Primates (2013) 54:89–98
DOI 10.1007/s10329-012-0320-8

ORIGINAL ARTICLE

Epidemiological study of zoonoses derived from humans in captive chimpanzees

Takanori Kooriyama · Michiko Okamoto · Tomoyuki Yoshida · Toshisada Nishida · Toshio Tsubota · Akatsuki Saito · Masaki Tomonaga · Tetsuro Matsuzawa · Hirofumi Akari · Hidekazu Nishimura · Takako Miyabe-Nishiwaki

Received: 15 January 2012 / Accepted: 18 July 2012 / Published online: 14 August 2012

Abstract Emerging infectious diseases (EIDs) in wildlife are major threats both to human health and to biodiversity conservation. An estimated 71.8 % of zoonotic EID events are caused by pathogens in wildlife and the incidence of such diseases is increasing significantly in humans. In addition, human diseases are starting to infect wildlife, especially non-human primates. The chimpanzee is an endangered species that is threatened by human activity such as deforestation, poaching, and human disease transmission. Recently, several respiratory disease outbreaks that are suspected of having been transmitted by humans have been reported in wild chimpanzees. Therefore, we need to study zoonotic pathogens that can threaten captive chimpanzees in primate research institutes. Serological surveillance is one of several methods used to reveal infection history. We examined serum from 14 captive chimpanzees in Japanese primate research institutes for antibodies against 62 human pathogens and 1 chimpanzee-borne infectious disease. Antibodies tested positive against 29 pathogens at high or low prevalence in the chimpanzees. These results suggest that the proportions of human-borne infections may reflect the chimpanzee's history, management system in the institute, or regional epidemics. Furthermore, captive chimpanzees are highly susceptible to human pathogens, and their induced antibodies reveal not only their history of infection, but also the possibility of protection against human pathogens.

Keywords Chimpanzee · Serology · Captive · Human-borne infection

Electronic supplementary material The online version of this article (doi:10.1007/s10329-012-0320-8) contains supplementary material, which is available to authorized users.

T. Kooriyama (✉)
Rakuno Gakuen University, 582 Bunkyodai-Midori, Ebetsu, Hokkaido 069-8501, Japan
e-mail: kooriyam@rakuno.ac.jp

T. Kooriyama · T. Nishida
Japan Monkey Centre, Inuyama, Japan

T. Kooriyama · T. Tsubota
Hokkaido University, Sapporo, Japan

M. Okamoto · H. Nishimura
Sendai Medical Center, Sendai, Japan

M. Okamoto
Tohoku University, Sendai, Japan

T. Yoshida · A. Saito · M. Tomonaga · T. Matsuzawa · H. Akari · T. Miyabe-Nishiwaki
Kyoto University, Inuyama, Japan

Introduction

Emerging infectious diseases (EIDs) in wildlife, which may arise as a result of complex relationships between social and environmental factors, are a major threat both to human health and biodiversity conservation (Daszak et al. 2000, 2001; Jones et al. 2008; McMichael 2004; Morens et al. 2004). Such diseases often reduce wildlife populations in isolated communities (e.g., an Ebola outbreak in gorillas and chimpanzees in Gabon and Congo), increasing their probability of extinction, especially in frequency-dependent outbreaks (De Castro and Bolker 2005; Gerber et al. 2005; Bermejo et al. 2006; Nunn et al. 2008). Most EID events have been caused by zoonotic pathogens from a non-human animal source (Taylor et al. 2001; Woolhouse and Gowtage-Sequeria 2005; Jones et al. 2008). Moreover, it is estimated that 71.8 % of such zoonotic events are

caused by pathogens that originate in wildlife, for example the emergence of Nipah virus in Perak, Malaysia, and severe acute respiratory syndrome (SARS) in Guangdong Province, China. Pathogens originating from wildlife have also increased significantly with time (Jones et al. 2008). This supports the suggestion that zoonotic EIDs are an increasing, very significant, threat to global health (Morens et al. 2004; Weiss and McMichael 2004; King et al. 2006). It also emphasizes the importance of understanding the factors that increase contact between wildlife and humans for developing predictive approaches to disease emergence (Daszak et al. 2000; Patz et al. 2004).

Human infectious diseases are also being increasingly transmitted to wildlife, especially non-human primates. Chimpanzees and humans are closely related species evolutionarily and genetically, not only in their anatomical and physiological characteristics but also in their immunological features, which are much more similar than those between other animals (Brack 1987; Woodford et al. 2002; Clark et al. 2003). Wild chimpanzees may be susceptible to human infectious diseases because their immune system is naïve to them, so they may not be protected against human pathogens. According to recent reports, in addition to poaching and habitat fragmentation by deforestation, human-borne disease epidemics spread by ecotourism have contributed to the decline in wild chimpanzee populations (Kaiser 2003; Whitfield 2003; Kondgen et al. 2008). Unknown respiratory diseases have also been reported in other chimpanzees residing in protected areas, including Bossou, Guinea, and Gombe and Mahale, Tanzania; these have resulted in chimpanzee deaths and are suspected of having been transmitted by humans (Goodall 1986; Hosaka 1995; Ferber 2000; Nishida et al. 2003; Matsuzawa et al. 2004; Hanamura et al. 2008). Infectious disease transmission is possible without close contact via sneezing, coughing, etc. Muehlenbein and Ancrenaz (2009) reported that 39 species of pathogens were recovered from throat swabs of tourists viewing orang-utans, revealing the possibility that many human-borne agents may be transmitted to apes by several modes of infection. Therefore, continuous health monitoring and investigation of human-borne infectious diseases in apes is needed for risk management.

In this study we sought serological evidence of zoonoses in captive chimpanzees at the Kyoto University Primate Research Institute (KUPRI) in Japan to obtain basic epidemiological information on zoonoses affecting wild chimpanzees in Africa and to prevent pandemic outbreaks. The chimpanzees examined in this study had been reared for 10–30 years since birth at KUPRI or approximately 40 years after introduction from other zoos or western Africa. They had not been in individual cages, but rather in social groups, as in the wild; they were, therefore, a good model for wild chimpanzee studies.

