Fatal Measles Virus Infection in Japanese Macaques (*Macaca fuscata*)

*Vet Pathol* 1999 36: 594
DOI: 10.1354/vp.36-6-594

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What is This?
Fatal Measles Virus Infection in Japanese Macaques (Macaca fuscata)

Y. K. Choi, M. A. Simon, D. Y. Kim, B. I. Yoon, S. W. Kwon, K. W. Lee,
I. B. Seo, and D. Y. Kim

Korea Research Institute of Bioscience and Biotechnology, Taejon, Korea (YKC); New England Regional Primate Research Center, Harvard Medical School, Southborough, MA (MAS); Department of Pathology, College of Veterinary Medicine, Seoul National University, Suwon, Korea (DYK, BIY, DYK); Everland Zoological Gardens, Yongin, Korea (SWK, KWL); and Department of Oriental Medicine, Se Myung University, Korea (IBS)

Abstract. An outbreak of natural measles virus infection occurred in a group of Japanese macaques (Macaca fuscata). Over a period of 4 months, 12 of 53 Japanese macaques died following a 2–23-day history of anorexia, diarrhea, and dermatitis. The monkeys were kept in outdoor exhibits but had been moved temporarily into indoor caging and then transferred to new outdoor exhibits. Ten monkeys died while they were in temporary caging, and two monkeys died after they were moved to new outdoor exhibits. The diagnoses were made based on the results of histopathology, immunohistochemistry (IHC), in situ hybridization (ISH), and electron microscopy. Measles virus antigens were detected in the lung, stomach, skin, salivary gland, spleen, and lymph nodes. Tangled, tubular nucleocapsids compatible with paramyxovirus were noted in the lung tissue. As a result of immunosuppression following measles virus infection, various secondary infections including disseminated cytomegalovirus infection, adenoviral and bacterial pneumonia, and Candida albicans-associated gingivitis and esophagitis were noted. The primary infective source or the mode of infection could not be determined in this outbreak, but measles virus may have been transmitted to the monkeys from human visitors while the monkeys were on exhibit.

Key words: Candida albicans; cytomegalovirus; Japanese macaques; measles; pneumonia.

The measles virus is a member of the genus Morbillivirus, family Paramyxoviridae.4–6 Measles virus is highly contagious through aerosolization in humans and several nonhuman primates; rhesus monkeys (Macaca mulatta) are most commonly affected. The monkeys are usually free of measles virus in their natural environment but are exposed in captivity when they come into contact with humans carrying measles virus.15,21 Several measles outbreaks following shipment of monkeys or introductions into an established colony have been described.14,15,18,21,23 The pathogenesis of measles virus infection in nonhuman primates is similar to that in humans.5,13 Measles virus is known to induce immunosuppression in affected human and nonhuman primates by disrupting both cellular and humoral immunity, which can result in various secondary opportunistic infections.7,8,16 Erythematous maculopapular skin rash and presence of multinucleated syncytial cells in the lungs are regarded as pathognomonic for simian measles virus infection.9,17 Endometritis, cervicitis, and abortion associated with measles virus has been described in rhesus monkeys.19

Here, we describe an outbreak of natural measles virus infection with 21% mortality in Japanese macaques (Macaca fuscata) at a Korean zoo. Various secondary infections, including disseminated cytomegalovirus (CMV) infection, were similar to those seen in other immunodeficiency conditions.

Materials and Methods

Case histories

Over a period of 4 months, 12 of 53 Japanese macaques housed at Everland Zoological Gardens in Yongin, Korea, died following a 2–23-day history of anorexia, diarrhea, and/ or dermatitis (Table 1). Three affected monkeys also showed seizures, and one of these monkeys had tetraparesis. The ages of affected monkeys ranged from 7 months to 22 years (Table 1). The monkeys had been imported from Japan over 20 years prior to the outbreak of disease and had been maintained as a closed group since then at Everland Zoological Gardens. They were kept in outdoor exhibits (concrete and metal gang cages) and fed homemade food and water ad libitum.

