Natural Occurrence of Primate SLAM-Adapted Canine Distemper Virus, Brazil, 2018

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We analyzed canine distemper virus (CDV) from a 2018 marmoset disease outbreak in Brazil. CDV hemagglutinin protein bound to the marmoset SLAM (signaling lymphocytic activation molecule) receptor through 2 amino acid substitutions within hemagglutinin, which also enhanced human SLAM receptor usage. Continued surveillance will be critical to monitor CDV cross-species transmission.

During 2006–2008, canine distemper virus (CDV) disease outbreaks occurred among macaques (*Macaca* spp.) in China and Japan (*1*–*3*). Despite efficient transmission and high illness and death rates, overt neurologic disease was uncommon, although infection of the central nervous system and histopathologic lesions occurred in some animals (*1*–*3*). Wild-type CDV from dogs can use both canine and macaque signaling lymphocytic activation molecule (SLAM) to enter cells; however, human SLAM is used inefficiently (*1*,*4*).

SLAM V domains in macaques and humans differ by only 2 amino acids. At position 28, macaque SLAM contains histidine, whereas in other primates, including humans, position 28 contains arginine; that amino acid residue determines whether typical wild-type CDV can use macaque or human SLAM (*4*,*5*).

Hemagglutinin (H) protein substitutions R519S, D540G, and P541S in CDV enhanced SLAM-dependent CDV binding to Vero cells that overexpressed human SLAM (*6*,*7*). However, whether those substitutions enable efficient SLAM usage in primary human immune cells remains unknown. Host switching by morbilliviruses, including CDV, requires more than SLAM adaptation. Morbilliviruses must evade or suppress innate immunity and efficiently use nectin-4 for epithelial spread and shedding (*8*). Moreover, morbillivirus infections generally induce long-lasting immunity, and infection or vaccination can provide some cross-protection against related morbilliviruses, creating additional barriers to CDV establishment in humans.

During 2018–2020, multiple lethal CDV disease outbreaks occurred in free-ranging marmosets (*Callithrix* spp.) in Brazil (*9*–*12*). Unlike measles virus infections in humans, CDV infections in carnivores are associated with a high risk for acute encephalitis. In CDV-infected marmosets, marked virus localization was observed not only in systemic lymphoid and epithelial organs but also within the central nervous system (*9*–*12*), which is believed to account for the high pathogenicity and mortality rates observed in that species. Phylogenetic analysis indicated independent introduction of ≥2 distinct virus strains (*11*). In one 2018 outbreak, 5 free-living black-tufted marmosets (*C. penicillata*) died in urban Central Brazil near neighborhoods with unvaccinated domestic dogs, suggesting possible transmission from those dogs (*11*).

Using mammal cell expression systems, we analyzed SLAM receptor usage by marmoset- and dog-derived CDV strains in different animal species. Furthermore, we evaluated whether adaptive mutations in those CDV strains affected human SLAM usage. We did not require ethics approval for this study because no experiments involving humans or animals were performed.

The Study

We reconstructed H genes by using the genomic sequences from the CDV NHP1 strain isolated from a marmoset (GenBank accession no. PP847352.1)

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and the Ac96I strain isolated from a dog (accession no. AB753775). We introduced mutations in the H genes that produced amino acid substitutions at positions 519 and 530 and evaluated functional characteristics of those H proteins in mammal cell expression systems. Vero cell lines constitutively expressing canine, macaque, and human SLAM have been described previously (*4*), whereas we established the Vero cell line expressing marmoset SLAM in this study by using the same method (Figure 1). We transfected those Vero cell lines with plasmids expressing both the H (with and without mutations) and fusion (F) proteins to induce receptor-mediated cell–cell fusion. For direct comparison of receptor use capacities of the different H protein variants, we used the F protein derived from the Ac96I strain throughout the assays. That method enabled assessment of H protein–mediated receptor use capacities of canine, marmoset, macaque, and human SLAM, as described previously (*1*,*4*).

Functional assays revealed that the H protein from the marmoset-derived CDV NHP1 strain not only recognized canine SLAM but also had adapted to bind to marmoset SLAM, unlike the conventional dog-derived Ac96I strain (Figure 2, panels A, B). We observed a similar overall SLAM use pattern when the homologous F protein from strain NHP1 was used. Furthermore, detailed molecular phylogenetic analysis demonstrated that the marmoset-derived NHP1 strain belongs to the Europe 1/South America 1 lineage, which is distinct from the lineages containing CDV strains that previously caused outbreaks in macaques (Figure 3, panel A). Eleven amino acid positions showed marked divergence among CDVs from marmosets and other species (Figure 3, panel B). The marmoset isolates harbored unique amino acid mutations at or adjacent to the specific residues on the H protein that we previously identified as critical for human SLAM adaptation (*6*) (Figure 3, panel C).

