

# Nonhuman Primate Quarantine: Its Evolution and Practice

*Jeffrey A. Roberts and Kirk Andrews*

## Abstract

Nonhuman primates (NHPs) are imported to the United States for use in research, domestic breeding, and propagation of endangered populations in zoological gardens. During the past 60 years, individuals responsible for NHP importation programs have observed morbidity and mortality typically associated with infectious disease outbreaks. These outbreaks have included infectious agents such as tuberculosis, *Herpesvirus* sp., simian hemorrhagic fever, and filovirus infections such as the Ebola and Marburg viruses. Some outbreaks have affected both animal and human populations. These epizootics are attributable to a variety of factors, including increased population density, exposure of naïve populations to new infectious agents, and stress. The practice of quarantining animals arriving in the United States was first applied by individual research programs to improve animal health and ensure the quality of animals entering research programs. The development of government regulations for nonhuman primate quarantine accompanied the recognition that imported NHPs could pose a risk to public health. This article briefly reviews the history of US NHP importation and the factors behind the development of NHP quarantine regulations. The focus is on regulations concerned with infectious disease, public health, and the health of domestic primate colonies. These regulations have had the dual benefit of protecting public health as well as reducing animal morbidity and mortality during importation and quarantine. We review current practices and facilities for nonhuman primate quarantine and identify challenges for the future.

**Key Words:** biohazards; CDC; filovirus; importation; non-human primates; quarantine; tuberculosis

## Quarantine History

Quarantine is an established practice in human and veterinary medicine to prevent the spread of infectious disease. The word quarantine is derived from the French word *quarantaine* and is defined as “1: a period

of 40 days; 2a: a term during which a ship arriving in port and suspected of carrying contagious disease is held in isolation from the shore” (Merriam Webster 2007). In human medicine quarantine has long served to isolate patients with infectious diseases such as tuberculosis (TB<sup>1</sup>), measles, or influenza from the general population in order to prevent the spread of infectious agents. The development of infectious agents that are resistant to currently available antibiotics has seen a return of mandatory—and controversial—quarantine for public health reasons, such as the recent incarcerations of individuals with drug-resistant TB (Moszynski 2007) and of individuals traveling in Asia during the outbreak of severe acute respiratory syndrome (SARS) (CDC 2003a).

The quarantining of nonhuman primates (NHPs<sup>1</sup>) began in the 1940s and 1950s as increased numbers of NHPs were imported for disease research (Ruch 1959, 232-233). Because many animals at that time were wild caught, there existed the potential for an animal or a group of animals to bring in novel agents that could pose a threat to human and animal health. This was particularly a concern for animals imported for the pet trade and for research animals incorporated directly into colonies or immediately used in research projects.

To appreciate the value of the current quarantine program in the United States, it is helpful to review the various factors that contributed to its development and lessons learned along the way.

## US Nonhuman Primate Importation and Quarantine Practices

### Importation and Infectious Disease

The basic principles of nonhuman primate quarantine were identified in the 1950s as the national effort to develop a polio vaccine required the importation of more than 200,000 rhesus monkeys annually for 6 years (Eudey and Mack 1984). Many of these imported NHPs were caught wild in their natural habitat (NAS 1970), a practice that provided a ready source of research animals but that also, because of the methods used at the time, resulted in high levels of

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Jeffrey A. Roberts, DVM, DACLAM, is Chief Executive Officer and Kirk Andrews, DVM, is Facility Veterinarian of Valley Biosystems in West Sacramento, California.

Address correspondence and reprint requests to Dr. Jeffrey A. Roberts, Chief Executive Officer, Valley Biosystems, 1265 Triangle Court, West Sacramento, CA 95605 or email [j.roberts@valleybiosystems.com](mailto:j.roberts@valleybiosystems.com).

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<sup>1</sup>Abbreviations used in this article: NHP, nonhuman primate; TB, tuberculosis

morbidity and mortality among these animals. The stress of capture, transport, and exposure to human pathogens resulted in epizootics of infectious disease in newly imported populations (NAS 1973). Animals that were already immunocompromised from stress encountered novel viral, bacterial, and parasitic agents such as measles, *Shigella*, and tuberculosis (Kalter 1970), and were therefore subject to disease outbreaks that caused the loss of animals, poor animal quality, and compromised research programs (DeValois 1960; Kalter 1970). To address these problems, government programs and research facilities in the United States began developing conditioning programs in source countries as well as quarantine programs at facilities receiving animals to help detect the presence of infectious disease and prevent its spread in captive primate populations (NAS 1973; Whitney et al. 1967).

Scientists also worked to control the spread of tuberculosis in their research colonies and began to articulate the foundations of quarantine policy and practice (Ruch 1959). A basic premise of animal management is the philosophy of “all in and all out” in which populations of animals are not mixed or combined with each other due to the risk of disease spread. Karl Habel in 1947 stated (referring to rhesus macaque importation) that “All large groups of fresh stock should be left as homogeneous as possible. All animals in a room should be used before more are moved in” and further recommended that “New monkeys should be isolated and released into the colony only if they have passed two tuberculin tests one month apart” (Habel 1947). Despite this advice, disease outbreaks occurred in domestic colonies as a result of the mixing of primate species in transit or inadequate biological separation of species at the receiving facility (DeValois 1960; Kalter 1970). The mixing of individuals of the same species but different geographic origins always has the potential to cause a disease outbreak, as different infectious agents may have a different prevalence in different populations (Hunt 1970; Kalter 1970). In addition, the exposure of naïve young animals without maternal antibody protection resulted in rapid infection and spread in those populations.

