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Chronological and Spatial Analysis of the 1996 Ebola Reston Virus Outbreak in a Monkey Breeding Facility in the Philippines

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Abstract: To describe the transmission pattern of natural infection with Ebola Reston (EBO-R) virus in a breeding colony, the chronological and spatial analysis of mortality during the 1996 EBO-R virus outbreak was done in this study. The EBO-R virus infection among monkeys in the facility was widespread. Over a period of 3 months, 14 out of 21 occupied units were contaminated with antigen positive animals. A large number of wild-caught monkeys were involved in this outbreak suggesting that wild-caught monkeys have a high susceptibility to EBO-R virus infection. In this outbreak, morbidity patterns for individual animal units were very different regardless of the type and size of cages, individual or gang cages. The results suggest that not only the cage size but also poor animal husbandry practices may be risk factors for the spread of EBO-R infection.

Key words: cynomolgus monkey, Ebola Reston virus, epidemiology

Until now, the epidemic of macaques infected with Asian Ebola virus, Ebola-Reston (EBO-R), has been reported only in developed countries during quarantine in animal facilities. These were during the 1989 Reston, Virginia, 1990 Alice, Texas, 1992 Siena, Italy and 1996 Alice, Texas outbreaks. Through epidemiological studies it became clear that all these infected monkeys were derived from the same monkey breeding facility in the

Philippines, but no detailed report on the outbreak of EBO-R in this breeding and exporting facility had been recorded, except for one epidemiological report on 2 breeding facilities in the Philippines in 1990 [1]. This report summarizes the outbreak investigation in the source facility in the Philippines in 1996. An overview of EBO-R infection in monkeys and the staff of various monkey breeding facilities has been described elsewhere

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Fig. 1. Monkey mortality surveillance from January to July 1996 in the source facility. Only the number of dead and moribund monkeys was recorded weekly. Euthanatized healthy monkeys antibody and/or antigen positive were not included in this figure. Apparently an outbreak of EBO-R infection started in the last week of June 1996.

[2].

In April 1996, the US Centers for Disease Control and Prevention (CDC) confirmed EBO-R infection of cynomolgus monkeys exported from the source facility. An epidemiologic investigation was started on May 3 in the Philippines in collaboration with the Department of Health, Department of Agriculture, Department of Environment and Natural Resources and CDC.

Figure 1 shows the weekly mortality trend from January to July 1996 in the source facility. The trend was relatively stable until the end of June. The total number of dead animals in the four to five weeks in each month ranged from 15 (April) to 28 (May), but in July a total of 109 deaths or moribund cases were recorded.

After starting the EBO-R surveillance, 8 out of 28 dead macaques were positive for EBO-R antigen in May (from May 5 to June 1), 18 out of 51 (June 2 to July 6) in June and 44 out of 109 in July (July 7 to August 3). The trend to both mortality and antigen positivity diminished in August and continued to diminish until surveillance was stopped in September

(Data not shown).

The breeding colony in the source facility consists of 23 animal building units as shown in Fig. 2. Three quarantine buildings (Q1, Q2 and Q3) with about 100 individual cages in each are in the northwest corner. Eight breeding buildings (BA to BH) are primarily composed of gang cages with a capacity of 10 to 50 animals, but BA was empty throughout the investigation period. There are 2 incubator buildings (IncA and B), 1 nursery (N) and 1 growing building (G2), all of which contain gang cages. Four conditioning buildings (C1 to C4) also contain gang cages. Three buildings (CEX1 to 3) each containing 100 individual cages are used for conditioning animals for export. A hospital (Hosp) containing 100 individual cages is located on the northeast edge of the facility. Table 1 shows the monkey population just before the start of the investigation on April 30.

The EBO-R infection among monkeys in the source facility was widespread. Over a period of 3 months, 14 out of 21 occupied units (excluding the Hosp and

Farm Lay-out

Fig. 2. Map of the source facility. The facility consists of 23 animal building units. All cages including individual and gang cages were hanging or high floor style. Q: Quarantine Bldgs. C: Conditioning Bldgs. B: Breeding Bldgs. G: Growing Bldg. N: Nursery Bldg. Inc: Incubator Bldgs. Hosp: Hospital. CEX: Conditioning Bldgs Using Animals for Export. Staff: Staff Bldgs or Work Areas.

