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RESEARCH ARTICLE

# Considering Human–Primate Transmission of Measles Virus Through the Prism of Risk Analysis

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Measles is a respiratory virus that is endemic to humans. Human–nonhuman primate (NHP) transmission of the measles virus has been shown to cause significant morbidity and mortality in NHP populations. We investigated serological evidence of exposure to measles virus in two free-ranging populations of macaques at the Bukit Timah (BTNR) and Central Catchment Nature (CCNR) reserves in Singapore and the Swoyambhu Temple in Katmandu, Nepal. At BTNR/CCNR none of the 38 macaques (*Macaca fascicularis*) sampled were seropositive for antibodies to measles virus. In contrast, at Swoyambhu 100% ($n = 39$) of the macaques (*M. mulatta*) sampled were seropositive for antibodies to the measles virus. Here the contrasting seroprevalences of the two sites are analyzed using risk analysis. These case studies show how risk analysis can be used to approach the phenomenon of cross-species pathogen transmission. Am. J. Primatol. 68:868–879, 2006.


**Key words: risk analysis; nonhuman primates; disease transmission; measles; Singapore; Nepal**

## INTRODUCTION

Measles has been recognized as a significant public health problem for over 1,000 years. The 10th-century Persian physician Abu Bakr considered it ‘‘more to

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be dreaded than the small pox'' [Anonymous, 2001]. Over the next 10 centuries the pathogenesis, phylogenetics, and epidemiology of measles were well described. A member of the genus Morbillivirus, measles is highly communicable through airborne respiratory droplets, nasal and throat secretions, and fomites [Benenson, 1990]. The disease most commonly afflicts children, with the bulk of mortality occurring in very young children and immunocompromised individuals.

Though an effective vaccine against measles was developed over 40 years ago, significant segments of the population in some developing countries remain unvaccinated. As a result, measles still infects 30–40 million people annually, with a worldwide mortality approaching 800,000 per year [World Health Organization, 2003]. Over 90% of global measles-related deaths occur in Africa and southeast Asia, which coincidentally are geographic areas in which the majority of the world's free-ranging nonhuman primate (NHP) populations are found (Fig. 1).

Small epidemics of measles virus still occur in the United States and other developed countries, usually in areas where children remain undervaccinated. Herd immunity greatly reduces the infection rates in developed countries. The World Health Organization (WHO) estimates that 80 million cases of measles are prevented annually; however, when vaccination levels drop below 94%, the likelihood of epidemic measles increases [Swartz, 1984].

## Measles in NHPs

In addition to humans, measles has been shown to infect many other primate species, including *Macaca mulatta*, *M. fascicularis*, *M. radiata*, *M. cyclopis*, *Papio cristatus*, *Cercopithecus aethiops*, *Saimiri sciureus*, *Colobus quereza*, *Pan troglodytes*, *Callithrix jacchus*, *Saguinus oedipus*, *S. fuscicollis*, and *Aotus trivirgatus* [Lowenstine, 1993; Mansfield & King, 1998]. Much of the data on measles infection in NHPs have been gathered from epizootics in laboratory settings. In laboratory NHPs, measles often causes mild gastrointestinal signs or may be asymptomatic [Habermann & Williams, 1957; Potkay et al., 1966].