Some of the most dramatic effects of mixing populations resulted from the mingling of New World primate species from the same geographic region. For example, the squirrel monkey (*Saimiri sciureus*) is a carrier of *Herpes tamarinus*.<sup>2</sup> Although members of different taxonomic groups may be sympatric in the wild, their natural behaviors and ecological niches typically prevent the close contact necessary to transmit this infectious agent. In captivity, however, these barriers are eliminated and the behaviors altered, allowing the proximity necessary for transmission

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<sup>2</sup>The species name doesn't derive from the origin of this agent but rather the unique pathogenicity that this endemic virus of squirrel monkeys presents to other New World primates such as tamarins and owl monkeys (Hunt 1970; Hunt and Melendez 1966).

and devastating epizootics of this agent in susceptible New World primate populations.

The disease risk increases sharply when mixing different species of animals from different continents (Hunt 1970; Kalter 1970), as occurred with NHP populations of African and Asian origin. During the 1960s and early 1970s African species including baboons, vervets, and Patas monkeys were imported for infectious disease research; at the same time Asian species, predominantly macaques, were imported for other areas of primate research. A variety of disease outbreaks occurred in the imported Asian macaques after exposure to African primates. This experience soon led to the recognition that many African primate species carry a specific arterivirus, simian hemorrhagic fever. This highly infectious positive-strand RNA virus agent is easily transmitted by aerosol, direct contact, or fomites (Renquist 1990); it has an incubation of 3 to 7 days in aberrant hosts; and it results in a severe hemorrhagic crisis, leading to organ failure and death (Mansfield and King 1998). The mixing of primate populations of different provenance provided primate veterinarians with very dramatic examples of epizootics and added to researchers' knowledge of the presence of latent viruses in primate species.

## Institutional Quarantine

Nonhuman primates in the United States are subject to *primary* and *secondary* quarantine. Primary, or international, quarantine refers to the isolation of animals arriving in the United States from a foreign country. Secondary, or domestic, quarantine refers to the subsequent isolation of animals upon transfer between institutions within the United States.

Until fairly recently the length of quarantine was an institutional choice. Reviews of the primate literature from the 1950s to 1970s reveal a wide range of recommended quarantine periods. Some authors recommended 40 days, others 90 days, and one author recommended a quarantine period of 6 months for a large captive population that may be at risk (Gisler et al. 1960; NAS 1973). At a minimum, the practice of requiring three negative TB skin tests 2 weeks apart—at the beginning, midpoint, and end of the quarantine—necessitated a period of 31 days of isolation and observation (Whitney et al. 1967). In addition to allowing time for serological or skin test conversion, this time period also allowed for observation of any latent clinical disease that might become apparent.

## Government Regulation

Concern about the possible exposure of humans to nonhuman primates carrying zoonotic diseases prompted at least one state to implement quarantine regulations that predate those established by the Centers for Disease Control and Prevention (CDC). California developed its own set of quarantine regulations in 1970 in response to concerns about the

importation of wild animals that could carry rabies or other infectious diseases. The regulations included nonhuman primates as a group, but the hazard posed by monkeys imported for use as pets was cited as a specific concern (Emmons et al. 1970). The California importation regulations (California Health and Safety Code Title 17 Subchapter 3.1) required

- two negative tuberculin skin tests 30 days apart;
- two physical exams, one at the beginning of quarantine and one at the end, and a report with the signature of the veterinarian certifying that the animals were healthy;
- no visible oral ulcers at the end of the quarantine period;
- no clinical evidence of dysentery or diarrhea, emesis, emaciation, contagious skin lesions, central nervous system disturbances, jaundice, or abnormal respiratory signs at the end of the quarantine period; and
- no evidence of zoonotic disease traced back to the quarantined animals.

Shortly before the California regulations went into effect, the importation in 1967 of African green monkeys to a laboratory in Marburg, Germany, resulted in the transmission of a previously unrecognized filovirus to a population of laboratory workers (BMJ 1967). Twenty-seven primary human cases occurred, with seven deaths, and six additional individuals were infected by secondary contact. The animals had been housed briefly and then euthanized for the collection of tissues to establish cell cultures. All of the primary human cases were in laboratory workers who did not wear protective gloves while participating in necropsy and tissue harvest. None of the animal caretakers were affected.

The high morbidity and mortality associated with this disease outbreak demonstrated the risks to public health from newly imported nonhuman primates. The Marburg animals were used shortly after arrival, without quarantine (Kalter 1970). Transmission studies immediately after the outbreak showed that the virus was lethal to African green monkeys and guinea pigs. The short times from experimental exposure to death in the animals, and from laboratory exposure to death in the laboratory workers, suggest that the animals were infected either in Uganda before shipment or at an airport holding facility (BMJ 1967; Muchmore 1987).

This occurrence, and concerns about the risks of other potential zoonotic agents (Kalter 1970), prompted the US Public Health Service (USPHS) to consider a national quarantine program. The USPHS, which had been responsible for oversight of nonhuman primate import since 1948 (NAS 1970), determined that national regulations for animal quarantine were highly preferable to individual state regulations that might result in varied practices and confusion (Parker 1975). The focus of the new regulations, enacted on October 10, 1975, was primarily on the provision of a health certificate for animals from countries where yellow fever is endemic and on the requirement that animals be shipped in mosquito-resistant enclosures in order to exclude yellow

fever vectors. The regulations also established basic quarantine requirements and required that the CDC be notified of the occurrence of Marburg virus, yellow fever, or monkeypox in any group of imported nonhuman primates (42 CFR 71.53). The regulations prohibited the importation of nonhuman primates for use as pets and required facilities that import nonhuman primates to

- register with the Centers for Disease Control and Prevention;
- certify that imported nonhuman primates will be used only for bona fide scientific, educational, or exhibition purposes (as defined in the regulations);
- isolate shipments of imported nonhuman primates upon arrival for 31 days (the time required for three tuberculin skin tests at 2-week intervals and the incubation period for known serious infectious zoonoses);
- maintain records for each shipment of nonhuman primates; and
- report suspected zoonotic illness in animals or workers.

Other regulatory oversight of NHP importation was provided by the Endangered Species Conservation Act of 1969 (Public Law 91-135, June 3, 1970).

