Potential pathogen transmission risk in non-human primate ecotourism: A case study at Mt. Huangshan, China

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Abstract: Ecotourism involving feeding wildlife poses potential threats to public health and has raised public attention, especially concerning non-human primates. This study aimed to assess disease's emergence in macaques and patterns of human-macaque contacts at Tibetan macaque ecotourism site at Mt. Huangshan, China. Using all-occurrence sampling, we collected aggressive behavior initiated by macaques. A total of 282 tourists were surveyed. During the study period, 16 macaque blood samples were collected and analyzed by enzyme linked immunosorbent assay (ELISA) for the seroprevalence of immunoglobulin antibodies to Herpes B Virus, Hepatitis A virus, Simian foamy virus, Simian pox virus, Simian retrovirus and Simian T-cell lymphotrophic virus-1. The results indicate that Tibetan macaques tested positive for 6 types of virus antibodies. Most aggressive behaviors initiated by macaques did not result in physical contact with humans. The main type of aggressive behavior with physical contact was scratching (92%). Among the participants that have physical contact with monkeys, 13.79% were scratched and 6.9% were bitted by monkeys. Of the injured, 89.36% were treated by doctors at a medical clinic. This study provides evidence that the people who come into contact with macaques at ecotourism site are at risk for exposure to the virus when interacting with macaques. Our study may aid in the management of human-macaque interaction to minimize potential disease emergence risk.
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1. Introduction

Ecotourism involving non-human primates (NHPs) is a recent and growing trend in NHPs habitat countries. NHPs ecotourism has contributed to species conservation by increasing public awareness, preserving the natural heritage and raising much needed funds to achieve conservation goals as well as financial and educational benefits for local communities (Berman et al., 2007; Muehlenbein et al., 2010). However, habituation of animals to human presence can increase the likelihood of close contact during the ecotourism management, such as Formosan macaques (*Macaca cyclopis*) in Taiwan, Barbary macaques (*Macaca sylvanus*) in Gibraltar, Long-tailed macaques (*Macaca fascicularis*) in Bali and Tibetan macaques (*Macaca thibetana*) at Mt. Emei and Huangshan (Zhao, 2005; Loudon et al., 2006; Hsu et al., 2009; McCarthy et al., 2009; Ruesto et al., 2010).

Interactions between humans and NHPs raising the concern that potential pathogen transmission risk might be heightened. The close phylogenetic relationship between humans and NHPs may thus increase risks of interspecies disease transmission (Wolfe et al., 1998; Gillespie et al., 2008). Long periods of separate evolutionary divergence have left humans immunologically naive to enzootic NHPs pathogens, and some of the infectious agents that are relatively harmless in NHPs may be lethal to humans (Travis et al., 2006). For example, most macaques carry herpes B virus without obvious signs of disease, but infection in humans is known to result in serious neurologic impairment or fatal encephalomyelitis (Huff and Barry, 2003). Researchers showed that park staff and tourists at the Sangeh Monkey Forest in Indonesia probably experience a high risk of infection with herpes B virus due to high seroprevalence in the monkey population and humans' frequent exposure to it via monkeys' bites and starches (Engel et al., 2002).

The use of wild NHPs for ecotourism is a recent and growing tread in China. Like most NHPs in China, Tibetan macaques live in small isolated populations (Berman and Li, 2002). The interaction between tourists and Tibetan macaques are at two main sites in China: Mt. Emei and Huangshan. At Mt. Emei, a Buddhist community where visitors come primarily to visit temples, there is little regulation and no instruction of tourists. As a result, religious pilgrims and some tourists regularly hand feed the monkeys and suffer monkey attacks, some injured by the macaques (Zhao, 2005). In contrast, the tourists are restricted to viewing pavilions and

also given brief lectures about monkey biology and behavior by the local staff at Mt. Huangshan. Park rules prohibit tourists from feeding the macaques, but some tourists use food to entice the macaques to come closer, perhaps for photo opportunities. Many tourists place food items directly into the macaque's hand or even mouth, and thus also possibly subject to zoonotic disease transmission. In such settings, contact between humans and macaques cannot be safety controlled, and workers and tourists are at risk. Thus, understanding the infectious agents in Tibetan macaque is important not only for animal conservation, but also for mitigating future disease-related threats to humans.

Potential pathogen transmission from non-human primates to humans pose a serious and increasing threat to human public health and welfare in the world, but there has no enough attention in public about this in China. With the faster developing of primate ecotourism in China, this problem is particularly important. In our study, we evaluated behavioral patterns between humans and macaques at free-ranging macaque ecotourism site at Mt. Huangshan China. Focusing on selected viruses, we measured the seroprevalence of antibodies in macaques. We also carried on the questionnaire surveys to tourists. Our aim was to provide critical pieces of information for future assessing potential pathogen transmission risk.