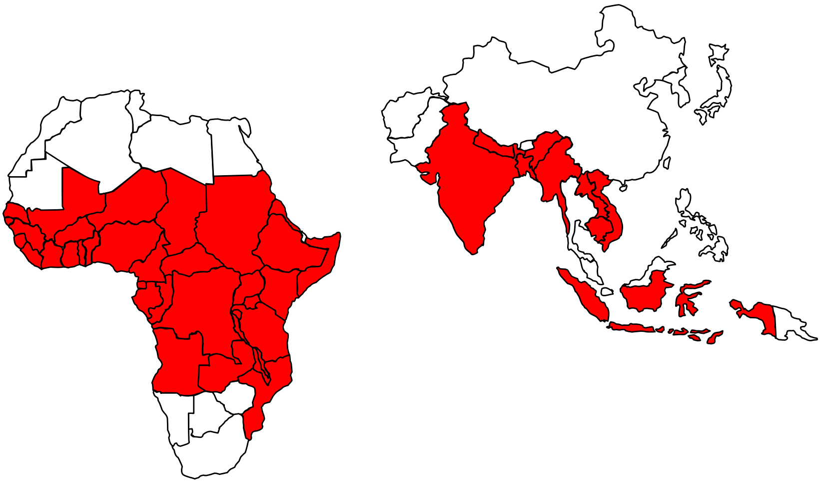

Fig. 1. >90% of measles related deaths among humans occur in Africa and South East Asia.

Stressed and immunocompromised animals are more likely to suffer severe sequelae, including secondary bacterial infection, pneumonia, encephalitis, abortion, or death [Levy & Mirkovic, 1971; MacArthur et al., 1979; Renne et al., 1973]. Epidemics among newly captured monkeys have been associated with mortality rates of 100% [Remfry, 1976]. Infection is believed to confer lifetime immunity against reinfection.

Serological evidence of measles infection in free-ranging populations of NHPs has been documented in previous studies [Bhatt et al., 1966; Jones-Engel et al., 2001; Meyers et al., 1962; Shah & Southwick, 1965]. These studies include evidence of measles infection in NHPs with frequent contact with human populations, as well as in wild NHP populations with minimal human contact. Although the morbidity and mortality of measles infection in free-ranging NHP populations are unknown, its pathological potential makes it a threat to NHP populations. This has implications for the conservation of NHP populations, especially endangered species. Because human populations represent the largest reservoir of the measles virus, it is most likely that measles epizootics in NHP populations are initiated by human–NHP transmission and subsequently spread by animal–animal transmission. Because of their relatively small numbers, it is unlikely that natural populations of NHPs are significant reservoirs of the measles virus.

## Applying Risk Analysis to Measles Infection in NHP Populations

Given the conservation implications of measles infections in NHP populations in particular, and cross-species transmission of endemic human pathogens in general (especially other pathogens spread via the respiratory system, such as tuberculosis and influenza), it is important to consider the ways in which NHP populations can be protected from endemic human diseases. One approach to this issue is to use the risk analysis paradigm described by Travis and colleagues in this issue. In the present article we use the risk analysis framework to organize a discussion of what is known about human–NHP transmission of measles, what directions future research on the subject should take, and how these data can be used to reduce the risk of human–NHP disease transmission (Fig. 2). Two NHP populations with contrasting patterns of measles antibody seroprevalence will be used to illustrate the main points with respect to risk analysis.

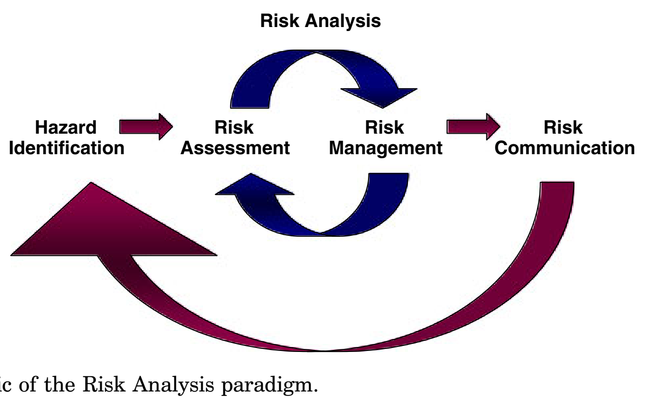


Fig. 2. Schematic of the Risk Analysis paradigm.