The standard method for screening nonhuman primates for tuberculosis was established by a report from the National Research Council's Committee on the Nonhuman Primate (NRC 1980); facilities that did not comply with these guidelines were not allowed to import nonhuman primates. The committee's publication expanded on earlier recommendations (Whitney et al. 1967) and specified that "A suspension (0.1 ml) containing 1,500 or more tuberculin units (TU) of USDA veterinary tuberculin (mammalian derivative) should be injected intradermally into the edge of the upper eyelid with a sterile 25-27 gauge needle," followed by daily visual evaluation for 3 days with a skin reaction rating of 1 to 5. The CDC used this document to establish the recommendation of a minimum of three tuberculosis skin tests, each 2 weeks apart, until the acquisition of three consecutive negative tests (NRC 1980). Any animal that tested positive was euthanized, and the remaining animals in the shipment were required to complete five additional negative tuberculin tests before being released. The CDC's 1975 regulations directed quarantine procedures for the importation of nonhuman primates for the next 15 years.

## Lessons Learned in Primate Importation and Infection Control

Awareness of the risk of zoonotic disease from nonhuman primates was already well established when a series of events in the late 1980s reinforced this potential public health hazard. Although monkey B virus (*Cercopithecine herpesvirus 1*) had long been recognized as a serious zoonotic threat (NRC 2003; Ruch 1959, 410-411), its seriousness was reinforced in 1987 by a cluster of four human B

virus cases, including two deaths, at a military research facility in Pensacola, Florida, that used nonhuman primates (CDC 1987). Notably, there was evidence of human-to-human transmission of the infection from an animal care worker to his spouse. Investigation of the cases revealed that, although the source animals had completed quarantine and were in an established colony, the population included B virus–positive monkeys, they had been handled without personal protective equipment, and employees had been bitten and scratched repeatedly.

This incident reinforced the necessity of certain safety precautions for those who work with and handle nonhuman primates and it illustrated the importance of appropriate infection control practices (beyond quarantine) for imported NHPs. The CDC responded with a set of guidelines to prevent the transmission of B virus infection to humans and to improve infection control in nonhuman primate facilities (CDC 1987).

Two years later, in 1989, there was another disease outbreak, this time with animals in quarantine. A large NHP facility experienced increased morbidity and mortality in a group of recently imported cynomolgus macaques (Renquist 1990). The severity of the disease, which presented as a hemorrhagic disorder and was eventually identified as simian hemorrhagic fever, prompted the involvement of the CDC, the National Institutes of Health (NIH), and the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). The outbreak was controlled only after implementing “Stringent management techniques in excess of those normally used for quarantine, [which] limited the outbreak to about 400 macaques out of a population of over 2000” (Renquist 1990). The number of animals lost in the outbreak (20%) was due in part to spontaneous death and in part to aggressive infection control procedures that entailed the depopulation of rooms with infected animals.

This disease outbreak caused concern for the safety of both human and animal populations. The clinical presentation of simian hemorrhagic fever (SHF) in nonhuman primates is very similar to that observed in animals infected with agents such as the Ebola and Marburg viruses. SHF is not a zoonotic agent but its presence in the animal facility at the time raised concerns about the possible presence of a more serious human pathogen. The fact that the outbreak spread from one group of primates to another during quarantine suggested lapses in facility design features and infection control practices.

The principal incident that prompted a CDC review of quarantine practices occurred later in 1989, when a group of cynomolgus macaques quarantined in an animal facility in Reston, Virginia, showed increased levels of morbidity and mortality. As in the earlier case, several of the animals exhibited clinical signs consistent with a hemorrhagic disorder. The clinical workup revealed possible infection with not only simian hemorrhagic fever but also Ebola virus, one of the most lethal filoviruses (Jahrling et al. 1990). The combined efforts of primate clinicians, virologists, and epi-

demiologists determined that both viruses, previously associated with outbreaks in Africa, were now associated with a group of monkeys that had originated in the Philippines (Dalgard et al. 1992), although the specific source of the infections remains unknown. Further epidemiological research indicated a broad distribution of filoviruses (Miranda et al. 1999).

In response to the publicity surrounding these events, the major international air carriers halted NHP shipments to the United States. Furthermore, the detection of a Biosafety Level 4 pathogen within 23 miles of the nation’s capital resulted in a nationwide review by CDC of primate populations in quarantine. Personnel in regular contact with quarantined or nonquarantined nonhuman primates were surveyed to determine the potential for zoonotic transmission (CDC 1990a). Seropositive humans, including an animal handler at the Reston facility, were identified but no individuals had signs of clinical disease.

## CDC’s Response to the Outbreaks

The most important discovery was that the “Reston agent” was an Ebola-like virus but not identical to the African strain. Based on this finding, CDC surveyed other NHP quarantine facilities and found one in Pennsylvania that reported increased morbidity and mortality in cynomolgus macaques (CDC 1990b). The agency conducted an investigation of this facility and found that these animals also were infected with the new Ebola-like agent, morphologically the same as the one identified in Reston. CDC then initiated a comprehensive review of quarantine facility practices and standard operations. Teams of public health veterinarians and quarantine officers visited facilities nationwide, and in a public meeting in Atlanta CDC sought input from commercial importers, universities, regional primate centers, the research community, and government agencies.

CDC modified the quarantine program to address some of the issues that emerged in its review. In January of 1990 the Centers issued Interim Guidelines for Handling Nonhuman Primates during Transit and Quarantine, which supplemented the existing quarantine regulations to address specific issues both in the transportation of nonhuman primates to quarantine facilities and in the quarantine procedures once the animals arrived (CDC 1990b). CDC also established a Special Permit for Importation program with additional control measures (CDC 1990c) for the importation of rhesus macaques, cynomolgus macaques, and African green monkeys (DeMarcus et al. 1999). The measures focus on these species because they demonstrated seroreactivity to filoviruses and represented the vast majority of NHPs entering the country. The goal of the Special Permit requirements is to protect public health and optimize occupational health and safety during the transport of nonhuman primates. (The permit regulations cover all aspects of animal transport, but focus on procedures once the animals have arrived in the United States and are under CDC authority.)