Table 1. Building units and number of animals just before the start of the investigation (April 30)

Quarantine & Hospitals		Breeding		Incubator, Nursery, Growing		Conditioning, Export	
No. Anim		No. Anim		No. Anim		No. Anim	
*Q1	48	BA	0	*IncA	16	C1	22
*Q2	37	BB	84	IncB	17	*C2	95
*Q3	98	*BC	58	*N	59	C3	36
Hosp	80	BD	47	*G	73	*C4	91
		BE	51			*CEX1	80
		*BF	74			*CEX2	100
		*BG	86			CEX3	28
		*BH	52				

*Contaminated buildings with antigen positive monkeys.

empty breeding building BA) were confirmed to be contaminated with EBO-R antigen positive animals (Table 1).

The first known episode of EBO-R infection was demonstrated in February 1996. Twelve animals from one of the conditioning buildings were transported on

Fig. 3. Chronological and spatial analysis of the EBO-R outbreak in the source facility between May and September 1996. The number of monkeys with the EBO-R antigen positive including healthy euthanatized ones was recorded monthly.

February 22 to another laboratory in the Philippines. On February 29, 1 monkey had anorexia and died on March 2. By necropsy and serologic investigation, it was confirmed positive for EBO-R antigen. Eleven other monkeys in the same group were used for experiments and 5 of them were autopsied. They did not show any lesions suggesting infection with EBO-R. Sera collected from 3 of these monkeys were negative for detectable EBO-R IgG antibodies and antigen. The remaining 6 animals were still alive without any illness as of February 1998.

In March, as previously documented, 100 monkeys kept in one of the CEX buildings were exported to Alice, Texas. One monkey became ill on March 27 and died on March 30. On April 10, another animal became ill and was sacrificed on April 13. Both were diagnosed to be EBO-R antigen positive [3].

On May 3 to 4, sera were obtained from a systematic sample of animals at the source facility to determine by IgG antibody ELISA if past infection had occurred here. The sampling plan was designed to detect a minimum antibody prevalence of at least 5% among defined caging groups and life stages (breeders and captive-bred growers) [2]. Three out of 303 (1%) had detectable antibody to EBO-R. Two cases were from the Hosp (#1408B and #1436W) and 1 was from BG (#1263W). One animal from the Hosp was born in the facility (B) and the others were wild-caught (W). Second serum samples collected from two animals (#1408B and #1436W) in the Hosp on May 16 were negative. On May 29, these animals were sacrificed and tested. They were negative for both antibody and antigen. No further information was collected from the third monkey (#1263W).

Hereafter, to determine ongoing infection, the same serum samples for the antibody survey were also tested with antigen capture ELISA [2]. Two monkeys had detectable EBO-R antigen in the serum (Fig. 3a). Both were born in the facility. One animal located in C2 (#1082B) was born in October 1994. This monkey was eventually sacrificed on July 11 and tested negative for antigen and antibody. The other (#1109B) was born in February 1994 and was moved to CEX2 in February 1996. No further information was recorded for this animal.

Mortality surveillance was started at the beginning of May and continued until September. Surveillance

for deaths among monkeys was instituted to determine if any mortality was associated with EBO-R virus. Liver and skin specimens were collected from all dead monkeys, regardless of the cause of death. All monkeys in the housing unit where animals were found Ebola antigen positive were euthanatized after July 8 1996. Liver, skin and blood samples were collected from the sacrificed monkeys. All samples were tested for the presence of Ebola antigens with the antigen capture ELISA. A limited number of monkeys were also tested for virus genome by RT-PCR [9]. By this time the unit Q2 had become a temporary hospital to accommodate the growing number of sick animals.

