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Implications for Primate Population Management and the Occupational Safety of Primate Handlers: A Natural, Zoos and Captive Environment Comparative Study in China

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YZ presented the concept and designed the study. YZ and QZ collected the data. XY and RT analysed and interpreted the results. YZ wrote the manuscript. QZ reviewed the manuscript.

Key words
Primate management, Occupational safety, Virus detection, Behavior, Macaque, Interaction

ABSTRACT

Nonhuman primates can be naturally infected with a plethora of viruses with zoonotic potential viruses. Relative to the other animals, the close phylogenetic relationship between human and nonhuman primates results in a high potential for pathogen exchange. Therefore, understanding pathogen emergence status in primates and interactive patterns between humans and primates will facilitate improving primate population management and disease prevention. In this study, we collected macaque aggressive behavior to humans in three representative human-macaque interaction environments: natural macaque ecotourism site (Tibetan macaque), zoological park (Japanese macaque), and captive breeding environment (Rhesus macaque) in Anhui, China. Results showed that, the ratio of macaque aggressive behavior was more frequently at the natural macaque ecotourism site. Macaque blood samples for antibodies of herpes B virus (HBV), hepatitis A virus, simian foamy virus, simian pox virus (SPV), simian retrovirus (SRV) and simian T-cell lymphotropic virus-1 (STLV-1) were collected. The Tibetan, Rhesus, and Japanese macaques tested positive for antibodies to six, five (except for STLV-1), and three (SPV, SRV and HBV) types of viruses, respectively. Conclusively, people directly involved in caretaking and managing macaque populations, particularly at the macaque ecotourism site, are at risk for exposure to pathogens. The health implications for occupational safety of primate handlers as well as managed primate populations were discussed, and suggestions for working safely with nonhuman primates were provided.

INTRODUCTION

Zoonotic pathogens often infect people in close contact with animals (Steneroden *et al*., 2011). Of the 175 known species of emerging infectious diseases that affect humans, 132 (75%) are estimated to have originated from other animals (Greger, 2007; Jones *et al*., 2008). These pathogens pose serious and increasing threats to human public health and welfare because they potentially cause substantial and widespread diseases in human populations (Pedersen and Davies, 2009). Outbreaks of zoonotic diseases are accelerating at an unprecedented rate in the current era of globalization, with substantial impacts on the global economy, public health, and sustainability (Gibb *et al*., 2020; Zhang *et al*., 2022).

Relative to the other animals, the close phylogenetic relationship between human and non-human primates (NHPs) results in a high potential for pathogen exchange (Wolfe *et al*., 1998; Gillespie *et al*., 2008). NHPs can spread pathogens to humans via body fluids, contaminated food or water, and insect vectors (Kaewchot *et al*., 2022). Long periods of separate evolutionary divergence have left humans immunologically naive to enzootic NHPs pathogens, and some infectious agents relatively harmless in NHPs may be lethal to humans (Travis *et al*., 2006; Medkour *et al*., 2021). For example, most macaques carry the herpes B virus (HBV) without disease symptoms, but infection in humans results in serious neurologic impairment or fatal encephalomyelitis (Huff and Barry, 2003). The HBV carried by macaques has caused 23 cases of encephalitis and 18 fatalities in 24 known human infections between 1932 and 1972 (Palmer, 1987;

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Ostrowski *et al*., 1998). Macaques (genus *Macaca*), in particular, have a high overlap rate with humans (Fuentes, 2006). Close contact and range overlap between humans and macaques introduce a significant and potentially dangerous situation for disease transmission. Humans risk exposure to several simian viruses, including simian T-cell lymphotropic virus-1 (STLV-1), simian retrovirus (SRV), simian foamy virus (SFV), and HBV, in addition to other infectious agents (Wolfe *et al*., 2004; Jones-Engel *et al*., 2005; Jun *et al*., 2022).

Humans in areas of overlap with macaques can be classified into three general groups: locals, staff, and tourists. Recent research has shown that hunters, zoo, laboratory, and park staff and people who live near wild macaques were infected with viruses shared with NHPs, such as SFV, HBV, STLV-1, and simian immunodeficiency viruses (Khabbaz *et al*., 1994; Kalish *et al*., 2005; Calattini *et al*., 2007). For instance, park staff and tourists at the Sangeh Monkey Forest in Indonesia probably experienced a high risk of infection with HBV due to high seroprevalence in the monkey population and frequent human exposure via bites and scratches from macaques (Engel *et al*., 2002). Contexts in which contact between NHPs and humans is more likely may thus increase risk of interspecies disease transmission.