2. Material and Methods

2.1 Study site and animals

This study was conducted at Mt. Huangshan National Reserve located in Anhui Province, China. The reserve is a World Culture and Nature Heritage site that is well-known as a tourist destination and research site for the study of Tibetan macaques. Researchers began to observe one of the groups (Yulinkeng, latter called Yulinkeng A1) in 1986. The other group, Yulinkeng 2 (YA2) group, which fissioned from the Yulinkeng 1(YA1) group in 1996. In 1994, the local government drove the YA1 group to an area adjacent to their natural range for tourism. And YA2 group was managed for tourism in 2002. Two of these groups are part of an ecotourism program that providing tourists with the opportunity to see the macaques from a human-constructed viewing pavilion (Berman and Li, 2002). To facilitate observation, the park staff provisioned 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 of day (10:00, 13:30, 15:30, 17:30). Park rules prohibit tourists from feeding, shouting at and contacting the macaques directly, but these rules are not consistently enforced. The macaques often threaten tourists and staff, and some macaques have occasionally jumped into the pavilion to attack people.

2.2 Behavioral data collection

All behavioral data were collected during an intensive study between July, 2009 and May, 2010 (286 days: mean ± SE = 25.2 ± 1.26 days/month, range = 20 - 28). According to the intensity of attack, macaques' aggressive behaviors to tourists 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 1). All-occurrence sampling was used to record the frequency of the monkeys' aggressive behaviors (Altmann, 1974).

Table 1. Classification of aggression behavior recorded from the macaques

Classify	Behavior	Description
AGGI	Stare	Staring at people as a threat
	Facial threats	Facial threats or ground slaps directed toward people
AGGII	Lunge	Monkey lunges at a person without contact
	Chase	Monkey chases a person without contact
AGGIII	Scratch	Monkey scrapes human's skin with its nails
	Bite	Monkey nips or cuts into human's skin with its teeth

2.3 Questionnaire survey

Our questionnaire surveys were collected in 2009-2010. The respondents were tourists at the viewing pavilion site. The survey was designed to focus on the contexts of macaques and tourists, including respondent's age, sex, religion, ethnicity, education and marital status. Health information relating to interaction experience with macaques and bite or scratch wounds inflicted by macaques was also collected.

2.4 Blood samples and detection of antibodies

We collected blood samples from a total of 16 Tibetan macaque individuals (proportion: 16 out of 65 individuals) in 2010. The blood samples were centrifuged at 3000 g for 5 min to obtain serum and then stored at −20°C until further analysis. We entrust Anhui Medical Science Research Institute in Hefei for serological examination. The kits were produced by R&D Syestems Company and commercially available. Samples were analyzed by enzyme-linked immunosorbent assay (ELISA) for the presence of immunoglobulin antibodies to Herpes B Virus (BV), Hepatitis A virus (HAV), Simian foamy

virus (SFV), Simian pox virus (SPV), Simian retrovirus (SRV) and Simian T-cell lymphotrophic virus-1 (STLV-1). This study was approved by animal care committees of the Wildlife Protection Society of Anhui Province, China (permit number: 201022). All the work was carried out under the Wildlife Protection Law of the People's Republic of China. No macaques sustained injury during this study.

2.5 Statistical analysis

We used Chi-square (χ^2) tests to test the difference of aggression behaviors from macaques to tourists. Mann-Whitney U-Test was used to test the significant difference among different groups in questionnaire surveys. All statistical analyses were two tailed and were carried out using the Statistical Package for the Social Sciences version 13.0 (SPSS Inc., Chicago, Illinois, USA), with the significance level set at 0.05.

3. Results

3.1 Behavioral data

During the study period we recorded 1150 interactions between humans and macaques. Most monkey aggressive behaviors did not result in physical contact with humans (χ^2=6.720, *df*=2, *P*<0.05; i.e., categorized as AGGI and II). AGGI accounted for 9.83% (113/1150) of interactions, AGGII rate accounted for 11.39% (131/1150), and AGGIII rate accounted for 2.17% (25/1150) (Figure 1). In terms of AGGIII, 80% (20/25) of attacked object were tourists, and only 20% (5/25) were workers. The main AGGIII type was scratching behavior (92%, 23/25), and only two events were bite behavior (Figure 2). 88% (22/25) of AGGIII was caused by tourists feeding macaques or by tourists moving to close proximity with macaques for photo opportunities. Of the 25 injured, one tourist and one worker were severely bitten or scratched sufficiently to induce bleeding.

