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Lethal Canine Distemper Virus Outbreak in Cynomolgus Monkeys in Japan in 2008

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Canine distemper virus (CDV) has recently expanded its host range to nonhuman primates. A large CDV outbreak occurred in rhesus monkeys at a breeding farm in Guangxi Province, China, in 2006, followed by another outbreak in rhesus monkeys at an animal center in Beijing in 2008. In 2008 in Japan, a CDV outbreak also occurred in cynomolgus monkeys imported from China. In that outbreak, 46 monkeys died from severe pneumonia during a quarantine period. A CDV strain (CYN07-dV) was isolated in Vero cells expressing dog signaling lymphocyte activation molecule (SLAM). Phylogenic analysis showed that CYN07-dV was closely related to the recent CDV outbreaks in China, suggesting continuing chains of CDV infection in monkeys. *In vitro*, CYN07-dV uses macaca SLAM and macaca nectin4 as receptors as efficiently as dog SLAM and dog nectin4, respectively. CYN07-dV showed high virulence in experimentally infected cynomolgus monkeys and excreted progeny viruses in oral fluid and feces. These data revealed that some of the CDV strains, like CYN07-dV, have the potential to cause acute systemic infection in monkeys.

Canine distemper virus (CDV) causes acute systemic infection in dogs and other Canidae, with symptoms of fever, coughing, vomiting, diarrhea, ataxia, and paralysis. It has long been thought that only animals in the family Canidae are susceptible to CDV infection in nature. However, during the last 2 decades, animals of many other species, such as Ailuridae (1), Mustelidae (2), Viverridae (3, 4), Procyonidae (5), Phocidae (6), and Felidae (7, 8), have been infected with CDV in nature.

CDV belongs to the genus *Morbillivirus* within the family Paramyxoviridae (9). Signaling lymphocyte activation molecule (SLAM) is a principal receptor of CDV. Other members of the *Morbillivirus* genus, namely, measles virus (MV), rinderpest virus, and peste des petits ruminants virus, are also known to utilize human, bovine, and goat SLAM, respectively, as a receptor. These viruses preferentially use the SLAM of their host animals but have the ability to use other SLAMs of nonhost animals with reduced efficiency (10). Recently, human nectin4 and dog nectin4 have been identified as epithelial cell receptors for MV (11, 12) and CDV (13), respectively.

Importantly, CDV outbreaks have recently emerged with a high mortality rate in nonhuman primates. The first outbreak occurred in 1989 in Japan (14). Twenty-two Japanese monkeys (*Macaca fuscata*) in the wild were captured and later shown to have CDV infections. Two of them developed neurological symptoms, and one died of encephalitis (14). Recently, large CDV outbreaks have occurred in rhesus monkeys (*Macaca mulatta*) at a breeding farm in Guangxi province, China, with a mortality rate of 5 to 30% (15). In 2008, an animal center in Beijing, China, experienced another CDV outbreak in rhesus monkeys (16). This outbreak was likely associated with the Guangxi outbreaks. Following these outbreaks in China, a CDV outbreak occurred in cynomolgus monkeys (*Macaca fascicularis*) in Japan in 2008. These monkeys were imported from China, and some 46 cynomolgus monkeys out of 432 imported were euthanized or died from severe pneumonia, diarrhea, and anorexia during a quarantine period. A CDV strain was isolated from a moribund monkey, and phylogenic analysis of its genome sequence showed that the CDV strain was closely related to the CDV strains associated with recent outbreaks in rhesus monkeys in China, suggesting continuing chains of CDV infection in monkeys. In the present study, we analyzed the pathogenicity of the CDV strain in cynomolgus monkeys. We also investigated whether the CDV strain utilized macaca SLAM and macaca nectin4 as its receptors.

MATERIALS AND METHODS

Cells. Vero cells constitutively expressing dog SLAM (Vero.DogSLAMtag) and dog nectin4 (Vero/dNectin4) were used (13, 17). Vero cells expressing human SLAM (Vero/hSLAM) (10) were also used. Vero cells constitutively expressing macaca SLAM (Vero/macSLAM) and macaca nectin4 (Vero/macNectin4) were generated in the present study. Total RNAs obtained from peripheral blood mononuclear cells (PBMCs) and a kidney from a cynomolgus monkey were used to synthesize cDNAs of macaca SLAM and macaca nectin4, respectively. The nucleotide sequences of cynomolgus SLAM and cynomolgus nectin4 were deposited in GenBank with accession numbers AB742520 and AB742522, respectively. The macaca SLAM and macaca nectin4 cDNA fragments were inserted into the pCXN2 vector (18), generating pCXN2-macSLAM and pCXN2-macNectin4, respectively. Vero/macSLAM and Vero/macNectin4 cells were generated by transfecting Vero cells with pCXN2-macSLAM and pCXN2-macNectin4, respectively, and were selected in Dulbecco's modified Eagle's medium (DMEM) supplemented with 7% fetal bovine serum (FBS) and 0.5 mg/ml Geneticin (G418; Invitrogen). Expression of macaca SLAM and nectin4 in Vero cells was confirmed by immunofluorescence

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staining using a goat anti-human SLAM and nectin4 polyclonal antibody, respectively.

Virus isolation. Tissue samples obtained from the spleens of moribund or dead monkeys were suspended in phosphate-buffered saline (PBS) supplemented with antibiotics and were homogenized. The homogenates were centrifuged at 10,000 × *g* for 5 min, and supernatants were inoculated to monolayers of Vero.DogSLAMtag cells.

RNA extraction and RT-PCR. Viral and total RNAs were extracted from culture media and cells, respectively, using ISOGEN-LS (Nippon Gene). Reverse transcription (RT) was carried out with Superscript III (Invitrogen) using primers of random nucleotide hexamers (TaKaRa Bio Inc.). Then, PCR was performed to amplify CDV-specific cDNA fragments.

Sequencing and phylogenic analysis of the CDV isolate. PCR amplicons were used as templates for sequencing on an Applied Biosystems 3130 automated DNA sequencer using a BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems Japan). The entire genome nucleotide sequence was determined using overlapping PCR amplicons. The nucleotide sequence of each extremity was determined by the rapid amplification of cDNA ends (RACE) method. The sequence was further confirmed by using a Roche GS Junior sequencer. Nucleotide and amino acid sequence identities were calculated using the pairwise distance algorithm (*p* distance) with MEGA 4 software (19). Phylograms were reconstructed using a neighbor-joining algorithm with MEGA 4 software. The robustness of the resulting branching patterns was tested using the bootstrap method with 1,000 replicates. Sequence relatedness is shown as percentage identity.

Histopathological examination of monkeys infected with CDV during the 2008 outbreak. Three cynomolgus monkeys (11, 12, and 13) infected with CDV during the 2008 outbreak were euthanized by exsanguination under excess ketamine anesthesia and autopsied for histopathological examination. Tissue samples were immersed in 10% phosphate-buffered formalin. Fixed tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Immunohistochemical analysis for the detection of the CDV antigens was performed on paraffin-embedded sections using EnVison/HRP Systems (Dako). After deparaffinization with xylene, the sections were rehydrated in ethanol and immersed in PBS. Antigens were retrieved by hydrolytic autoclaving for 15 min at 121°C in a sodium citrate-sodium chloride buffer (10 mM, pH 6.0). Endogenous peroxidase was blocked by incubation in 1% hydrogen peroxide in methanol for 30 min. The sections were incubated with a monoclonal antibody against CDV nucleoprotein (NP) (VMRD Inc.) and then with biotin-conjugated anti-mouse IgG. Peroxidase activity was detected by development with diaminobenzidine containing hydrogen peroxide, and then the nuclei were counterstained by hematoxylin.

Double immunofluorescence stainings were also performed for the various tissues of the CDV-infected cynomolgus monkey 11. Rabbit anti-wide-spectrum cytokeratin antibody (ab9377; Abcam), rabbit anti-neuron-specific β III tubulin antibody (ab56110; Abcam), rabbit anti-CD3 antibody [SP7] (ab21703; Abcam), goat anti-nectin4 polyclonal antibody (R&D Systems), and the monoclonal antibody against CDV NP were used as primary antibodies. Normal rabbit, goat, and mouse sera were used as negative-control antibodies (Dako). The sections were deparaffinized, rehydrated, and immersed in PBS. Antigens were retrieved by hydrolytic autoclaving in the retrieval solution (pH 9.0; Nichirei) for 15 min at 121°C. After the sections were cooled, to block background staining, normal goat or donkey sera were used. The sections were incubated with the monoclonal antibody against CDV NP overnight at 4°C. The sections were washed and incubated with antibodies to the cell markers for 60 min at 37°C. The sections were washed and incubated with goat anti-rabbit IgG-Alexa Fluor 568 (Invitrogen) and goat anti-mouse IgG-Alexa Fluor 488 (Invitrogen) antibodies or donkey anti-goat IgG-Alexa Fluor 568 (Invitrogen) and donkey anti-mouse IgG-Alexa Fluor 488 (Invitrogen) antibodies for 60 min at 37°C. After being washed, the sections were mounted with SlowFade Gold antifade reagent with 4′,6-diamidino-2-phenylindole (DAPI) (Invitrogen). The images were captured by a fluorescence microscopy (IX71; Olympus) equipped with a Hamamatsu high-resolution digital black and white charge-coupled-device (CCD) camera (ORCA2; Hamamatsu Photonics).

Experimental infection of cynomolgus monkeys. Five cynomolgus monkeys of 5 to 11 years of age were obtained from the Tsukuba Primate Research Center (National Institute of Biomedical Innovation, Ibaraki, Japan). They were free from simian retrovirus type 4 (SRV) and were confirmed to be free from MV and CDV antibodies. Four of them (no. 4450, 4571, 4965, 4969) were male, while one (no. 4970) was female. Their cages were placed in negatively pressurized glove boxes. They were anesthetized with ketamine (0.1 ml/kg) and inoculated intranasally with 5×10^5 PFU of CDV in 0.5 ml of DMEM using a spray (0.25 ml in each nostril; Keytron). On the day of inoculation, and at 3, 7, 10, and 15 days after inoculation, body weight and body temperature were measured, and throat and rectal swabs and peripheral blood were obtained. PBMCs were isolated using Percoll gradients (GE Healthcare), adjusted to a concentration of 10^5/ml, and divided into 2-fold serial dilutions. Then, a 500-μl aliquot of each diluted PBMC sample was inoculated into Vero. DogSLAMtag cells. On the assumption that one CDV-infected PBMC was contained in the maximum diluted PBMC sample that induced syncytium, the number of CDV-infected PBMC/10^5 PBMCs was calculated. All monkeys were euthanized 15 days after inoculation by exsanguination under excess ketamine anesthesia, and tissue samples were collected for histopathological examination and virus isolation. For virus isolation, tissue homogenates were prepared in PBS containing antibiotics and clarified by centrifugation. These samples were inoculated to Vero. DogSLAMtag cells. When no cytopathic effect (CPE) was observed, RT-PCR was performed for the detection of CDV-specific RNAs. When no CDV-specific cDNA was amplified, samples were determined as being negative for CDV. Total numbers of blood cells were measured using an autoanalyzer (Cell Tuck; Nihon Koden). Numbers of neutrophils, lymphocytes, monocytes, eosinophils, and basophils were determined by microscopic analysis. A virus-neutralization test for CDV was performed using a plaque reduction method with a constant amount of virus and various serum dilution. Sample sera were serially diluted 4-fold and mixed with equal volumes of 100 PFU of CDV units. The neutralizing antibody titer was calculated at the 50% plaque reduction point by the Behrens-Kaerber method.