## MATERIALS AND METHODS

### Study Sites

*Singapore, Bukit Timah Nature (BTNR), and Central Catchment (CCNR) nature reserves*

Singapore, a tiny island state of 636 km$^2$, is located at the southern tip of the Malay Peninsula. It is one of only two cities in the world that has primary rain forest within its boundaries. Singapore's population of 4,235,000 people is almost entirely urban and the country is renowned for its cleanliness, orderliness, and economic prosperity. In the center of the island are the adjacent BTNR and CCNR areas, which together total more than 3,000 ha. BTNR and CCNR are both managed by the National Parks Board, Singapore (NParks), which has built an elaborate visitor's center and maintains a system of trails that run through both reserves. BTNR alone serves 380,000 visitors annually who make use of the reserves extensive hiking opportunities. BTNR/CCNR is marketed to Singaporeans who are interested in nature. Though Singapore receives millions of tourists each year, relatively few visit BTNR/CCNR.

Approximately 2,000 *M. fascicularis* range freely throughout the reserves. The presence of these macaques was first recorded in the 1800s. A multilane expressway transecting the two reserves was built in 1986 and is believed to limit contact between the macaques in BTNR and CCNR. The only other NHPs found in Singapore are remnant populations of *Presbytes femoralis and Nycticebus coucang*. NParks has erected signs urging visitors not to feed or touch the NHPs (Fig. 3), and stiff fines are posted to discourage these activities. Occasional conflicts arise between the macaques and homeowners whose land borders the reserves, when the macaques raid their gardens. These conflicts are managed by the NParks.

*Swoyambhu Buddhist Temple, Kathmandu, Nepal*

Nepal is a country of 177,181 km$^2$ situated between India and China. The population of the capital, Kathmandu, is approximately 1.2 million people. The mean per capita income is $1,370 USD, which makes Nepal one of the poorest countries in Asia. Swoyambhu is one of two temple sites in the Kathmandu Valley that has a large population of free-ranging rhesus monkeys (*Macaca mulatta*). Situated atop a hill in the center of densely populated Kathmandu, it is one of the region's oldest Buddhist holy places, dating back almost 2000 years, and has been designated a world heritage site. The site's extensive complex of stupas is intermixed with shops and residences. Swoyambhu is a vibrant part of Kathmandu's cultural life. In addition to the Tibetan monks, Brahmin priests, and Newar nuns who live on the site, a brisk flow of local worshippers and visitors from around the world pass through Swoyambhu. People who live and work in and around Swoyambhu share common water sources with the monkeys and report that the rhesus macaques frequently invade their homes, gardens, and refuse piles in search of food. The monkeys at Swoyambhu have become a tourist attraction in their own right, and many visitors interact with the macaques, often by feeding or teasing them. These activities bring people into close proximity and often physical contact with macaques. There are no regulations against feeding the macaques.

The rhesus macaques at Swoyambhu number approximately 400 individuals, which are distributed nearly equally across seven or eight groups with overlapping home ranges [Chalise & Ghimire, 1998]. Physical contact between macaque groups is common. Natural forage is extremely limited at Swayambhu.

## Biological Sample Collection

The trapping and sampling protocols used for the Singapore and Nepal macaques were identical. In Singapore, over a 2-week period in January and February 2003, 38 *Macaca fascicularis* from five different groups in BTNR/CCNR were trapped, sampled, and released. In May 2003, over a 4-day period, 39 rhesus macaques from three different groups at Swoyambhu were trapped, sampled, and released. The macaques were trapped in a cage measuring $2.5\,m \times 2.5\,m \times 1.5\,m$ and sedated with 3 mg/kg of intramuscular Telazol® (tiletamine HCl/zolazepam HCl). To avoid stressing very young animals, infants were not anesthetized or sampled as part of this protocol. All anesthetized macaques were given a complete physical exam. Then, with the use of a sterile technique and with universal precautions taken, 10 ml of blood were collected via venipuncture of their femoral vein. Eight milliliters of blood were centrifuged to extract serum. The remaining blood was aliquotted into a vacutainer vial containing EDTA. Serum and whole-blood samples were frozen in the field and then stored at $-70^{\circ}C$. Following sample collection, the animals were placed in a recovery cage and allowed to recover fully from the anesthesia before they were released as a group back into their home range. In Nepal, each macaque was tattooed on the inner right thigh with a unique identifier to avoid resampling and to facilitate future follow-up. Each macaque's weight and dental formula were collected and recorded for age assessment. This data collection protocol was reviewed and approved by the University of Washington's Institutional Animal Care and Use Committee (3143-03).