The Special Permit for Importation regulations required the implementation of the Interim Guidelines for Handling Nonhuman Primates during Transit and Quarantine, which have three sections that cover general guidelines and specific recommendations on transit and quarantine. We reproduce the guidelines here (CDC 1990c).

### General Guidelines for Handling Nonhuman Primates during Transit and Quarantine

1. Management of transportation and quarantine facilities should ensure that personnel are instructed as to the hazards of handling nonhuman primates, that protective apparel is available, and that the need for its use is understood. Management should provide periodic retraining as well as reinforcement of these procedures.
2. Persons working with nonhuman primates should not drink, eat, or smoke while handling animals, cages, crates, or materials from such animals.
3. Access to animal holding areas should be restricted to essential personnel. The number of persons involved in the care, transport, and inspection of nonhuman primates should be the minimum necessary to expedite efficient and humane handling.
4. All staff in direct contact with animals should wear protective clothing (i.e., gloves and surgical masks and gowns) when opening crates, removing foreign materials from crates, feeding the animals, removing dead animals, or handling bedding materials. These persons should remove disposable protective clothing before leaving the animal holding facilities; this clothing should be autoclaved or incinerated. Nondisposable contaminated clothing should be disinfected on site before laundering.
5. Separate nonglass water bottles should be provided for each nonhuman primate during transit and quarantine. Reusable items should be adequately decontaminated between uses.
6. All animal waste, bedding, uneaten food, and other possibly contaminated items should be treated with appropriate disinfectant before removal from the animal holding facilities. All cages, feeding bottles, and other possibly contaminated items should be disinfected between each use or before disposal. Glass items should not be used.
7. A separate disposable needle and syringe (and, if required, infusion equipment) should be used for each animal, then autoclaved or incinerated. A clean needle should be used for any access to multidose vials (e.g., of ketamine) to avoid contamination. After each use on a group of quarantined animals, multidose vials must be autoclaved and discarded. Disposable supplies should be used whenever possible and must not be reused. Nondisposable equipment should be thoroughly disinfected.
8. Caution must be used to prevent infection from potentially contaminated needles, scalpels, or other sharp instruments, particularly during disposal of needles. Used needles should not be recapped by hand; removed from disposable syringes by hand; or bent, broken, or otherwise manipulated. Only one set of disposable syringes, needles, and scalpels should be used per animal. Used disposable syringes and needles, scalpel blades, and other sharp items should be placed in puncture-resistant containers kept as close to the work site as practical.

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9. Nonquarantined animals should never be placed in, or permitted access to, areas with quarantined animals. This includes unrestrained pets, feral animals, and animals temporarily boarded for overseas travelers or destined for export.
10. Management should keep records of all serious febrile illnesses (fever  $>101.3$  F [sic] (greater than  $38.5$  C°[sic]) for  $>2$  days) in persons having direct contact with nonhuman primates in transit or in quarantine and should promptly notify CDC if such an illness occurs. Management should ensure that the physician providing care is informed that the patient works with and/or has been exposed to nonhuman primates.

### Additional Guidelines for Handling Nonhuman Primates during Transit

1. Persons who handle crates or pallets containing nonhuman primates should be protected with elbow-length reinforced leather gloves, long-sleeved shirts and trousers of sufficient thickness to resist minor tears, and sturdy waterproof shoes or boots. The gloves should be of a thickness that prevents penetration of splinters or other crating debris. During warm weather, garments may be of lightweight materials to minimize discomfort. Disposable coverall suits can be used for added protection.
2. Crates should be free of sharp projections that can cause scratches or wounds to workers. Handles should be present on the sides of crates, and mechanical lifting and transporting devices should be used whenever possible.
3. Crates containing nonhuman primates should be separated by a physical or spatial barrier from all other animals and cargo at all times.
4. Wherever possible, nonhuman primates should not be handled directly. Live animals should be removed from cages only when staff can be supervised by a qualified veterinarian. Procedures that may result in bites or scratches should be avoided.
5. Management of holding facilities should maintain records to document the removal of dead animals; documentation should include the date, shipment number, country of origin, species, importer, and disposition of the removed animal. The carcass must be placed in waterproof double bags and incinerated. The Division of Quarantine, Center for Prevention Services (CPS), CDC, should be notified.
6. Temporary holding facilities should document all injections or parenteral infusions administered to nonhuman primates.
7. If animals are removed from a shipment while in transit, facilities retaining these animals should ensure full compliance with these guidelines and should maintain records on the care and disposition of animals. Temporary facilities holding animals in this way must be registered as importers of nonhuman primates.

### Additional Guidelines for Care of Nonhuman Primates during Quarantine

1. Quarantine facilities should be secure, with access limited to authorized, trained, and informed personnel.

2. Quarantine facilities should be designed to be adequately disinfected. Management and staff should refer to the *Guide for the Care and Use of Laboratory Animals* and the CDC/National Institutes of Health *Biosafety in Microbiological and Biomedical Laboratories*, second edition (Animal Biosafety Level 2, p. 52) (4), for information on design and operation of animal holding facilities. Staff should use protective clothing, gloves, and masks at all times when in the animal holding facilities; these items should be disinfected or disposed of properly.
3. Staff should use fresh clothing when going from room to room.
4. Adequate equipment and space should be available for discarding and disinfecting all equipment, clothing, and caging.
5. Care should be taken to avoid scratches and bites of animals. All handling of individual animals should be done while the animals are anesthetized or tranquilized, and animals should be maintained in squeeze-back cages wherever possible.
6. Different lots of primates should not be mixed while in quarantine (minimum 31 days).
7. Management should notify the Division of Quarantine, CPS, CDC, of severe illnesses and deaths in recently imported primates. CDC will advise management on collection of specimens for investigation of cause of death.