Multiplex analysis of cytokines and chemokines in monkey sera. Monkey sera were subjected to multiplex cytokine analysis using the human cytokine 25-plex antibody bead kit (Invitrogen) with Luminex 100 (Luminex Co.) according to the manufacturer's instructions. Enzyme-linked immunosorbent assays (ELISAs) were performed in duplicate, and the averages of each assay are shown.

Replication kinetics of the CDV isolate in Vero cells expressing SLAM and nectin4 of various animal species. Vero, Vero.DogSLAMtag, Vero/macSLAM, Vero/hSLAM, Vero/dNectin4, and Vero/macNectin4 cells (2×10^5 cells/well) were cultured in 24-well plates and infected with the CDV isolate at a multiplicity of infection (MOI) of 0.01. The cells were adsorbed with the virus for 1 h at 37°C, and then the virus inoculum was removed and the cells were rinsed twice with DMEM. The cells were cultured in DMEM supplemented with 1% fetal calf serum (FCS) at 37°C. The cells and culture supernatants were harvested every 12 h until 3 days postinfection (p.i.). The harvested samples were stored at −80°C until use. The samples were centrifuged at 1,000 × *g* and titrated with plaque assay.

Cell-to-cell fusion assay. DNA fragments encoding H and F protein of the CDV isolate were amplified by RT-PCR and cloned into the pCAGGS vector (18). Vero, Vero.DogSLAMtag, Vero/macSLAM, Vero/hSLAM, Vero/dNectin4, Vero/macNectin4, and Vero cells in 24-well plates were transfected with the F protein-expressing plasmid together with or without the H protein-expressing plasmid. To detect syncytia clearly, a fluorescent protein-expressing plasmid, pEGFP-C1 (Clontech Laboratories),

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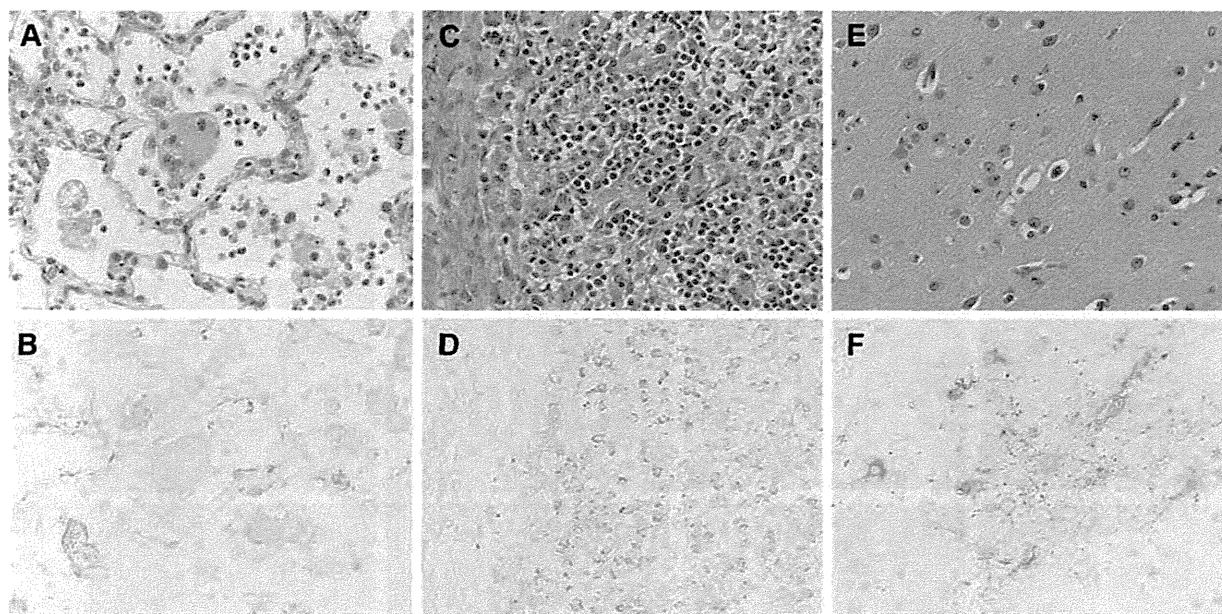

FIG 1 Histopathological analyses of cynomolgus monkeys naturally infected with CDV in the 2008 outbreak. Tissue sections obtained from cynomolgus monkey 11 were examined by hematoxylin and eosin staining (HE) and immunohistochemistry (IHC) using anti-CDV-NP monoclonal antibody. Giant cell pneumonia (A) and CDV antigen in the syncytial pneumocytes (B) were seen in the lung. Lymphocyte depletion (C) and CDV antigen in the mononuclear cells (D) were observed in the lymph node. Focal and slight microglia cell infiltration (E) and CDV antigen in neurons and glia cells (F) were observed in the cerebrum. HE, original magnification ×20; IHC, ×40.

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was cotransfected. The cell monolayers were observed using an Axio Observer.D1 microscope at 16 and 24 h posttransfection.

Entry assay using pseudotyped viruses. To analyze the efficiency of virus entry using SLAM and nectin4 in more detail, a vesicular stomatitis virus (VSV) pseudotype system (17) was employed (VSVΔG* was kindly provided by M. A. Whitt, The University of Tennessee Health Science Center). A VSV pseudotype bearing the H protein and the F protein of the CDV strain on the surface of the virion (referred to as VSVΔG*-F-dVH) was constructed. VSV pseudotype bearing only the F protein (referred to as VSVΔG*-F) was also constructed. Vero, Vero.DogSLAMtag, Vero/macSLAM, Vero/dNectin4, and Vero/macNectin4 cells were infected with the VSV pseudotypes. Infectivity titers of the VSVΔG*-F-dVH and VSVΔG*-F were determined at 24 h p.i.

Ethics statement. The experiments with animals were performed at animal biological safety level 2 in strict accordance with the Animal Experimentation Guidelines of the National Institute of Infectious Diseases. The protocol was approved by the Institutional Animal Care and Use Committee of the institute (permit number 611001). Collection of the specimens from the monkeys was performed under ketamine hydrochloride anesthesia, and all efforts were made to minimize suffering.

RESULTS

Clinical and pathological features of cynomolgus monkeys naturally infected with CDV in the outbreak in Japan. Forty-six monkeys out of 432 cynomolgus monkeys died or were euthanized during a quarantine period after import from China in Japan in 2008, resulting in a fatality rate of 10.6% (46/432) if euthanized animals were considered to be fatal. Clinical signs of sick monkeys were characterized by eye mucus, nasal mucus, rhinitis, coughing, anorexia, diarrhea, fever, and generalized rash, which are similar to those observed in acute measles in humans and in monkeys in recent CDV outbreaks in China. Swelling of the footpads was also observed in sick monkeys. Three moribund monkeys were autopsied for histopathological examination. Histopathologically, two monkeys (animals 11 and 12) were in the acute phase of systemic CDV infection. One monkey (animal 13) was considered to be in a convalescent phase. Various stages of giant cell pneumonia were found in all three monkeys (Fig. 1A). CDV antigen-positive syncytial pneumocytes were seen in the lungs of two monkeys (animals 11 and 12) (Fig. 1B). In the thymus, spleen, tonsils, and lymph nodes of all monkeys, almost all lymphocytes were depleted, suggesting severe immune suppression. The remaining mononuclear cells were positive for CDV antigen in the lymph tissues of two monkeys (animals 11 and 12) (Fig. 1C and D). Focal gliosis and demyelination were found in the cerebrum and/or cerebellum of all monkeys. In these lesions, some neurons and glia cells were positive for CDV antigen in all monkeys (Fig. 1E and F). In other organs, including the skin, small and large intestines, kidneys, salivary glands, and testes, giant cells and/or CDV antigen-positive cells were observed. Interestingly, CDV antigen-positive cells were not observed in the tissues except for the brain and testis of monkey 13. The types of cells with CDV antigens in the tissues were identified by dual staining with antibodies to CDV NP and various cell markers (Fig. 2). CDV antigens were detected in some of the cytokeratin-positive giant cells in the bronchi and bronchiole (Fig. 2). The bronchiolar epithelial cells with CDV antigens were also necitin4 positive (Fig. 2). In addition, the virus antigens were also detected in the $CD3^+$ lymphocytes in the lymph node and the βIII tubulin-positive neurons in the brain (Fig. 2). Sera of the three monkeys (no. 11, 12, and 13) were subjected to multiplex cytokine analysis. Compared with eight normal monkeys from Tsukuba Primate Research Center, naturally CDV-infected monkeys in the outbreak showed upregulated levels of proinflammatory cytokines and chemokines, such as interleukin 1β (IL-1β), IL-6, macrophage inflammatory protein 1α (MIP-1α), MIP-1β, monocyte chemoattractant protein 1

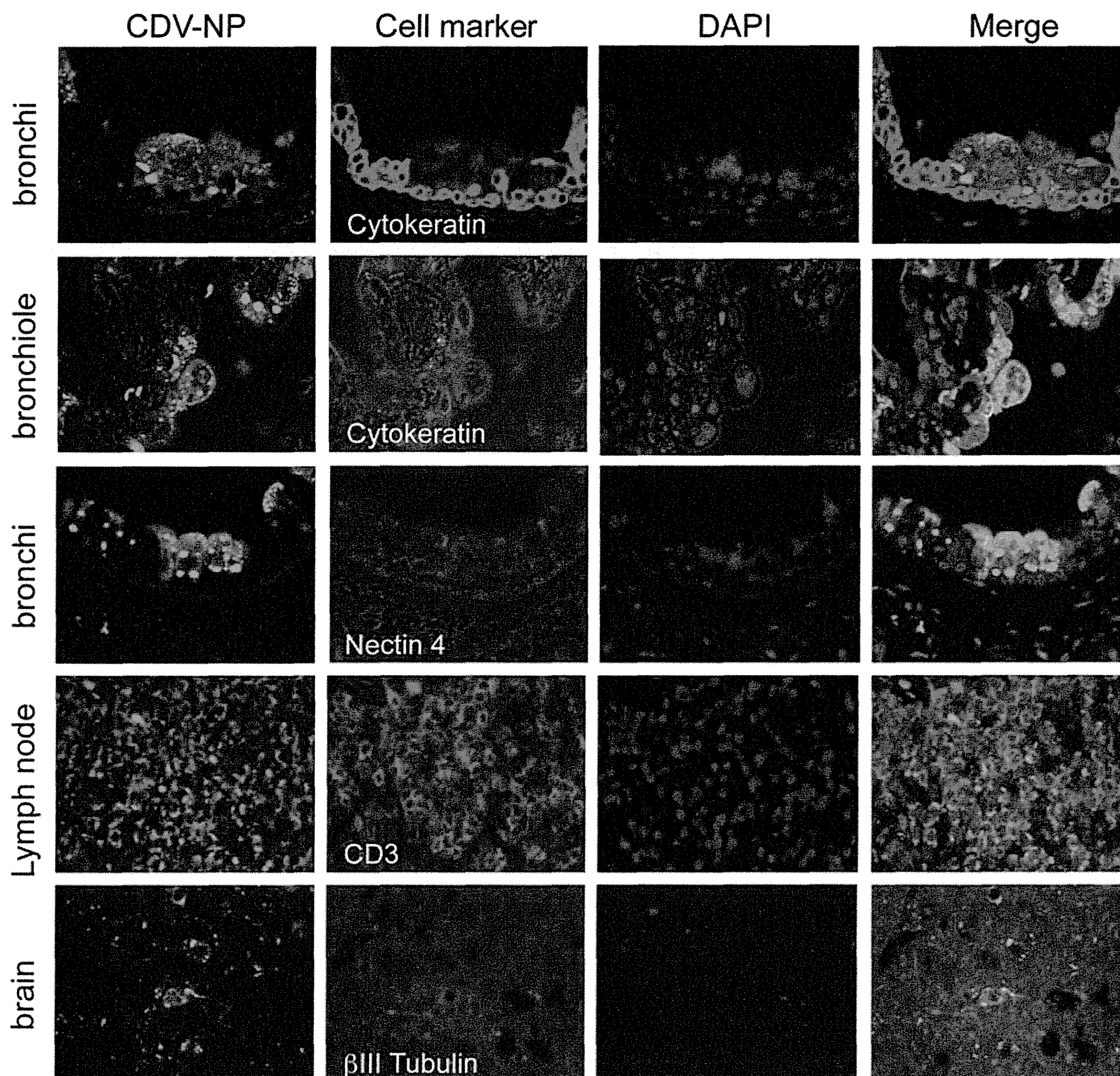

FIG 2 Double immunofluorescence staining of tissues of cynomolgus monkey 11 naturally infected with CDV in the 2008 outbreak. Tissue sections obtained from cynomolgus monkey 11 were examined by double immunofluorescence staining with anti-CDV-NP antibody and rabbit anti-cytokeratin, rabbit anti-neuron-specific β III tubulin, rabbit anti-CD3, or goat anti-nectin4 antibody. CDV-NP antigens were detected in the bronchi, bronchiole, lymph node, and brain. Some CDV-NP antigen-positive cells were positive for cytokeratin, nectin4, or CD3. A few CDV-NP antigen-positive neurons were positive for βIII tubulin.

