

## RESEARCH ARTICLE

# Human quarantine: Toward reducing infectious pressure on chimpanzees at the Taï Chimpanzee Project, Côte d'Ivoire

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Due to their genetic relatedness, great apes are highly susceptible to common human respiratory pathogens. Although most respiratory pathogens, such as human respiratory syncytial virus (HRSV) and human metapneumovirus (HMPV), rarely cause severe disease in healthy human adults, they are associated with considerable morbidity and mortality in wild great apes habituated to humans for research or tourism. To prevent pathogen transmission, most great ape projects have established a set of hygiene measures ranging from keeping a specific distance, to the use of surgical masks and establishment of quarantines. This study investigates the incidence of respiratory symptoms and human respiratory viruses in humans at a human-great ape interface, the Taï Chimpanzee Project (TCP) in Côte d'Ivoire, and consequently, the effectiveness of a 5-day quarantine designed to reduce the risk of potential exposure to human respiratory pathogens. To assess the impact of quarantine as a preventative measure, we monitored the quarantine process and tested 262 throat swabs for respiratory viruses, collected during quarantine over a period of 1 year. Although only 1 subject tested positive for a respiratory virus (HRSV), 17 subjects developed symptoms of infection while in quarantine and were subsequently kept from approaching the chimpanzees, preventing potential exposure in 18 cases. Our results suggest that quarantine—in combination with monitoring for symptoms—is effective in reducing the risk of potential pathogen exposure. This research contributes to our understanding of how endangered great apes can be protected from human-borne infectious disease.

## KEYWORDS

great apes, habituation, prevention, quarantine, respiratory disease

## 1 | INTRODUCTION

In 1979, primatologists Christophe Boesch and Hedwige Boesch-Achermann instituted one of the first long-term field studies of chimpanzees: the Taï Chimpanzee Project (TCP) situated in Côte d'Ivoire's Taï National Park. They set out to habituate chimpanzees—to accustom them to human presence—in order to study their behavior (Boesch & Boesch-Achermann, 2000). Their work has significantly contributed to our understanding of chimpanzee culture and cognition as well as human evolution. Moreover, Boesch and Boesch-Achermann made important

observations on illness, on the disappearance of individual chimpanzees, and obtained biological samples that paved the way for future investigations of disease (Boesch, 2008; Leendertz et al., 2006).

These later investigations have led to the identification of numerous pathogens. Not only do they account for pathogens occurring naturally in the chimpanzees (e.g., STLV and SFV) and in the habitat (e.g., Ebola and *Bacillus cereus* *bv anthracis*), but also those introduced by humans, such as the human respiratory syncytial virus (HRSV) and human metapneumovirus (HMPV) (Boesch, 2008; Calvignac-Spencer et al., 2012; Formenty et al., 1999; Gogarten et al., 2014; Köndgen et al., 2008; Köndgen, Schenk, Pauli, Boesch, & Leendertz, 2010; Le Guenno et al., 1995; Leendertz, Boesch, Junglen, Pauli, & Ellerbock, 2003; Leendertz et al., 2004, 2008).

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Since 1999, TCP experienced at least six major respiratory disease outbreaks of human origin and with losses of up to 19% of the chimpanzee communities (Köndgen et al., 2008). Other projects have also published data on human respiratory pathogens infecting and, ultimately, killing several wild, habituated great apes (Grützmacher et al., 2016; Kaur et al., 2008; Palacios et al., 2011). Such findings emphasize the inherent risk of proximity. In all of the reported outbreaks, the viruses detected were either HRSV or HMPV: two common human paramyxoviruses that generally cause only mild symptoms in healthy human adults (Falsey and Walsh, 2000; Webe, Mulholland, & Greenwood, 1998). People can easily be unaware of their infectiousness because they may still feel physically fit enough to visit the great apes. In short, the risk of transmission is often overlooked.