The implementation of these requirements has enhanced infection control practices and improved quarantine operations at facilities throughout the United States.

CDC conducted a further review of nonhuman primate quarantine practices in 1993 after 11 of 16 cynomolgus macaques imported from Mauritius tested positive for tuberculosis (CDC 1993). The review, which included 249 NHP importations from June 1990 through May 1993, drew attention to the prevalence and risk of tuberculosis in nonhuman primate populations and the need for improved surveillance and diagnostic procedures. As a result of the review findings, CDC issued the following additional guidelines to update TB surveillance and disease reporting during quarantine (CDC 1993):

#### Interim Guidelines for Tuberculin Skin Testing of Nonhuman Primates During Quarantine

1. All imported nonhuman primates (NHPs) should be administered a minimum of three tuberculin skin tests (TSTs), with at least 2 weeks between tests, before release from import quarantine. All cohorts containing animals with positive or suspicious reactions should remain in quarantine and be administered at least five additional TSTs following removal of the last affected animal.
2. Records of all TSTs performed during the lifetime of each imported NHP should be maintained at the facility housing the NHP and should accompany the NHP during moves to other facilities.
3. Necropsies of imported NHPs should be performed only by qualified veterinary pathologists or laboratory-animal veterinarians accredited by the American Association for Accreditation of Laboratory Animal Care. Necropsies of tuberculosis (TB)-suspect animals (NHPs with positive or suspicious TST results or NHPs from cohorts within which TB-infected ani-

- mals have been identified previously) should be performed under Animal Biosafety Level 3 conditions. Regardless of gross necropsy findings, fresh and formalin-fixed tissue specimens—to include tracheobronchial lymph node, liver, lung, and spleen—from all such NHPs should be collected for laboratory examination. Granulomatous lesions found in any NHP at necropsy, regardless of whether TB was previously suspected, should be submitted both for laboratory examination for acid-fast bacilli and for mycobacterial culture. Necropsy reports should address all major organ systems and should incorporate clinical history and laboratory findings.
4. All NHPs with positive or suspicious TST results, necropsy findings, or laboratory results should be reported to CDC (telephone [404] 639-8108; fax [404] 639-2599) within 48 hours, along with a copy or summary of the individual NHP's health records.
  5. All facilities that house NHPs should adhere to the Institute for Laboratory Animal Research recommendations regarding baseline and (at a minimum) annual TST screening of employees and routine safe work practices involving NHPs. Results of employee TSTs should be maintained and reviewed by the occupational health professional responsible for the employee health program. Skin-test conversions among employees may suggest transmission of TB in the facility. Workers exposed to NHPs with laboratory-confirmed TB should receive a post-exposure TST and, if negative, a TST 3 months after exposure. Workers with a reactive skin test should be referred for medical evaluation.
  6. All persons directly involved in the transportation and quarantine of imported NHPs should adhere to the importer's standard operating procedures approved by CDC under the special permit process.
  7. In addition to the protective clothing requirements described in previously published guidelines (3), inspection personnel and other transit workers who handle crates or pallets containing imported NHPs should wear disposable dust/mist respirator masks and be trained in their proper use. Because of the potential for aerosol transmission of certain pathogenic bacteria (e.g., TB) and viruses, face shields or eye protection should be worn by workers whose faces may come within 5 feet of the NHPs.
  8. All NHPs should be individually identified with a unique number or alphanumeric code permanently applied to the animal by tattoo. Health certificates, shipping documents, and animal health records should always include this number or code and the age, sex, and species of the NHP.

#### Impacts of the CDC Guidelines

The goal of the CDC regulations has been the protection of human health, and by that standard the CDC nonhuman primate quarantine regulations have been a remarkable success (Rollin et al. 1999). In terms of NHP importation and quarantine, the impacts have also been beneficial. At the time of the agency's review in 1989 there were more than 140 CDC-registered importation facilities in the United States and the number of nonhuman primates imported annually approached 24,000 animals. The review process and the required changes caused many facilities to reevaluate

the necessity of maintaining their CDC quarantine registration. As a result, by 1999 the number of such facilities had decreased to 27 and the number of animals imported annually had fallen to approximately 8,500, although the latter number subsequently rose again, to more than 27,000 in 2006 (Mullan 2006).

Similarly, before the implementation of the Special Permit process, nonhuman primate mortality rates during quarantine and shipment reportedly reached 20% (DeMarcus 2002), whereas by 1999 the rates had decreased to less than 1% (DeMarcus et al. 1999). This trend has continued to 2006 (the latest year for which data are available) when, despite the large number of imported NHPs, mortality associated with transportation and quarantine remained below 1% (Mullan 2006). The decrease in NHP mortality associated with transit and importation is due in part to improved procedures and in part to a change in the sources of imported animals.

## NHP Sources and Quarantine Facilities

Since the occurrence of the Ebola-like (subtype Reston) virus, the number of nonhuman primates imported into the United States has increased significantly (DeMarcus 2003). As advances in the understanding of NHP genetics have stimulated new lines of research (Pennisi 2007), the global increase in demand for nonhuman primates is likely to continue based not only on research needs in the United States and in other countries but also on the needs of countries that supply animals to other countries (Robinson and Beattie 2003).

Many primate source countries now export only the offspring of animals maintained in captive breeding programs. These animals may be in breeding enclosures or from island free-range breeding colonies where animals have been introduced and do not represent endemic fauna (Ervin and Palmour 2003; Stanley 2003). In either case the animals are held in captivity and complete standard conditioning protocols before shipment to other facilities (Hsu and Jia 2003). Such conditioning programs, coupled with the screening of animals for microbiological pathogens, decrease the rate of morbidity and mortality in transported animals and improve the quality of animals used in research. These outcomes are an obvious benefit to both animal welfare and the quality of research.