The animals had received no vaccinations since arriving from Japan. In December 1992, they were moved temporarily into indoor metal cages and then transferred to new outdoor exhibits in February 1993. Clinical signs were first noticed in the original outdoor housing and continued to appear during indoor housing and then after transfer to the new
outdoor exhibit. Ten monkeys died while they were in temporary caging, and two monkeys died after being moved to new outdoor exhibits.

Necropsy, histopathology, and electron microscopy

Complete postmortem examinations were performed on 10 of the 12 monkeys that died. Tissues were fixed in 10% neutral phosphate-buffered formalin, routinely processed, and stained with hematoxylin and eosin (HE) for histopathologic examination. Replicate sections of the lung, stomach, esophagus, liver, and brain of all 10 monkeys having lesions were also stained with periodic acid–Schiff (PAS), acid-fast, Gram, or Gomori methenamine silver (GMS) methods.

Areas of interest were cut from tissues that had been fixed in 10% formalin and embedded in paraffin blocks, were embedded in Epon resin, sectioned, stained with uranyl acetate and lead citrate, and examined with a JEOL 100S transmission electron microscope.

Immunohistochemistry

Immunohistochemical identification of CMV, adenovirus, herpes simplex, or measles virus was performed on replicate sections of the lung, stomach, liver, kidney, salivary gland, esophagus, intestine, skin, and/or brain as previously described. Sections were placed on Superfrost Plus slides (VWR Scientific, West Chester, PA), and unlabeled antibodies directed against rhesus CMV (Dr. P. Barry, University of California, Davis, CA), herpes simplex (BioGenex, San Ramon, CA), measles (Chemicon International, Temecula, CA), and adenovirus (Chemicon International) were used as primary antibodies. The standard avidin–biotin–peroxidase complex (ABC) method was used according to the manufacturer’s protocol (Vectastain kit, Vector Laboratories, Burlingame, CA) to demonstrate antigen, using 3,3-diaminobenzidine (DAB) as the chromogen (Sigma, St. Louis, MO). Control procedures included omission of the primary antibody and substitution of an isotype-matched irrelevant antibody.

In situ hybridization

In situ hybridization (ISH) was performed on selected formalin-fixed, paraffin-embedded tissue sections mounted on Superfrost Plus slides. The probes for simian immunodeficiency virus (SIV), CMV, and simian virus 40 (SV40) were labeled with digoxigenin-dUTP by random priming and quantified by blotting as described previously. Hybridizations were performed in a humidified chamber overnight at 37°C under denaturing conditions to localize both DNA and RNA. Probes were applied at 0.2–0.5 ng/ isotype-matched irrelevant antibody.

Results

Gross pathology

Well-circumscribed areas of an erythematous rash, which were often covered by scales, were noted on the ventral abdomen of seven animals before clinical illness was evident (Fig. 1). Significant gross findings observed at necropsy were confined to the lung, oral cavity, skin, esophagus, and gastrointestinal tracts. The lungs were generally reddened and firm, with sublobular consolidation in four of the 10 monkeys examined. Numerous 1–4-mm discrete, firm, tan foci were scattered throughout the lung lobes in four monkeys (Fig. 2). The fundic portion of the gastric mucosa of

