MULTIPLE VIRAL INFECTIONS IN CONFISCATED WILD BORN SEMI-CAPTIVE CHIMPANZEES (*Pan troglodytes schweinfurthii*) IN A SANCTUARY IN UGANDA: IMPLICATIONS FOR SANCTUARY MANAGEMENT AND CONSERVATION

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Abstract

Infectious agents/diseases from non-primates in particular have caused a number of new human diseases leading to calls for international surveillance to monitor the human-nonhuman primate (NHP) interface. Hence, confiscated 42 wild-born semi-captive chimpanzees living in a sanctuary on Ngamba Island, Uganda were screened for a broad range of viral pathogens to determine the prevalence of specific viral infections some of which may be cross reacting with human viruses using specific polymerase chain reactions (PCR) and serologic assays. The viral infection prevalence in chimpanzees was 82.4%, 73%, 60.5% and 32.4% for adenoviruses, simian foamy viruses, gammaherpesviruses and hepatitis B virus, respectively. They were negative for simian/human immunodeficiency virus (SIV/HIV), human T-lymphotropic virus types I and II (HTLV-I and HTLV-II) antibodies, hepatitis C virus (HCV), hepatitis E virus (HEV), flavivirus, human-metapneumovirus (HMPV), and Chikungunya viruses. These results indicate that wild-born captive chimpanzees are infected with multiple viral pathogens with potential for inter- and intra-species transmission. The data has implications for sanctuary management and conservation efforts and implies the usefulness of incorporating sanctuary primates into emerging infectious disease research programs.

Newly emerging diseases can become major threats to public health and animal reservoirs are implicated as the major sources of these emerging diseases[1,4,11,24]. Indeed, approximately 75% of emerging human infectious diseases are zoonotic from wildlife, livestock or their products.[26,27] Primate-associated zoonotic diseases have received dramatic global attention after emergency of HIV 1 and 11 from a simian variant of the virus SIVcpz[7,10,12,19] and caused global HIV/AIDs pandemic. More recently, a novel HIV-1 lineage (HIV-1 group P) distinct from HIV-1 groups M, N, and O closely related to gorilla simian immunodeficiency (SIVgor) was discovered in a Cameroonian woman.[20]. At the same time great ape populations are being driven to extinction by emergence of infectious diseases in great ape populations some of which are of human origin.[8,14] Human respiratory syncytial virus (HRSV) and meta-pneumovirus (HMPV) caused mortalities in chimpanzees in Tai Forest in Cote d'Ivoire[13] following habituation for research. 49% of chimpanzee deaths documented during 47 yr of study at Gombe, Tanzania, were attributed to respiratory diseases.[25] Other confirmed transmissions of potential pathogens from humans and

domestic animals to wild primates have been documented. For example, gorillas and chimpanzees in Uganda have recently been shown to be infected with human strains of bacteria.[9, 21]

The unprecedented high levels of primate-human interactions through forest destruction, bush meat; illegal trade in infant apes is implicated in recorded emerging infectious diseases. The high levels of interactions between human and NHPs primates is even higher in established 20 Pan Africa Sanctuaries Alliance (PASA) sanctuaries in Africa with continuous high influx of orphaned/rescued primates at an average of 56 arrivals per year.[6] PASA sanctuaries house a variety of primates with 15 sanctuaries caring for the endangered chimpanzees with population of more than 855 chimpanzees with numbers projected to increase to 1800 individuals in 20 yr.[6] The primate orphans in sanctuaries are result of bush meat trade where adults are killed for meat and the surviving infants sold as pets and other commercial business, the majority of which are confiscated by relevant authorities and brought to sanctuaries.[2] This presents different levels of primate-human interactions with potential risks of disease transmission. Disease management is not or not well addressed in sanctuaries often overshadowed by other ethical and welfare requirements of the rescued primates and other conservation roles. Hence the management of these sanctuaries presents a risk of disease transmission that exposes naïve population of primates to human pathogens and vice versa. The study was undertaken on chimpanzees on Ngamba Island to understand the dynamics of diseases transmission and highlight the role of sanctuaries in monitoring potential pathogens that may lead to emergence of infectious diseases and cross-species transmission of dangerous pathogens to humans.

Blood and fecal samples were taken from 42 captive wild-born chimpanzees on 43 hectares of forest on Ngamba Island on L. Victoria during the annual health checks under general aneasthesia. All samples were stored at –80^{o}C until transported on dry ice to the Robert Koch Institut, Berlin, Germany for analysis.

Qualitative enzyme immunoassay kits were used for detection of antibodies against human T-lymphotropic virus types I and II (HTLV-I and HTLV-II) on 42 chimpanzee serum samples (Murex Biotech Limited, Central road, Temple hill Dartford DA1 5LR, UK) as previously shown to detect STLV-1 effectively in wild chimpanzees. SIV/HIV antibody detection was perfomed using Murex HIV-1.2.0 kit (Abbot Murex Boitech limited) and GENESCREEN HIV 1.2 version 2 (BIO-RAD) by enzyme immunoassay while Enzygnost Anti-HBc monoclonal (Dade Behring Marburg GmbH, Germany) was used for qualitative determination of antibodies to Hepatitis B (core)-Antigen in serum as per manufacturer's instructions.