Methods

Animals

This study was conducted under the guidelines of KUPRI. After obtaining the approval of the Institutional Animal Welfare and Care Committee, serological surveillance was conducted on 14 chimpanzees between 10 and 44 years old kept in an indoor–outdoor enclosure at KUPRI (see Matsuzawa 2003, 2006 for further information about the animals). Information about the chimpanzees is summarised in Table 1. The chimpanzees had been subjects for behavioural, psychological, and evolutionary studies. As summarised in Table 1, Pendesa suffered from allergic dermatitis, Mari and Reiko had severe colds in 1984 and 1980, respectively, and Reo developed tetraparesis resembling acute transverse myelitis in 2006 (Miyabe-Nishiwaki et al. 2010). Pal, Cleo, Ayumu, Pan, Reo, and Popo were born at KUPRI; Pendesa was born in another institution in Japan and transferred to KUPRI when she was 2 years old. Mari and Akira were born in Africa and reared at other institutes in Japan, then transferred to KUPRI; Chloe was born in a French Zoo; Puchi and Gon were born in Africa and reared as pets in Japan; Ai and Reiko were transported directly from Africa. The health of each animal was monitored daily by their keepers, and each individual underwent a periodic health examination every 1–2 years. None of these chimpanzees had been vaccinated against any pathogens.

Sample collection

Samples were collected between April 2007 and February 2010, when each chimpanzee was anaesthetised for research purposes or for a periodic health examination. The chimpanzees were anaesthetised with a combination of 3.5 mg/kg ketamine hydrochloride (Ketalar; Sankyo Parke Davis, Japan) and 0.035 mg/kg medetomidine hydrochloride (Domitor; Meiji Seika Kaisha, Tokyo, Japan) with or without premedication with oral midazolam (1 mg/kg) or droperidol (0.2 mg/kg). Anaesthesia was maintained with isoflurane (Isoflu; Dainippon Sumitomo Pharma, Osaka, Japan) when necessary. Blood samples were collected in plain tubes with a coagulant, and the serum or plasma was separated by centrifugation at 3000*g* for 20 min and then analysed within 1 day or stored at −80 °C until serological tests were performed.

Human infectious microbiological agent tests

Human respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) serological analyses were conducted at the Virus Research Centre of Sendai Medical

Table 1 Information on each chimpanzee

Name (abbr.)	Sex	Age[a]	History[b]	Introduction[c]	Medical record
Pal (Pal)	F	10	D: Pendesa, S: Akira	2000	
Cleo (Cle)	F	10	D: Chloe, S: Reo	2000	
Ayumu (Ayu)	M	10	D: Ai, S: Akira	2000	
Pan (Pan)	F	27	D: Puchi, S: Gon	1983	
Reo (Reo)	M	28	D: Reiko, S: Gon	1982	Total paralysis from 2006[d]
Popo (Pop)	F	28	D: Puchi, S: Gon	1982	
Chloe (Chl)	F	30	Paris Zoo-KUPRI	1985	
Pendesa (Pen)	F	33	JMC[e]-KUPRI	1979	Atopic dermatitis
Ai (Ai)	F	34	Africa-KUPRI	1977	
Mari (Mar)	F	34	Africa-JMC-KUPRI	1978	Severe cold in 1984[f]
Akira (Aki)	M	34	Africa-KUPRI	1978	
Reiko (Rei)	F	44	Africa-KUPRI	1968	Severe cold in 1980[f]
Puchi (Puc)	F	44	Africa-Pet in JPN-KUPRI	1979	
Gon (Gon)	M	44	Africa-Pet in JPN-KUPRI	1979	

F female, *M* male

[a] Age in December 2009

[b] The parents are shown for the chimpanzees born at KUPRI (D, dam; S, sire); relocation history is shown for transferred chimpanzees

[c] The year transferred or the years Pal, Cleo, Ayumu, Pan, Reo, and Popo were born at KUPRI

[d] Reo contracted tetraparesis resembling acute transverse myelitis in 2006

[e] Japan Monkey Centre

[f] These chimpanzees were separated from the others and hospitalized for therapy

Centre, Sendai, Japan (Okamoto et al. 2010). The other analyses were outsourced to the Tokai Chuo Laboratory (ISO15189: 2003) at Falco Biosystems, Kyoto, Japan, and The Corporation for Production and Research of Laboratory Primates, Tsukuba, Japan. The HBV test was conducted with Espline HBs-N (Fujirebio Diagnostics, Tokyo, Japan), an immunochromatographic test that uses serum and gives a visible result. These tests were repeated, and positive and negative controls were prepared to reduce non-specific reactions.

The antibodies analysed targeted causative agents of respiratory diseases and hepatitis in humans in addition to retroviruses, encephalitis virus, and chimpanzee foamy virus (CFV). The specific antibodies examined reacted against *Mycoplasma pneumoniae*; *Chlamydia pneumoniae*; *Bordetella pertussis* (Japanese Higashihama or Yamaguchi strains); influenza A virus; influenza B virus; human parainfluenza virus types 1–4 (hPIV-1–4); hMPV; RSV; mumps virus; measles virus (MV); adenovirus (ADV)-1 through 8 and 11, 19, and 37; coxsackievirus types A5–7, 9, 10, and 16 (CVA-5–7, 9, 10, and 16) and B1–6 (CVB-1–6); echovirus types 3, 6, 7, and 13; enterovirus 71; poliovirus types 1–3 (PV-1–3); herpes simplex virus-1 and 2 (HSV-1 and 2); cytomegalovirus (CMV); varicella zoster virus (VZV); Epstein–Barr virus (EBV); human herpesvirus 6 (HHV-6); hepatitis A virus (HAV); hepatitis B virus (HBV); hepatitis C virus (HCV); rubella virus; reovirus; rotavirus; human parvovirus B19 (HPV-B19); Japanese encephalitis virus (JEV); human immunodeficiency virus type I (HIV-1); human T cell lymphotropic virus type I (HTLV-1); CFV; filovirus; and *Entamoeba histolytica*.

For statistical analysis, Student's *t* test was used to compare the average antibody titres between chimpanzees reared since birth (SB) and chimpanzees reared after birth (AB). A P value of <0.05 was considered to indicate statistical significance of the results (Figs. 2, 3).

Results

We investigated the prevalence of antibodies against human-borne pathogens in chimpanzees at Japanese primate institutes using standard procedures for human serological analysis.

Table 1 summarises the name, sex, age, history, year of arrival at KUPRI, and medical notes for each chimpanzee. Six chimpanzees were born at KUPRI: Pal, Cleo, Ayumu, Pan, Reo, and Popo. Eight chimpanzees were born in Europe or Africa: Chloe, Pendesa, Ai, Mari, Akira, Reiko, Puchi, and Gon.