Beyond the CDC core requirements of a primate quarantine program, each facility determines the specific elements of its quarantine program based on the intended use of the animals after quarantine and the size of the on-site NHP population that may be put at risk by the introduction of new animals. The operation and design of a quarantine facility vary depending on whether the intent is to import a single male to a zoological garden for a captive breeding program or large numbers of macaques to meet critical research needs.

## Elements of a Nonhuman Primate Quarantine Program

The challenges of operating an NHP quarantine facility remain the same as those first encountered in the importation of nonhuman primates. Planning for the worst possible scenario is the best approach in quarantine design and operation. In this section we outline the principal components of an effective quarantine program.

### Quarantine Facility

The most important element of a quarantine program is a suitable animal facility that allows physical segregation of the quarantined population—including air space, wastes, body fluids, and tissues—from all other animals and personnel in proximity. The ideal is complete physical separation: a separate, dedicated, controlled-access building with a dedicated high-efficiency particulate air (HEPA)-filtered heating, ventilation, and air conditioning (HVAC) system and infectious waste disposal. Unfortunately, separate facilities and equipment dedicated for quarantine use are rarely an option because of expense and space limitations. If the quarantine area is located in a multiuse building, the following measures are essential to contain or minimize the risk of infection (NRC 1996, 73-78):

- Limit access to the quarantine area to appropriate personnel.
- Provide anterooms to quarantine rooms for personnel to don protective clothing before entering the animal room.
- Install doors with windows to allow observation of the room before entering in the event that an animal has escaped from its enclosure.
- Ensure that all animal rooms meet the standard design requirements for NHP housing, with smooth impervious surfaces, adequate lighting, and appropriate equipment for cage sanitation.

Although not required, the ideal physical layout is a “clean/dirty corridor system” that enables the entry of personnel and materials to the quarantine room from a “clean” anteroom, and the exit of personnel and potentially infectious materials to a “dirty” corridor that leads to facilities for waste disposal and personal hygiene. If such a layout is not possible, each quarantine room should have an anteroom that serves as a transition zone, where protective clothing can be donned or removed according to rigid procedures to contain and properly dispose of any potentially infectious materials.

The size of a facility’s quarantine rooms depends in large part on the institutional program—they may accommodate a single animal or as many as 200 animals. There are advantages and disadvantages to the number and size of

quarantine rooms. Dividing quarantine shipments into subgroups housed in separate smaller rooms and treating them as isolated cohorts may provide the twin benefits of reducing the number of animals at risk in the event of a disease outbreak and limiting secondary spread of infection. But this benefit may be limited as quarantine will likely be extended for all animals in the shipment to account for the possibility that the entire shipment had direct contact immediately before shipment and therefore will have had the same pathogen exposure. Furthermore, smaller quarantine rooms decrease the space efficiency of the quarantine facility. Animal vivariums in general and quarantine rooms in particular are some of the most expensive research spaces to construct, and the additional walls, doors, and air handling equipment required by smaller rooms can dramatically increase the cost of the total square footage (Ruys 1990, 555-563).

The number and size of the quarantine rooms is only one element of the proper design of a quarantine facility. At a minimum, the containment facility design must meet Animal Biosafety Level 2 “plus” requirements, indicating the need for ABSL-3 personal protective equipment and procedures (DHHS 1999). Ideally, there should be support areas such as clinical pathology laboratories, necropsy facilities, and if appropriate a radiology facility in the same containment area as the animal rooms.

Whether designing a quarantine space from the ground up or remodeling existing space for primate quarantine, it is important to plan for the disposition of infectious waste, biological samples, and, in the event of an animal trauma or death, animal remains. Careful planning is essential to determine how these items will be transported to appropriate areas and, just as importantly, how they will be handled in the laboratory or necropsy facility once they arrive. It is important to decide in advance how best to handle sample processing and necropsy while maintaining biological containment. A simple but helpful exercise in assessing the suitability of any animal room, laboratory, necropsy suite, or support space for quarantine is to mentally review how one would completely disinfect the entire room and its contents in the event that a BSL-4 pathogen outbreak occurred in your quarantine facility.

The same issues are present when determining how to receive animals in quarantine. The facility design should include loading docks or shipping areas that allow the vehicle transporting nonhuman primates to approach as close as possible to the animal entrance door for quarantine. It is important to ensure a direct and fully enclosed transport route between the truck and quarantine area to prevent the escape of an animal in the event of a broken crate or open door. Animal crates should meet the specifications of the Special Permit program as well as the Animal Welfare Act, the Lacey Act, and the International Air Transport Association (CFR Title 9b; Lacey Act 18 USC 42 [50 CFR Part 14 Subpart J]; IATA Live Animal Regulations, 33<sup>rd</sup> ed., 2006).

## Health Screening and Animal Husbandry

Quarantine programs are the basis for detecting disease in incoming shipments of animals and for either treating animals as appropriate or euthanizing those with infectious agents that cannot be treated. The quarantine period provides the opportunity to conduct a physical exam of the animals, perform general health screening, and also screen for the presence of specific viruses by testing for viral-specific antibody or antigen or by culturing the virus. Perhaps the most important assessment tool in quarantine is the daily observation of the animal itself and of its stool, urine, and food consumption, all of which are important factors in assessing animal health. In addition to these physical observations, a primary quarantine program should include, at a minimum, the following health screens:

- three consecutive negative tuberculin skin tests (TSTs) at 2-week intervals (but cohorts containing animals with positive or suspicious reactions should remain in quarantine and undergo at least five additional TSTs after removal of the last affected animal);
- isolation of the arriving animals for a period of 31 days, with daily observation for signs of illness (e.g., weight loss, dyspnea, diarrhea, lethargy);
- physical exam, with additional weighings during immobilization for the TST;
- baseline blood values;
- parasitology exam; and
- rectal culture.

