**NOTE** *Pathology*

# Herpes Simplex Virus Type 1 Infection in Two Pet Marmosets in Japan

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ABSTRACT. An 8-month-old common marmoset (*Callithrix jacchus*) was presented with tic-like symptoms, and a 2-year-old pigmy marmoset (*Callithrix pygmaea*) was presented with dyspnea and hypersalivation. Both monkeys died within a few days, and necropsies were performed. Histopathological examinations revealed ulcerative stomatitis with epithelial cell swelling and eosinophilic intranuclear inclusion bodies in the oral epithelium of both cases. In the central and peripheral nervous systems, neuronal cell degeneration with intranuclear inclusion bodies was observed. Immunohistochemical examination using anti-herpes simplex virus type 1 antibody revealed virus antigens in both cases. Both animals had been kept as pets with limited exposure to the ambient environment except via their owners. Therefore, herpes simplex virus type-1 was probably acquired from close contact with their owners.
KEY WORDS: herpes virus, marmoset, zoonosis

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Human is the natural host of herpes simplex virus type 1 (HSV-1; human herpes virus type 1). Individuals with active infection exhibit oral ulcers and gingivitis, which usually transit to latent infection in the nervous system with no or intermittent manifestation and virus production. Herpes simplex virus type 1 infection has been reported in apes, such as orangutan (*Pongo pygmaeus*) [13], gorilla (*Gorilla gorilla*) [9] and white-handed gibbon (*Hylobates lar*) [6, 14], and new world monkeys, such as common marmoset (*Callithrix jacchus*) [10, 12], black-tufted marmoset (*Callithrix penicillata*) [3, 5], night monkey (*Aotus trivirgatus*) [16] and white-faced saki (*Pithecia pithecia pithecia*) [17].

Though the prevalence of HSV-1 infection in non-human primates is not clear, it is considered that HSV-1 infection causes fatal disease especially in new world monkeys [8, 15]. This virus is most likely to be transmitted through direct contact with an active lesion, and therefore, cross-transmission between human and new world monkeys is of particular concern in veterinary clinical practice. Here, we report two cases of fatal HSV-1 infection in common marmoset and pigmy marmoset in Japan with clinical and pathological investigations.

In both cases, a veterinary clinician soaked the carcasses in 10% neutral buffered formalin (NBF) soon after these monkeys had died for biosafety concerns. Formalin-fixed tissues were embedded in paraffin after dehydration, and 4 $\mu$m thick sections were stained with hematoxylin and eosin (HE). Immunohistochemistry was performed using anti-HSV-1 antibody (rabbit polyclonal, Dako-Japan, Kyoto, Japan) and anti-rabbit EnVision+ System (Dako-Japan).

*Case 1*: An 8-month-old male common marmoset was presented to a veterinary clinic with complaint of anorexia and intermittent convulsions, and of subsequent tic-like symptoms (unusual blinking and facial twitching). The owner had purchased the monkey a month before and since then raised it at home. Physical examination revealed redness in the right commissure of the mouth and white vesicular lesion on the tongue. Slight gastrointestinal gas retention was observed on X-ray. Blood test revealed an increased white blood cell count (12,100/$\mu$*l*) and hypoglycemia (66 mg/d*l*). Despite symptomatic treatments, the level of consciousness decreased, and the incidence of convulsion increased during the next day. At the third day, the animal died, and necropsy was performed.

By gross examination after mild NBF-fixation, vesicular glossitis and petechial hemorrhage of the cerebrum were observed (Figs. 1 and 2). Histological examination revealed erosive glossitis with epithelial cell swelling and moderate inflammatory cell infiltration (mainly lymphocytes) in the submucosa (Fig. 3), and erosion of the esophageal mucosa. Cerebral meningitis with lymphocyte, macrophage and neutrophil infiltration (Fig. 4) was observed together with multifocal neuronal cell death (Fig. 5). Immunohistochemistry using anti-HSV-1 antibody revealed viral antigens in the epithelial cells and peripheral nerve tissue of the tongue (Fig. 6), neuronal cells of the cerebrum, cerebellum, medulla oblongata, trigeminal nerve (Fig. 7) and abducens nerve, epithelial cells of the pancreas, peripheral nerves of the heart and monocytes in the mandibular and cervical lymph nodes [1].

