

# Reston ebolavirus in Humans and Animals in the Philippines: A Review

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The 2008 *Reston ebolavirus* infection event in domestic pigs has triggered continuing epidemiologic investigations among Philippine health and veterinary agencies in collaboration with international filovirus experts. Prior to this, there were only 3 known and documented *Reston ebolavirus* outbreaks in nonhuman primates in the world, all traced back to a single geographic source in the Philippines in a monkey breeding/export facility. The first one in 1989 was the first-ever Ebola virus that emerged outside of Africa and was also the first known natural infection of Ebola virus in nonhuman primates. When it was first discovered among laboratory monkeys in the United States, the source was immediately traced back to the farm located in the Philippines. The second outbreak was in 1992–93. The third episode in 1996 was the last known outbreak before *Reston ebolavirus* reemerged in pigs in 2008. The isolated outbreaks involving 2 animal species bring forth issues requiring further investigations, and highlight the significance of intersectoral collaboration to effectively address zoonoses prevention and control/response in the interest of minimizing public health risk.

The Philippines is still the only known geographic source of *Reston ebolavirus* (REBOV). Until the 2008 re-emergence of REBOV infection in domestic pigs in the Philippines, only 1 species, the *Macaca fascicularis* or cynomolgus macaques in 1 laboratory animal breeding facility, was known to be naturally infected [1]. Although serologic and epidemiologic evidence suggest that the virus can be transmitted to occupationally exposed individuals, it has not caused the same overt serious illness in humans as the African ebolaviruses [2–4]. When the virus was detected in a different domestic animal species, the likelihood of further infection and the potential pathogenicity for humans cannot be discounted. Following the isolation of REBOV infection from domestic pigs in 2008, epidemiologic investigations were initiated

by Philippine health and veterinary agencies in collaboration with international filovirus experts. Prior to this, there were only 3 known, documented REBOV outbreaks in nonhuman primates (NHPs) in the world, all traced back to a single source in the Philippines. The first outbreak in 1989 was the first-ever ebolavirus that emerged outside of Africa [5, 6]. It was also the first known natural infection of ebolavirus in NHPs. When it was first discovered among laboratory monkeys in the United States, the source was immediately traced back to the Philippines. The second outbreak was in 1992–93. The third episode in 1996 was the last known outbreak before REBOV reemerged in pigs in 2008.

The business of exporting purpose-bred NHPs in the Philippines has been occurring since the early 1980s. These animals are used for preclinical research, disease modeling, drug development, experimental infections, and biological production and testing. *M fascicularis* is the only indigenous simian species in the Philippines; therefore, collection sites and quantities are government regulated. Breeders are collected from wildlife trapping areas mostly in the southern Philippines, and brought to holding and rearing facilities in or around Metro Manila. In the 1980s and 90s, >5000 animals per year were exported mainly to Japan, United States, and Europe. Currently, these have been reduced to <1500 annually.

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National authorities (the Departments of Agriculture and Environment and Natural Resources) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) allow only export of laboratory-conditioned or captive-bred animals. The primate breeders and exporters are mostly self-regulated in terms of animal care and use, though they follow all the official trade regulations, including CITES. Indonesia, Vietnam, and Mauritius are other major sources of this same species of laboratory monkey.

### 1989–1990 OUTBREAK

The original outbreak was first detected among imported NHPs in a quarantine facility in Reston, Virginia, USA [5, 6]. This outbreak was initially suspected to be simian hemorrhagic fever (SHF), another viral disease caused by an arterivirus, and indeed SHF virus was isolated from the animals. However, Ebola virus was also noted by electron microscopy and indirect fluorescent antibody assays in the cultures.