Animals shipped on international carriers will have been transported in multiple conveyances over lengthy periods before arriving at the receiving facility—total time in the shipping crate may range from 12 to 72 hours or longer. Thus animals entering quarantine are typically stressed and may also be dehydrated or hypoglycemic. It is important to let them acclimate to their new surroundings and ensure that they eat and drink (it may be necessary to train them to use automatic watering devices). The animals should have an acclimation period of at least 2 to 3 days before their immobilization for physical exams and their initial TB skin test. In addition, weighing the animals at each immobilization is another means to assess their acclimation.

If possible, animals should be singly housed in squeeze-back cages to facilitate safer observation and chemical immobilization. During quarantine, the cages should be cleaned on a regular basis and thoroughly sanitized every 2 weeks in compliance with the *Guide for the Care and Use of Laboratory Animals* (NRC 1996) and USDA regulations (CFR Title 9a). Cages may be sanitized in place with chemical disinfectants while the animals are immobilized and moved from their enclosures for examination, weighing, and TB testing. In the event of a disease outbreak in quarantine, it may be necessary to alter the cage sanitation schedule to implement specific infection control measures. If daily hosing is a part of routine husbandry the hoses



should be used in a manner to avoid the creation of aerosols when cleaning the cage. If permitted by state and local authorities, biological waste may be disposed of in the sanitary sewer system with disinfectant. Solid waste removed from the room should be autoclaved or appropriately bagged for transport to a medical waste treatment facility. In addition to CDC regulations, local, community, and state agencies should be consulted to determine appropriate disposal procedures.

## Animal Illness and Necropsy

In the event that an animal develops illness while in quarantine, it will be necessary to determine the availability of appropriate diagnostic procedures and options for treatment. Animals typically are not removed from the room during quarantine due to the potential for cross contamination of animals from one enclosure to another and the risk of exposure of nonquarantined animals. This restriction may limit treatment options because diagnostic or therapeutic equipment brought into a quarantine room must be chemically disinfected or autoclaved before leaving the room; and multiple dose vials or containers must be bagged for disposal as infectious waste before removal from the room. Additionally, outside clinical laboratories are often not equipped to handle diagnostic specimens from animals in CDC quarantine. The ideal option is to have dedicated clinical equipment in the biocontainment area for analysis of samples. Otherwise, arrangements for strict containment procedures must be documented before transport.

## Personnel Training and Occupational Health

The integrity of a biocontainment operation depends on appropriate standard operating procedures (SOPs) and personnel training. All quarantine personnel should be familiar with exposure control methods (NRC 2003, 98-119).

Staff entering animal quarantine rooms should wear appropriate personal protective equipment (PPE) to prevent exposure to potentially infectious agents; standard PPE includes moisture-resistant protective clothing, double protective gloves, a NIOSH-approved N95-rated disposable particulate respirator, protective footwear, and a face shield to prevent splash contact with the eyes and mucous membranes.

All staff should receive training in infection control procedures and the proper methods for donning protective clothing when entering quarantine rooms, with a fit testing for the respirator and training on how to wear the mask properly. It is equally important to train staff in the appropriate methods for removing protective clothing when exiting the rooms. This training should integrate engineering controls (i.e., quarantine facility design elements such as clean/dirty corridors and air pressure differentials) with cor-

rect work practices to provide an optimal level of infection control.

In addition to requiring personnel to read SOPs and quarantine procedures it may be helpful to provide classroom demonstrations and workshops that include active discussions and staff involvement in the development, review, and revision of quarantine procedures. Proper procedures in quarantine husbandry should be second nature to personnel. Such knowledge requires extensive training, particularly for staff who handle quarantine responsibilities infrequently. Training methods can include creative exercises, such as spraying individuals dressed in protective clothing with a colored substance (e.g., ketchup) and observing how they remove the soiled protective clothing without “contaminating” themselves. Practical demonstrations like this help engage staff in training and can often result in helpful input on infection control procedures.

All personnel should be familiar with universal precautions and the proper use and disposal of sharps (e.g., needles, catheters, and scalpels) (NRC 2003, 98-119). Training should address common pathogens encountered in nonhuman primates and the different potential routes of transmission—fecal-oral or respiratory transmission, needle sticks, other injuries, and exposure of skin and/or mucous membranes. Efficacy of training and SOPs can be assessed using either written quizzes or observation of employee performance of tasks. The best-designed quarantine facility can be compromised by improper procedures in infection control and failure to properly use protective clothing.

Individuals working in nonhuman primate quarantine should enroll in the facility’s occupational health program (requirements for such programs are available in several resources; cf. NRC 1997, 197-121; 2003, 98-119). All new employees should complete an initial health evaluation that includes a TB skin test or chest radiograph and appropriate immunizations. There should also be follow-up health assessments and TB skin tests on a regular basis (e.g., every 6 months or annually), as determined by the facility management and occupational health staff. All personnel working in quarantine should be informed of the potential hazards in the workplace, including both infectious agents and chemicals used in daily husbandry. In addition, the occupational health program should monitor and record illness and injuries among personnel working in quarantine, including the occurrence of fever ( $>101.3^{\circ}\text{F}$  or  $38.5^{\circ}\text{C}$ ) or other symptoms that may be associated with exposure to nonhuman primates.

Workers should receive annual training to report to management all workplace injuries or potential exposures. Management must maintain an injury log and a record of follow-up on all injury reports. Such a record can be a valuable tool for evaluating workplace practices and modifying procedures if it becomes apparent that some aspect of the work environment is frequently associated with employee injuries.