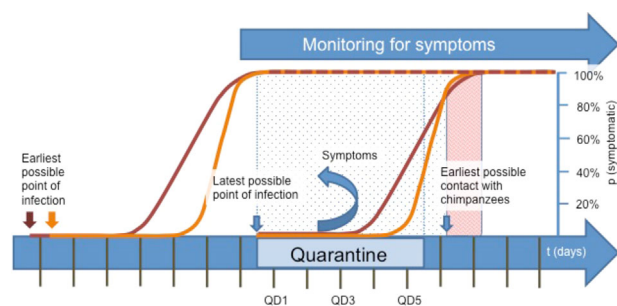
Although the issue of whether humans should interfere with naturally occurring disease outbreaks in protected species is controversial, it is commonly understood that disease transmission from humans should be mitigated (Gilardi et al., 2015). It is the ethical responsibility of humans entering great ape habitats to help minimize the costs of habituation, including potential changes in behavior, alterations of the habitat, and the sickness and loss of individuals. Indeed, since 1992, the TCP has gradually implemented preventative steps to reduce the risk of exposing chimpanzees to human pathogens. The first steps included rules not to enter the forest when ill and to remove all garbage. Two years later, additional rules to bury human feces and to bring back all food remains from the forest were established and complemented by veterinary collaboration. In 1999, a minimum viewing distance of 5 m was implemented (later extended to 7 m) alongside requirements that all humans be vaccinated against yellow fever, tuberculosis, measles, and poliomyelitis. Vaccination against meningococcal disease was added in 2006. In addition to being under constant veterinary attendance since 2000, in 2002, the TCP installed a “hygiene barrier” outside the camps—clothes are changed, boots are disinfected whenever entering or leaving the forest, and human feces are carried back to camp. In 2004, preventive measures were once again strengthened. Spitting in the forest and family members in the camps became prohibited. And, simultaneously, the wearing of surgical masks became obligatory when in the presence of chimpanzees. The step toward quarantine was first taken in 2008. An 8-day quarantine was introduced for travelers arriving in the country, wherein the last 3 days of quarantine were completed in the TCP camps (Boesch, 2008). Following the last major outbreak in 2009, the quarantine rules were adjusted to a 5-day period of quarantine in a separate quarantine camp for all humans intending to visit the chimpanzees (Gilardi et al., 2015). Additionally, international travelers have to spend a minimum of 2 days in Côte d'Ivoire before entering quarantine.

Researchers and field assistants alike now enter the quarantine on Day 1 (QD1). They must remain in a designated area in the quarantine camp without direct contact to people who have already cleared quarantine, anybody from the outside, or anybody in a different quarantine stage (the quarantine camp is divided into two separate sections to allow for two independent parties). After four nights, should no symptoms develop, the respective person clears quarantine

on the 5th day (QD5). However, if any symptoms are detected, the person exits quarantine and starts over with QD1 after all symptoms have subsided. If anyone falls sick during quarantine, then everyone in quarantine at the same time also has to start over with QD1 (see Figure 1). However, 5 days do not cover the longest possible incubation periods—the time between infection and symptom onset—of most human respiratory pathogens (see Table 1). But, with the exception of measles (against which humans have to be vaccinated in order to visit habituated chimpanzees at TCP), at least 50% of this period is covered for all other relevant viruses. Against this backdrop, the goal of this study is to determine the number of humans falling ill during quarantine. Furthermore, the risk of potential exposure to human pathogens is assessed by testing sick humans to detect common human respiratory viruses they brought to the habituation site, and by randomly testing apparently healthy humans in the beginning and at the end of quarantine to assess the possibility of excreting HRSV and HMPV, the two most relevant viruses for wild great apes.

## 2 | METHODS

The current study was performed at the TCP in Côte d'Ivoire. At the TCP, the quarantine camp is separate from the research camps with a designated kitchen, shower, and toilet. A driver supplies subjects in quarantine with food, wearing a facemask on delivery and leaving the food outside the building with no personal contact (for a more detailed description of quarantine protocols at TCP see Gilardi et al., 2015). When a subject falls ill, he or she is taken home to their village, or in case of international visitors (including researchers and film-makers), the subject is physically separated within the quarantine facilities until symptoms cease and quarantine can begin again at QD1. If symptoms are more severe, subjects are taken to the local hospital for treatment.



**FIGURE 1** Overview of the quarantine system in the Tai Chimpanzee Project and time from infection to observation of symptoms for HRSV and HMPV. Y-axis: Parametric estimates of the incubation period;  $p$  (symptomatic) = cumulative percentage of cases developing symptoms, and thus, shedding HRSV or HMPV by a given day under the estimates for the log-normal distribution (Lessler et al., 2009). The red line represents HRSV and the orange line represents HMPV to exemplify the earliest and latest possible scenario. Whereas the blue dotted area illustrates the window covered through the quarantine, the red area covers the remaining risk.