The NHPs were traced back to a Philippine exporter that shipped infected monkeys to Virginia, Pennsylvania, and Texas. As soon as the alert was received from US authorities, the Philippine Department of Health Field Epidemiology Training Program (FETP) and the Research Institute for Tropical Medicine (RITM) mounted a joint outbreak investigation with the US Naval Medical Research Unit No. 2 and the US Army Medical Research Institute of Infectious Diseases. Soon after, the US Centers for Disease Control and Prevention (CDC) Special Pathogens Branch and Quarantine and Border Health Services Branch (known at the time as the Division of Quarantine, National Center for Infectious Diseases, Centers for Disease Prevention and Control) joined the investigation. The objectives of the outbreak investigation in humans and animals were to trace back to the source monkey facility, trace back to wildlife collection sites, and conduct epidemiologic investigations in the other monkey breeding facilities in the Philippines.

Only the source facility had infected animals [7]. Daily morbidity and mortality surveillance over 2.5 months revealed that the REBOV antigen was positive in 53% of 161 deaths. The case fatality rate was 82%. Clinical manifestations included diarrhea (50%), respiratory illness (34%), and hemorrhage (1%). Infection was documented in 52 of these monkeys by either seroconversion (>4-fold increase in immunofluorescence titer) or detection of antigen in the blood or liver. This was the first-ever Ebola virus isolation outside Africa and the first documented natural infection in NHPs.

In response to this disease emergence, the US Quarantine and Border Health Services Branch initiated stricter disease control measures for handling NHPs during the process of importation, emphasizing the protection of occupationally exposed individuals, use of containment facilities and procedures,

measures to prevent spread of infection among animals, and laboratory testing of animals that die or become ill during quarantine [8].

### 1992–1993 OUTBREAK

The REBOV was again introduced into an NHP quarantine facility in Sienna, Italy. Infected monkeys were traced back to the same Philippine farm and exporter. No humans became ill, although 2 had immunoglobulin G (IgG) antibodies [9]. Some collections of bats and rodents in the farm yielded negative antigen (Ag) and antibody (Ab) results (unpublished).

### 1996 OUTBREAK

Infected macaques were again imported into the United States from the Philippines in March 1996 [10]. Joint investigations with the CDC Special Pathogens Branch were initiated readily with RITM and FETP in the Philippines [3]. The objectives were to identify the source of the virus among monkey-breeding and export facilities, to reestablish surveillance and testing, and to assess the risk and significance of REBOV infections in humans who work in these facilities.

Over a 5-month period, acutely infected animals were found at only 1 facility using Ebola antigen detection enzyme-linked immunosorbent assay (ELISA) protocol from the CDC. Virus transmission documented through daily morbidity and fatality surveillance among monkeys in the source facility showed that 14 of 21 occupied animal houses had infected monkeys.

Three of 1732 monkeys had detectable antibodies, and 132 of 1011 dead, dying, or sick monkeys were antigen positive. Active transmission among the animals was facilitated by poor infection control practices, which continued for several months and was stopped only when the facility was depopulated. One of 251 animal handlers had antibodies detected by Ebola antibody detection ELISA protocol from the CDC. None of the 251 employees of the facilities or the 4 contacts of previously antibody-positive individuals reported any Ebola-like illness.

From 1989 to 1996, a total of 458 persons who were occupationally exposed, including animal handlers, trappers and administrative support personnel involved in the monkey export facilities, were tested. Five (1%) had detectable IgG antibodies (Table 1). All seropositives were from the only facility where the animal outbreaks occurred. Animal handlers in the hospital of this facility had significantly higher titers than the rest of the facility, indicating high-risk virus exposures. This review affirmed that human REBOV infection is rare.

The mandatory quarantine and laboratory testing requirements imposed after the 1989–1990 outbreak were effective in the early detection and containment of REBOV infection in newly imported animals, thus reducing potential public health risks.

**Table 1. Summary of Ebola Reston Virus Serological Studies on Persons Occupationally Exposed to Monkeys in Breeding and Export Facilities in the Philippines, 1989–1996**

Year of report	Place	IgG positive	No. tested	%
1989–1990	Philippines, Calamba	3	18	17%
	Philippines, other sites	0	168	0%
1993	Philippines, Calamba	2	21	10%
1996	Philippines, Calamba	1 <sup>a</sup>	21	0%
1996	Philippines, other sites	0	230	0%
	Total	5	458	1%

**NOTE.** IgG, immunoglobulin G.

<sup>a</sup> Same person already IgG positive in 1993.