## Measles Enzyme-Linked Immunosorbent Assay (ELISA) Methodology

Serum samples from the Singapore macaques were initially screened at Washington National Primate Research Center (WaNPRC) in April 2003 for antibodies to measles using a commercially available IgG ELISA from DIA Diagnostic Automation (Calabasas, CA). The initial measles ELISA screening for the Nepal samples was conducted on-site in Kathmandu in May 2003, also using a measles ELISA kit from DIA. The Nepal and Singapore samples were retested at WaNPRC in November 2003 with another IgG measles ELISA kit (IBL ImmunoBiological Laboratories, Hamburg, Germany) to confirm the results. Positive and negative controls were included with the kits. Serological results were considered positive if their OD value was greater than the cutoff values calculated for each assay.

# RESULTS

Table I presents the demographic distribution of the Singapore and Nepal macaque study populations. Of the 38 *M. fascicularis* sampled in Singapore, 36.8% were adults, 7.9% were subadults, and 55.3% were juveniles. Adults accounted for 48.7% of the 39 *M. mulatta* sampled at Swoyambhu, 17.9% of the sampled population were subadults, and 33.3% were juveniles. In Singapore, an equal number of males ($n = 18$) and females ($n = 18$) were sampled. At Swoyambhu, 17 of the macaques sampled were male and 22 were female. No significant differences in age class ($\chi^2 = .12$) or sex distribution ($\chi^2 = .57$) between the two study populations were detected.

**TABLE I. Age-Sex Composition and Measles Seroprevalence of the Study Groups**

| | | Sex | | Age classes | | | |
|---|---|---|---|---|---|---|---|
| | N | Males | Females | Adults | Subadults | Juveniles | Prevalence (%) of antibodies to measles |
| Singapore BTNR/CCR *M. fascicularis* | 38 | 19 | 19 | 14 | 3 | 21 | **0%** |
| Nepal Swoyambhu *M. mulatta* | 39 | 17 | 22 | 19 | 7 | 13 | **100%** |

The results of the measles ELISA obtained using both the DIA and IBL kits were in complete concordance. All 39 of the serum samples from the Swoyambhu macaques tested positive by ELISA for antibodies to measles virus. In contrast, ELISAs of serum from all 38 macaques at BTNR/CCNR were negative for antibodies to measles virus.

## DISCUSSION

The risk analysis paradigm discussed by Travis and colleagues in this issue of *AJP* consists of four components: hazard identification, risk assessment, risk management, and risk communication (see Fig. 2).

### Hazard Identification

For decades it has been known that measles virus can cause significant mortality and morbidity in NHPs that come into contact with humans. As early as 1962, studies showed that previously unexposed NHPs captured from the wild often seroconverted once they were exposed to humans [Meyers et al., 1962]. More recently, several populations of free-ranging NHPs in Indonesia with varying degrees of contact with human populations have been shown to demonstrate serological evidence of prior measles infection [Jones-Engel et al., 2001; Jones-Engel et al., unpublished data].

### Risk Assessment

The contrast in measles antibody seroprevalence between that measured at BTNR/CCR (0%) and that measured at Swoyambhu (100%) invites an examination of the factors that can potentially explain these observations. The risks associated with measles infection can be broadly divided into three categories: 1) the likelihood that visitors to the site will be shedding the measles virus, 2) the likelihood that an NHP will come into close contact with a shedding individual (human or NHP) or infectious fomites, and 3) the likelihood that an NHP will be nonimmune to measles and therefore vulnerable to infection.