Understanding the infectious status of pathogen and behavioral interaction patterns will help assess human-NHP interconnections in behavioral, epidemiological, ecological, and cultural contexts. In this study, we selected three representative contexts with a high potential for human-macaque interaction; a natural macaque ecotourism site (Tibetan macaque), a zoological park (Japanese macaque), and a captive breeding environment (Rhesus macaque). These three settings represent possible sites for behavioral interaction and potential disease emergence. Contact between these macaques and humans are common, and such contacts create opportunities for cross-transmission of pathogens. We measured the seroprevalence of antibodies for detecting the disease emergence status in macaques at each location and compared rates of macaque aggressive behavior in each context. Thus, this study aimed to provide critical information for primate population management and the occupational safety of primate handlers.

MATERIALS AND METHODS

Study sites

The study was conducted in Anhui province, China, in three different settings where human-macaque interactions are known to occur. Huangshan Valley of the Wild Monkeys (HVWM), Hefei Wildlife Zoo (HWZ), and Qimen Laboratory Rhesus Macaque Center (QLRMC).

Huangshan valley of the wild monkeys (HVWM)

HVWM is located at Mt. Huangshan National Reserve in southern Anhui province. Mt. Huangshan is a United Nations Educational, Scientific and Cultural Organization (UNESCO) World Natural and Cultural Heritage site well-known as a tourist destination and is home to several groups of wild Tibetan macaques (*Macaca thibetana*). Two of these groups are part of an ecotourism program which, since 1994, has provided tourists the opportunity to see the macaques from a human-constructed viewing pavilion. To facilitate observation, park staff provides the macaques with ca. 6 kg of whole corn per day in an open area by a stream. Tourists climb a stairway up a hill to the open pavilion and observe the macaques in the provisioning area for 30-45 min at a time, usually at 3-4 set times per day (10:00, 13:30, 15:30, 17:30). Park rules prohibit tourists from feeding, shouting at or contacting the macaques directly; however, these rules are not consistently enforced. The macaques often threaten tourists and staff, and some macaques have occasionally jumped into the pavilion and attacked people (Berman *et al*., 2007).

Hefei wildlife zoo (HWZ)

HWZ is located in Hefei, Anhui province. In 1997, a group of Japanese macaques (*Macaca fuscata*) was brought to the zoo from Japan. In the early years of their display at the zoo, the macaques were separated from tourists only by a barbed-wire rail fence, and zoo patrons could hand-feed them through the rails. Because many tourists were bitten or scratched by the monkeys, in 2006, the zookeepers put the macaques into iron cages that separated the monkeys from tourists by a distance of approximately 1 m. During our study, the zookeepers cleaned the cages daily at 08:30 and fed the macaques twice daily at 09:00 and 16:00.

Qimen laboratory rhesus macaque center (QLRMC)

QLRMC, established in 1999, is located at Qimen in southwest Anhui province. The staff has close contact with the Rhesus macaques (*Macaca mulatta*) housed in the laboratory and has been occasionally bitten or scratched while conducting physical examinations on the macaques. During our study, the laboratory staff cleaned cages daily at 08:00 and fed the macaques thrice daily at 08:30, 12:00, and 16:00.

Behavioral data collection

All behavioral data were collected from Aug. 2010 to Jun. 2011 and Mar. to Jun. 2019 (HVWM data collection: Aug. to Oct. 2010 by YZ, Mar. to Jun. 2019 by QXZ;

HWZ data collection: Nov. 2010 to Feb. 2011 by YZ; QLRMC data collection: Apr. to Jun. 2011 by YZ). When human and macaques were involved in conflicts, we used behavior sampling to record human-macaque interactions (Altmann, 1974). According to the intensity of attack, macaque aggressive behaviors were rated on a scale of I–III. Aggression behavior I (AGGI) was defined as simple threats (such as stare and facial threats), AGGII as lunging at and chasing without body contact, and AGGIII as physical contact, including scratching and biting. Table I provides an ethogram of macaque aggressive behaviors. To record the behavioral data in real-time and accurately, we recorded all of our behavioral data by a voice recorder (ICD-AX412F, SONY).

Blood sample collection

Blood samples were collected from a total of 46 macaques: 16 from Tibetan macaques (proportion: 16 out of 65), 8 from Japanese macaques (proportion: 8 out of 38), and 22 from Rhesus macaques (proportion: 22 out of 106). Wild macaques were trapped in a locally constructed metal stick and bamboo cage measuring 1.5 m × 1.5 m × 1.5 m, with a 1.5 m × 0.8 m chute at one end. Fruits were placed as bait at the center of the cage to attract the macaques. The door to the trap could be closed remotely by observers when macaques entered the trap. After capture, the macaques were injected with Sumianxin II (narcotic) at < 0.3 mL/kg. Three milliliters of blood were drawn from the femoral vein and transferred into a serum separator tube. After blood collection, the monkeys were injected with Luxingning (anesthetics antidote; dose 1:1 to Sumianxin II), and they were released fully conscious. No macaque sustained any injury during this study. The blood samples were centrifuged at 3000 g for 5 min to obtain serum and then stored at −20 °C until further analysis.