The seroprevalence of human and chimpanzee-originating pathogens in the chimpanzees are listed in Supplementary Table S1. Antibodies against 29 of 62 human pathogens were detected by use of standard procedures for

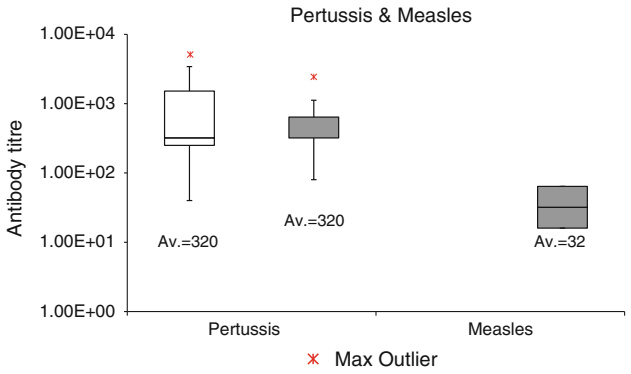

Fig. 1 Seroprevalence and titres of *B. pertussis* and MV antibodies, obtained by use of BAT and HI, respectively, for 14 chimpanzee serum samples. The range of antibody titre against *B. pertussis* was broad (40–5120×), and the average titre for the SB group (chimpanzees reared in KUPRI since birth) was higher than that for the AB group (chimpanzees reared in KUPRI after birth). MV antibody titre ranged from 16× to 64×, and most positive chimpanzees were in the AB group (in the SB group only one chimpanzee was positive: 64×). The *hollow box* indicates SB, and the *solid box* indicates AB. The *bars* indicate the average (Av.)

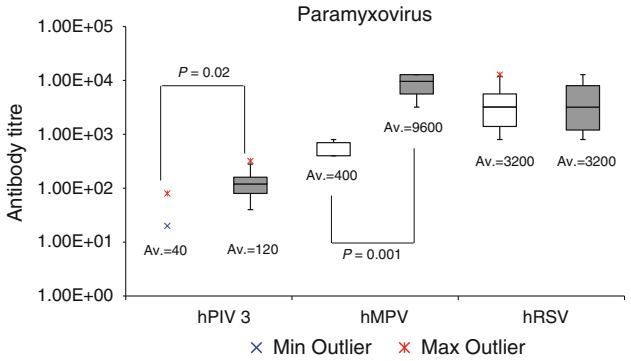

Fig. 2 Seroprevalence and titres of hPIV-3, hMPV, and RSV antibodies obtained by use of HI and ELISA (second two) for 14 chimpanzee serum samples. The titre against hPIV-3 for SB chimpanzees ranged from 20× to 80×, and that for the AB chimpanzees ranged from 80× to 160×. The average titre for the SB group was lower than that for the AB group. The titre against hMPV for SB chimpanzees ranged from 400× to 800×, which was lower than that for AB chimpanzees (3200–12800×). The average titre for the SB group was also lower than that for the AB group. The titre against hRSV ranged broadly from 800× to 12800× for both SB and AB chimpanzees. Average titres for SB chimpanzees were not much higher than those for AB chimpanzees. The *hollow box* indicates SB, and the *solid box* indicates AB. The *bars* indicate the average (Av.)

serological analysis of humans. Briefly, more than 50 % of the chimpanzees were positive (high prevalence) for 14 human pathogens: pertussis, hPIV-3, hMPV, RSV, ADV-1, ADV-2, ADV-4, ADV-5, ADV-6, CVA-7, CMV, VZV, EBV, and HHV-6. In addition, 15 human pathogens were found in some chimpanzees (low prevalence): influenza A

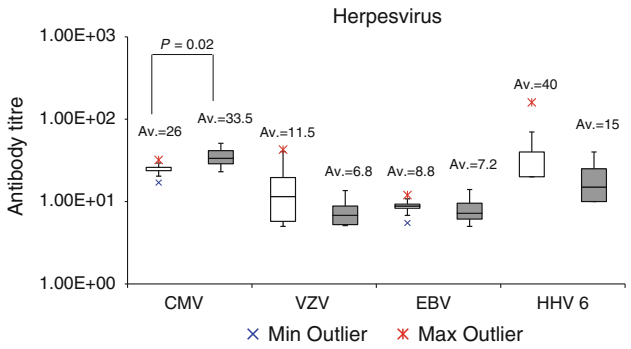

Fig. 3 Seroprevalence and titres of CMV, VZV, EBV, and HHV6 antibodies obtained by use of EIA for 14 chimpanzee serum samples. The antibody titre against CMV ranged from 17× to 32× for SB chimpanzees and from 26× to 51× for AB chimpanzees. The average titres in SB chimpanzees were lower than those in AB chimpanzees. The antibody titre against VZV in SB chimpanzees ranged from 5.0× to 42.7×; that in AB chimpanzees ranged from 5.1× to 13.6×. The antibody titre against EBV ranged from 5.5× to 12× for the SB group, which was not much higher than that for the AB group (5×–14×). The antibody titre against HHV6 ranged from 20× to 160× for SB chimpanzees and from 20× to 40× for AB chimpanzees. Differences in the average titres of VZV, EBV, and HHV6 were not statistically significant between SB and AB chimpanzees. The *hollow box* indicates SB, and the *solid box* indicates AB. The *bars* indicate the average (Av.)

(H3N2), MV, ADV-3, ADV-19, CVB-2, CVB-3, CVB-6, echovirus-6, CVA-5, CVA-9, HAV, reovirus, rotavirus, HPV-B19, and JEV. CFV was also detected in chimpanzees. Antibodies for the following 26 pathogens were not detected in any chimpanzee: *M. pneumonia*, *C. pneumonia*, influenza A (H1N1), influenza B, hPIV-1, hPIV-2, mumps virus, ADV-8, ADV-11, CVB-1, CVB-4, CVB-5, echovirus-7, echovirus-13, CVA-10, CVA-16, enterovirus 71, PV-2, PV-3, HSV, HBV, HCV, rubella virus, HIV-1, HTLV-1, and filovirus. In addition, hPIV-4, ADV-7, ADV-37, echovirus-3, CVA-6, PV-1, and *E. histolytica* were positive, but their positive sample antibody titres were equal to the cut-off titre (asterisk in Supplementary Table S1).