• **Likelihood that visitors to the site will be shedding the measles virus.** Several factors influence the likelihood that a shedding individual will visit the site. Because the human population is the reservoir of measles virus, one must focus on the likelihood that the visitors will be infectious. Important determinants of this include the prevalence of measles among visitors, both from within and outside the community, and the demographics of the visiting population.

The measles vaccination rate plays a very important role in determining the prevalence of shedding individuals.

• **Likelihood that an NHP will come into close contact with a shedding individual (human or NHP) or infectious fomites.** The frequency of interspecies contact is influenced by the habituation of NHPs to humans, practices and policies regarding feeding and provisioning, and the availability of natural food sources. The presence of infectious fomites in the environment depends on waste disposal practices at the site and on individual hygiene practices (e.g., spitting).

• **Likelihood that an NHP will be nonimmune to measles and therefore vulnerable to infection.** Because measles vaccinations are not given to macaques at either site, immunity to measles can be assumed to result from prior measles infection. Species differences in susceptibility to measles virus is an unlikely explanation for the contrasting seroprevalences, since laboratory studies to date have not yielded evidence that *M. mulatta* is more likely than *M. fascicularis* to become infected with measles. Health differences between the two populations could also lead to a differential susceptibility to measles. Nutritional and immunological data were not available to test this hypothesis.

The foregoing analysis suggests several factors that may explain the contrasting seroprevalence of measles antibodies in the two populations: First, Table II presents select human demographic variables for Singapore and Nepal (data pertaining only to Kathmandu were not available). The demographic structures of Singapore and Nepal are dramatically different, with nearly 15% of Nepal's population under the age of 5 years. This is in contrast to Singapore, where less than 6% of the population is under 5 years old. Because children under the age of 5 are the most likely segment of the population to become infected with measles, these contrasting demographic patterns may help explain the observed differences in measles prevalence between the macaques at BTNR/CCNR and Swoyambhu.

Second, in the human population, the number of reported cases of measles is far higher and the measles vaccination rate for the population is lower in Nepal

**TABLE II. Selected Demoeraohic Variables for Singapore and Nepal***

| | Singapore | Nepal |
|---|---|---|
| Land area (square km) | 636 | 177,181 |
| Demographic profile | | |
| Population | 4,253,000 | 25,164,000 |
| Population <5 yrs | 242,000 | 3,688,000 |
| Population <15 yrs | 880,000 | 10,066,000 |
| GDP per capita | 24,040.00 USD | 1,370.00 USD |
| Infant mortality (per live 1000 births) | 3 | 70 |
| Child mortality (per live 1000 births) | 4 | 97 |
| Fertility rate | 1.4 | 4.3 |
| Vaccinations | | |
| Measles | 88% | 75% |
| DTP (3rd dose) | 92% | 78% |
| BCG | 97% | 91% |
| Active surveillance for measles | Y | Y |

*http://www9.who.int/vaccines/globalsummary/immunization/.

**TABLE III. Rates of Vaccination Coverage and Reported Cases of Measles for Singapore and Nepal***

| | Year cases reported | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Country | 2003 | 2002 | 2001 | 1999 | 1998 | 1997 | 1996 | 1995 |
| Vaccination coverage (%) | | | | | | | | |
| Nepal | 75 | 71 | 71 | 81 | 73 | 85 | 80 | 71 |
| Singapore | 88 | 91 | 89 | 93 | 96 | 90 | ND | 88 |
| Number of reported cases | | | | | | | | |
| Nepal | 13,344 | 6,749 | 10,849 | 5,771 | 12,677 | 7,213 | 4,810 | 1,401 |
| Singapore | 33 | 211 | 408 | 65 | 114 | 1,413 | | 185 |

*Measles incidence, WHO: http://www.who.int/vaccines/globalsummry/timeseries/tsincidencemea.htm; vaccine coverage: http://www.who.int/vaccines/globalsummry/timeseries/tscoveragemcv.htm.
ND, no data.

than in Singapore (Table III). This makes it much more likely that a macaque at Swoyambhu will come into contact with a person shedding measles compared to a macaque at BTNR/CCNR.