Serological examination

Macaque blood samples for antibodies of herpes B virus (HBV), hepatitis A virus, simian foamy virus, simian pox virus (SPV), simian retrovirus (SRV) and simian T-cell lymphotropic virus-1 (STLV-1) were collected (Table II) (Weber *et al*., 1999). Serological samples were examined at Anhui Medical Science Research Institute in Hefei. Samples were analyzed by enzyme-linked immunosorbent assay (The kit is solid phase sandwich ELISA assay, R & D Systems, Lot: 10–02) for the presence of HBV, HAV,

Table I. Macaque aggressive behavior classification and behavior definition.

Behavior type/ Behavior catalog		Behavior definition
Aggressive behavior		
AGGI	Stare	Macaque staring at people as a threat.
	Threat	Macaque facial threats or ground slaps directed toward people.
AGGII	Lunge	Macaque lunges at a person without contact.
	Chase	Macaque chases a person without contact.
AGGIII	Scratch	Macaque scrapes human's skin with its nails.
	Bite	Macaque nips or cuts into human's skin with its teeth.

Table II. Disease, transmission, zoonotic potential and treatment possibilities in human and NHP.

Agent	Clinical symptoms in NHPs	Zoonotic potential symptoms in human	Transmission
HBV	Most asymptomatic, occasionally lesions in mouth	Neurological/lethal	Saliva and body fluids in bites and scratches, mucosal contamination
HAV	Asymptomatic, hepatitis rare	Hepatitis	Contact with excreta
SPV	Mostly asymptomatic, fever, exanthema	Pox lesions	Contact with excreta, aerogenic
SFV	Usually asymptomatic	Antibodies found in humans but unknown pathogenecity	Possibly contact with excreta
SRV	Simian AIDS	Antibodies found in humans but unknown pathogenecity	Possibly bites and scratches contaminated with saliva
STLV-1	Asymptomatic but occasionally associated with lymphoproliferation	Resembles HTLV-1 which may cause leukemia	Possibly bites and scratches

Table III. Seroprevalence of antibodies among three species of macaques.

Virus category	*Macaca thibetana*			*Macaca fuscata*			*Macaca mulatta*		
	Samples	Seropositive	Seroprevalence	Samples	Seropositive	Seroprevalence	Samples	Seropositive	Seroprevalence
Herpes B virus (HBV)	16	1	6.3%	8	3	37.5%	22	6	27.3%
Hepatitis A virus (HAV)	16	2	12.5%	8	0	0.0%	22	3	13.6%
Simian pox virus (SPV)	16	2	12.5%	8	1	12.5%	22	6	27.3%
Simian foamy virus (SFV)	16	3	18.8%	8	0	0.0%	22	2	9.1%
Simian retrovirus (SRV)	16	3	18.8%	8	2	25.0%	22	1	4.5%
Simian T-cell lymphotrophic virus-1 (STLV-1)	16	1	6.3%	8	0	0.0%	22	0	0.0%

SFV, SPV, SRV, and STLV-1 antigens. In brief, the test samples, reference sera (positive, negative and weak positive controls) and blank controls were run in duplicates for each test plate. The optical densities (ODs) of samples were measured at 450 nm using a microplate reader. On the ELISA detector, the O•D value of each hole is measured with the blank control hole after zero adjustment. As recommended by the manufacturer, if it is greater than 2.1 times of the specified negative control OD value, the sample was classified as positive.

Data analysis

During the study period, 1150 human-macaque interaction events were recorded at HVWM, 460 at HWZ, and 380 at QLRMC. Differences in the ratio of human-macaque interactions and aggressive behaviors in macaques were analyzed using a chi-square test using the Statistical Package for the Social Sciences (SPSS, version 13.0; SPSS Inc., Chicago, Illinois, USA). All significant levels were set at $P < 0.05$.