It is important that employees who report an animal-related injury not receive negative reinforcement as the con-

sequence may be a failure to report injuries. Studies in the research setting have shown that a significant number of injuries, including those involving exposure to blood or body fluids, go unreported, in part because of concern about negative feedback from supervisors (bin Zakaria et al. 1996). The time to provide feedback and corrective action is when employees are observed either not following safe work practices or improperly wearing, using, or disposing of personal protective equipment. The goal is to reinforce correct infection control practices and at the same time remove any negative reinforcement associated with reporting animal-related exposures.

## Quarantine Disease Surveillance

Many NHP quarantine programs now address not only agents that cause clinical diseases—most of which are still associated with tuberculosis or enteric pathogens (Mullan 2006)—but also those that represent a potential confound in the research animal.

### Tuberculosis

Nonhuman primate quarantine has had a long-term focus on tuberculosis and the devastating impact it can have on captive colonies (CDC 1993; Ruch 1959), where animals are put at risk from exposure to humans and increased density in captivity (Kalter 1970). TB also is an established human health hazard and is a primary concern of CDC in monitoring the condition of animals in quarantine.

The tuberculin skin test has been the central element in TB screening for over 60 years in nonhuman primates (Ruch 1959). It has aided in the detection and elimination of tuberculosis both in quarantine and in colonies where the infection may have been introduced by human contact or by a latently infected animal. At the same time, the test has significant limitations as, for example, poor technique in its administration can lead to false positives and issues of sensitivity and specificity (Garcia 2004; Vervenne 2004). Issues associated with TB diagnostics are addressed elsewhere in this issue (Lerche et al. 2008), but it is important to note here that although the perception of risk from clinically significant viral infections remains high, the most significant challenge to primate importation facilities is the screening process for tuberculosis. The development of rapid, cost-effective *in vitro* diagnostic assays of an infection with mycobacterial agents of significance is needed to improve the efficiency of nonhuman primate quarantine. The development of these assays could also benefit colony management by allowing differentiation of exposure to atypical mycobacterial agents rather than agents such as *M. tuberculosis* or *M. bovis*.

### Enteric Pathogens

A comprehensive quarantine program should outline procedures to screen for bacterial, protozoan, and metazoan

pathogens such as *Shigella* sp., *Salmonella*, *Yersinia* sp., *Entamoeba histolytica*, *Balantidium coli*, and metazoan parasites. These agents represent potential hazards both to colony health and to humans if appropriate infection control procedures are not rigidly followed. But if they are detected, it is usually possible to treat them in quarantine. Some facilities routinely treat animals prophylactically with antihelminthics and antiprotozoal compounds in addition to conducting standard parasitology exams (Fegley and Sauer 1960; Pucak et al. 1977).

## Viral Infections

The challenge in the health screening of imported nonhuman primates is to improve the diagnostic tools available for effective and reliable identification of animals infected with undesirable viral pathogens. Nonhuman primate quarantine has historically focused on excluding viral agents with significant zoonotic potential, such as yellow fever or Marburg virus. After the identification of the Ebola-like agent in imported nonhuman primates, CDC initially required serum sampling and testing of all imported Special Permit Species for filovirus infection, but subsequently modified the requirements so that the liver tissue of any suspect illness or death of a Special Permit species is tested for the presence of filovirus antigen (Tipple 1996). If any animals in a shipment exhibit signs suggestive of a hemorrhagic disorder, serum must be collected from all animals in that shipment for an immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) antibody test for filovirus.

Many institutions have expanded viral screening in their domestic breeding colonies to include viral pathogens that may be asymptomatic but represent potential research confounds; these include simian retroviruses and herpesviruses such as B virus and cytomegalovirus. For macaque species the primary retroviral infections are simian retrovirus (SRV), simian T cell lymphotropic virus (STLV-1), simian foamy virus (SFV), and simian immunodeficiency virus (SIV) (Mansfield and King 1998). These viral agents are present in wild primate populations and are not usually associated with clinical disease in their natural host, with the exception of simian retrovirus, which is frequently associated with spontaneous disease in its natural host, macaque species, in their country of origin (Lerche et al. 1991). But any of these retroviral agents can be a potential confound for many research fields (Lerche and Osborn 2003).

These agents have not been demonstrated to cause disease in humans, although asymptomatic human infections have occurred with some agents (Switzer et al. 2004). The human health risk associated with exposure to B virus in macaques and the potential research confounds represented by retroviral infections have resulted in institutional and national programs working to create specific pathogen-free (SPF) primate colonies (Robinson and Beattie 2003). Most of these colonies have been established through the testing and selection of NHPs from domestic breeding colonies to

ensure breeding animals that are free of the disease agent in question (Lerche et al. 1991).

## Future Directions

The importation of nonhuman primates continues to represent a critical element in meeting research needs for NHP models. The number of animals imported has increased steadily over the past 4 years and will continue to expand to meet research needs in areas such as genetics, infectious disease, and neuroscience (Mullan 2006; Robinson and Beattie 2003). But the exclusion of animals with latent viral or even bacterial infections that are not health risks may limit the availability of certain populations or species and complicate effective genetic management of domestic breeding colonies.

The use of primary quarantine to prevent the introduction of disease-causing agents is an appropriate and valuable step in protecting human health as well as the health of both research and breeding colonies. And while the goal of the CDC regulations is to protect public health, the regulations and infection control programs that have developed in response to the CDC requirements have also dramatically reduced morbidity and mortality of animals during shipment and upon their arrival at quarantine facilities.

The biggest challenges now facing nonhuman primate importation are the need for improved diagnostic tools to screen for diseases such as tuberculosis and the ability to respond rapidly when a new agent is identified. The 2003 SARS outbreak in China disrupted the international NHP supply when the Chinese government, in an effort to control the outbreak, banned all animal movement. Primate exports resumed after surveys of animal handlers and the species they worked with implicated the palm civet as a primary source of infection (CDC 2003b).

The continued demand for nonhuman primates increases the likelihood that new unknown agents will be identified. The best response to such an occurrence will be the continued rigorous application of the lessons learned about nonhuman primate quarantine over the past 60 years.

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