both humans and bonobos; and may provide a distinguished model for how to address increasingly complex human-animal disease dynamics.

Appendix A: Sample Close Contact CRF Form (Cohort Registration Form)

LOLA YA BONOBO COHORT REGISTRATION FORM

Bonobo Close Contact Questionnaire

Bonobo Name_____

Index ID # _____

Today's date: __ __ / __ __ __ / 2014
Day (##) Month (JAN)

For internal use only

Sample Collection Information:

Plasma	☐ No	☐ Yes
Serum	☐ No	☐ Yes
RNA	☐ No	☐ Yes
NP Swab	☐ No	☐ Yes
OP Swab	☐ No	☐ Yes
Urine	☐ No	☐ Yes
Stool	☐ No	☐ Yes

Collection Time: ☐☐:☐☐ (24 Hour Clock) Collected By: _____

Venipuncture Site: ☐ Right ☐ Left

Has subject had blood collected before? ☐ No ☐ Yes

If yes, when? __ __ / __ __ __ / __ __ __ __
Day (##) Month (JAN) Y Y Y Y

Comments/Notes:

Protocol Title: Detecting Interspecies Disease Transmission and Novel Pathogen Detection at Lola Ya Bonobo Sanctuary, Democratic Republic of Congo

IUCUC Approval: A261-13-10

LOLA YA BONOBO COHORT REGISTRATION FORM

Bonobo Close Contact Questionnaire

Bonobo Name_____

Index ID # _____

Subject Behavior Characteristics

Has the subject had any close contact with any humans displaying cold/flu symptoms in the past **TWO** days?

☐No ☐Yes

Has the subject had any close contact with any bonobos displaying cold/flu symptoms on the past **TWO** days?

☐No ☐Yes

Please list the people or bonobos the subject has had the most contact with in the past **TWO** days:

_____

_____

Close Contact Symptoms

Is the subject experiencing any symptoms today? ☐No ☐Yes

☐N/A If they are experiencing symptoms, when did they start? __ __ / __ __ __ / __ __ __ __
Day (##) Month (JAN) Y Y Y Y

☐N/A If they are experiencing symptoms, please select which ones they are feeling today:

Physical Symptoms

	None				Moderate			Severe
1. Nasal discharge ("Runny nose")	0	1	2	3	4	5	6	7
2. Coughing	0	1	2	3	4	5	6	7
3. Sneezing	0	1	2	3	4	5	6	7
4. Facial Swelling	0	1	2	3	4	5	6	7
5. Diarrhea	0	1	2	3	4	5	6	7

Other:

_____

_____

_____

_____

_____

LOLA YA BONOBO COHORT REGISTRATION FORM

Bonobo Close Contact Questionnaire

Bonobo Name_____

Index ID # _____

Behavioral Changes

	None			Moderate				Severe
6. Lethargy	0	1	2	3	4	5	6	7
7. Activity Avoidance	0	1	2	3	4	5	6	7

Other: _____

_____

_____

_____

_____

Diet Changes

Did the subject eat today? ☐ No ☐ Yes

Did the subject eat fruit today ☐ No ☐ Yes

Describe any differences in diet/appetite in the subject:

_____

_____

_____

Is the subject currently taking or have they taken any antibiotics in the past 7 days?

☐ No ☐ Yes

If so, please list and describe: _____

_____

_____

_____

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