Figures 1a, b, 2a–c, 3a–d, and 4a, b show the antibody titres against pertussis, MV, hPIV-3, hMPV, RSV, CMV, VZV, EBV, and HHV-6 grouped by the birthplace of the chimpanzee. SB indicates that chimpanzees were reared in KUPRI since birth; AB indicates that chimpanzees were introduced and reared in KUPRI after birth. The AB group was older than the SB group. Their birthplaces are listed in Table 1.

Pertussis is caused by *B. pertussis* infection. The antibody titres against pertussis varied from 40 to 5120×, and the average titre in SB chimpanzees (320×) was equal to that in AB chimpanzees (320×) (Fig. 1). Antibody titres against MV were 16–64×, and the average titre in AB chimpanzees was 32× (Fig. 1). Only one chimpanzee was

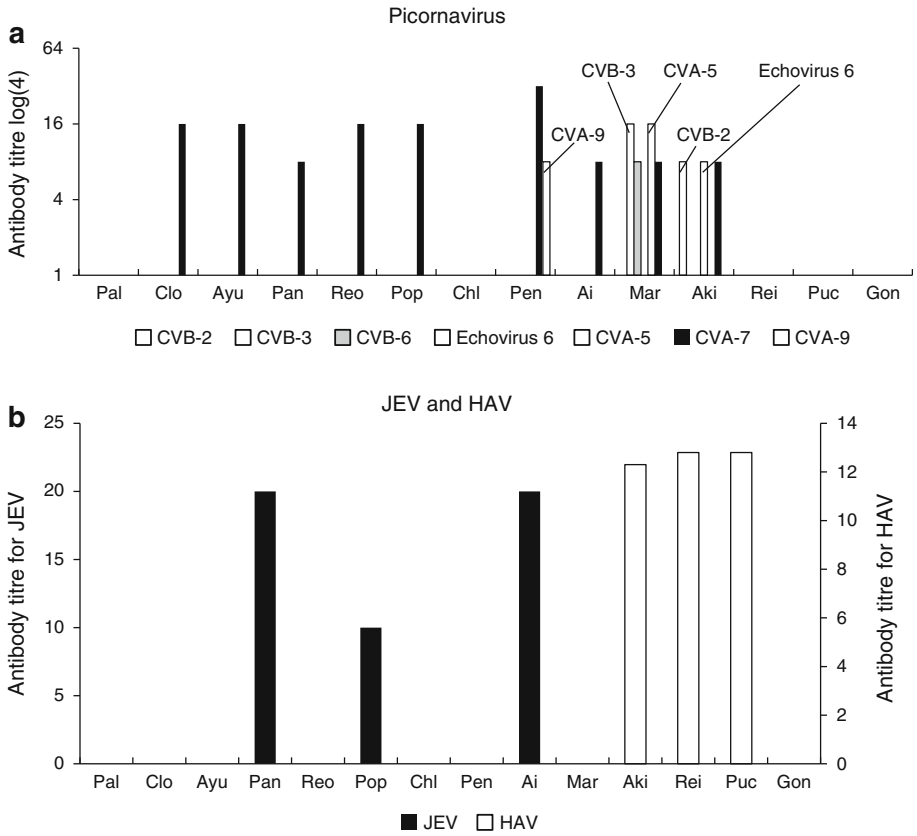

Fig. 4 **a** Seroprevalence of antibodies against picornaviruses, CVB-2, CVB-3, CVB-6, echovirus-3, echovirus-6, CVA-5, CVA-6, CVA-7, CVA-9, and PV-1 for each chimpanzee. The antibodies in serum were detected by use of NT. The AB female Mari had antibodies against four picornaviruses (CVB-3, CVB-6, CVA-5, and CVA-7); the AB male Akira had antibodies against CVB-2, echovirus-6, and CVA-7. Abbreviations of each chimpanzee name are listed in Table 1. **b** The seroprevalence of antibodies against HAV and JEV for each chimpanzee detected by use of CLIA for HAV and CF for JEV. Antibody against JEV was detected in both SB and AB chimpanzees, but HAV was detected in AB chimpanzees only

positive against MV in the SB group. The average antibody titre against hPIV-3 in SB chimpanzees (40×) was lower than that in AB chimpanzees (120×) (Fig. 2). The range of titre against RSV was broad, from 800–12800×, and the average titre for SB chimpanzees (3200×) was equal to that for AB chimpanzees (3200×) (Fig. 2). The titre against hMPV in SB chimpanzees was 800–12800×, and the SB animals (400–800×; average: 400×) had lower levels than the AB chimpanzees (3200–12800×; average: 9600×) (Fig. 2). Titres of PIV3 and hMPV increased with age ($P < 0.05$).

We examined specific antibodies against herpesviruses in the captive chimpanzees. Surprisingly, the chimpanzees had high prevalence of antibodies against the four herpesviruses CMV (100 %), VZV (100 %), EBV (100 %), and HHV-6 (75 %), but not against HSV-1 or 2 (Supplementary Table S1). The titres against CMV, VZV, EBV, and HHV-6 are shown in Fig. 3. Titres of antibodies against CMV, only, increased with age ($P < 0.05$).

In humans, ADVs are common causes of respiratory, eye, urologic, and gastrointestinal diseases. ADV-8, 19, and 37 (species D ADV) are the causative pathogens of epidemic keratoconjunctivitis in humans; in this study, however, antibodies against ADV-19 and 37 were detected in three chimpanzees and one chimpanzee, respectively, without specific symptoms. ADV-1 to 7 (mainly ADV3) cause pharyngoconjunctival fever in humans; in this study, ADV-1, 2, 4, 5, and 6 were detected with comparatively high seroprevalence (57.1, 78.6, 100, 100, and 85.7 %, respectively), whereas ADV-3 was detected in one chimpanzee only (Supplementary Table S1). There were no significant differences in prevalence between SB and AB chimpanzees.

Figure 4a shows antibody titres and chimpanzee seroprevalence against picornaviruses; CVB-2, 3, and 6; echovirus-6; and CVA-5, 7, and 9. The titres of antibodies against CVA-7 varied; no other picornavirus antibodies were found in SB chimpanzees. Mari, an AB female, had antibodies against four picornaviruses: CVB-3 and 6 and CVA-5 and 7. Akira, an AB male, had antibodies against CVB-2, echovirus-6, and CVA-7, although he had relatively low titres.

Figure 4b shows antibody titres and chimpanzee seroprevalence for mosquito-borne encephalitis virus (JEV) and HAV. The antibody against JEV was detected in SB and AB chimpanzees. Antibody against HAV was detected in AB chimpanzees only.