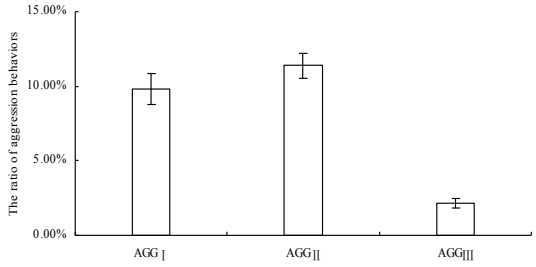

Figure 1. Ratio of macaques' aggressive behavior to tourists on a scale of I-III

3.2 Demographics of questionnaire survey

Demographics data for the human study participants are summarized in Table 2. In total, 282 persons volunteered to participate in the study. The mean age of the surveyed population was 33.5 years (standard deviation 10.57, range 16-65 years). 63.83% of respondents were male. About the education of respondents, 40.43% of respondents were university and above, and 37.94% were senior high school. The two most common occupations were government employee (31.91%) and students (22.34%).

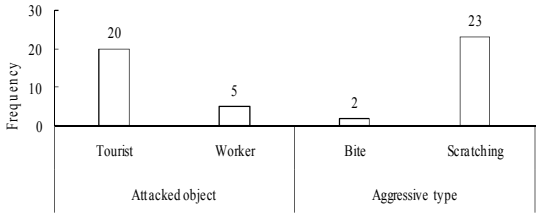

Figure 2. Frequency of macaques' aggressive type and attacked object

Table 2. Demographic characteristic of respondents

Variable	No. of Respondents	Percentage (%)
All Tourists	282	
Age group (yrs)		
<20	21	7.45%
20-29	107	37.94%
30-39	79	28.01%
40-49	51	18.09%
>49	24	8.51%
Sex		
Male	180	63.83%
Female	102	36.17%
Education		
Grade school or none	22	7.80%
Junior high school	39	13.83%
Senior high school	107	37.94%
University and above	114	40.43%
Religion		
Buddhism	39	13.83%
Christianism	9	3.19%
Islamism	2	0.71%
Other	232	82.27%
Occupation		
Industry	37	13.12%
Agriculture	13	4.61%
Commerce	28	9.93%
Government employee	90	31.91%
Student	63	22.34%
Other	51	18.09%

3.3 Interaction behavior

Prevalences of contacting with monkeys are summarized in Table 3. The participants that had the experience of feeding monkeys are high to 57.8%. 9 (3.19%) participants or their friend had the experience of petting the monkeys, and 6 (2.13%) participants or their friend had the experience of eating monkeys. Results from the survey showed that 8.16% of participants had touched the monkeys and 10.28% of participants been touched by monkeys. Among the participants been touched by monkeys, 13.79% (4/29) were scratched and 6.9% (2/29) were bitted by monkeys.

Table 3. Questionnaire survey on respondents contacting with monkeys

Variable	No. of Respondents	Percentage (%)
All Tourists	282	
Feed monkeys		
Yes	163	57.80%
No	119	42.20%
Pet monkeys		
Yes	9	3.19%
No	273	96.81%
Eat monkeys		
Yes	6	2.13%
No	276	97.87%
Touch monkeys		
Yes	23	8.16%
No	259	91.84%
Used the same water with monkeys		
Yes	40	14.19%
No	242	85.81%
If use, for what?		
Drink	2	5.00% (2/40)
Wash hand	31	77.50% (31/40)
Wash food	1	2.50% (1/40)
Other	6	15.00% (6/40)
Contacted with monkeys		
Yes	29	10.28%
No	253	89.72%
If contacted, in what way?		
Be touched	13	44.83% (13/29)
Be climbed	6	20.69% (6/29)
Be scratched	4	13.79% (4/29)
Be bitted	2	6.90% (2/29)
Be urine splashed	4	13.79% (4/29)

3.4 Wound treatment

The respondents on treatment of if were touched by monkeys or splashed body by monkey's urine, most participants chose washing by clean water (47.87%) and soapy water (39.01%), but only 6.03% chose daubing antibacterial agent (6.03%) and treated by doctors (7.09%). In terms of wound treatment, 4.26% washed the wound with clean water and 6.38% washed with soapy water. Of the injured, 89.36% were treated by doctors at a medical clinic (Table 4). The results show that most respondents will choose the appropriate safeguard measures when scratched or bitted by monkeys (Z=-1.964, P=0.050, Mann-Whitney U-Test).