We focused on 2 specific amino acid substitutions at positions 519 (R519I) and 530 (G530S) and analyzed whether those unique mutations found in the marmoset-derived CDV strain were responsible for their adaptation to marmoset SLAM. We demonstrated that R519I, but not G530S, marginally enhanced the use of marmoset SLAM; however, coexistence of both mutations further increased marmoset SLAM usage (Figure 2, panel A). Conversely,

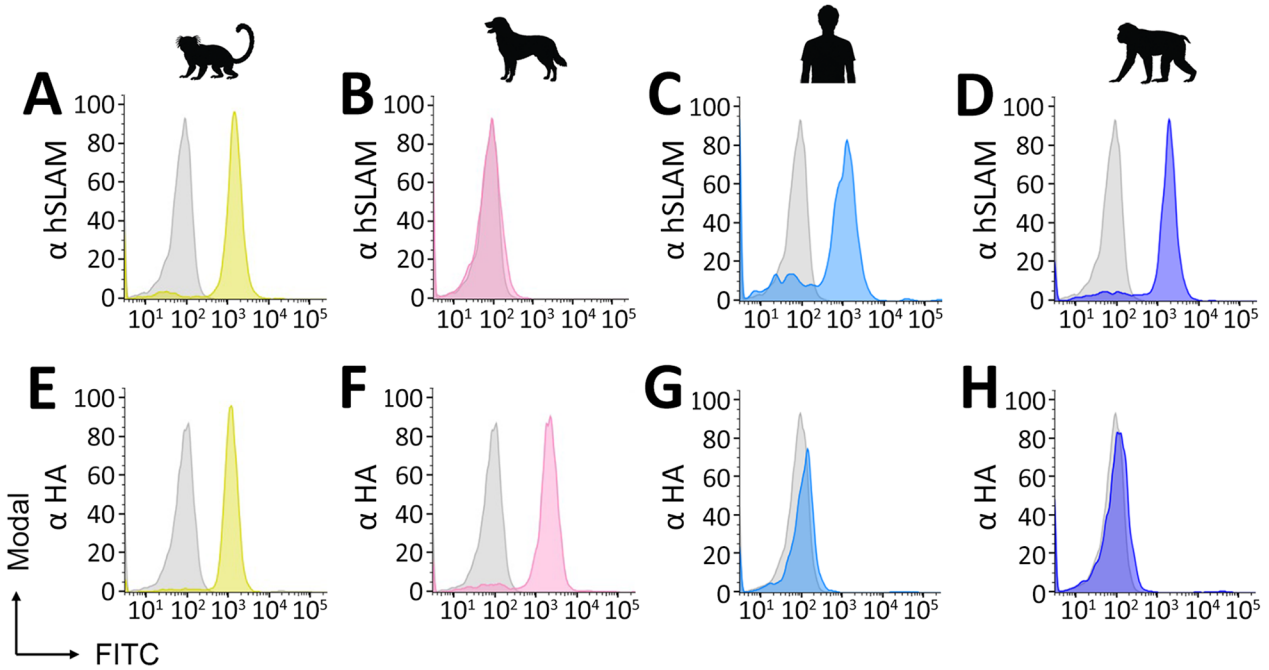


Figure 1. Expression of SLAM in Vero cells in study of natural occurrence of primate SLAM-adapted canine distemper virus, Brazil, 2018. We assessed the expression of marmoset, canine, human, and macaque SLAM in Vero cell lines used for cell–cell fusion assays by using flow cytometry. A–D) Vero cells were untransfected (gray histogram) or transfected (colored histogram) with SLAM constructs derived from marmoset (A), canine (B), human (C), and macaque (D) cells. Cells were stained with human SLAM antibody (α hSLAM, clone IPO-3; ViroGen Corporation, https://www.virogen.com). E–H) Vero cells were untransfected or transfected with HA-tagged SLAM derived from marmoset (E) and canine (F) cells; canine SLAM was not detected by the human SLAM antibody. Human (G) and macaque (H) SLAM constructs did not contain an HA tag and were used as negative controls. FITC, fluorescein isothiocyanate; HA, hemagglutinin; SLAM, signaling lymphocytic activation molecule.