Discussion

We surveyed whether captive chimpanzees have specific antibodies against human-borne infectious pathogens. We tested for antibodies against 62 infectious diseases in serum from 14 captive chimpanzees and found high or low

prevalence for 29 antibodies. Therefore, standard procedures for human serological analysis may be very useful for detecting specific antibodies against human-borne infectious pathogens in captive chimpanzees.

Data for older and younger chimpanzees can be compared to assess hygiene conditions at an institute. This study found fewer positive pathogens than in a study of US primate centres conducted by Kalter and Heberling (1971) more than 30 years ago. This may be because the infectious agents had been removed from the chimpanzee environment with improved knowledge about infectious diseases, and antibody titres of chimpanzees had been reduced to undetectable levels over time.

Serological tests against human-borne pathogens

The serological tests selected were conducted at commercial laboratories. The methods used for each test varied, and the types of detectable immunoglobulin (Ig), and test sensitivity, differed. Hence, the ideal serological screening against several diseases is an assay that can detect as many Igs as the chimpanzees can produce (i.e., IgM, IgA, IgG, and IgE). However, monitoring these human-borne pathogens should not be complicated. Easily available test methods are required for public zoological gardens and primate research institutes.

We could determine most of the history of infection by use of conventional serological analyses, but we could not determine when the antibody titre had decreased to a barely detectable level. Other serum sample-related factors affected the tests, for example cross-reaction of antibodies between chimpanzee viruses and human viruses, effect of haemolytic samples, and contamination. In this study, seven pathogens were detected, and all of their titres were equal to the standard cut-off titre. Among the pathogens, ADV-7, ADV-37, hPIV-4, echovirus-3, and CVA-6 are viruses of common human diseases, and thus it is highly possible they may infect chimpanzees. On the other hand, the West Pacific area has been free from PV-1 (poliomyelitis) since October 2000 (WHO/WPRO Kyoto conference, Kyoto, Japan), and a false-positive case of *E. histolytica* infection was reported despite the fact that titres were equal to the cut-off level (Tachibana et al. 2000). Thus, we conservatively assumed that PV-1 and *E. histolytica* are indeterminate, although their titres are normally deemed positive.

Implications of seroprevalence against human-borne infections

Chimpanzee foamy virus is a common virus in chimpanzees that is transmitted by a variety of routes. The 100 % prevalence of CFV indicates that the rearing conditions in KUPRI provide an environment for spread among chimpanzees. Therefore, human pathogens may not only be directly transmitted to chimpanzees from humans, but may also spread among chimpanzees.

Pertussis infection of chimpanzees was reported in a zoological garden in Sweden, and the affected chimpanzees had typical clinical signs of *B. pertussis* infection (Gustavssona et al. 1990). However, no clinical signs have been detected in the KUPRI chimpanzees despite their higher antibody titres. On the basis of their high antibody titres the chimpanzees at KUPRI may have been infected recently and may have developed immunity against pertussis. Pertussis in humans is common in children and the efficacy of the vaccine is proved, but antibody titre decreases after 2–5 years. Hence, permanent immunity is not established, and adults may be reinfected as antibody levels decrease. For example, some previously vaccinated adults in Japan were recently re-infected with pertussis (NIH 2008).

Measles virus antibody was found in six chimpanzees in this study. Interestingly, only one of the chimpanzees born in Japan (reared since birth in KUPRI: SB) had specific antibodies against MV, compared with five chimpanzees that were positive among those born in Europe or Africa (reared after birth in KUPRI: AB). More than 80 % of people have an antibody titre against MV, and antibody production is believed to indicate that the person has acquired immunity against MV (Taya et al. 2011). Chimpanzees with lower antibody titres are still at risk of MV infection.

PIV3, RSV, and hMPV antibody prevalence in humans increases with age. The prevalence of PIV-3 (HI test) antibody in humans peaks at 64–128× (Kishi et al. 1978), whereas that of hMPV (ELISA) peaks at 800–1600× (Okamoto et al. 2010). Antibody titres against RSV in chimpanzees did not vary with age, in contrast with humans, who normally acquire immunity by adulthood (Bhattarakosol et al. 2003). In humans, PIV-3 and the paramyxoviruses hMPV and RSV cause severe diseases of childhood and mild diseases of adults, who have acquired immunity after repeated infection in the process of growing. Therefore, the high prevalence of these viruses in chimpanzees may indicate that KUPRI chimpanzees have developed, or are developing, immunity against PIV-3, hMPV, and RSV. Increasing antibody prevalence with age was similar to that in humans with PIV-3 and hMPV. RSV and hMPV outbreaks in wild chimpanzees have, however, been causes of death or severe diseases (Kaur et al. 2008; Kondgen et al. 2008). RSV was identified as the chimpanzee coryza agent when it was isolated for the first time at a primate institute in the USA; hence, chimpanzees are highly susceptible to RSV (Morris et al. 1956).

Adenoviruses are common in humans, but not all infections lead to disease, and people develop adequate

immunity against reinfection by the same serotype. In this study, 9 of 11 ADV serotypes were found in chimpanzee serum, and the seroprevalence of ADV-1, 2, and 4 to 6 were high (Supplementary Table S1). Chimpanzee adenoviruses have also been identified, and the chimpanzee ADV Pan 9 neutralises human ADV-4 (Willimzik et al. 1981). More recently, 30 novel great ape ADVs from chimpanzees, bonobos, and gorillas were detected in captive non-human primates held in facilities and zoological gardens in North America (Roy et al. 2009). Typically, each ADV has a narrow host range that is restricted to one animal species or to closely related host species (Wold 2007).

Herpesviridae is highly infectious in its host animals, persistently or latently. EBV, CMV, HHV-6, and HSV-2, like viruses in chimpanzees, have been reported previously, so a neutralising antibody test is needed to distinguish among viruses of human and chimpanzee origin (Gerber et al. 1976; Swinkels et al. 1984; Lacoste et al. 2005; Luebcke et al. 2006). In this study 100 % of the chimpanzees were positive for EBV, CMV, and VZV and 75 % were positive for HHV-6, but no antibodies against HSV-1 or 2 were found (Supplementary Table S1). In humans, antibody prevalence against herpesviruses increase with age, with acquisition of immunity; hence, antibody prevalence against CMV is similar to that in humans. Repeat reactivation of the other herpesviruses, EBV, VZV, and HHV-6, may occur in the host animals, or human herpesviruses may be transmitted by chimpanzees in KUPRI. Human VZV infection has been reported in chimpanzees; the affected chimpanzees had a mild skin rash over the entire body (Cohen et al. 1996). In humans, VZV infection sometimes causes herpes zoster, which is thought to be triggered by stress or weak immunity. A case of severe haemorrhagic symptoms as a result of reactivation of its original VZV (simian varicella virus) has been reported in a cynomolgus monkey (Takasaka et al. 1990). However, although all of the KUPRI chimpanzees had the antibodies, the chimpanzees never developed symptoms. The original great ape VZV has not been identified, but the high incidence of infection may indicate the existence of a VZV-related virus. HSV is a common virus not only in humans but also in other primates. However, no transmission has been observed in KUPRI chimpanzees.