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(MCP-1), and eotaxin. Proinflammatory cytokines associated with T cell activation, gamma interferon (IFN-γ) and IL-15, were also found. Anti-inflammatory responses of IL-1 receptor antagonist (IL-1ra) were also upregulated in the monkeys (Table 1).

Isolation of CDV from monkeys using Vero.DogSLAMtag cells. Typical syncytia developed in monolayers of Vero. DogSLAMtag cells at as early as 2 days p.i. of spleen homogenates obtained from dead or moribund monkeys. However, syncytia were not observed in Vero cells inoculated with the spleen homogenates (data not shown). One of the CDV isolates was named CYN07-dV.

Relationship between CYN07-dV and Chinese CDV strains associated with monkey outbreaks. The entire genome nucleo-

TABLE 1 Blood chemokine/cytokine levels

	Monkeys naturally infected with CDV			Normal monkeys			
Cytokine	Median (pg/ml)	SD	No. positive/ no. tested	Median (pg/ml)	SD	No. positive/ no. tested	P value[a]
IL-1β	120	66	2/3	<17		0/8	0.023*
IL-6	94	72	3/3	<9		0/8	0.011*
MIP-1α	228	78	3/3	84	48	8/8	0.000**
MIP-1β	157	61	3/3	40	36	7/8	0.001**
MCP-1	3,917	2,286	3/3	461	245	8/8	0.043*
Eotaxin	2,121	1,096	3/3	413	203	8/8	0.023*
IFN-γ	509	150	3/3	261	183	3/8	0.002*
IL-15	89	65	2/3	64	23	1/8	0.115

[a] Asterisks indicate statistically significant differences between monkeys naturally infected with CDV and normal monkeys (*, $P < 0.05$; **, $P < 0.001$).

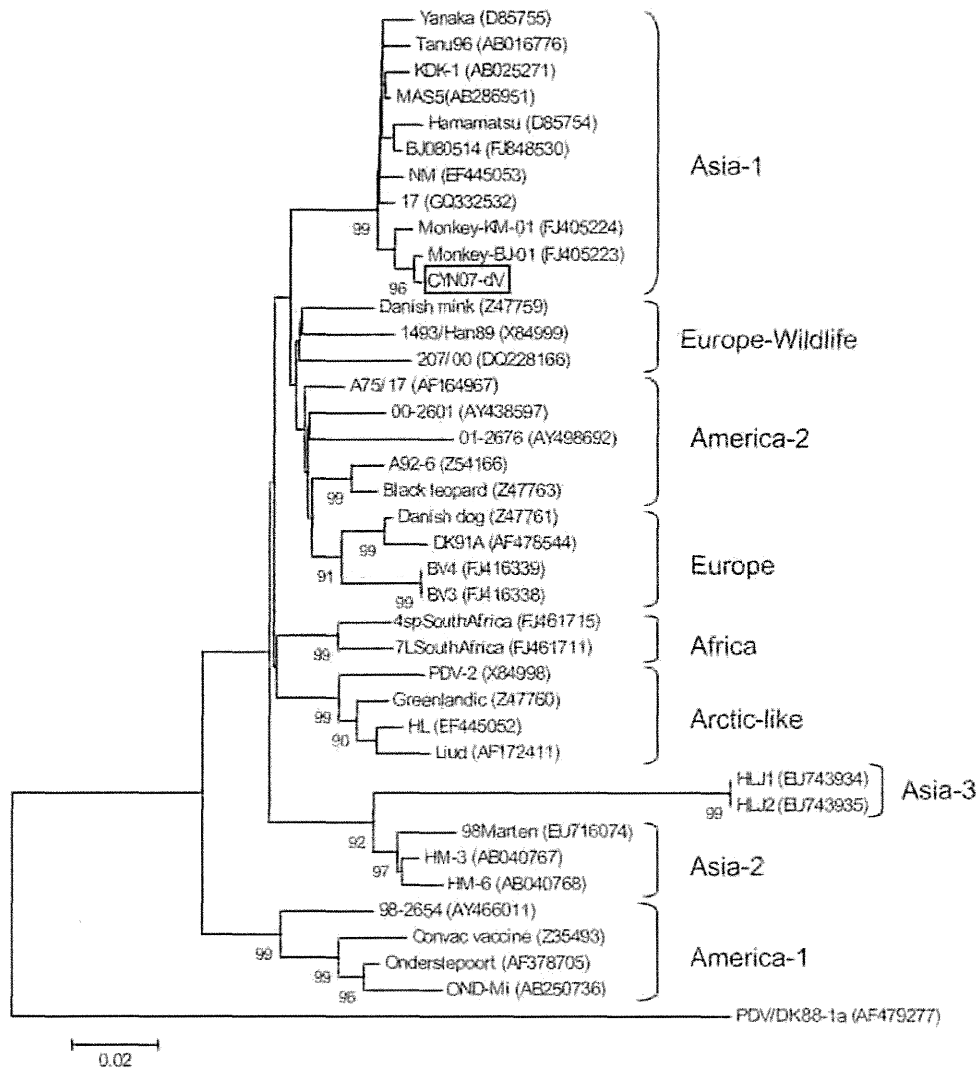

FIG 3 Phylogenetic tree of CDV based on the CYN07-dV sequence. Phylogenetic analysis of the H protein of the CDV showed that the CYN07-dV was closely related to the CDV strains isolated from rhesus monkeys in China. Scale bars indicate phylogenetic distance between CDV isolates.

tide sequence of the CDV strain CYN07-dV was determined (DDBJ/GenBank accession number AB687720). A phylogenic analysis of H protein indicated that CYN07-dV belongs to the Asia-1 clade and is closely related to monkey-BJ-01 and monkey-KM-01 strains (GenBank accession numbers FJ405223 and FJ405224, respectively) isolated from rhesus monkeys in China in 2008 (15) (Fig. 3). Comparative analyses throughout the genomes of CDV strains revealed that CYN07-dV showed the highest homology to the monkey-KM08 strain isolated from a rhesus monkey in China in 2008 (GenBank accession number HM852904) (15) (99.6%; 15,632/15,690 nucleotides). A phylogenic analysis indicated that CYN07-dV showed high homology with CDV isolates from different animal species in China (NM strain isolated from foxes in China and 17 strains isolated from dogs in China), suggesting a Chinese source of the CYN07-dV strain.

Experimental infection of cynomolgus monkeys with CYN07-dV. Five cynomolgus monkeys were infected intranasally with CYN07-dV. In these animals, no lethal infection was observed during an experimental period of 15 days, and clinical symptoms were less severe than those observed in the moribund and dead monkeys during the outbreak. However, all five monkeys had appetite loss at 7 to 12 days p.i. Body weight was decreased in three of the five monkeys (Fig. 4A). The rectal temperature was transiently increased at 3 to 7 days p.i. in some monkeys (Fig. 4B). In all five monkeys, the numbers of white blood cells and lymphocytes were decreased (Fig. 4C and D), as the numbers of CDV-infected PBMCs were increased (Fig. 4E). Neutralizing antibodies against CDV were detected in the monkey sera at 7 days p.i., and then the titers of antibodies were raised at 10 days p.i. (Fig. 4F). Infectious CDV was isolated from various tissues of autopsied specimens at 15 days p.i., including local lymph nodes, lungs, liver, kidneys, intestinal tracts, and central nervous tissues of monkeys (Table 2, 3). In monkey 4965, giant cells were observed in the alveolar area of the lungs where the syncytial pneumocytes were positive for CDV antigen (Fig. 5A and B), and lymphocytes were depleted in the lymph nodes where the follicular cells and mononuclear cells were positive for CDV antigen (Fig. 5C and D). These histopathological findings were also observed in

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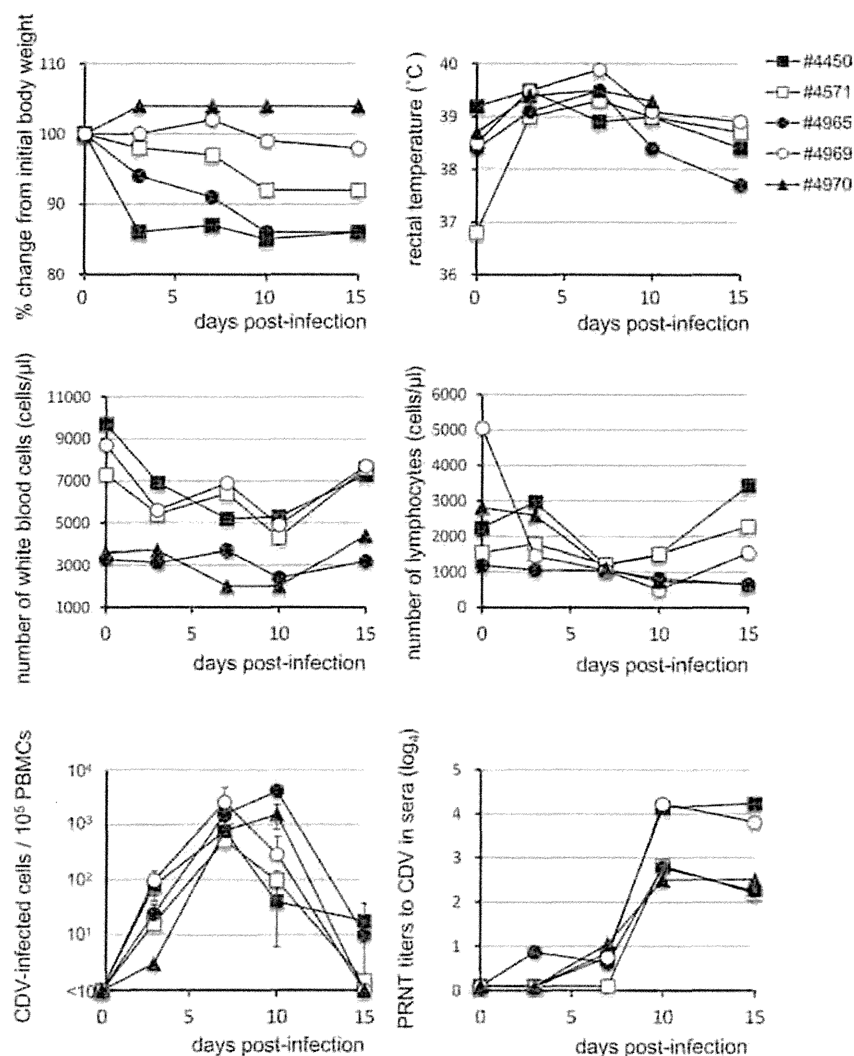

FIG 4 Changes in body weight, rectal temperature, and the numbers of white blood cells, lymphocytes, and CDV-infected PBMCs and neutralization antibody level in the experimentally CDV-infected monkeys. (A) Body weight; (B) rectal temperature; (C) white blood cell count; (D) lymphocytes count; (E) CDV-infected PBMC count; (F) neutralizing antibodies against CDV.

other monkeys but were less severe than those in monkey 4965 (data not shown). The experimentally CDV-infected monkeys showed upregulated levels of IFN-α and IL-1ra at 7 to 10 days p.i. Some monkeys showed upregulated levels of MIP-1β, MCP-1, eotaxin, IFN-γ, and IL-15 (data not shown).