Table 1. Clinical findings in the Japanese macaques fatally infected with measles virus.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Age</th>
<th>Sex</th>
<th>Clinical Signs</th>
<th>Clinical Course (days)</th>
<th>Date of Death*</th>
</tr>
</thead>
<tbody>
<tr>
<td>92501</td>
<td>3 years</td>
<td>F</td>
<td>Dermatitis</td>
<td>22</td>
<td>12/27/92</td>
</tr>
<tr>
<td>92502</td>
<td>7 years</td>
<td>M</td>
<td>Dermatitis, diarrhea</td>
<td>23</td>
<td>12/31/92</td>
</tr>
<tr>
<td>93001</td>
<td>14 years</td>
<td>F</td>
<td>Dermatitis, diarrhea</td>
<td>14</td>
<td>1/05/93</td>
</tr>
<tr>
<td>93002</td>
<td>7 months</td>
<td>F</td>
<td>Dermatitis</td>
<td>11</td>
<td>12/30/92</td>
</tr>
<tr>
<td>93008</td>
<td>5 years</td>
<td>M</td>
<td>Dermatitis, anorexia</td>
<td>5</td>
<td>1/09/93</td>
</tr>
<tr>
<td>93009</td>
<td>9 years</td>
<td>F</td>
<td>Dermatitis, diarrhea</td>
<td>15</td>
<td>1/08/93</td>
</tr>
<tr>
<td>93018</td>
<td>22 years</td>
<td>M</td>
<td>Anorexia, diarrhea</td>
<td>4</td>
<td>1/21/93</td>
</tr>
<tr>
<td>93019</td>
<td>4 years</td>
<td>M</td>
<td>Dermatitis, anorexia</td>
<td>13</td>
<td>1/24/93</td>
</tr>
<tr>
<td>93035</td>
<td>8 months</td>
<td>F</td>
<td>Diarrhea</td>
<td>10</td>
<td>1/30/93</td>
</tr>
<tr>
<td>93043</td>
<td>9 months</td>
<td>M</td>
<td>Seizures</td>
<td>2</td>
<td>2/08/93</td>
</tr>
<tr>
<td>93063</td>
<td>16 years</td>
<td>F</td>
<td>Seizures, tetraparesis</td>
<td>7</td>
<td>3/07/93</td>
</tr>
<tr>
<td>93064</td>
<td>6 years</td>
<td>F</td>
<td>Seizures</td>
<td>16</td>
<td>3/08/93</td>
</tr>
</tbody>
</table>

* Month/day/year.
three monkeys was reddened and contained multifocal to coalescing, firm, gray to white, slightly elevated nodules with occasional central craters (Fig. 3). In three monkeys, the esophageal mucosa was covered by yellowish white, friable, crusty material. The small intestine of four monkeys contained semifluid ingesta, and the mucosa was congested or had petechial hemorrhage. A well-circumscribed 7 × 3-mm area of ulceration was noted in the gingiva of one monkey.

**Histopathology**

Histologically, the lungs were most frequently affected (8/10 monkeys) and showed similar changes characteristic of interstitial pneumonia with occasional secondary bacterial bronchopneumonia. The alveolar septa were thickened due to infiltration of mixed mononuclear cells and fibroblasts and were lined by hypertrophic type II pneumocytes. Alveolar spaces were filled with mononuclear cells, multinucleated giant cells, and a few neutrophils (Fig. 4). Inclusions were not seen in the multinucleated cells. In two monkeys, bacterial clumps, fibrin, necrosis, and hemorrhage were noted in the terminal bronchioles and associated alveoli. Where the pulmonary lesions were grossly extensive, necrosis and proliferation of fibroblasts indicating organization were also observed. Cytomegalic cells with eosinophilic to amphophilic intranuclear inclusions were seen in five monkeys, both in alveolar septa and in perivascular spaces in association with necrosis of the vascular walls (Fig. 6). Acid-fast, PAS, and GMS stains were not useful for identifying the infectious agents.

The nodules recognized grossly in the gastric fundic region were characterized by severe necrosuppurative gastritis. A focally extensive area of the mucosa, submucosa, and muscle layer was necrotic, hemorrhagic, and heavily infiltrated with neutrophils and fewer lymphocytes, plasma cells, and macrophages. Fibrinoid necrosis accompanied by neutrophilic infiltration and perivascular edema was noted in the blood vessels. Inclusion-bearing cytomegalic cells were also observed in the perivascular space in the submucosa (Fig. 7). Gastric epithelium occasionally contained round basophilic inclusion bodies in the nuclei.

The oral and esophageal mucosa were thickened due to parakeratotic hyperkeratosis and/or were covered by a mixture of desquamated cellular debris, neutrophils, bacterial colonies, and budding yeast characteristic of...
Fig. 6. Lung; Japanese macaque, No. 93002. Alveolar walls contain cytomegalic cells with eosinophilic to amphophilic intranuclear inclusions (arrows). HE. Bar = 100 μm.