JEV is an arbovirus transmitted by the mosquito *Culex tritaeniorhynchus*, which feeds on the blood of host pigs. Its seroprevalence in a pig population in a prefecture, Aichi, neighbouring that where KUPRI is located, suggests it is not rare, and there is even a report of a human resident suffering from JEV infection there (NIID 2008; Sato et al. 2009). Pan and Popo, who were born in KUPRI, were infected with JEV, suggesting that JEV occurs in the region around KUPRI and that chimpanzees might be bitten by mosquitoes carrying the virus. However, this is the first report of detection of an antibody against JEV in chimpanzees.

HAV is transmitted via the faecal–oral route. Outbreaks of human HAV spreading from chimpanzee to human and chimpanzee to chimpanzee have been reported elsewhere (Dienstag et al. 1976). The human is the only host for HAV, and normally humans in developing countries are infected during childhood and acquire immunity against it. The three seropositive chimpanzees were born in Africa, and might have been infected in their childhood (Fig. 4b). HAV infection is now rare in Japan, and most cases are imported; therefore, no transmission might ever have occurred in the other chimpanzees at KUPRI.

Among the picornaviridae infections, poliomyelitis by poliovirus is the most clinically important disease among enteroviruses. In this study, the chimpanzees were positive, with high incidence, for CoxA7 virus only. This causes herpangina in human children. Reiko and Akira were seropositive for several picornaviruses, indicating they may have been infected by different persons without chimpanzee-to-chimpanzee transmission.

Influenza virus is prevalent in humans of all ages, but only Ayumu had antibody against H3N2. The severe symptoms of influenza infection may force workers not to enter the rearing zone, and then the chances of transmission may decrease.

Managing captive chimpanzees and zoonotic risks to humans

An important issue to examine in future studies is whether the caretakers have specific antibodies against the same human-borne infectious pathogens as our captive chimpanzees.

One of the tasks of zoos and research institutes is to protect and maintain endangered species, including chimpanzees. Therefore, it is important to keep the chimpanzees healthy under conditions that are quite different from their native habitats. It may be difficult for some small zoos or research institutes to establish their own examination systems. We successfully detected chimpanzee antibodies against human pathogens by use of commercial tests developed for humans. This should facilitate routine monitoring and surveillance of captive chimpanzees. We will continue serological examinations to analyse antibody levels and other respiratory pathogens. Furthermore, our data are limited to one institute, and more information should be gathered that will be useful for great ape conservation. In this study, we performed one-point serological surveillance, which provides only the history of each chimpanzee. Therefore, a phase examination should be performed to analyse disease prevalence in the future.

This study focussed on the transmission of human-borne infections to chimpanzees, but the reverse should also be considered. Of human emerging diseases, 75 % are

zoonotic and originate from wildlife via direct or indirect contact. For example, a case of Ebola virus infection resulted from contact with an infected wild chimpanzee (Morell 1994; Le Guenno et al. 1995; Taylor et al. 2001). Chimpanzee caretakers and researchers are at risk of exposure to unknown chimpanzee infectious pathogens. In addition to basic hygiene, keepers and researchers need appropriate vaccinations and should wear masks, gloves, and protective clothing during quarantine periods.

Disease prevention management for wild chimpanzees

Our study provides important information for hygiene management in wild chimpanzee conservation by adding information about possible human–chimpanzee zoonotic diseases. Infectious agents newly identified by their antibodies as agents possibly transmittable to chimpanzees (ADV-1, 2, 3, 5, 6, 19, CVA-5, 7, and JEV) or previously detected pathogens might cause the next outbreak in wild chimpanzees, not only in primate institutes, but also zoological gardens. Furthermore, we should consider agents that were not seropositive in the chimpanzees because the chimpanzees do not possess immunity against them, or at least not antibodies.

For chimpanzees at KUPRI, unlike some groups of wild chimpanzees, a previous infection of RSV or hMPV did not cause a severe respiratory disease. Captive chimpanzees might have more opportunity to be exposed to human pathogens compared with wild populations, but their environment and diet might not be as harsh as in the wild, which could alter the incidence and effects of the same pathogens in captive and wild populations. In addition, captive chimpanzees are at risk of new infectious diseases to which they have never been exposed and against which they have not established immunity. Consequently, monitoring results should be analysed carefully at each research institute or zoo, and care should be taken with all pathogens, including those to which chimpanzees are highly susceptible and those for which only a few or no chimpanzees were seropositive. Ultimately, without direct surveillance of wild populations, we cannot elucidate the prevalence of human-borne infectious diseases in the wild, but our data may still be used as a model. Our data suggest that a means of detecting antibodies in faeces should be developed to facilitate further studies in the wild.

In recent years, a vaccination program to protect wild chimpanzees against Ebola virus has been planned and is in preparation (Walsh 2009) after vaccine challenge against polio virus in wild chimpanzees at Gombe and against measles virus in gorillas at Virunga (Whittier et al. 2001). Furthermore, its effectiveness has been reported in one case of intervention (Robbins et al. 2011). This study shows that groups of chimpanzees under captive conditions produced specific antibodies against human diseases and that the chimpanzees were probably protected by their acquired immunity. Therefore, pre-immunity probably effectively protects wild chimpanzees from the human infectious diseases that tourists or researchers unknowingly transmit. However, the vaccination campaign needs careful consideration in terms of negative side effects for wild chimpanzees and nature. Recently, Ryan and Walsh (2011) reviewed the positives and negatives of the intervention and described the available vaccines against human pathogens.

Conclusion

We conducted serological surveillance for human-borne zoonoses in chimpanzees, and revealed the possibility of disease transmission between humans and chimpanzees. To reduce the chance of transmitting disease to captive chimpanzees in research institutes and zoos and to prevent disease transmission among researchers, animal caretakers, and chimpanzees, it is necessary to evaluate the risk of disease transmission. The serology of captive chimpanzees provides important information for hygiene management in ecotourism involving wild chimpanzees and other great apes.