Third, the demographic profile of typical visitors to Swoyambhu differs from that seen in BTNR/CCNR, and although no data on this are available, the authors estimate that more families, including young children, come into contact with NHPs in and around Swoyambhu than at BTNR/CCNR. Young children represent the demographic group most likely to be infected with measles.

Fourth, the Swoyambhu macaques enter homes in the communities around the temple. This increases their risk of contact with a person infected with measles, since a person infected with measles is more likely to stay home. The macaques at BTNR/CCNR do occasionally raid the gardens of homes located near the perimeter of the reserves, but the NParks management is very active in managing and limiting these kinds of contact.

Fifth, differences in NHP–human interactions at the temple sites themselves may help explain the observed seroprevalence patterns. BTNR/CCNR has erected large signs admonishing visitors to refrain from feeding and touching the macaques. In fact, a fine is levied against violators of these rules. Undoubtedly, these rules are often flouted, but in general they may prevent many people from coming into close contact with the macaques. At Swoyambhu, in contrast, food hawkers encourage visitors to feed the macaques, and there are no rules or signs advising against the practice. Perhaps as a consequence, the macaques at Swoyambhu are generally more habituated to humans than those at BTNR/CCNR; however, no formal data supporting this assertion have been gathered. Populations may also play a role, as higher macaque population density at Swoyambhu would be expected to favor a more rapid spread of the virus among macaques.

Finally, fomites are a potentially more significant factor favoring the spread of measles at Swoyambhu. Refuse is generally well contained in designated containers at BTNR/CCNR (Fig. 4), while open trash heaps are scattered about Swoyambhu and macaques are frequently seen on the open refuse heaps, searching for food. The possible role of poor nutrition or toxic insult should also be considered as contributory factors in the likelihood of infection/transmission of measles.

Fig. 3. Example of the signage used at BTNR/CCNR to discourage feeding of the monkeys. (Photo by Randall C. Kyes)


Fig. 4. The photo on the left is of the specially designed rubbish bins used in BTNR/CCNR. These bins are truly 'monkey-proof'. The photo on the right, taken at Swoyambhu, illustrates the difficulties in keeping monkeys out of open rubbish bins/piles. (Photo on the left by Benjamin Lee) (Photo on the right by Randall C. Kyes)

Sampling bias may also explain the observed differences in measles seroprevalence in Swoyambhu and BTNR/CCR. Because not all groups of macaques at BTNR/CCR were sampled, it is possible that evidence of measles infection went undetected. However, this is unlikely because the macaques sampled were from groups with the most human contact. Furthermore, regular intragroup contact occurs at BTNR/CCNR, increasing the likelihood that measles infection in one group would spread to other groups and be detected by our serosurvey. It could also be suggested that measles actually does affect the BTNR/CCR macaques, but that it causes high mortality among affected monkeys. This explanation is less likely, given serological data from other *M. fascicularis* populations at monkey temples with a high seroprevalence of antibodies to measles when tested with the DIA or IBL ELISA kits (Jones-Engel, unpublished data).