*Case 2*: A 2-year-old female pigmy marmoset was presented to a veterinary clinic with a complaint of persisting

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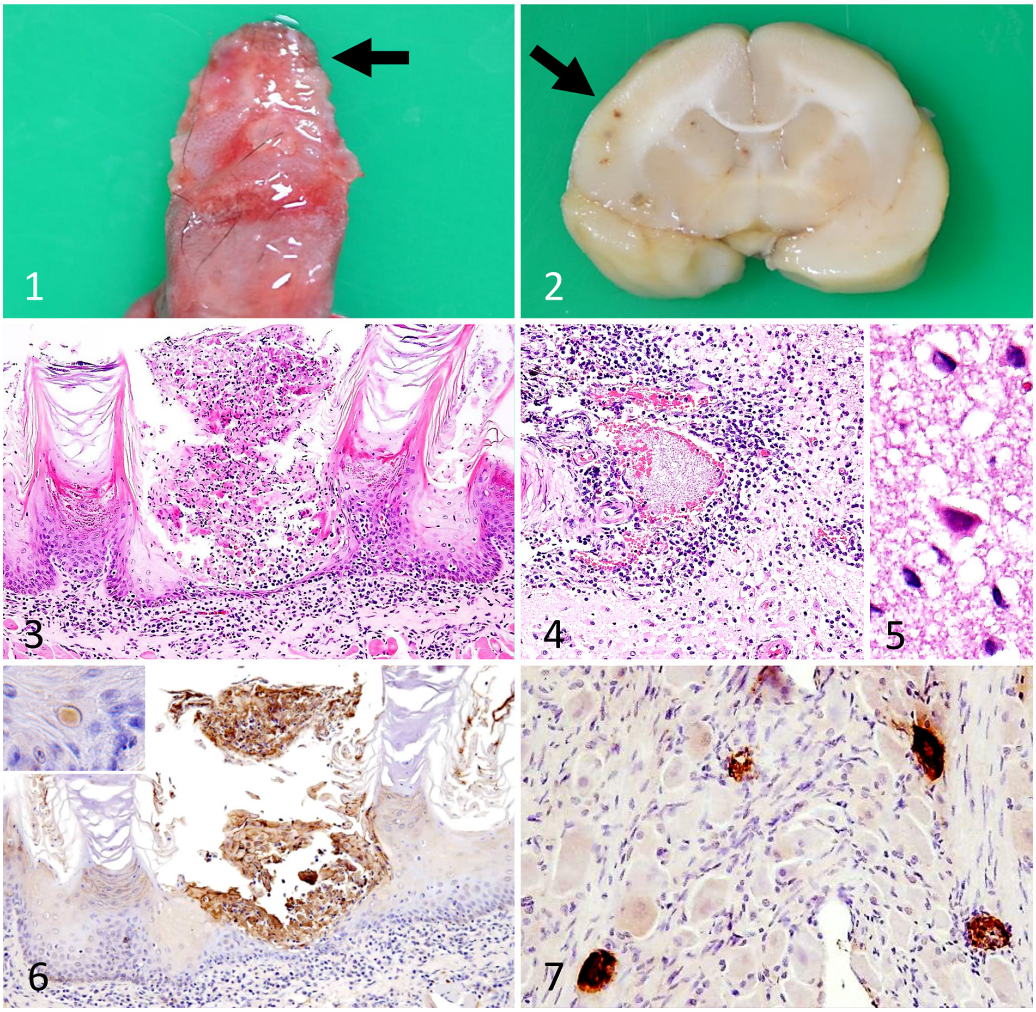


Fig. 1. Vesicular or erosive lesions at the tip of the tongue (arrow). Case 1.
Fig. 2. Petechial hemorrhages in the cerebral cortex (arrow). Case 1.
Fig. 3. Erosive glossitis with epithelial cell swelling. HE, × 40. Case 1.
Fig. 4. Cerebral meningitis. Inflammatory cells (mainly lymphocytes) infiltrate into the meninges. HE, × 40. Case 1.
Fig. 5. Neuronal cell death in the cerebral cortex. Atrophic neurons with eosinophilic cytoplasm are observed. HE, × 200. Case 1.
Fig. 6. Detached epithelial cells in the tongue are positive for HSV-1 antigen (sequential section of Fig. 3). Immunohistochemistry, × 40. Case 1. Inset shows an epithelial cell with intranuclear viral antigen. Immunohistochemistry, × 400.
Fig. 7. Ganglion cells in the trigeminal nerve are stained with anti-HSV-1 antibody. Immunohistochemistry, × 200. Case 1.