Acknowledgments This study was supported by the Global Environmental Research Fund (F-061) and the Environment Research and Technology Development Fund (D-1007) of the Japanese Ministry of Environment, MEXT KAKENHI (20002001, 24000001, 24770234), the Cooperation Research Program of KUPRI (2009-B-38, 2010-C-11), and the Global COE Program of the Japan Society for the Promotion of Science. We thank Shohei Watanabe, Norihiko Maeda, Kiyonori Kumazaki, Akino Watanabe, Akihisa Kaneko, and Juri Suzuki for help with blood sampling.

References

Bermejo M, Rodriguez-Teijeiro JD, Illera G, Barroso A, Vila C, Walsh PD (2006) Ebola outbreak killed 5000 gorillas. Science 314(5805):1564

Bhattarakosol P, Pancharoen C, Mungmee V, Thammaborvorn R, Semboonlor L (2003) Seroprevalence of anti-RSV IgG in Thai children aged 6 months to 5 years. Asian Pac J Allergy Immunol 21(4):269–271

Brack M (1987) Agents transmissible from simians to man. Springer, Berlin

Clark AG, Glanowski S, Nielsen R, Thomas PD, Kejariwal A, Todd MA, Tanenbaum DM, Civello D, Lu F, Murphy B, Ferriera S, Wang G, Zheng X, White TJ, Sninsky JJ, Adams MD, Cargill M (2003) Inferring nonneutral evolution from human–chimp–mouse orthologous gene trios. Science 302(5652):1960–1963

Cohen JI, Moskal T, Shapiro M, Purcell RH (1996) Varicella in chimpanzees. J Med Virol 50:289–292

Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife–threats to biodiversity and human health. Science 287(5452):443–449

Daszak P, Cunningham AA, Hyatt AD (2001) Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Trop 78(2):103–116

De Castro F, Bolker B (2005) Mechanisms of disease-induced extinction. Ecol Lett 8:117–126

Dienstag JL, Davenport FM, McCollum RW, Hennessy AV, Klatskin G, Purcell RH (1976) Nonhuman primate-associated viral hepatitis type a: serologic evidence of hepatitis a virus infection. JAMA 236:462–464

Ferber D (2000) Primatology. Human diseases threaten great apes. Science 289(5483):1277–1278

Gerber P, Pritchett RF, Kieff ED (1976) Antigens and DNA of a chimpanzee agent related to Epstein–Barr virus. J Virol 19:1090–1099

Gerber LR, McCallum H, Lafferty KD, Sabo JL, Dobson A (2005) Exposing extinction risk analysis to pathogens: is disease just another from of density dependence? Ecol Appl 15:1402–1414

Goodall J (1986) The chimpanzees of Gombe: patterns of behavior. Belknap Press of Harvard University Press, Cambridge

Gustavssona OEA, Rökenb BO, Serrandera R (1990) An epizootic of whooping cough among chimpanzees in a zoo. Folia Primatol (Basel) 55:45–50

Hanamura S, Kiyono M, Lukasik-Braum M, Mlengeya T, Fujimoto M, Nakamura M, Nishida T (2008) Chimpanzee deaths at Mahale caused by a flu-like disease. Primates 49(1):77–80

Hosaka K (1995) A single flu epidemic killed at least 11 chimps. Pan Afr News 2:3–4

Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P (2008) Global trends in emerging infectious diseases. Nature 451(7181):990–993

Kaiser J (2003) Conservation biology. Ebola, hunting push ape populations to the brink. Science 300(5617):232

Kalter SS, Heberling RL (1971) Comparative virology of primates. Bacteriol Rev 35:310–364

Kaur T, Singh J, Tong S, Humphrey C, Clevenger D, Tan W, Szekely B, Wang Y, Li Y, Alex Muse E, Kiyono M, Hanamura S, Inoue E, Nakamura M, Huffman MA, Jiang B, Nishida T (2008) Descriptive epidemiology of fatal respiratory outbreaks and detection of a human-related metapneumovirus in wild chimpanzees (Pan troglodytes) at Mahale Mountains National Park, Western Tanzania. Am J Primatol 70(8):755–765

King DA, Peckham C, Waage JK, Brownlie J, Woolhouse ME (2006) Epidemiology. Infectious diseases: preparing for the future. Science 313(5792):1392–1393

Kishi N, Ota H, Maeda H, Obata M, Hayashi H, Takasugi N (1978) A serological study on parainfluenza viruses. Sapporo City Institute of Public Health, Research and Investigation annual report no. 5

Kondgen S, Kuhl H, N'Goran PK, Walsh PD, Schenk S, Ernst N, Biek R, Formenty P, Matz-Rensing K, Schweiger B, Junglen S, Ellerbrok H, Nitsche A, Briese T, Lipkin WI, Pauli G, Boesch C, Leendertz FH (2008) Pandemic human viruses cause decline of endangered great apes. Curr Biol 18(4):260–264

Lacoste V, Verschoor EJ, Nerrienet E, Gessain A (2005) A novel homologue of human herpesvirus 6 in chimpanzees. J Gen Virol 86:2135–2140

Le Guenno B, Formentry P, Wyers M, Gounon P, Walker F, Boesch C (1995) Isolation and partial characterisation of a new strain of Ebola virus. Lancet 345:1271–1274

Luebcke E, Dubovi E, Black D, Ohsawa K, Eberle R (2006) Isolation and characterization of a chimpanzee alpha herpesvirus. J Gen Virol 87(Pt 1):11–19

Matsuzawa T (2003) The Ai project: historical and ecological contexts. Anim Cogn 6(4):199–211

Matsuzawa T (2006) Sociocognitive development in chimpanzees: a synthesis of laboratory work and field work. I. In: Matsuzawa TT (ed) Cognitive development in chimpanzees. Springer, Tokyo, pp 3–33

Matsuzawa T, Humle T, Koops K, Biro D, Hayashi M, Sousa C, Mizuno Y, Kato A, Yamakoshi G, Ohashi G, Sugiyama Y, Kourouma M (2004) Wild Chimpanzees at Bossou-Nimba: deaths through a flu-like epidemic in 2003 and the green-corridor project. Primate Res 20:45–55

McMichael AJ (2004) Environmental and social influences on emerging infectious diseases: past, present and future. Philos Trans R Soc Lond B Biol Sci 359(1447):1049–1058