## 2008 VIRUS DETECTION IN PIGS

From July 2007 to June 2008, there was a noticeable increase of porcine reproductive and respiratory syndrome (PRRS) cases in the Philippines. The disease was characterized by high mortality in sows and piglets, abortion in sows, and respiratory signs. These PRRS cases were suspected of being caused by an atypical, highly virulent PRRS virus similarly being experienced in China and Vietnam [11]. The Philippine Department of Agriculture (DA) wanted to determine the strain of this disease to decide on the appropriate vaccine to control the disease [12]. The Philippine DA sent pig tissue and serum samples to the Foreign Animal Disease Diagnostic Laboratory (FADDL) of the Plum Island Animal Disease Center in Greenport, New York, USA for analysis. PRRS virus (arterivirus) that was isolated from these samples were close to the North American strain and 96–98% identical to the Chinese atypical strain. REBOV was coincidentally detected in 9 tissue samples using PCR, which were traced to 2 commercial farms in the Philippines. Samples were also submitted to the CDC and were confirmed by PCR and virus isolation.

There has been some evidence from serological testing in humans indicating possible pig-to-human transmission [4]. Between December 2008 and April 2009, a total of 102 people have been tested using the CDC ELISA protocol. These include 47 pig farm workers from the 2 affected farms of whom 4 tested positive for IgG antibodies; 48 pig slaughterhouse workers of whom 2 tested positive for IgG antibodies; and 7 contacts of seropositive persons, all of whom tested negative. All antibody-positive persons denied any bat or monkey contact. The 6 seropositive individuals had daily occupational exposure to pigs. The most likely zoonotic routes of transmission were direct contact with blood, secretions, organs, and other body fluids.

## DISCUSSION

A number of veterinary public health policy issues have come out of these investigations. In the local primate breeding

industry, there were improvements in the occupational health and safety program, biosafety, and biosecurity measures and standard operating procedures. These resulted in better quality laboratory animals as well. Surveillance of morbid and fatal cases was established for monkeys in all export facilities and in monkey holding facilities in the wildlife sources. The established local testing in the Veterinary Research Department of RITM of the Department of Health sustained in collaboration with the CDC was responsible for the initial local laboratory response to the pig outbreak in 2008.

Few human seropositive cases with no overt illness have developed after occupational exposures to animals infected with REBOV. However, consistent risk reduction measures such as personal protection and animal quarantine should be applied to prevent human infections not only due to REBOV but to other infectious disease agents as well, provided adequate precautions are taken.

The detection of REBOV in monkeys and (after more than a decade) in pigs signifies the ability of REBOV to infect multiple species. There are a number of issues for further investigation. In relation to the recent pig infections, there are many questions that arise. Could the initial monkey outbreak in 1989 have originated from contact of monkeys with infected pigs or contaminated pig offal? And, if this were the case, why has REBOV never been detected in monkeys outside the single source? Has surveillance in feral monkeys been thoroughly conducted to provide clues to the possible natural history of REBOV? How long has REBOV been circulating in swine populations before it was accidentally detected in 2008? And to what extent has it circulated in both backyard and commercial pigs? Have banked pig serum samples been thoroughly tested to help determine this? The tracing of the sources or natural reservoir of both the monkey and pig infections should continue, aiming to establish the relationship of all the isolated outbreaks.

Intersectoral cooperation of medical and veterinary services and community involvement and participation are required for targeted response and control of emerging zoonoses. Public health and veterinary authorities are urged to implement control measures that reduce the risk of transmission to humans, determine the extent of infection in animals, and reduce infection in domestic animals from possible wildlife reservoir. The work on REBOV in the Philippines showed the value of integration and the coordinated response and control measures for zoonotic diseases to determine the extent of infection in the animal populations and reduce the risk of transmission to humans.

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