The nucleotide and amino acid sequences of SLAM and nectin4 of cynomolgus monkeys. The nucleotide and deduced amino acid sequences of SLAM and nectin4 of cynomolgus monkeys were determined (DDBJ/GenBank accession numbers AB742520 and AB742522, respectively). Cynomolgus monkey

TABLE 2 CDV isolation from various organs in the experimentally infected cynomolgus monkeys at 15 days p.i.

	Result[a]									
Animal no.	Skin	Respiratory	Liver	Kidney	Intestine	Spleen	Lymph node[b]	Thymus	Tonsil	Central nervous system
4450	−	+	−	−	+	−	−	−	−	−
4571	−	+	−	−	+	+	+	+	+	−
4965	−	+	+	+	+	+	+	+	+	+
4969	−	−	−	−	−	−	−	−	−	−
4970	−	+	−	−	−	−	−	+	+	−

[a] −, CDV negative; +, CDV positive.
[b] Cervical and intestinal lymph node.

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TABLE 3 CDV isolation from throat swabs, rectal swabs, and feces in the experimentally infected cynomolgus monkeys

	Result by day p.i.[a]																
	Throat swabs					Rectal swabs					Feces						
Animal no.	0	3	7	10	15	0	3	7	10	15	1–9	10	11	12	13	14	15
4450	–	–	–	–	–	–	–	+	+	–	–	–	+	+	–	–	–
4571	–	–	–	+	–	–	–	–	+	–	–	+	+	+	+	+	+
4965	–	–	–	+	+	–	–	–	+	–	–	+	+	–	+	+	+
4969	–	–	–	–	–	–	–	–	+	–	–	–	+	+	–	–	–
4970	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[a] –, CDV negative; +, CDV positive.

SLAM showed high levels of identity to rhesus monkey SLAM (DDBJ/EMBL/GenBank accession no. XM_001117605) and human SLAM (DDBJ/EMBL/GenBank accession no. U33017), with 99.9% (100%) and 97.6% (96.7%) nucleotide (amino acid) identity, respectively. Amino acid sequences of the SLAM were completely conserved among three macaques: cynomolgus monkey, rhesus monkey, and pig-tailed monkey (AB742521) (data not shown). On the other hand, cynomolgus monkey SLAM showed a lower level of identity to dog SLAM (DDBJ/EMBL/GenBank accession no. AF390108), with 76.5% (65.0%) nucleotide (amino acid) identity.

Cynomolgus monkey nectin4 showed identity to dog nectin4 (DDBJ/EMBL/GenBank accession no. AB755429), rhesus monkey nectin4 (DDBJ/EMBL/GenBank accession no. XM_001117709), and human nectin4 (DDBJ/EMBL/GenBank accession no. NM_030916), with 89.0% (94.1%), 100% (100%), and 98.3% (99.4%) nucleotide (amino acid) identity, respectively.

CDV strain CYN07-dV utilizes macaca SLAM and macaca nectin4 as receptors. The replication kinetics of CYN07-dV was analyzed in Vero, Vero.DogSLAMtag, Vero/macSLAM, Vero/hSLAM, Vero/dNectin4, and Vero/macNectin4 cells. In Vero and Vero/hSLAM cells, CYN07-dV replicated inefficiently (Fig. 6A). On the other hand, it replicated efficiently in Vero.DogSLAMtag, Vero/macSLAM, Vero/dNectin4, and Vero/macNectin4 cells. The peak titer and replication kinetics of the virus in Vero/macSLAM cells were comparable to those in Vero.DogSLAMtag cells (Fig. 6A). The peak titer of the virus in Vero/macNectin4 cells was also comparable to that in Vero/dNectin4, although virus production was slightly delayed in Vero/macNectin4 cells (Fig. 6A). The wild-type CDV strain Ac96I (13), isolated from

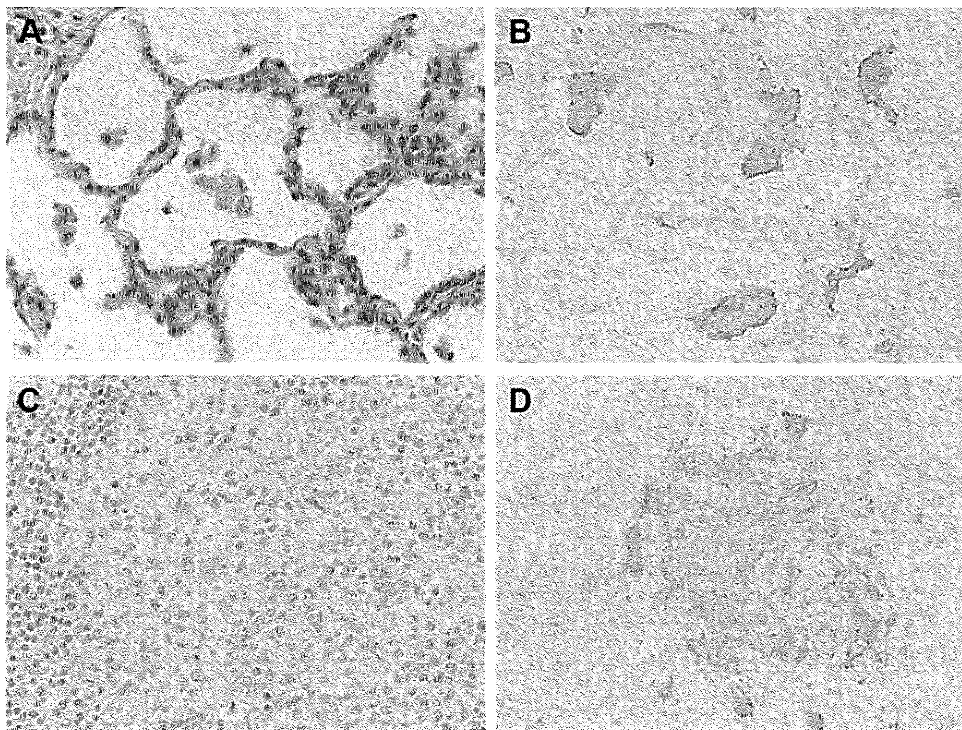

FIG 5 Histopathological analyses of cynomolgus monkeys experimentally infected with CDV. Tissue sections obtained from cynomolgus monkey 4965 were examined by hematoxylin and eosin staining and immunohistochemistry using anti-CDV-NP monoclonal antibody. Giant cells (A) with CDV antigen (B) were observed in the alveolar area in the lung. Lymphocyte depletion (C) and CDV antigen-positive cells were observed in the follicular area (D) in the lymph node of CYN07-dV-infected monkey 4965. HE, ×20; IHC, ×40.

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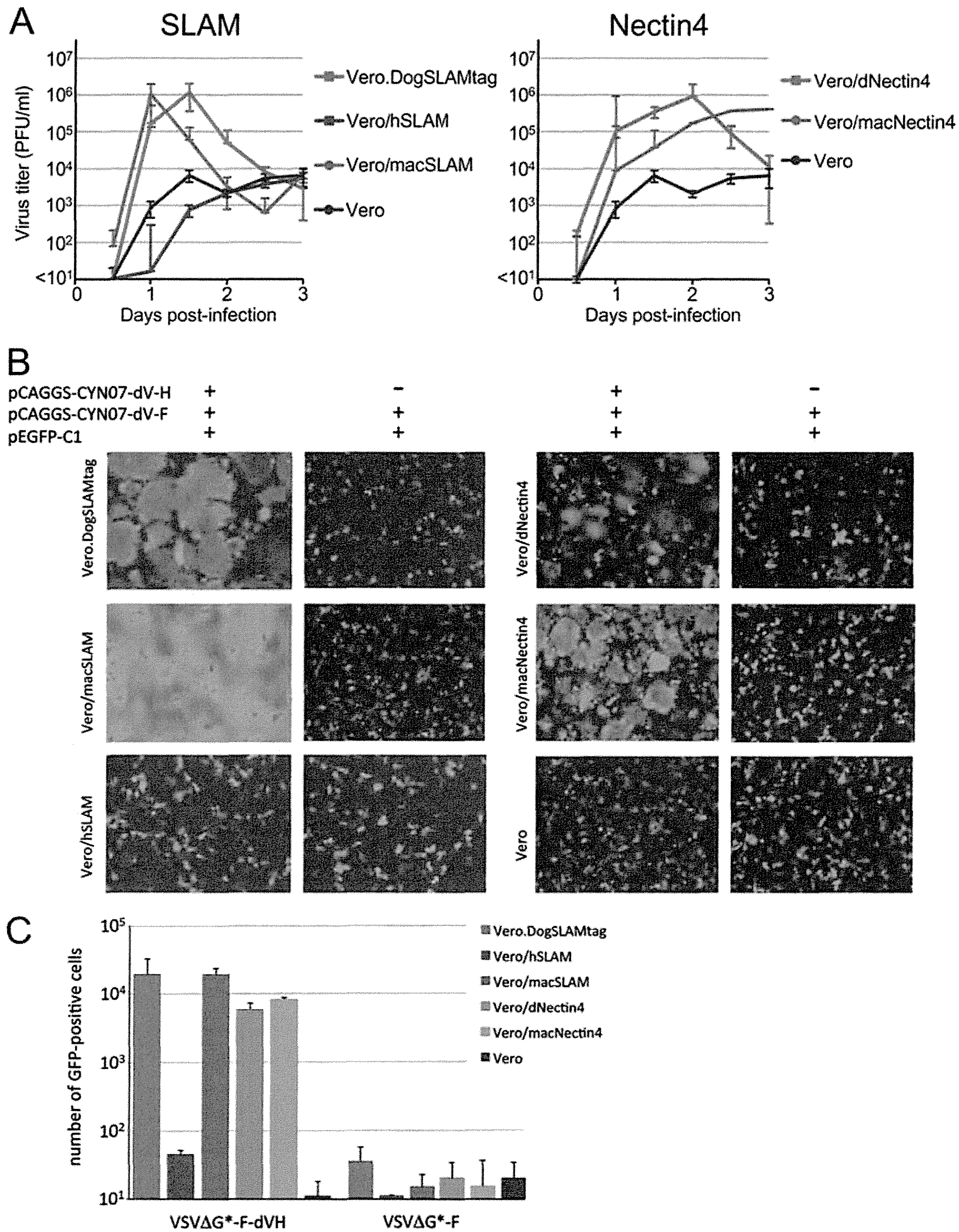

FIG 6 CYN07-dV utilizes macaca SLAM and macaca nectin4 as receptors. (A) Growth kinetics of CYN07-dV in Vero.DogSLAMtag, Vero/hSLAM, Vero/macSLAM, Vero/dNectin4, Vero/macNectin4, and Vero cells. The cells were infected with the virus at an MOI of 0.01, and titers at the indicated points were shown. (B) Induction of syncytium upon transfection with a mixture of plasmids expressing enhanced green fluorescent protein (EGFP) and CDV F with or without a plasmid expressing CDV H. (C) Infectivity of VSV pseudotype bearing H and F proteins of CYN07-dV (VSVΔG*-F-dVH) or that bearing the F protein (VSVΔG*-F) in Vero.DogSLAMtag, Vero/hSLAM, Vero/macSLAM, Vero/dNectin4, Vero/macNectin4, and Vero cells.

a sick dog, also replicated in the Vero/macSLAM cells; however, the appearance of synsytia was delayed in the Vero/macSLAM cells compared with that in the Vero.DogSLAMtag cells, and the peak titer of the virus in the Vero/macSLAM cells was significantly lower than that in the Vero.DogSLAMtag cells (data not shown).