**TABLE 1** Overview of incubation periods of the most important human respiratory viruses, central tendency (e.g., median, mean); data derived from Lessler et al. (2009)

Respiratory pathogen	Incubation period (days)	Central tendency	Incubation period covered by 5-day quarantine (in %)
Adenovirus	4–8	6	62.5
Human coronavirus (non-SARS)	2–5	3	100
SARS-associated	2–10	5	50
Influenza A	1–4	2	100
Influenza B	0.3–1.1	0.6	100
Human metapneumovirus	4–6	5	83.33
Measles <sup>a</sup>	8–14	10	35.7
Parainfluenza	2–6	4	83.33
Respiratory syncytial virus	3–7	5	71.43
Rhinovirus	2–4	2	100

<sup>a</sup>Measles vaccination is mandatory for humans approaching great apes in the Tai Chimpanzee Project.

The staff health program of TCP includes yearly health checks at the local hospital, were a tuberculosis skin test and vaccinations against yellow fever, measles, poliomyelitis, and meningococcal disease are conducted. Informed consent was obtained from all participants and no animal samples were tested in this study. All study procedures were in compliance with the American Society of Primatologists' Principles for the Ethical Treatment of Primates.

Over a period of 1 year (from August 2012 to August 2013), throat swabs were collected from all subjects (staff and researchers) entering the TCP and, thereby, quarantine. A total of 223 subjects entered quarantine during the year comprised of 67 international visitors and 156 local staff. When possible, samples were collected on both QD1 and QD5. In total, 262 were selected for testing. This sample selection was systematized to achieve an even seasonal distribution, wherein a minimum of 20 samples were tested for each month of the 1-year research period, and to best assess the risk of potential pathogen exposure. Thus, the selection included 14 samples, collected from subjects with symptoms of illness ( $N = 18$ ) plus randomly selected subjects with corresponding samples from QD1 ( $N = 110$ ) and QD5 ( $N = 101$ ) (when possible), and samples from subjects tested on a different day of quarantine (due to various constraints) or who had been in contact with sick individuals ( $N = 33$ ). Signs of illness were generally mild respiratory symptoms, including sore throat, runny nose, or cough. Subjects in quarantine are expected to self-report symptoms; however, the respective camp manager and "veterinarian in charge" also oversee the quarantine process.

Swabs were stored and transported in liquid nitrogen and later kept in  $-80^{\circ}\text{C}$  freezers. Three hundred microliters of nuclease-free water were added to each sample and vortexed thoroughly before extraction. DNA/RNA was extracted using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. cDNA was synthesized with a Superscript Kit (Invitrogen, Waltham, MA) and random hexamer primers (TIB Molbiol, Berlin, Germany), and subsequently screened for HRSV and HMPV using generic PCR protocols as described by Reiche and Schweiger (2009) and Reiche et al. (2014), targeting the N or F protein gene, respectively, with expected amplicon sizes of 142 and 161 bp. Amplification was

conducted for 5 min at  $95^{\circ}\text{C}$ , followed by 45 cycles of  $95^{\circ}\text{C}$  for 15 s, and  $60^{\circ}\text{C}$  for 30 s. PCR products were analyzed by electrophoresis in a 2% agarose gel. Bands were cut from gel and extracted, if multiple bands occurred. Positive samples were purified using Exo-SAP (USB Europe GmbH, Staufien, Germany) and sequenced, using the ABI Big Dye Termination Kit (Applied Biosystems, Weiterstadt, Germany). qPCR positive samples were tested for confirmation with an additional hemi-nested pan-pneumovirus PCR assay, targeting the L protein gene as detailed by Tong, Chern, Li, Pallansch, and Anderson (2008). For individual confirmation of HRSV A and B, respectively, a hemi-nested PCR was performed targeting the hypervariable region of the G protein gene as previously described by Sato et al. (2005); for confirmation of HMPV a hemi-nested confirmation PCR was performed targeting the P protein gene, as described by Mackay et al. (2004). If samples from symptomatic individuals tested negative for HRSV and HMPV, then an array of PCR-based screening assays were performed, targeting common human respiratory viruses, including adenovirus, coronavirus, enterovirus, influenza A and B, measles, parainfluenza, and rhinovirus (Chmielewicz, Nitsche, Schweiger, & Ellerbrok, 2005; Jang, Lee, Kwon, Chung, & Lee, 2005; Nitsche unpublished data; Pusch et al., 2005; Santibanez, Heider, Gerike, Agafonov, & Schreier, 1999; Schulze, Nitsche, Schweiger, & Biere, 2010). Positive samples were analyzed and sequenced as detailed above. All obtained sequences were compared to the non-redundant nucleotide sequence database of the National Center for Biotechnology Information (NCBI) using BLAST. The sample was considered positive only when a sequence was obtained and identified via BLAST.