Figure 3b shows the mortalities recorded from May 5 to June 1. A total of 9 deaths that included 3 sick animals sacrificed and 6 deaths were necropsied and tested positive for EBO-R antigen. In Q2, 1 animal (#2271W) derived from BH was found dead on May 31. In BF, 4 monkeys (#2386W, 2381W, 2385W, 0956W) became sick from May 24 to 27. All four were moved to the Hosp; 2 died and 2 were sacrificed. In BG, 1 monkey (#2223W) became ill on May 20 and was moved to the Hosp and sacrificed on the same day. In C4, 2 animals (#2377W, 2384W) died on May 25 and 26. The latter animal (#0038B) was born in the facility in September 1994. It was originally from CEX1 and had been hospitalized since March 1996. It died on May 16. There was one monkey (#2228W) which was wounded in BC and was showing signs of gastrointestinal infection on May 25. This was sacrificed in the Hosp on May 27 and tested antibody positive but antigen negative (omitted from Fig. 3b).

Figure 3c summarizes the results of the surveillance from June 2 to 30. Seven out of 20 animals which died were EBO-R antigen positive as follows. In BF, one sick animal (#2103W) and 3 apparently healthy animals (#2102W, 2104W, 2182W) were euthanatized and tested antigen positive. One other monkey (#2939W) had diarrhea and was moved from BF to the Hosp and sacrificed. One animal (#2938W) in BG became ill and was moved to the Hosp on June 26 and died on June 28. The last case was an animal in C4 (#2941W) which died on June 27. All these wild-caught monkeys were introduced into the source facility from March 1992 to January 1995, over one year before this EBO-R outbreak.

A total of 83 animals out of 105 which died were

EBO-R antigen positive during July 1 to 28 as shown in Fig. 3d. During this period several animal units were closed as follows: C4 on July 8, the Hosp and BF on July 9 and BG on July 10 and C2 on July 11.

In the breeding buildings, a total of 23 animals which were sacrificed or died were EBO-R antigen positive. In BF, 1 animal became ill on July 3 and was sacrificed in the Hosp and was antigen positive. On July 9, all 35 apparently healthy monkeys in this building were sacrificed. Four wild caught animals which were moved from quarantine buildings to BF in February 1996 were antigen positive. The other 31 animals were antigen negative. In BG, 16 out of 53 animals were antigen positive. Five out of 16 antigen positive monkeys died between July 1 and 7. The remaining 11 animals were euthanatized. All 16 animals were wild-caught and arrived at the source facility between June 1991 and January 1993. In BH, 2 wild caught animals died (July 8 and July 22) and were antigen positive.

In the incubation, nursery and growing buildings, 2 wild-caught monkeys in IncA, in which one monkey died on July 1 (#3065W) and another became sick and was sacrificed at the Hosp on July 2 (#3052W), were antigen positive. In G2, one monkey (#2348W) which died on July 22 was also antigen positive.

The animals in the conditioning buildings were relatively free from infection at the time of the closing unit. In C2, one animal (#3054B) died on July 2 and was antigen positive. Another 64 animals euthanatized on July 10 and 11 were all negative. In C4, 1 animal (#3074W) showed paralysis and was sacrificed at the Hosp on July 1. Another 6 wild caught animals became sick or moribund and were sacrificed between July 2 and 8. All these animals were antigen positive. One apparently healthy monkey (#2656W) sacrificed on July 8 was antigen positive but the other 21 animals were negative.

The most severe outbreak occurred in CEX1 in this period. A total of 31 animals (1 facility born; #2312B, and 30 wild-caught) died and all were antigen positive. The peak of animal deaths was biphasic: the first peak was from July 6 to 10 ($n=9$) and the second was from July 23 to 24 ($n=9$).

In the quarantine buildings, 17 animals including 2 in Q1, 4 in Q2 and 11 in Q3 which were all wild caught died between July 2 and 23. The peak of deaths in this area occurred from July 4 to 11 when 13 (76.5%) ani-

mals out of 17 died.

Figure 3e shows the results of the surveillance between August 1 and 31. Twenty-one monkeys were antigen positive at necropsy. In BH, 2 monkeys died, one on August 2 (#2877W) and one on August 6 (#2874W). Both were antigen positive. One in N (#2873B) died on August 7 and was antigen positive. A small outbreak occurred in G2. Nine wild-caught monkeys became ill and were sacrificed. The peak of deaths was concentrated on August 21 when 3 monkeys died and 3 were moribund and were sacrificed. All 9 monkeys were antigen positive.