Single and Multiplex PCR were performed on extracted nucleic acids (DNA, RNA) extracted from chimpanzee blood and feces to indentify a number of viruses: Simian Foamy viruses, Herpesviruses, adenoviruses, Hepatitis Viruses (B,C, E), Flaviruses, Human-metapnuemovirus, and Chikungunya viruses. Established non-invasive methods were also used for analysis both genomic DNA and proviral RNA extracted from fecal samples of individual chimpanzees.

Our results revealed that the wild-born semi-captive chimpanzees screened in study were infected with multiple viruses including novel viruses in some individual chimpanzees. We report for the first time results of extensive study using both PCR and ELISA methods for detection of viral pathogens and antibodies from chimpanzees in sanctuaries in Africa with viral prevalence of 82.4%, 73%, 60.5% and 32.4% for adenoviruses, simian foamy viruses, gammaherpesviruses and hepatitis B virus respectively. The screened samples were negative for SIV/HIV, human T-lymphotropic virus types 1 and 2 (HTLV-1 and HTLV-2) antibodies and Hepatitis C virus (HCV), Hepatitis E Virus (HEV). Interestingly, all samples were negative for adenovirus on PCR from genomic DNA extracted from blood compared 82.4% positive on PCR performed on DNA extracted from fecal samples. For gammaherpesviruses, only three (3/21) were detected on DNA extracted from feces. For HBV, Mika was both infected on the analysis of genomic DNA from feces and blood. This confirms applicability of some non-invasive diagnostics/methods for the investigation of the viral pathogens in great apes. Statistical results using 1-sided Fisher's exact test and Exact logistic regression did not reveal any effect/ association of identified viral infections with age, sex and origin except for HBV (1-sided fishers exact analysis (P=0.004)) where HBV was more prevalent in adults than non-adults. There was also no link or effect of infection of one virus on another virus indentified in the same individuals. No evidence of SIV and STLV infections was found chimpanzees in contrast to other studied captive and wild chimpanzees mainly from central and west Africa in which these viruses are known to be enzootic.[10,23] Hence it is surprising that all 42 wild-born chimpanzees of eastern *P t. schweinfurthii*, were negative for both SIV and STLV antibodies. This can probably explained by the fact that most of the apes in sanctuaries were taken out of the forests when they were still young, well protected by their mothers and most likely not have encountered any aggressions risky for transmission of these viruses. This may not be true with other sanctuaries and care needs to be taken to establish the status of all primates in sanctuaries. With the most recent findings that SIVcpz, the immediate precursor of HIV-1, is pathogenic in free-ranging chimpanzees[12] and identification of a new human immunodeficiency virus (HIV-I group P) in a Cameroonian woman closely related to SIVgor[20] highlights the significance testing all rescued primates on arrival and presents new challenges to the management of sanctuaries. Our study reveals multiple viral infections in most of the individual chimpanzees screened with at least more than one virus including a novel herpes virus.[16] This shows that chimpanzees and other apes harbor multiple viruses some of which are still unknown. This is exemplified by recent documentation many novel viral pathogens in primates: 2 novel gammaherpesvirus in captive primates[5]; 10 betaherpesviruses in wild great apes[15]; 30 adenoviruses from apes[5]; 3 adenoviruses from macaques[22] and a novel gammaherpesvirus in chimpanzee.[16]

The documented viruses in chimpanzees in this study with zoonotic potential[17,18] plus other already described viral pathogens in primates might be transmitted to humans given the current levels of interactions. Contact between the donor and receipt hosts is a precondition for virus transfer affected by geographic, ecological and behavioral factors. Trade in wildlife, bushmeat hunting, human population expansion, environmental factors like deforestation and agriculture expansion promote viral emergence and viral host switching from animals to humans and vice versa. Animal husbandry practices in sanctuaries are critical in this case to disease transmission as daily routine welfare brings about daily interactions with staff especially through feeding and

rehabilitation processes. In some sanctuaries like Ngamba, human interaction has extended to tourists through walking/carrying infant chimpanzees through the forest as part of rehabilitation, raising awareness and funding for the sanctuary. This promotes high level of interaction during rehabilitation of newly rescued infant apes and in other sanctuary cases with domesticated animals like dogs and cats. All these behavioral/management practices are being done by individuals without the knowledge of the potential risks of infectious disease acquisition and transfer between the animal and persons undertaking rehabilitation process.

Periodic health checks post quarantine should be undertaken preferably 2 to 3 yr and should include complete physical examination, body measurements, full blood count, kidney and liver functional tests, urinalysis, TB screening, screening for helminthes and protozoa and samples submitted to appropriate laboratories for screening major viral and bacterial pathogens of concern. The information generated from these examinations will help in performing disease risk analysis, management of group dynamics (re-socialization and integration) and planning for conservation programs like re-introductions. Likewise, all employees working in direct contact with primates should be vaccinated and monitored for infectious pathogens of zoonotic potential. The results from this study presents the first extensive study for viral pathogens carried by wild-born-captive chimpanzees within sanctuaries and provides baseline data for a wide range of diseases to be monitored in sanctuaries during the entire process of quarantine, routine management, re-introduction and post-reintroduction and by wild great ape health monitoring projects.

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