To clarify whether the H and the F proteins of the virus induce syncytia in the cells expressing either SLAM or nectin4, cell-to-cell

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fusion assay was performed upon transfection of the plasmids expressing the F and the H proteins of CYN07-dV. No syncytium formation was detected when the F protein of CYN07-dV alone was expressed in the cells (data not shown). On the other hand, many syncytia were observed in Vero.DogSLAMtag, Vero/macSLAM, Vero/dNectin4, and Vero/macNectin4 cells, but not in Vero and Vero/hSLAM cells, when the F and the H protein of CYN07-dV were expressed together (Fig. 6B). The syncytium formation was most remarkable in Vero/macSLAM cells (Fig. 6B). Although syncytia in nectin4-expressing Vero cells (Vero/dNectin4 and Vero/macNectin4) were smaller than those in SLAM-expressing cells (Vero.DogSLAMtag and Vero/macSLAM), syncytia in Vero/macNectin4 and Vero/dNectin4 cells were comparable (Fig. 6B).

To clarify the function of the H and the F proteins on entry of the virus via the SLAM and the nectin4, the infectivities of the VSV pseudotype bearing the H and the F protein of CYN07-dV, VSVΔG*-F-dVH, in various cells were compared. The VSV pseudotype bearing the F protein alone, VSVΔG*-F, did not infect any cells tested, whereas the VSVΔG*-F-dVH efficiently infected Vero/macSLAM cells and Vero.DogSLAMtag cells (Fig. 6C) but did not infect Vero/hSLAM cells. The VSVΔG*-F-dVH also efficiently infected Vero/macNectin4 cells and Vero/dNectin4 cells (Fig. 6C). These data showed that the CYN07-dV H protein efficiently utilizes macaca SLAM and macaca nectin4 as receptors.

DISCUSSION

Viruses in the genus *Morbillivirus* often cause severe diseases in animals and humans. Generally, symptomatic infection with each morbillivirus occurs in specific animal species or humans. Among the morbilliviruses, MV is the one that causes an acute febrile and systemic infection in humans. Although CDV also shows host specificity and causes acute infections primarily in dogs, it often affects animals in different species, including nonhuman primates, showing a high mortality rate. In the CDV outbreak that occurred among Japanese monkeys in Japan in 1989, only one monkey out of 34 died (14). However, in recent outbreaks in China and Japan, higher mortality rates were recorded: 4,250 monkeys out of ~10,000 died in Guangxi Province in 2006, 12 out of 20 died in Beijing in 2008, and 46 out of 432 died in Japan in 2008. Most authorized animal suppliers in China receive monkeys from a Guangxi farm and distribute monkeys to researchers throughout the mainland of China. Laboratory investigations of clinical specimens from moribund and/or dead monkeys in the present study and in earlier studies (15, 16) fulfilled the two criteria of Koch's postulates: (i) detection of CDV in sick animals and (ii) isolation of CDV in cultured cells. The experimental infection in the study further fulfill the remaining postulates: (iii) induction of a comparable disease in the original host and (iv) reisolation of CDV from experimentally infected animals. These findings proved that CDV is the primary cause of the outbreak in monkeys. Moreover, the numbers of monkeys infected with CDV in the Guangxi farm decreased greatly after the introduction of attenuated CDV vaccination in early 2009, even though a few cases have still been reported every year (15).

In the present study, mortality was not observed in experimentally CDV-infected monkeys. However, many monkeys, especially those that eventually became CDV antibody-positive at the outbreak, showed mild or no symptoms. Severe symptoms were observed only in some moribund and dead monkeys in the outbreak. Thus, mortality might have been observed if more monkeys were experimentally infected, even though we could not rule out the possibility of enhanced pathogenicity of CDV by coinfection of some other agent during the outbreak. To date, no other particular agents were detected in the monkeys. However, we could not rule out the possibility that isolation and passage of the virus in Vero/dog.SLAM could have caused a partial attenuation of the virus. This may be clarified in future to analyze the quasispecies of genome sequences of the virus in the original clinical samples of the monkey and to compare them to the sequence of the isolated virus.

In the present study, three moribund monkeys in the 2008 CDV outbreak showed upregulated levels of proinflammatory cytokines and chemokines, such as IL-1β, IL-6, MIP-1α, MIP-1β, MCP-1, eotaxin, IFN-γ, and IL-15. Anti-inflammatory responses of IL-1ra were also upregulated. In the rhesus monkeys infected with measles virus, suppression of IL-12 in the sera was reported (20). The induction of IFN-γ, IL-2, and MCP-1 in the sera of the measles virus-infected cynomolgus monkeys was also reported (21). However, comparable levels of upregulation in proinflammatory cytokines, chemokines, and anti-inflammatory responses of IL-1 receptor antagonist observed in the CDV-infected monkeys were not reported for the measles virus-infected monkeys. These observations are rather similar to rhesus monkeys infected with a lethal dose of Zaire ebolavirus (22). The mixed anti-inflammatory response syndrome in Zaire ebolavirus-infected monkeys is characterized by highly elevated levels of IL-13 and IL-1ra, which are similar to the CDV-infected monkeys in the outbreak. Thus, unbalanced responses of cytokines and chemokines may have contributed to the pathogenesis of fatal cases of CDV infection in monkeys in the outbreak.

The CYN07-dV efficiently infected Vero cells expressing dog and macaca SLAM but not the cells expressing human SLAM. This was confirmed by syncytium induction upon transient expression of the H and the F proteins of the virus and also by infectivity of the VSV pseudotype bearing the H and the F proteins of the virus. On the other hand, the CYN07-dV efficiently utilized nectin4 of dog and macaca. Thus, the CYN07-dV is capable of utilizing macaca SLAM and macaca nectin4 as receptors, as efficiently as dog SLAM and dog nectin4, respectively. These findings were consistent with the experimental infection of the CYN07-dV to cynomolgus monkeys, in which the virus infected PBMCs and epithelial cells expressing macaca SLAM and nectin4, respectively. Interestingly, the CYN07-dV did not efficiently utilize human SLAM as a receptor even though the SLAMs of human and macaca are highly conserved. Thus, at the moment, CDVs like CYN07-dV may not be a direct threat to humans. However, the expansion of host animal species of CDV to include primates might be a global threat in the future. Wild-type CDV strains isolated from dogs with distemper were recently shown to efficiently utilize both dog SLAM and dog nectin4 as receptors (13, 17). However, the wild-type CDV strain Ac96I also replicated in the Vero/macSLAM cells even though less efficiently than in the Vero.DogSLAMtag cells. This suggested that some wild-type CDV strain is capable of utilizing macaca SLAM as a receptor *per se*, even though the CYN07-dV utilizes macaca SLAM more efficiently. Thus, the CYN07-dV is considered to be adapted to spread among monkeys using macaca SLAM and macaca nectin4.

Nucleotide and amino acid sequence accession numbers. The complete nucleotide sequence of the CYN07-dV has been

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deposited in DDBJ/GenBank under accession number AB687720, while the amino acid sequence of hemagglutinin protein has been deposited in DDBJ/GenBank under accession number BAM15593. The complete nucleotide sequences of mRNAs of cynomolgus monkey SLAM, pig-tailed monkey SLAM, and cynomolgus monkey nectin4 have been deposited in DDBJ/GenBank under accession numbers AB742520, AB742521, and AB742522, respectively.

ACKNOWLEDGMENTS

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新興・再興・輸入感染症

加藤康幸

わが国は四方を海に囲まれ，有史以来，感染症の国外からの流入に絶えず気を配ってきた。この30年間にHIV感染症，出血性大腸菌感染症などすでに国内に定着した新興感染症もあるが，主に海外渡航者が罹患する輸入感染症として捉えられる疾患も多い。本稿では，日常臨床で経験することが比較的多い輸入感染症を中心に，新興・再興感染症について概説する。

19世紀後半からの微生物学，化学療法，予防接種の発展により，1970年代ごろまでに感染症の時代は終わったとする楽観的な風潮が広まった。1980年の痘瘡根絶が象徴的である。しかし，このころから新たに認識される感染症(新興感染症)が次々と見つかるようになった。また，これまでにも知られていたが，常在地とは考えられなかった地域で伝播したり，薬剤耐性を示すようになったりすることで再認識される感染症(再興感染症)も出現するようになった(**図1**)。これら感染症を取り巻く状況の変化は，近年の航空機による大量輸送などにより加速している可能性もあるが，人類の歴史では絶えず起こってきた現象とも考えられる(**表1**)。

わが国においても，過去30年あまりに，さまざまな新興・再興感染症が経験されている(**表2**)。1887年に制定された伝染病予防法は，これらの新たな感染症の脅威に対応できず，1999年に「感染症の予防及び感染症の患者に対する医療に関する法律(感染症法)」が施行された。その後も，米国同時多発テロでの炭疽菌事件(2001年)，中国を中心とした重症急性呼吸器症候群(SARS)の流行(2003年)，東南アジアでの鳥インフルエンザ(H5N1)常在地の拡大などを契機に法改正が行われてきた。

輸入感染症とは

ある感染症の罹患率は世界一様ではなく，さまざまな要因により地理的に大きな差を示す場合がある。温帯に位置するわが国にとって，マラリア，デング熱などの蚊媒介性の熱帯感染症は代表的な輸入感染症である。また，本来は世界中に分布しうる疾患であるが，衛生の改善，予防接種の普及などにより，わが国では発生が目立たなくなった疾患がある。A型肝炎，腸チフス・パラチフス，麻疹などがあげられる。**輸入経路として，旅行者，媒介動物，汚染された物品などがあげられるが，臨床上は前者が最も重要な経路である(旅行者関連感染症)**。これらの輸入感染症は，その定義から国内ではまれな疾患であるため，医師の鑑別診断にもあげられず，診断や治療に遅れが生じることになりやすい。

輸入感染症の疫学

先進工業国から開発途上国への渡航者の約8%(年間約400万人)が，渡航中や帰国後に医療を受ける必要が生じると推定されている。渡航先により罹患しやすい疾患は異なるが，主訴では下痢，発熱，呼吸器症状の順に頻度が高い。

国際旅行医学会と米国疾病管理センター(CDC)