The EBO-R outbreak continued in CEX1 from July 4 to August 12. Seven wild-caught monkeys died or became sick and were sacrificed between August 2 and 12 when the last monkey in this unit was sacrificed. All these monkeys were antigen positive. In the quarantine units, 1 monkey (#2872W) in Q1 died on August 12 and another (#2882W) in Q2 died on August 8. Both animals were antigen positive.

In September (Fig. 3f), 3 monkeys in G2 which died or became ill and were sacrificed on September 1 were antigen positive. In CEX2, 100 monkeys were maintained. Among them, a monkey, later omitted from this group was tentatively antigen positive on May 3 but became negative for both antigen and antibody on June 26. Another monkey was only antibody positive on June 26 and remained positive until September 9. This group of monkeys were isolated from the rest of the colony and cared for independently (both cases were excluded from Fig. 3f), but 2 eventually became sick. On September 1, one died (#1791B) and the other (#1792B) was sacrificed on September 4. Both were confirmed to be antigen positive.

A large number of wild-caught monkeys were involved in this outbreak (127 antigen positive monkeys involving 114 wild-caught, 7 facility-bred and 6 non-recorded monkeys), suggesting that wild-caught monkeys have a high susceptibility to EBO-R virus infection. The majority of these monkeys had been introduced into the facility one to two years prior to the outbreak of EBO-R. This suggests that these monkeys were likely free of the virus infection when they were introduced into the facility. There are 2 possibilities for the cause of the outbreak. First, the animal infected with EBO-R in February may have transmitted the virus to a limited number of animals including the infected

ones exported to the US in March, until the outbreak peaked in July. Secondly, since there was no previous evidence of persistent infection with EBO-R in cynomolgus monkeys, newcomers might have brought infection into the colony before the outbreak. Even though the index case of this outbreak was not confirmed, it may be possible that the 2 antigen positive monkeys in quarantine (#2914W, 2931W) introduced into the facility on April 16, 1996 were potential sources of the outbreak in the colony. Because only these two animals were recorded to be antigen positive and had been introduced into the facility just before the outbreak. These animals were kept in Q1 in individual cages until they died in July.

There was no significant difference between males (11%, 41/388) and females (15%, 91/623) in antigen positivity. A similar phenomenon is described in the human Ebola outbreaks in Africa [4-8].

In this outbreak, morbidity patterns for individual animal units were very different regardless of the type of cages, individual or gang ones. In the quarantine buildings, 20 out of 53 animals (38%) caged individually were antigen positive. In the conditioning buildings containing small sized gang cages, 12 out of 123 (10%) animals were antigen positive. In the breeding buildings containing middle to large sized gang cages, 33 out of 129 (26%) animals were antigen positive. In CEX1, 38 out of 72 (53%) individually caged monkeys were antigen positive. On the other hand, in CEX2 where the animals were separated and independently maintained from May, two out of 99 (2%) animals became antigen positive. These results suggest that not only the cage size but also poor husbandry practices including reuse of needles for TB tests, administration of vitamins and antibiotics may be risk factors for the spread of EBO-R infection. There was no SOP for separation of clean and dirty areas and flow of work.

The majority of the animals infected with EBO-R became acutely ill and eventually died, but two animals (#1436W, 1408B) with antibodies on May 3 and 4 became free of both antigen and antibody on May 29. Moreover, one monkey (#1082B) which was antigen positive on May 3 became free of both antigen and antibody by July 11. These minority cases indicate the possibility that low virus load infection might occur in cynomolgus monkeys as in human beings. In addition, 6 monkeys in breeding units (#0049W, 0050W, 0051W,

0053W, 0956W, 2223W) were positive in the liver and other tissues such as oral, nasal, anal and vaginal fluids by the PCR method [9]. Further epidemiological, histopathological and experimental investigations are needed to clarify this.

In February 1997, this facility was closed down and depopulated by order of the Department of Environment and Natural Resources. All animals and all contaminated materials were incinerated. The vacated buildings were washed down and the floors, walls, ceiling, cages, accessories and other equipment were decontaminated. Two disinfection cycles with 1% lysol solution were done one day apart. Since then, no new evidence of EBO-R antigen or antibody positive animals has been found despite continued surveillance of other monkey export facilities and wildlife collection sites.

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