## Risk Management

The above risk assessment points the way to interventions designed to reduce the likelihood of human–macaque transmission of measles at Swoyambhu. Several strategies present themselves as options. First, increasing measles vaccination rates in human populations in Kathmandu, especially for those who live in and around the monkey temple itself, would decrease the likelihood that a macaque will come into contact with an infected human or fomites contaminated with measles virus. A second step would be to increase awareness of the potential for human–macaque as well as macaque–human cross-species pathogen transmission among visitors, and discourage people with symptoms of illness (especially respiratory illness) from coming into close proximity with macaques. Third, improving trash disposal and cleaning up open refuse heaps would decrease the risk of fomite-spread infections. Discouraging visitors from feeding the macaques would reduce the amount of close contact between humans and macaques, and decrease the risk of fomite-spread infection.

## Risk Communication

The goal of risk communication is to provide information about risk assessment and management to all groups that influence or are influenced by the identified hazard as well as the measures taken to address the hazard. This is an important but often overlooked aspect of the risk analysis continuum. The seroprevalence data presented in this report suggest that different issues confront conservationists at BTNR/CCNR and Swoyambhu. The macaques at BTNR/CCNR appear to have been protected against measles infection. However, it should be noted that their lack of immunity implies that they would be vulnerable

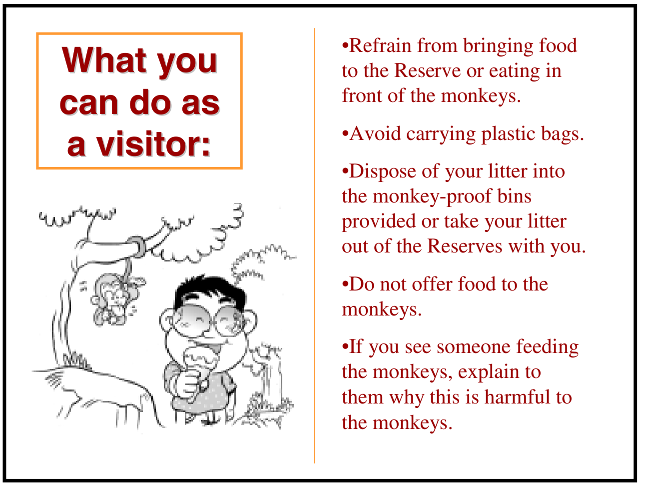


Fig. 5. Pamphlet used in recent "Don't Feed the Monkeys" campaign at BTNR/CCR designed to educate visitors about the negative consequences of feeding monkeys. The campaign consisted of a roving exhibition in neighborhood libraries and the handing out of car windshield decals with the "Do not feed the monkeys. Please help save them." message. (Pamphlet courtesy of National Parks Board, Singapore)

to measles if it were introduced. This makes it all the more important for the authorities to remain vigilant about the possibility of visitors transmitting infectious diseases to the macaques. NParks has taken a very active role in managing and conserving the macaque population at BTNR/CCNR. Extensive signage discouraging the feeding of macaques is evident throughout the reserves. In 2004, as part of the Chinese Year of the Monkey, the NParks began an information campaign designed to educate visitors to BTNR/CCNR about the negative consequences associated with feeding the monkeys (Fig. 5). This education program was extended to local libraries through traveling exhibits and information talks given by NParks employees. Brochures and car decals with the same message were also distributed to reach a wider populace. Additionally, signs requesting visitors with symptoms of infectious respiratory or gastrointestinal diseases to avoid close proximity with macaques might influence visitors to abstain from behaviors that could lead to disease transmission.

On the other hand, the Swoyambhu macaques show evidence of having been exposed to measles, which implies that measures designed to prevent disease transmission to macaques need to be strengthened. In addition, when diseases (especially respiratory diseases) are transmitted from people to macaques, the potential exists for disease transmission in the other direction [Jones-Engel et al., 2005, 2006; Engel et al., 2002; Schillaci et al., 2005]. Of course, crafting and communicating effective messages require a commitment of resources, which may be difficult to come by in a developing country. Furthermore, it should be emphasized that the message must be delivered in such a way as to help people appreciate the valuable resource that NHPs constitute for their community. Ultimately, effective risk communication can go a long way in ensuring the successful commensalism of humans and NPHs.

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