### 3 | RESULTS

Eighteen (8%) of the 223 subjects who entered quarantine developed symptoms after their arrival to TCP. In 17 instances, subjects developed symptoms during quarantine and in a single instance after quarantine. International visitors (two of which had only recently arrived in Côte d'Ivoire) represented 7 (39%) and local staff

represented 11 (61%) of symptomatic subjects. These subjects were subsequently sent back to the village or were isolated within camp until symptoms ceased. Illness occurred throughout the year with the exception of March, April, October, and December. The highest number of cases with illness ( $N = 5$ ) were observed in July (Dates of symptom occurrence: September 16, 22, and 23, November 8 and 28, 2012, January 8, two cases on February 3, May 20, June 8, two cases on July 6, July 8, and two cases on July 20, August 8, 21, and 31, 2013).

Of the 262 samples tested, the 211 (80%) were samples from subjects entering quarantine on QD1 ( $N = 110$ ) or clearing quarantine on QD5 ( $N = 101$ ). A total of 37 samples were taken on a different day of quarantine (i.e., QD2–QD8) due to constraints in the field, and originated from subjects who had been in contact with the subject testing positive for HRSV or in cases when quarantine was prolonged. The remaining 14 samples were obtained from the 18 subjects who developed symptoms. Only one of the samples from a subject with symptoms tested positive for HRSV (on November 8). In other words, no virus was found in 13 out of the 14 samples from symptomatic subjects. All samples taken from asymptomatic subjects tested negative for HRSV and HMPV.

## 4 | DISCUSSION

Anthropogenic respiratory disease poses a serious risk to habituated wild great apes with high morbidity and considerable mortality (Boesch, 2008; Kaur et al., 2008; Köndgen et al., 2010; Leendertz et al., 2006; Palacios et al., 2011). Its mitigation is therefore, an ethical responsibility for habituation projects (Gruen, Fultz, & Pruetz, 2013). However, preventing people from exposing wild habituated great apes to human pathogens is a challenge. In terms of potential pathogen exposure, both people from overseas who come to visit habituation sites as well as local staff on such projects routinely pose a risk to the health of wild great apes (Muehlenbein & Ancrenaz, 2009). People who come to visit wild great apes from overseas are more likely to have been exposed to a variety of pathogens throughout their travel (e.g., in airports or airplanes) (Mangili & Gendreau, 2005). Additionally, the stress of travel and changes in climate make them more susceptible to infection, leading to disease within few days after arrival at the destination where the great apes are encountered (Muehlenbein & Ancrenaz, 2009). Despite these different risk factors, eco-tourists frequently lack information about the relevance of their own health for the wildlife they intend to visit and, thus, may not take precautions or report illness (Muehlenbein & Ancrenaz, 2009). Finally, local project staff can also pose an increased risk when they come into frequent contact with children in their villages—a subpopulation with high respiratory disease prevalence (Walker et al., 2013). The results show that international visitors represented 7 (39%) and local staff represented 11 (61%) of symptomatic subjects. However, only two of the seven international visitors became ill shortly after their arrival in Côte d'Ivoire and the remaining five rather mirror the health of permanent project staff in contact with the local diversity of pathogens.

The detection of HRSV in only one out of 262 tested samples (including the 14 samples from people showing symptoms of respiratory

disease) points toward a rather low prevalence of humans excreting HRSV and HMPV, when entering the TCP research area. Although the possibility of false-negative results exists, it seems likely that if a person excretes these viruses in any relevant quantity—a quantity that would be sufficient for transmission to chimpanzees—it is likely to be detectable with the methods used here. Of course, the negative results for asymptomatic people are not surprising. For some respiratory viruses, however, pathogen excretion might start before the first symptoms occur. For example, 1–8% of influenza infectiousness occurs prior to illness onset (Lau et al., 2010). Cases of asymptomatic shedding have been reported for HRSV and HMPV—the most common causative agents of respiratory disease outbreaks in wild habituated great apes—but this is believed to be a rather rare occurrence (Falsey, Erdman, Anderson, & Walsh, 2003). That said, HMPV has recently been reported at two different great ape field sites in 1 out of 24 and 1 out of 42 apparently healthy staff members (Grützmacher et al., 2016). Although asymptomatic infections must be taken into account as a risk factor, the absence of symptoms also limits the physical spread (e.g., the absence of cough or nasal discharge limit the excretion of the pathogen). Thus, strict hygiene rules should be sufficient to counteract the potential risk posed by apparently healthy people, especially those who have passed through quarantine.