RESULTS

Prevalence of macaque aggressive behaviors

During the study period, we recorded 1,150 interactions between humans and macaques at HVWM, 460 at HWZ and 380 at QRLMC. At all three locations, most aggressive behaviors in monkeys did not result in contact with humans (i.e., AGGI and II). AGGI accounted for 9.83% (113/1,150) of interactions at HVWM, 2.61% (12/460) at HWZ, and 3.42% (13/380) at QRLMC. AGGII rate accounted for 11.39% (131/1,150) of interactions at HVWM, 1.3% (6/460) at HWZ, and 2.11% (8/380) at QRLMC. Contact aggression such as bites or scratches was relatively uncommon: AGGIII accounted for 2.17% (25/1,150) of interactions at HVWM, 0% at HWZ, and 1.58% (6/380) at QRLMC. Overall, the ratio of aggressive behaviors at HVWM was higher than that at HWZ or QRLMC ($\chi^2 = 5.956$, $df = 2$, $P < 0.05$).

During the study period, over 83.87% (26/31) of bites and scratches observed were minor and seldom resulted in breaking of the skin. Only one tourist and one worker at HVWM and three workers at QRLMC were severely bitten or scratched sufficiently to induce bleeding. No injury incidents to visitors (i.e., bites or scratches) were observed at HWZ. At HVWM, 88% (22/25) of AGGIII was caused by tourists feeding macaques or moving too close to the macaques for photo opportunities.

Seroprevalence of antibodies in macaques

Our results indicate that the Tibetan macaques at HVWM tested positive for antibodies to all six virus types, while the Rhesus macaques at QRLMC were positive for five virus types except for STLV-1. The Japanese macaques at HWZ were positive for three virus types, including SPV, SRV, and HBV (Table III).

DISCUSSION

Implications for primate population management

Our data indicate that one tourist and one worker at HVWM and three workers at QRLMC were severely bitten or scratched sufficiently to induce bleeding. The potential risk of AGGIII interactions was higher in HVWM than at the other two sites studied. Some tourists use food to bring the macaques closer, possibly for photography opportunities. Many tourists place food items directly into the hands or mouths of the macaques. At HVWM, the relatively high frequency of contact interactions contributes to the risk of pathogen transmission, especially of bacterial pathogens on the hands/feet of the macaques (which frequently have fresh or dry feces and urine on them) and of viral pathogens easily transmitted through mucosal contact. Similar situations occur at other NHP tourism sites in Singapore, Indonesia, Bali, and Gibraltar (Fuentes, 2006; Fuentes *et al.*, 2007, 2008). We recommend that HVWM park staff

should more consistently enforce rules against feeding the macaques to minimize the potential for human-macaque contact and proximity, thus reducing the risk of pathogen transmission.

Similar human-macaque interactions occur elsewhere in some areas of China. For example, at Mt. Emei, China, religious pilgrims, nuns, monks, and some tourists occasionally bring food to the local macaques (*Macaca thibetana*), who often respond aggressively while attempting to obtain food from humans. Visitors occasionally come in contact with macaques during visits for worship, recreation, or tourism. Some people hand-feed the macaques but are susceptible to macaque attacks, some of which have resulted in serious injury or death (Zhao and Deng, 1992; Zhao, 2005).

Implications for the occupational safety of primate handlers

Our serological analysis indicated positive results for antibodies to six virus types in the three species of macaques. Although an animal positive for a virus does not mean it is actively shedding the virus; however, it can provide current status consistent with a potential for future pathogen transmission. In 1999, the Federation of European Laboratory Animal Science Associations (FELASA) Working Group on Non-human Primate Health reported the clinical symptoms in NHPs and humans and zoonotic transmission routes (Table II). People can avoid HBV, HAV, SPV, SFV, SRV, and STLV-1 infection from Tibetan macaques by preventing body contact or proximity with them at HVWM. Tourists and park staff should note that SPV and perhaps other respiratory diseases can be air-borne and acquired through proximity to macaques. QLRMC staff should be cautious of potential exposure to HBV, HAV, SPV, SFV, and SRV viruses that can occur while conducting routine physical exams of Rhesus macaques or through mucosal contact with excreta of the macaques during cage cleaning. Laboratory staff should be trained in handling macaques safely to avoid bites or scratches. They should be educated about the proper use of personal protective equipment (i.e., masks, gloves, eye protection, and barrier clothing) and methods for cleaning cages. Institutions housing NHPs will need to continuously review and update their occupational health programs to help primate population management and prevent zoonotic transmission.

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IRB approval

The research was approved by the Animal Care Committees of Hefei Normal University and Wildlife Administrate Agency of Anhui Provincial Forestry Department (201022). This research was performed in accordance with the Guide for the Care and Use of Animals of the National Institute of Health, and all efforts were made to minimize suffering.

Ethical statement

The animal study was reviewed and approved by Institutional Animal Care and Use Committee, Hefei Normal University and Anhui University. The collection of samples from macaques was approved by the Wildlife Administration Agency, Anhui Provincial Forestry Department, China (Permit number: 201022).

Statement of conflict of interest

The authors have declared no conflict of interest.

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