Kato Y：国立国際医療研究センター，国際疾病センター国際医療支援室

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図1　新興感染症の世界地図

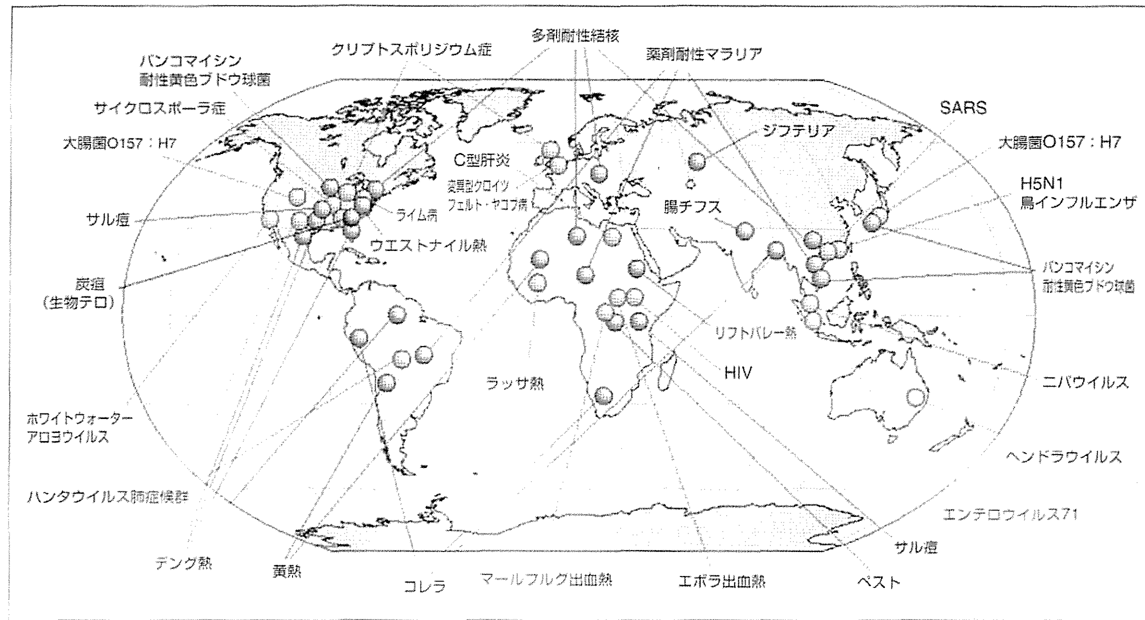


表1　感染症が新興・再興する要因

国際貿易
人口動態
感染症に対する感受性の変化
貧困
戦争・飢饉
公衆衛生の破綻
技術の発達
生態系の変化
気候・天候
生物テロ
政治的意思の欠如
微生物の変化
経済開発

表2　わが国からみた新興・再興感染症の歴史

1974年	わが国で最後の痘瘡症例
1987年	シエラレオネからラッサ熱の輸入例(東京)
	国内ではじめてAIDS患者の報告(神戸パニック)
1995年	バリ島への旅行者を中心にコレラの集団発生
1996年	堺市を中心に病原性大腸菌O157：H7による食中毒
1999年	感染症法が施行
	結核緊急事態宣言
2000年	東京や大阪などの大都市を中心に麻疹の流行
2001年	米国同時多発テロ(炭疽菌事件)をまねた不審郵便物の発見
2003年	SARSの流行(台湾人医師事例)
2005年	京都，山口，大分で鳥インフルエンザ(H5N1)の発生
2006年	フィリピンから狂犬病の輸入例2例が38年ぶりに報告
2009年	新型インフルエンザ(H1N1)の発生
2010年	都内大学病院で多剤耐性アシネトバクター菌による院内感染

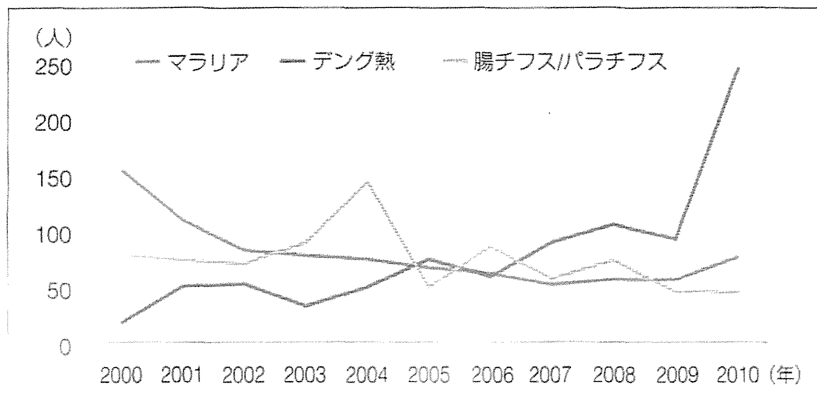
が共同で行っているGeoSentinel Surveillance Networkの調査によれば，帰国後に医療機関を受診した渡航者24,920人中，6,957人(28%)に発熱を認めた。発熱を主訴とした患者の最終診断は，全身性発熱疾患35%，急性下痢症15%，呼吸器疾患14%，泌尿器疾患4%，不明22%などとなっている。全身性発熱疾患では，マラリア21%，デング熱6%，腸チフス・パラチフス2%，リケッチア症2%の順となっている。マラリアの割合が最も高いが，渡航地による差が大きいことに注意する。

◎発熱のある渡航者の評価

海外渡航歴のある発熱患者では，まず，臓器症状・所見(下痢，呼吸器症状，神経症状など)の有無を評価する。マラリア，デング熱，腸チフス・パラチフスなどの比較的頻度の高い熱帯感染症は，局所症状・所見のない発熱(undifferentiated fever)として捉えられることが多い。

渡航先と渡航期間は診断のうえで決定的な役割を果たすことがある。渡航期間は，渡航先で感染した場合の潜伏期を計算するうえで不可欠の情報

図2　主な輸入感染症の年間報告数の推移



(感染症発生動向調査より)

表3　主な発熱性熱帯感染症の潜伏期(目安)

7日以内	8〜20日	21日以上
・細菌性下痢症 ・インフルエンザ ・デング熱 ・チクングニア熱 ・リケッチア症 ・黄熱	・マラリア(熱帯熱) ・腸チフス ・パラチフス ・ラッサ熱 ・レプトスピラ症 ・アフリカトリパノソーマ症	・急性A・E型肝炎 ・マラリア(非熱帯熱) ・赤痢アメーバ症 ・片山熱(急性住血吸虫症)

である(**表3**)。多くの熱帯感染症は曝露後1カ月以内に発症する。日本国内でも経験する感染症(肺炎，尿路感染症，菌血症，伝染性単核球症など)は，渡航者においても同じように経験されることに注意する

白血球分画を含めた全血算がスクリーニングとして有用性が高い。マラリア，デング熱においては，血小板減少の頻度が高い。また，寄生虫症では好酸球増多がみられることが多いが，マラリアや赤痢アメーバ症などの原虫症では，好酸球増多はみられないことが多い。一方，腸チフスやリケッチア症においては，好酸球減少がよくみられることも知っておくと役立つかもしれない。

診療にあたっては，咳エチケットを含めた標準予防策を遵守する。マラリア，デング熱，ウイルス性出血熱では，血液曝露による医療従事者の発症が報告されている。欧州では，数年に1回，ラッサ熱の輸入例が報告されているが，診断前の院内感染はまれである。

代表的な輸入感染症

旅行者下痢症とインフルエンザ様疾患を除くと，マラリア，デング熱，腸チフス・パラチフスが旅行者の最も罹患しやすい感染症である。いずれも局所症状・所見のない発熱(undifferentiated fever)として捉えられることが多い。国の感染症発生動向調査による年間報告数を**図2**に示した。

◎マラリア

マラリアはマラリア原虫の感染による急性発熱性疾患である。ヒトの赤血球に感染するマラリア原虫は5種(熱帯熱マラリア原虫，三日熱マラリア原虫，卵形マラリア原虫，四日熱マラリア原虫，二日熱マラリア原虫)知られているが，なかでも**熱帯熱マラリア原虫**(***Plasmodium falciparum***)は，免疫のない渡航者において致死的な重症マラリア(severe malaria)をきたすため，特に重要である。

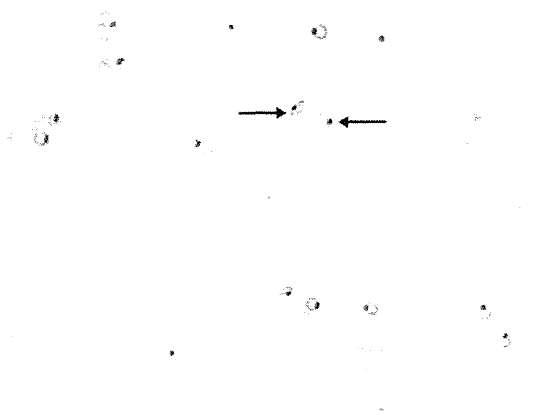


図3　熱帯熱マラリア患者の末梢血塗抹標本
赤血球内に熱帯熱マラリア原虫の輪状体がみられる。原虫が感染している赤血球は大きくなく，原虫が辺縁にみられ，1つの赤血球内に複数の原虫を認める（↑）のは熱帯熱マラリアの特徴である。免疫のない旅行者では，原虫寄生率（感染赤血球の割合）が2%を超えると重症マラリアの徴候を認めることが多い。本症例の原虫寄生率は12%であり，急性腎不全を合併した。

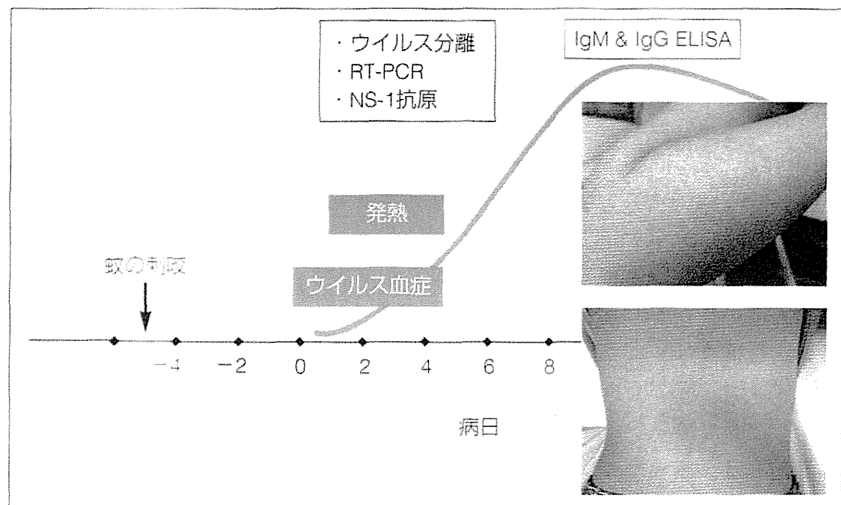


図4　デング熱の検査診断
第5病日ごろまでの有熱期にはウイルス血症を認めるため，この時期の検査診断では，ウイルス分離，RT-PCR法によるウイルス遺伝子の検出，ウイルスNS-1抗原の検出が有用である。特異的IgM抗体は第5病日ごろから検出されるようになる。解熱時に四肢，体幹に紅斑を認めることが多い。

感染ハマダラカの刺咬後，一定の潜伏期（原虫は肝細胞内で増殖する）を経て発症する。マラリアは高度流行地では無症候感染（貧血や脾腫は認める）も多いが，免疫のない渡航者では，ほとんど例外なく突然の高熱（体温39℃以上）と頭痛で発症する。三日熱や卵形マラリアでは48時間ごと（四日熱マラリアは72時間ごと）の周期熱になるが，熱帯熱マラリアでは稽留熱となることが多い。特徴的な症状はなく（頭痛，筋肉痛，倦怠感など），**診断には血液検査による原虫の確認が必須である（図3）**。