dyspnea, hypersalivation and anorexia. Two other pigmy marmosets that had been kept together died within the last three weeks also exhibited dyspnea and eye manifestation. At the clinic, the monkey was in severe respiratory distress displaying marked panting and showed eye discharge (Fig. 8). The monkey was immediately treated with oxygen inhalation and nebulizer therapy, but died soon after. On gross examination, the duodenal intussusception and hemorrhage in the adrenal glands were observed, whereas no significant oral lesions were detected. Histological examination revealed epithelial cell swelling in the lingual mucosa together with eosinophilic intranuclear inclusion bodies (Fig. 9). Smooth muscle cells in the invaginated duodenum were atrophied, and inclusion bodies were observed in nerve cells of the enteric plexus (Fig. 10). The adrenal glands were necrohemorrhagic, and eosinophilic intranuclear inclusion bodies were observed in the remaining cells. Inclusion bodies were also detected in hepatic cells. Immunohistochemistry using anti-HSV-1 antibody revealed viral antigens in the epithelial cells (Fig. 11) and peripheral nerve tissue of the tongue, neuronal cells of the cerebrum, cerebellum, medulla oblongata and gastrointestinal tract (Fig. 12), hepatocytes of the liver, epithelial cells of the pancreas and peripheral nerves of the kidneys.

The both monkeys died within four days after showing symptoms of dyspnea, salivation and anorexia. Histopathologically, severe necrosis and nuclear inclusion bodies were observed in the oral mucosa and nervous tissues, indicat-