Table 4. Questionnaire survey on respondents body contact with monkeys

Variable	No. of Respondents	Percentage (%)
All Tourists	282	
You were or if you were touched by monkeys or were splashed body by monkey's urine, how to handle?		
Washed by clean water	135	47.87%
Washed by soapy water	110	39.01%
Daub antibacterial agent	17	6.03%
Treated by doctors	20	7.09%
You were or if you were scratched or bitted by monkeys, how to handle?		
Washed by clean water	12	4.26%
Washed by soapy water	18	6.38%
Daub antibacterial agent	0	0%
Take antibiotics	0	0%
Treated by doctors	252	89.36%

3.5 Seroprevalence of antibodies in macaques

Our results indicate that Tibetan macaques tested positive for 6 types of virus antibodies. Three (18.8%) of the 16 sampled macaques were tested positive for antibodies to SFV and SRV, two (12.5%) of the 16 were tested positive for antibodies to HAV and SPV, and only one (6.3%) of the 16 were tested positive for antibodies to BV and STLV-1 (Table 5).

Table 5. Seroprevalence of antibodies in Tibetan macaques (*Macaca thibetana*) at Mt. Huangshan

Virus ategory	Samples	Seropositive	Seroprevalence
BV	16	1	6.3%
HAV	16	2	12.5%
SPV	16	2	12.5%
SFV	16	3	18.8%
SRV	16	3	18.8%
STLV-1	16	1	6.3%

4. Discussions

4.1 Exposure to macaque bites and scratches

The survey data presented in this study suggest that some tourists and workers in free-ranging macaque ecotourism site at Mt. Huangshan have been bitten and scratched by macaques. Most of AGGIII was caused by tourists feeding macaques or by tourists moving to close proximity with macaques for photo opportunities, and the participants that had the experience of feeding monkeys were high to 57.8%. Park rules prohibit tourists from feeding the macaques, but some tourists use food to entice the macaques to come closer, perhaps for photo opportunities. Obviously, the feeding behavior will enhance the chance of been bitten or scratched by macaques. The same situation also occurs at other NHPs tourism sites, such as in Singapore, Indonesia, Bali and Gibraltar (Fuentes, 2006; Fuentes et al., 2007; Fuentes et al., 2008). Bites and scratches provide an opportunity for infectious agents endemic/enzootic in a host population to infect individuals in a population that previously was uninfected (Engel and Jones-Engel, 2012). We recommend park staff more consistently enforce existing rules against feeding the macaques to minimize the potential for human-macaque contact interactions and potential close proximity, thus reducing the risk of pathogen transmission.

4.2 Wound treatment and the risk of pathogen transmission

We found prevalence of six different kinds of viral infection in macaques. Although an animal positive for virus does not mean that it is actively shedding virus, it can provide current status consistent with a potential for future pathogen transmission. Studies of captive NHPs indicate that transmission can occur via close direct contact, including situations where a NHPs bites or scratches a human; such contact is expected between hosts with overlapping ranges (Wolfe et al., 1998; Nunn and Altizer, 2006). These contact and transmission pathways may occur where tourists, field researchers, or forest workers contact NHPs during fieldwork or ecotourism (Wolfe et al., 2004a; Wolfe et al., 2004b; Nunn and Altizer, 2006). For instance, researchers showed that park staff and tourists at the Sangeh Monkey Forest in Indonesia probably experience a high risk of infection with herpes B virus due to high seroprevalence in the monkey population and humans' frequent exposure to it via monkeys' bites and starches (Engel and Jones-Engel, 2012). Therefore, wound treatment for bites and scratches injuries is particularly more important to reduce the risk of pathogen transmission.

4.3 Influence of the monkey culture in China

Cultural views of wildlife often supersede scientific claims when it comes to managing zoonotic disease (Peterson et al., 2006). Chinese culture shows a long tradition of monkey veneration, and monkeys are prominent in early Chinese folklore. The Monkey is the ninth animal in the Twelve Terrestrial Branches (the Chinese zodiac). Traditional respect for monkeys owes much to the role of the Monkey King, Sun Wukong (Mandarin), in the ancient novel Journey to the West. Chinese people respect the Monkey King because he is the embodiment of justice, and nearly everyone knows about him. Because of this fascination with monkey culture, most tourists want to get close enough to monkeys to take photo with them, or perhaps to even touch the monkeys. People's views of NHPs affect the types of interactions that occur, as well as their attitudes toward the macaques and the potential for pathogen transmission.

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