Fig. 7. Stomach; Japanese macaque, No. 93018. Necrosis and intranuclear inclusions in cytomegalic cells (arrows) were observed in the submucosa. HE. Bar = 100 μm. Inset: Immunohistochemistry for CMV demonstrates positive cells (brown). DAB with Mayer’s hematoxylin counterstain.

Fig. 8. Stomach; Japanese macaque, No. 93018. Note measles antigen in gastric epithelial cells and desquamated epithelium. DAB with Mayer’s hematoxylin counterstain. Bar = 100 μm.

Fig. 9. Cerebral cortex; Japanese macaque, No. 93063. Note measles antigen (arrows) in neurons adjacent to a focus of perivascular inflammation. DAB with Mayer’s hematoxylin counterstain. Bar = 100 μm. Inset: Neuron with cytoplasmic inclusions (arrows). HE.
Table 2. Summary of immunohistochemistry (IHC) and in situ hybridization (ISH) in the Japanese macaques fatally infected with measles virus.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Tissues, IHC*</th>
<th>Measles</th>
<th>CMV</th>
<th>Adenovirus</th>
<th>Tissue Positive, ISH</th>
<th>CMV</th>
<th>SIV</th>
<th>SV40</th>
</tr>
</thead>
<tbody>
<tr>
<td>93001</td>
<td>Lung, skin, stomach, spleen</td>
<td>Stomach</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Neg</td>
<td>ND</td>
</tr>
<tr>
<td>93002</td>
<td>Lung, LN, salivary gland</td>
<td>Lung</td>
<td>Neg</td>
<td>Lung</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Neg</td>
</tr>
<tr>
<td>93008</td>
<td>Lung, LN</td>
<td>Lung, LN</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>93009</td>
<td>Lung</td>
<td>Stomach</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>93018</td>
<td>Lung, stomach</td>
<td>Lung, stomach</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>93019</td>
<td>Lung, spleen</td>
<td>Lung</td>
<td>ND</td>
<td>Lung</td>
<td>Neg</td>
<td>ND</td>
<td>Neg</td>
<td>ND</td>
</tr>
<tr>
<td>93035</td>
<td>Lung, LN</td>
<td>Lung</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>93043</td>
<td>Lung</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>93063</td>
<td>Brain</td>
<td>Neg</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>93064</td>
<td>Brain</td>
<td>Neg</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* ND = not done; Neg = negative; LN = lymph node.

*Candida albicans.* In the oral cavity, hemorrhage and edema extended deeply into muscle layers similar to that seen in the gastric mucosa. Inclusion-bearing cytomegalic cells were also observed in the submucosa and muscle layer.

The small intestinal mucosa and submucosa of the monkeys with diarrhea (4/10 monkeys) had hemorrhage and mild to moderate infiltration of neutrophils with a few lymphocytes, along with villous atrophy and crypt dilation. Lymphoid depletion was present in the splenic white pulp and lymph nodes, although neither syncytial cells nor inclusion bodies were noted in lymphoid tissue. The epidermis in the regions with gross lesions was necrotic and thickened due to parakeratotic hyperkeratosis admixed with bacterial colonies and degenerate inflammatory cells. Neither inclusions nor multinucleated giant cells were found in the skin.

In the brain, perivascular cuffing of lymphocytes and plasma cells (monkey Nos. 93063, 93064) or demyelination (monkey No. 93043) was observed. Neuronal inclusions were also seen in 2 monkeys (Fig. 9).

**Immunohistochemistry and ISH**

The results of immunohistochemistry (IHC) and ISH are summarized in Table 2. IHC for measles antigen was performed on lungs of eight animals, and multinucleated giant cells and mononuclear cells in the alveoli stained strongly for measles antigen in all of these animals (Fig. 5). In the stomach, gastric epithelium with or without inclusions and desquamated epithelium also contained measles antigen (Fig. 8). Cells stained positively for measles antigens were also noted in the skin, lymph node, spleen, and salivary gland (Table 2). In the brain, neurons were stained positively for measles antigen in two animals (Fig. 9).

CMV was identified by IHC or ISH in cytomegalic cells and other mononuclear cells in multiple tissues from several animals (Fig. 7). IHC did not identify herpes simplex virus in any tissue studied, but in one monkey, adenovirus was identified in cells with intranuclear inclusions in the lung.