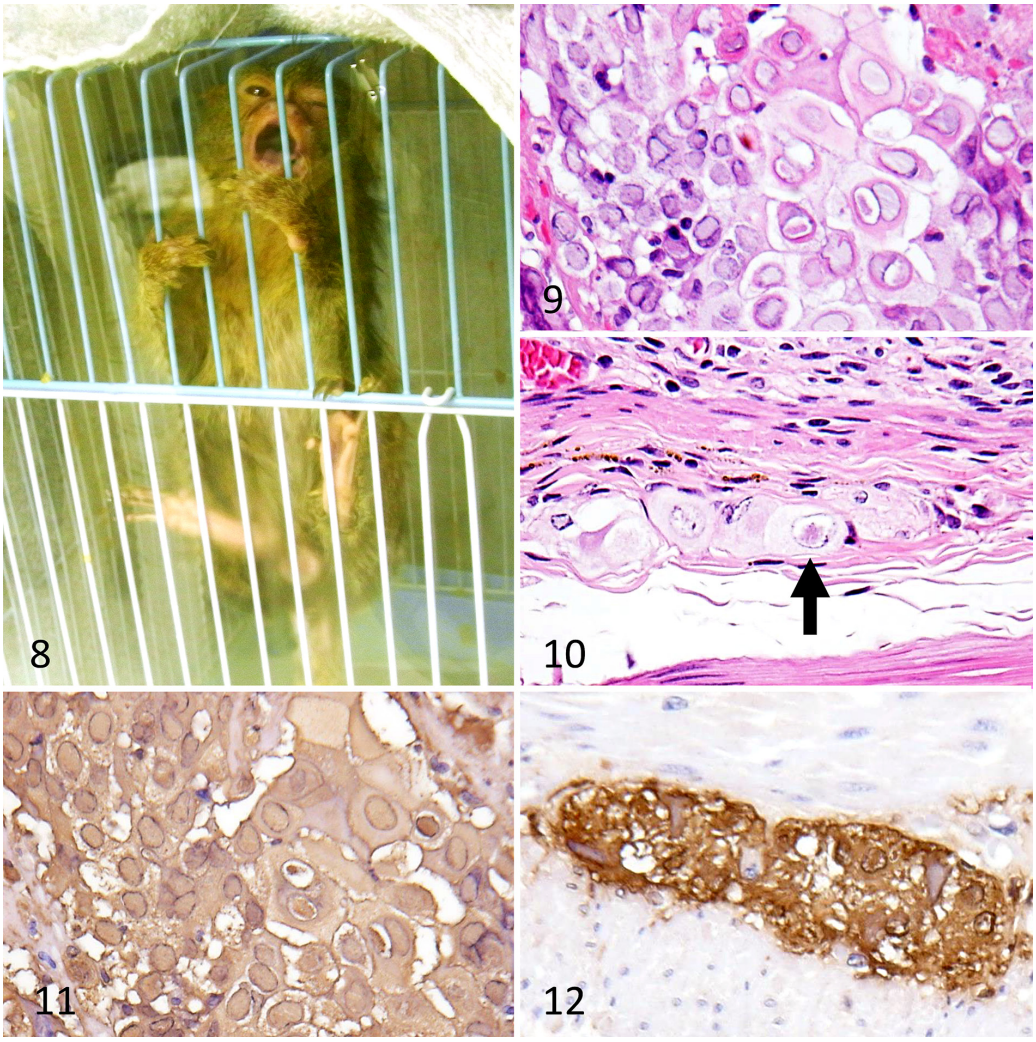


Fig. 8. Clinical manifestation of the monkey. Severe respiratory distress displaying marked panting. Case 2.
Fig. 9. Eosinophilic intranuclear inclusion bodies in epithelial cells of the tongue. Some are surrounded by a clear halo, and others are faintly stained and fill the nucleus. HE, × 400. Case 2.
Fig. 10. Eosinophilic intranuclear inclusion bodies in the enteric plexus ganglion cells of the invaginated duodenum. HE, × 400. Case 2.
Fig. 11. Epithelial cells of the tongue are positive for HSV-1 antigen. Immunohistochemistry, × 400. Case 2.
Fig. 12. Enteric plexus ganglion cells of the invaginated duodenum are positive for HSV-1 antigen. Immunohistochemistry, × 400. Case 2.

ing herpes virus infection. The primary antibody used in the present study reacts with HSV-1 specific antigens and with antigens common for HSV-1 and −2, but not with cytomegalovirus, Epstein-Barr virus and varicella zoster virus (information provided in the data sheet). In recent years, the seroprevalence of HSV-2 in Japanese population is very low, though the seroprevalence of HSV-1 remains high [7]. In general, HSV-1 is acquired during childhood and develops oral infection, while HSV-2 is transmitted sexually and develops genital infections. Other viral infections that should be differentiated in the present study include cercpithecine herpesvirus 1 (herpes simian B virus). However, cercpithecine herpesvirus 1 shows extensive antigenic cross-reactivity with HSV, and definitive diagnosis requires genetic analysis and/or epidemiological evidences [2]. In the present study, the carcasses of the monkeys were soaked in 10% NBF for biosafety purpose which made genetic analysis impossible. The natural host for cercpithecine herpesvirus 1 is considered to be macaques, and the virus may cause fatal encephalomyelitis in other primate species including humans [4, 11]. In Japan, cercpithecine herpesvirus 1 infection has not been identified except in macaques, thus acquiring cercpithecine herpesvirus 1 requires direct contact with a macaque with active viral shedding which was not the case in this study. From the pathological and immunohistochemical findings together with the environmental evidences, diagnoses of fatal HSV-1 infection were made in the present cases.

It has been reported that the new world monkeys are highly susceptible to HSV-1 infection and outbreaks have occurred in free-living and captive colonies [5, 15]. Most

common gross finding of HSV-1 infection in marmosets is vesicular or ulcerative stomatitis. Involvement of other organs, such as the adrenal glands, liver and central nervous system, may occur in case of systemic infection. In case 1, abnormal blinking and facial twitching were observed in the initial phase. Such symptoms have never been documented in the previous reports and may be characteristic clinical signs of HSV-1 infection in monkeys. Case 1 showed severe cerebral meningitis and viral infection in the trigeminal and abducens nerves, which may explain the neurological symptoms of the monkey. On the other hand, duodenal intussusception and hemorrhage in the adrenal glands were the major lesions in case 2. Virus infection in the peripheral nerves (enteric plexus and adrenal medulla) may be responsible for these lesions. As far as we know, this is the first report to describe the distribution of HSV-1 antigen in visceral organs together with histopathological findings. In conclusion, case 1 showed a severe cerebral involvement, and case 2 showed peripheral involvement.

Both animals had been kept with a limited exposure to the ambient environment. Infection of HSV-1 occurs through direct contact with virus shedding lesions, most likely the saliva. Therefore, the present cases probably acquired HSV-1 from their owners. In case 1, the monkey often licked the owner's lips. In case 2, though the route of infection was not clear, the monkey was infected directly from the owner or via other monkeys that had died probably of HSV-1 infection few weeks before. This is the first report of HSV-1 infection in a pigmy marmoset (case 2) and also of new world monkeys in Japan.

Since 2005, importing pet monkeys into Japan have been prohibited (http://www.maff.go.jp/aqs/animal/aq11-8.html). However, pet monkeys, especially new world monkeys such as marmosets and squirrel monkeys, can be purchased from breeders inside the country. In fact, both of the animals in this study had been purchased from breeders within the country. As mentioned above, the clinical manifestations and outcome of HSV-1 infection in marmosets are quite acute and lethal. Prophylactic strategies are important concerns, however, it may be difficult to prevent these incidences in captive monkeys since latent HSV-1 infection is common in humans. Animals exhibiting active HVS-1 infection and virus shedding are of serious concern as a source of infection to veterinary clinicians and veterinary pathologists.

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