熱帯熱マラリアが急速に進行する致死的な疾患であることから，**マラリア常在地（特にサハラ以南アフリカ）**より帰国した発熱患者では，マラリアの検査診断を行うことが勧められる。

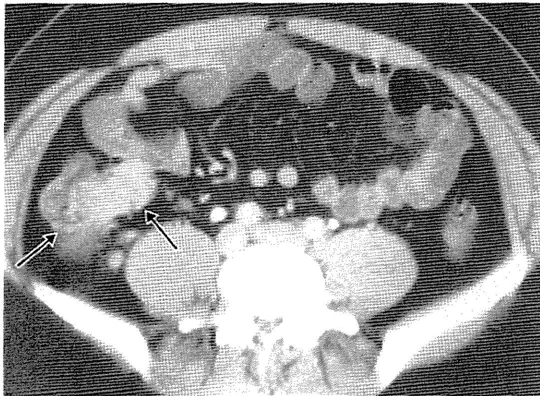
免疫のない渡航者の熱帯熱マラリアでは，第3病日以降，重症化の徴候（頻呼吸，意識障害，黄疸など）が出現するようになる。罹患年齢によって臨床像に差がみられるが，免疫のない成人患者では，**黄疸**，**急性腎不全（乏尿）**，**代謝性**アシドーシス，**意識障害**を合併することが多い。

合併症のないマラリアの治療は塩酸メフロキン錠，塩酸キニーネ末が使用される。重症マラリアでは，厚生労働科学研究班「熱帯病治療薬研究班」の薬剤使用機関において，未承認のグルコン酸キニーネ注，アーテスネート坐薬が使用できる。

◎デング熱

デング熱は，**主に東南アジア**，**南アジアや中南米**で流行しているヤブカ媒介性の急性発熱性疾患である。マラリアと異なり，**これら地域の都市部**でも流行している。4つの血清型をもつデングウイルスがその原因である。ほとんどの渡航者において，5〜7日程度で自然に回復する予後良好な疾患であるが，常在地では，その重症型の臨床像であるデング出血熱の発生に悩まされている（**図4**）。

図5　腸チフス



WBC：5,490/μL
Hb：16.0g/dL
Hct：42.4%
PLT：16.2万/μL
AST：69U/L
ALT：64U/L
LDH：500U/L
γ-GTP：90U/L
BUN：11.8mg/dL
Cr：1.14mg/dL
Na：126mEq/L
K：3.2mEq/L
CRP：24.97mg/dL

腸チフスは菌血症を伴うが，白血球数は正常範囲にあることが多い。また，白血球分画で好酸球減少を認める。トランスアミナーゼ，LDHの軽度上昇を伴うことが多い。

CT
回盲部の腸管壁肥厚を認める（↑）。

異なる血清型のデングウイルスには交差免疫が長期間維持されず，かえって再感染時に増悪しデング出血熱が起こりやすいというのが最も有力な仮説である

デング出血熱の病態は，血漿漏出によるショックであり，急性期の輸液療法が救命の鍵となる。特異的な抗ウイルス薬はない。診断は，血清からのウイルス分離，RT-PCR法による遺伝子検出とEIA法による特異的IgM抗体の検出による。行政検査として，国立感染症研究所ウイルス第一部や地方衛生研究所に依頼することができる。

出血傾向に注意しながら，毎日血球計算を行いつつ，経過を観察する。頭痛，関節痛にはアセトアミノフェンを使用する。

◯腸チフス・パラチフス

Salmonella enterica subsp. enterica serover Typhi Paratyphi A)による発熱性疾患である。腸チフスという名称から消化管感染症のイメージがあるが，原因菌は網内系のマクロファージ内で増殖し，菌血症を伴う　**汚染された飲食物の摂食が主な感染経路で，7～14日間の潜伏期を経て発症する。近年はインドおよびその周辺国での感染が多い。**回盲部のPeyer板に発症が起きるため同部の腸管壁肥厚を認めることが多い（**図5**）。

診断は血液培養による。第3病週になると血液培養の感度は低くなるため，骨髄や便の培養検査も行う。

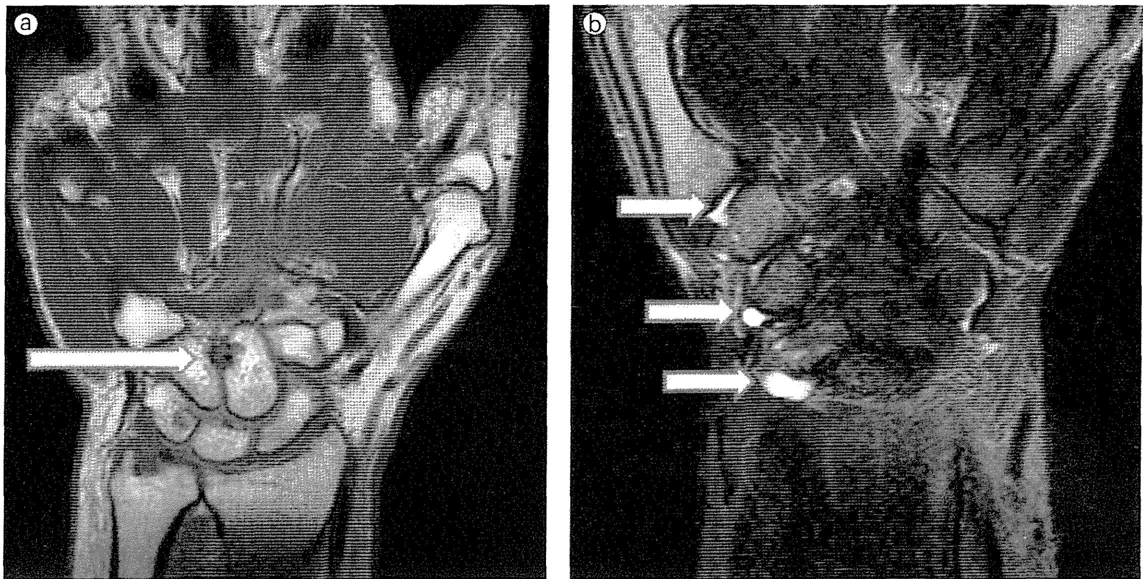
治療はフルオロキノロン薬が第一選択薬になるが，罹患率の高いインドおよびその周辺国では同薬に低感受性を示す菌が増加しているため，分離菌の薬剤感受性試験結果が判明するまではセフトリアキソンを選択する。

◯チクングニア熱

デング熱によく似た臨床像を示す疾患にチクングニア熱がある。トガウイルス科アルファウイルス属チクングニアウイルスによる急性の発熱性疾患である。ヤブカ（ネッタイシマカやヒトスジシマカ）によって媒介され，4～7日の潜伏期を経て発症することが多い。発熱，関節痛，発疹が3主徴である。多くは7日以内に症状は消失するが，多関節痛が長期間続くこともある（**図6**）。

チクングニア熱は基本的に熱帯感染症という性格をもつが，近年流行地は拡大しており，再興感染症として注目されている。わが国でも，**2011年2月から4類感染症，および検疫感染症として，国内での発生・蔓延を防ぐ体制が強化された。**2004年にケニアではじまったチクングニア熱の流行は，2005～2006年には仏領レユニオン（推定患者数27万人）やインド（推定患者数140万人），2009年までには東南アジアの広い地域に拡大した。これに伴い，海外旅行者による輸入症例が増加し，世界の一部の温帯地域でも国内伝播が報告されている。2007年にはイタリアで295例のチクングニア熱が確認され，インドからの旅行者が発端症例と推定されている。2010年にはフランスや中国でも国内伝播が報告された。このように広範な地域に流行が拡大した背景として，ヒトスジシマカでの増殖活性が増加したウイルス株の出現などがいわれている。

図6　チクングニア熱



a：T1強調冠状断像
手根骨において関節リウマチ様の骨びらんによる低信号を認める(↑)。

b：T2強調冠状断像
手関節に液体貯留を認める(↑)。

血液検査所見
デング熱と比較して，血小板減少が目立たないことが多い。

a	b
WBC：5,190/μL	WBC：4,020/μL
PLT：16.7万/μL	PLT：18.7万/μL
AST：2.5 IU/L	AST：23 IU/L
ALT：28 IU/L	ALT：15 IU/L
CRP：0.03mg/dL	CRP：0.28mg/dL

(文献6より転載)

新興呼吸器感染症

新興感染症のなかでも次にあげる呼吸器感染症は，

①ヒトからヒトに飛沫によって伝播し，流行が比較的早く拡大する可能性がある
②呼吸不全を合併し，患者に集中治療が必要になる

という点で重要である。これらの感染症は治療法のみならず，医療機器や施設の不足，医療従事者や患者間での院内感染への対応という解決の難しい問題も提起している。2009年に発生した新型インフルエンザ(H1N1)は記憶に新しいが，今後も警戒が必要なSARSと鳥インフルエンザ(H5N1)について述べる。

◎SARS(図7)

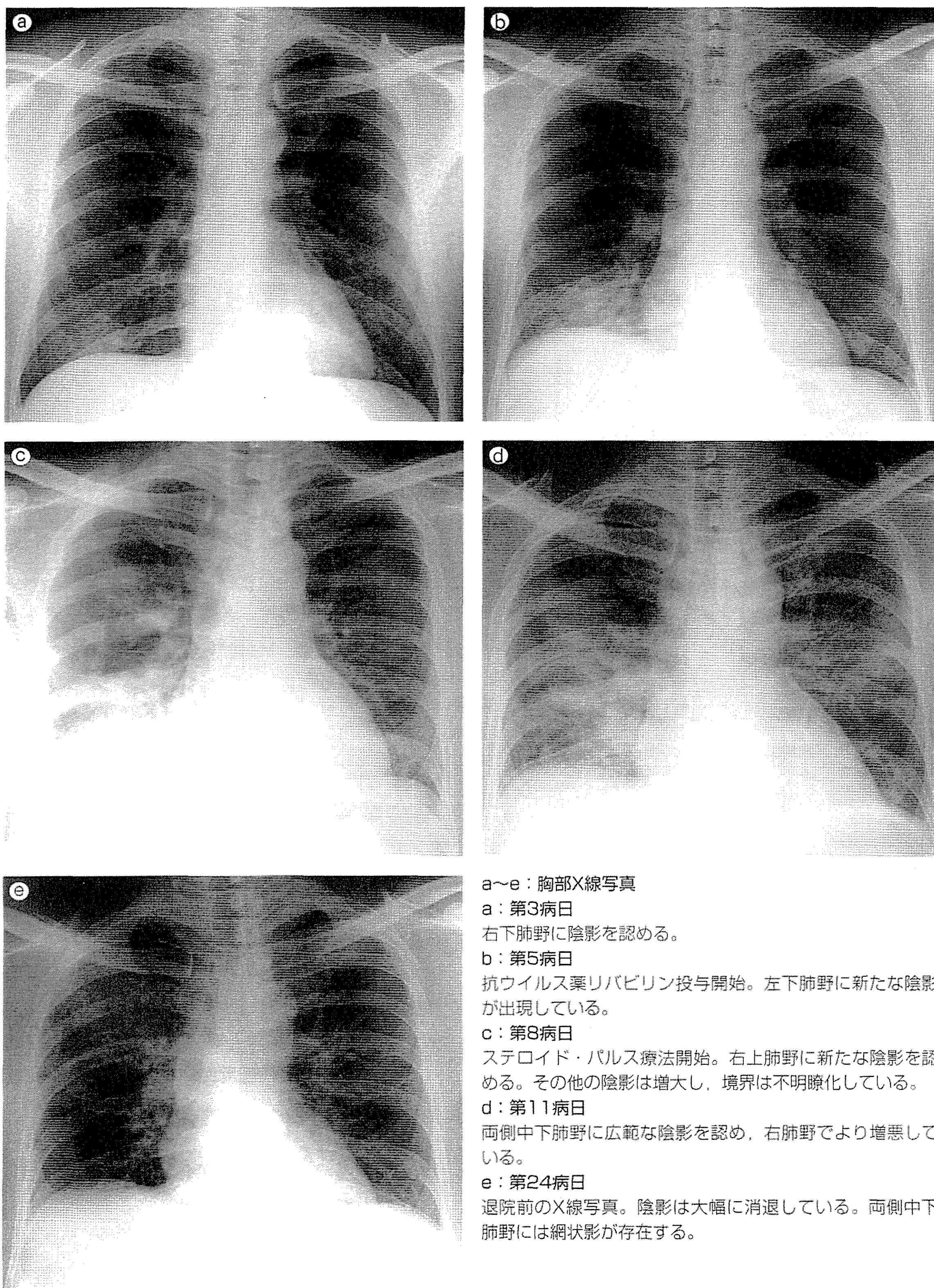
2002年11月に中国広東省仏山市で流行がはじまったと考えられるSARSコロナウイルスを病原体とする肺炎である。2003年2月にSARSを発症した医師が滞在した香港からベトナム，シンガポール，カナダなどに流行が拡大した。同年7月までに流行は終息したが，26カ国から8,098名の患者と774名の死亡者が報告された。このうち中国本土，香港，台湾が全体の90%を占め，わが国での発生報告はなかった。2004年4月に実験室内感染が北京市で報告されたが，以後の報告はない。