Miyabe-Nishiwaki T, Kaneko A, Nishiwaki K, Watanabe A, Watanabe S, Maeda N, Kumazaki K, Morimoto M, Hirokawa R, Suzuki J, Ito Y, Hayashi M, Tanaka M, Tomonaga M, Matsuzawa T (2010) Tetraparesis resembling acute transverse myelitis in a captive chimpanzee (Pan troglodytes): long-term care and recovery. J Med Primatol 39(5):336–346

Morell V (1994) Chimpanzee outbreak heats up search for Ebola origin. Science 268:974–975

Morens DM, Folkers GK, Fauci AS (2004) The challenge of emerging and re-emerging infectious diseases. Nature 430(6996):242–249

Morris JA, Blount RE Jr, Savage RE (1956) Recovery of cytopathogenic agent from chimpanzees with coryza. Proc Soc Exp Biol Med 92(3):544–549

Muehlenbein MP, Ancrenaz M (2009) Vaccination and health status of foreign travelers, and the roles of travel medicine specialists. Great ape health workshop 2009, 21–24 August 2009, Entebbe, Uganda

NIH (National Institute of Health, Japan) (2008) The topic of this month, vol 29, No. 3 (No. 337) "Pertussis, Japan, 2005–2007". Infect Agents Suveil Rep 29:65–66. (http://idsc.nih.go.jp/iasr/29/337/tpc337.html)

NIID (2008) Japanese encephalitis. Department of Virology I, National Institute of Infectious disease, Japan. http://www.nih.go.jp/vir1/NVL/JEVMeeting.htm

Nishida T, Corp N, Hamai M, Hasegawa T, Hiraiwa-Hasegawa M, Hosaka K, Hunt KD, Itoh N, Kawanaka K, Matsumoto-Oda A, Mitani JC, Nakamura M, Norikoshi K, Sakamaki T, Turner L, Uehara S, Zamma K (2003) Demography, female life history, and reproductive profiles among the chimpanzees of Mahale. Am J Primatol 59(3):99–121

Nunn CL, Thrall PH, Stewart, Harcour AH (2008) Emerging infectious diseases and animal social system. Evol Ecol 22:519–54

Okamoto M, Sugawara K, Takashita E, Muraki Y, Hongo S, Mizuta K, Itagaki T, Nishimura H, Matsuzaki Y (2010) Development and evaluation of a whole virus-based enzyme-linked immunosorbent assay for the detection of human metapneumovirus antibodies in human sera. J Virol Methods 164(1–2):24–29

Patz JA, Daszak P, Tabor GM, Aguirre AA, Pearl M, Epstein J, Wolfe ND, Kilpatrick AM, Foufopoulos J, Molyneux D, Bradley DJ (2004) Unhealthy landscapes: policy recommendations on land use change and infectious disease emergence. Environ Health Perspect 112(10):1092–1098

Robbins MM, Gray M, Fawcett KA, Nutter FB, Uwingeli P, Mburanumwe I, Kagoda E, Basabose A, Stoinski TS, Cranfield MR, Byamukama J, Spelman LH, Robbins AM (2011) Extreme conservation leads to recovery of the Virunga mountain gorillas. PLoS One 6(6):e19788

Roy S, Vandenberghe LH, Kryazhimskiy S, Grant R, Calcedo R, Yuan X, Keough M, Sandhu A, Wang Q, Medina-Jaszek CA, Plotkin JB, Wilson JM (2009) Isolation and characterization of adenoviruses persistently shed from the gastrointestinal tract of non-human primates. PLoS Pathog 5(7):e1000503

Ryan SJ, Walsh PD (2011) Consequences of non-intervention for infectious disease in African great apes. PLoS One 6(12):e29030

Sato H, Taya K, Okabe N, Takasaki T, Kurane I (2009) Seroprevalence rate against Japanese encephalitis in domestic pig in Japan. IASR 30:151–152 (Infectious disease surveillance center, National institute of Infectious disease, Japan. http://idsc.nih.go.jp/iasr/30/352/dj3522.html)

Swinkels BW, Geelen JLMC, Wertheim-van Dillen P, van Es AA, van der Noordaa J (1984) Initial characterization of four cytomegalovirus strains isolated from chimpanzees. Arch Virol 82:125–128

Tachibana H, Cheng XJ, Kobayashi S, Fujita Y, Udono T (2000) Entamoeba dispar, but not *E. histolytica*, detected in a colony of chimpanzees in Japan. Parasitol Res 86(7):537–541

Takasaka M, Sakakibara I, Mukai R, Suzuki M (1990) An outbreak of nonhuman primate varicella-like herpesvirus infection in the established breeding colony of cynomolgus monkeys. Tsukuba Primate Center News 9:5–11

Taya K, Sato H, Arai T, Kitamoto R, Okabe N (2011) Seroepidemiological survey and vaccination coverage against measles virus 2010: 2010 interim report forecasting survey for infectious disease epidemics. IASR 32:36–39 (Infectious disease surveillance center, National Institute of Infectious disease, Japan. http://idsc.nih.go.jp/iasr/32/372/dj3723.html)

Taylor LH, Latham SM, Woolhouse ME (2001) Risk factors for human disease emergence. Philos Trans R Soc Lond B Biol Sci 356(1411):983–989

Walsh PD (2009) VaccinApe: protecting wild apes against the disease threat. Great ape health workshop, Entebbe, Uganda, 21–24 August

Weiss RA, McMichael AJ (2004) Social and environmental risk factors in the emergence of infectious diseases. Nat Med 10(12 Suppl):S70–S76

Whitfield J (2003) The law of the jungle. Nature 421(6918):8–9

Whittier CA, Nutter FB, Stoskofp MK (2001) Zoonotic disease concerns in primate field settings. The apes: challenges for the 21st century, conference proceedings, May 10–13, 2000. Brookfield Zoo, Brookfield, pp 232–237

Willimzik H-F, Kalter SS, Lester TL, Wigand R (1981) Immunological relationship among adenoviruses of humans, simians, and nonprimates as determined by the neutralization test. Intervirology 15:28–36

Wold W (2007) Adenovirus fields virology, vol 2, 5th edn, p 2395

Woodford MH, Butynski TM, Karesh WB (2002) Habituating the great apes: the disease risks. Oryx 36:153–160

Woolhouse ME, Gowtage-Sequeria S (2005) Host range and emerging and reemerging pathogens. Emerg Infect Dis 11(12):1842–1847