SV40 and SIV DNA or RNA were not detected in any of the sections that were used for ISH.

**Electron microscopy**

Typical tangled, tubular nucleocapsids characteristic of paramyxovirus were present either in the nuclei or in the cytoplasm of cells stained positively for measles virus in the lung (Fig. 10). In addition, numerous round electron-dense viral capsids 120–140 nm in diameter, which were compatible with herpesvirus, were observed in the cytomegalic cells. Intranuclear tightly packed crystalline arrays and single adenoviral virions, 75–80 nm in diameter, were seen in the lung tissue of one animal.

**Discussion**

An outbreak of natural measles virus infection with 21% mortality occurred in a colony of Japanese macaques in a Korean zoo. Measles virus generally does not occur in monkeys in the wild but can become an important pathogen when nonhuman primates have contact either directly or indirectly with humans, the natural host for measles. Several nonhuman primates species can be experimentally infected with measles virus or can contract measles virus when they are exposed to infected humans. Among them, marmosets (*Saguinus mystax*) are highly sensitive to measles virus; however, the usually fatal disease in that species tends to be localized in the gastrointestinal tract and lacks the characteristic features of human
measles.\textsuperscript{1} In macaques, including cynomolgus (\textit{Macaca fascicularis}) and rhesus monkeys, both natural and experimental measles infection can cause disease of varying severity with pathogenesis similar to that of human measles virus infection.\textsuperscript{2,22}

SIV was considered as a possible etiologic factor in this disease outbreak because of the giant cell interstitial pneumonia without paramyxoviral inclusions and because of the opportunistic infections seen in these animals. ISH failed to demonstrate SIV nucleic acid in any monkey examined. Also, the course of SIV infection is characteristically months to years, completely unlike the epizootic observed here. Three of the five monkeys in the colony that survived the outbreak were seropositive for human measles virus, supporting measles virus as the primary etiologic agent in this outbreak. As with canine and phocine distemper viruses, measles virus is known to cause immunosuppression in human and nonhuman primates by depressing cellular immunity. Stresses associated with transportation, handling, capture, or environmental changes due to housing relocation may also have played a role in the high incidence of death observed in this outbreak. Previous descriptions of measles outbreaks in nonhuman primates have reported mortality rates ranging from 0 to 100\%.\textsuperscript{14}

The source or mode of measles infection could not be determined in this outbreak. No new monkeys had been brought into the colony, and no contact had been made with other nonhuman primate species. Because several monkeys developed clinical signs, including a rash, before transfer into indoor housing, we believe that measles virus may have been transmitted to the monkeys via aerosol from infected visitors to the zoo when the monkeys were in their original outdoor exhibit. Outbreaks of measles in free-living and laboratory primates are known to occur coincidentally with human outbreaks.\textsuperscript{14}

In children, bacterial pneumonia and mycoplasmosis are typical secondary infections following natural measles virus infection.\textsuperscript{8,16} Rarely, other opportunistic infections, such as CMV infection, have been described in immunodeficient human and nonhuman primates.\textsuperscript{2,27} In this outbreak, various secondary infections, such as disseminated CMV infection, adenoviral pneumonia, \textit{Candida albicans}-associated gingivitis, and esophagitis and bacterial bronchopneumonia, were noted in a high percentage (70\%) of the animals with
documented measles infection. Secondary infections in this outbreak are similar to those seen in SIV-infected monkeys and support the association of immunosuppression with measles virus infection.

Acknowledgements

We thank Dr. B. W. Ahn of the Department of Pathology, KFDA, John MacKey for electron microscopy, Kristen Toohey, for graphic services, and Dr. Fred Doddy for helpful case discussions. This study was supported in part by grants from the Ministry of Science and Technology, Korea (KB1140), and the US Public Health Service (RR00168).

References


Request reprints from Dr. D. Y. Kim, Department of Pathology, College of Veterinary Medicine, Seoul National University, Suwon 441-744 (Korea). E-mail: daeyong@plaza.snu.ac.kr.