In this study, 13 subjects with respiratory symptoms (including cough, nasal discharge, or a sore throat) were negative for all viruses tested. That pathogens were not detected despite the observed symptoms may indicate that the symptoms were caused by viruses not included in our PCR panel or be due to the fact that symptoms do not always correlate well with viral particle excretion. These negative results can also be due to the detection limit of diagnostic methods (here PCR). Furthermore, symptoms indicating respiratory disease can be caused by primary or secondary bacterial infections, such as with *Streptococcus pneumoniae*. Importantly, despite their involvement in mortalities as secondary infections (Chi et al., 2007; Köndgen et al., 2011; Palacios et al., 2011), we did not test for potentially pathogenic bacteria. We did not do so mainly because such bacteria are often carried within the commensal flora of healthy humans, which makes it impossible to link a positive finding to the symptoms observed. This is a common problem for diagnostics in human medicine as well (Bartlett, 2011; Bosch, Biesbroek, Trzcinski, Sanders, & Bogaert, 2013). Beyond viruses and bacteria, the most frequent causative agents for acute respiratory illness, certain parasites, or fungi are also capable of triggering symptoms. More generally, infectious agents are not the only cause of respiratory symptoms (Boutayeb, 2006). Non-infectious etiologies include allergies, asthma, chronic obstructive pulmonary disease (COPD), cancer, air pollution, wildfire smoke, and smoking. While most of these causes are unlikely sources for the symptoms observed in this study—there was no particular air pollution or forest fire during the study period and neither allergies, asthma, nor any other chronic respiratory disease were reported by any of the subjects—it is impossible to entirely rule them out.

From a practical point of view, however, preventing the spread of viruses is a bigger challenge because the particle size is smaller and aerosolization is more likely. Therefore, hygiene measures preventing viral spread will also likely prevent the transmission of bacterial pathogens.

Even though a causative pathogen could only be determined in a single case, the delays through quarantine led to development of symptoms within isolation in 17 cases. Without the quarantine procedure, these subjects would have already entered the forest, possibly approaching the chimpanzees and interacting with other people in the camps. Notably, the one incidence in which a person developed symptoms after clearing quarantine was the very same incidence where HRSV was found. Therefore, the infectious subject had not yet approached the chimpanzees and all contact persons were returned to quarantine. HRSV has a median incubation period of 4.4 days (95% CI: 3.9–4.9) and 95% of infected individuals will develop symptoms by 6.3 days (95% CI: 5.2–7.3) after infection (Lessler et al., 2009). While a 5-day quarantine does not cover the complete incubation period, it does cover 71% of the HRSV incubation period (83% for HMPV), and thus, lowers the risk considerably.

Quarantine has rather high costs and is a time intensive measure, so length needs to be decided carefully. Extra space must also be made available to have physically separated facilities. Additionally, people are very limited in the work they can do while in quarantine and lose time that could otherwise be spent doing valuable fieldwork—which is expensive for the project. Yet, from a purely preventive health point of view, a 6-day quarantine would be even better as it would cover 95% of people developing symptoms of HRSV, and 100% for HMPV—the only two viruses ever found in respiratory disease outbreaks among wild great apes (Kaur et al., 2008; Köndgen et al., 2010; Palacios et al., 2011). In addition, the challenging process of quarantine has the positive side effect of limiting exchanges with surrounding villages, which in itself lowers the risk of project staff becoming infected. If logistics allow, then adding this one additional day to the quarantine process should be strongly considered.

Observations of outbreak frequency at the TCP are not high enough to statistically show that the frequency has declined since the implementation of the described quarantine procedure. That said, the patterns strongly suggest beneficial impacts from this procedure. Before the implementation of quarantine, TCP experienced six major outbreaks between 1999 and 2006. Since its implementation, the TCP has only experienced one further outbreak (in 2009), one that started when the quarantine was ignored (Leendertz, unpublished data). This underscores the argument that following quarantine rules is essential. It also demonstrates that all measures taken only result in risk reduction and emergency protocols must be kept in place for any outbreak intervention. Our data and observations clearly suggest that quarantine does not replace monitoring for symptoms—they need to be used synergistically.

In conclusion, it is important to note that single measures are insufficient for obtaining ethically acceptable risk reduction. Several hygiene measures need to be combined: the implementation of quarantine and symptom monitoring with keeping a minimum distance of 7 m and wearing surgical masks (especially as chimpanzees do not follow distance rules) (Gilardi et al., 2015; Köndgen et al., 2008). Moreover, through monitoring and diagnostic investigation we can further characterize the real risk while assuring that the preventive measures are being followed.

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