潜伏期は2～10日間で，インフルエンザ様症状が1週間ほど続き，息切れ，呼吸困難などの肺炎に伴う症状がはっきりしてくる。下痢もみられることが多い。第3病週に回復に向かうが，約20%の患者で急性呼吸促迫症候群(ARDS)をきたした。確立した抗ウイルス療法はない。

◎鳥インフルエンザ(H5N1)

1997年5月にARDSのため死亡した3歳の男児から高病原性鳥インフルエンザウイルス(A/H5N1)

図7　SARS
40歳代，男性。



a～e：胸部X線写真
a：第3病日
右下肺野に陰影を認める。
b：第5病日
抗ウイルス薬リバビリン投与開始。左下肺野に新たな陰影が出現している。
c：第8病日
ステロイド・パルス療法開始。右上肺野に新たな陰影を認める。その他の陰影は増大し，境界は不明瞭化している。
d：第11病日
両側中下肺野に広範な陰影を認め，右肺野でより増悪している。
e：第24病日
退院前のX線写真。陰影は大幅に消退している。両側中下肺野には網状影が存在する。

Antonio GE, et al：Radiographic-clinical correlation in severe acute respiratory syndrome: study of 1373 patients in Hong Kong. Radiology, 237：1081-1090, 2005.より許可を得て転載

図8　鳥インフルエンザウイルス(H5N1)

30歳代，女性。農業，ベトナム北部在住。

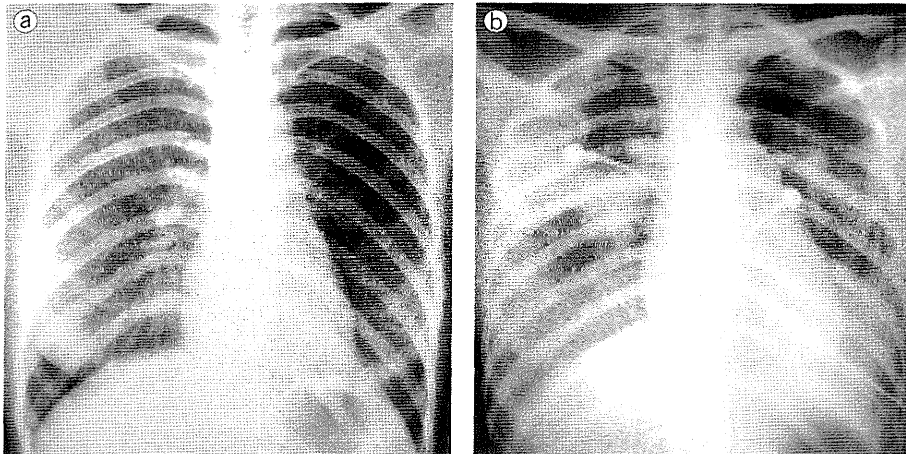
現病歴：2003年12月中旬から近所で多数のニワトリが死亡した。12月25日，12歳の娘が肺炎を発症し，30日に死亡した。娘の入院中はベッドサイドに付き添っていた。2004年1月1日より倦怠感，乾性咳嗽が出現した。4日，発熱，呼吸困難が出現し，5日，肺炎の診断で入院した。

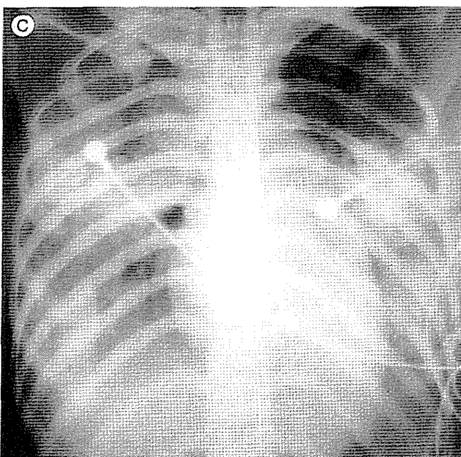
既往歴：特記すべきものなし。

入院時身体所見：体温38℃，血圧80/40mmHg，呼吸数30回/分，脈拍90回/分，チアノーゼあり，意識清明，両側肺でラ音を聴取。

入院時検査所見：WBC 1,700/μL，RBC 508×10⁴/μL，PLT 66,000/μL，AST 327 U/L，BUN 14.3mg/dL，Na 133 mEq/L，K 3.1 mEq/L，Cl 101 mEq/L。

入院後経過：セフェピム，ガチフロキサシン，アジスロマイシン，メチルプレドニゾロンが使用されたが，入院2日後，呼吸不全のため死亡した。抗インフルエンザウイルス薬は使用されなかった。死後，患者の気道吸引液と娘の肺組織からRT-PCR法でH5N1ウイルスが検出された。





a：第2病日の単純X線写真
b：第5病日の単純X線写真
c：第7病日の単純X線写真

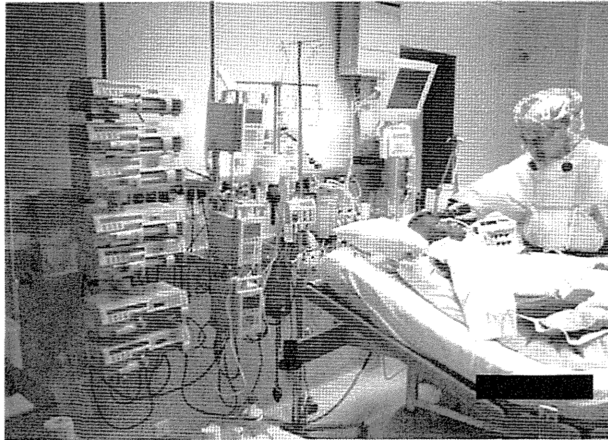
(ベトナム国立熱帯病感染症研究所のご厚意による)

が検出され，その年18例の症例が見つかったのがこの疾患が注目される最初である。その後，SARSが終息した2003年末までに東南アジアの広い地域で家禽の間にこのウイルスが定着していたことが判明した。

2011年3月25日現在，世界保健機関(WHO)に2003年以降報告された鳥インフルエンザ(H5N1)の累積症例数は535(死亡率59%)である。**患者の90%は40歳以下の若年者で性差はない。致死率は10〜19歳の群で最も高く，50歳以上の群で最も低い**が，その理由はわかっていない。無症候感染や軽症例はまれで，海外旅行者の感染は報告されていない。

感染家禽と接触で感染し，潜伏期間は2〜5日間と推定されている。初発症状は発熱，頭痛，筋肉痛などである。**東南アジアの常在地では，第4病日に医療機関を受診し，第10病日に呼吸不全で死亡するのがこれまで報告されている典型的な経過である。**家禽において高病原性H5N1ウイルスは，全身の臓器で増殖し，ウイルス性出血熱に類似した病態を示すが，ヒトにおいては肺炎が主病態である。二次的な細菌性肺炎はほとんどなく，ウイルス性肺炎と考えられる(**図8**)。

図9　ドイツ高度隔離施設（ゲーテ大学病院）におけるラッサ熱の集中治療



ラッサ熱などウイルス性出血熱の重症例は多臓器不全やショックを伴う。血液，体液にはウイルスが高度に含まれるため，医療従事者は自らが感染しないように細心の注意をはらう必要がある。個人用防護具を着用しながらの集中治療は困難な作業である。

（Brodt教授のご厚意による）

血液検査所見では，白血球減少，リンパ球減少，血小板減少，肝トランスアミナーゼの上昇が認められる。これらは，東南アジアに多いデング熱に類似した所見であり，ウイルス血症が示唆される。実際，多くの死亡例で，血液からH5N1ウイルスが検出されている。しかし，剖検にて肺や消化管以外の臓器でウイルス増殖が確認されるのはまれである。治療は抗ウイルス薬（ノイラミニダーゼ阻害薬）を含めた集学的治療が行われる。

今後注目される輸入感染症

◯麻疹

2000年に入り麻疹の大きな流行を経験したわが国は，麻疹含有ワクチン2回接種の定期接種化（2006年），麻疹予防指針の制定（2008年），地方衛生研究所での検査診断推進（2009年）など，2012年に麻疹の国内排除を目標に対策を進めてきた。すでに症例の約50％が成人となり，疫学が近年大きく変化している。2011年前半は相対的に輸入例が目立ち，フランスを中心とした欧州での流行を反映して，遺伝子型D4ウイルスが多く検出された。

輸入例を契機とした学校や病院での集団発生事例が目立つことが予想され，医療関係者における免疫の確認は今後も継続していく必要がある。

◎ウイルス性出血熱

ラッサ熱はアレナウイルス属ラッサウイルスによる急性発熱性疾患である。流行地は**西アフリカ（ナイジェリア，シエラレオネなど）**である。ラッサウイルスは，げっ歯類のマストミスの腎臓に無症候感染し，尿中に排泄される。尿に汚染された環境から感染が成立すると考えられている。このため，マストミスが増加する乾期に患者の増加することが知られている。マストミスは都市部より郊外に生息しているため，渡航者の感染事例は少ないが，英国やドイツを中心に欧州では数年に1例くらいの報告がある。2009年には英国で2例，2010年には米国で1例の報告があり，日本でも1987年にシエラレオネから帰国した渡航者の症例が報告されている。先に述べた新興呼吸器感染症と同様に感染防止に注意をはらいながら集中治療を提供する（**図9**）。

ラッサ熱は数週間の経過で出血傾向，意識障害などをきたす致死率が高い疾患で，国際保健規則により，WHO加盟各国に速やかな報告が義務づけられている。**わが国でも一類感染症に指定され，患者の入院勧告など公衆衛生対応が規定されている**。