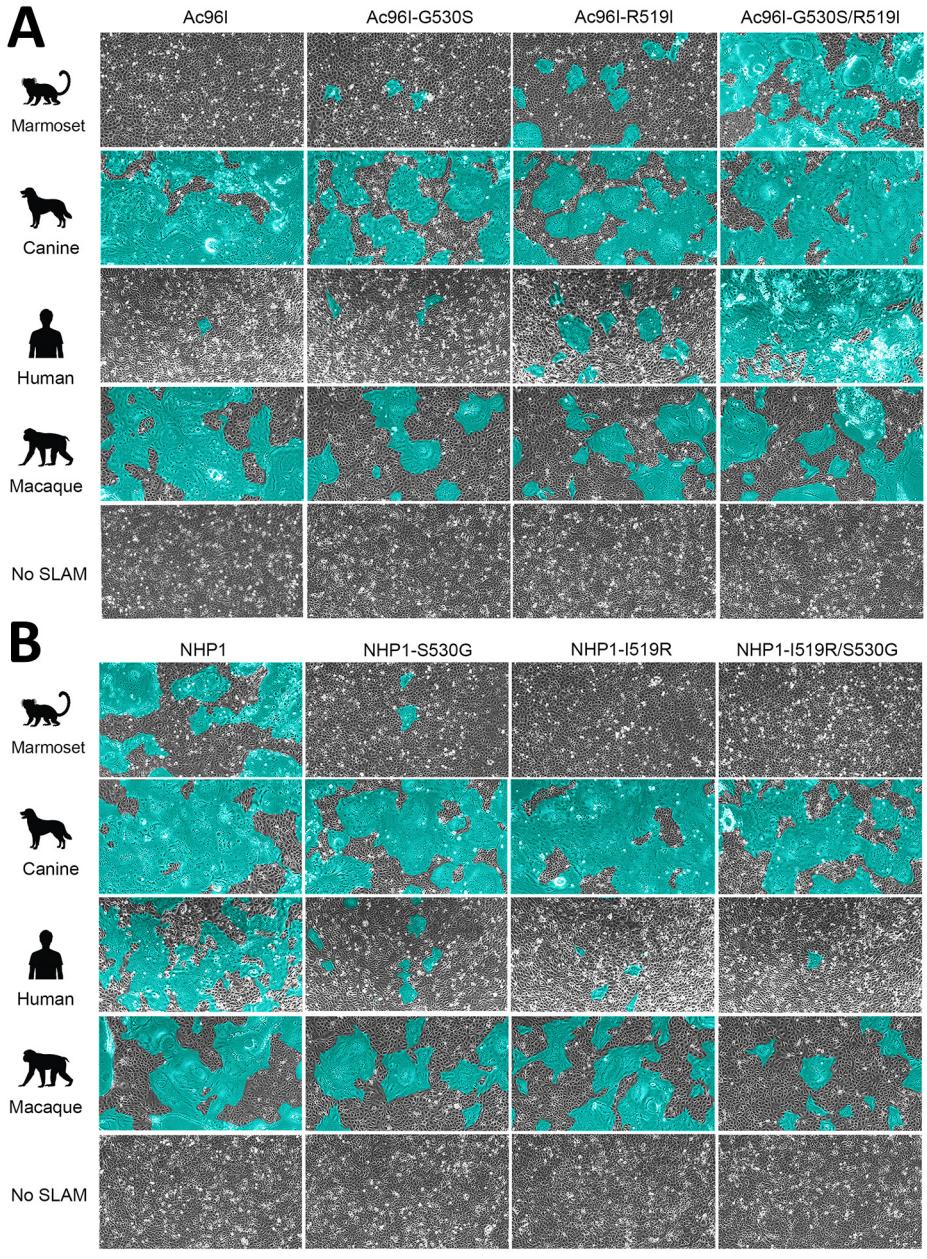


Figure 2. Cell fusion assay in study of natural occurrence of primate SLAM-adapted canine distemper virus, Brazil, 2018. Vero cells constitutively expressing marmoset (GenBank accession no. XM_002760176), canine (accession no. AF325357), human (accession no. NM_003037.4), or macaque (accession no. XM_001117605.3) SLAM, as well as parental Vero cells, were transfected with pCAGGS plasmids (RIKEN BioResource Research Center, https://dnaconda.riken.jp) expressing both canine distemper virus fusion and hemagglutinin (H) proteins. H genes were derived from the Ac96I strain (accession no. AB753775) (A) or NHP1 strain (accession no. PP847352.1) (B). Fusion gene was derived from the Ac96I virus strain. Mutations were introduced at positions 519 and 530 of the H genes, and amino acid substitutions are indicated. Syncytial areas showing cell–cell fusion are highlighted in green. Images represent 3 independent experiments. Original magnification ×100.

introduction of either the I519R or S530G mutation in the H protein from the NHP1 strain caused a lost or greatly reduced ability to use marmoset SLAM (Figure 2, panel B).

To quantitatively assess differences in SLAM usage, we performed dual-split reporter protein–based cell–cell fusion assays, as described previously (*4*). Fusion activity for each H protein was normalized to that obtained with canine SLAM, which was set to 100%. The quantitative results were consistent with microscopic observations. The R519I mutation of the H protein enhanced marmoset SLAM usage in the Ac96I strain background; a further increase in usage occurred when the R519I mutation was combined with G530S. The I519R or S530G reverse substitutions in the NHP1 H background reduced marmoset SLAM usage (Figure 4).

We investigated whether the R519I or G530S adaptive mutations affected human SLAM usage. The microscopic fusion assay showed those mutations affected human SLAM usage similar to that observed with marmoset SLAM (Figure 2, panel A). The quantitative fusion assay independently confirmed that pattern. The R519I mutation in the H protein enhanced human SLAM usage in the Ac96I background, and combination with G530S further

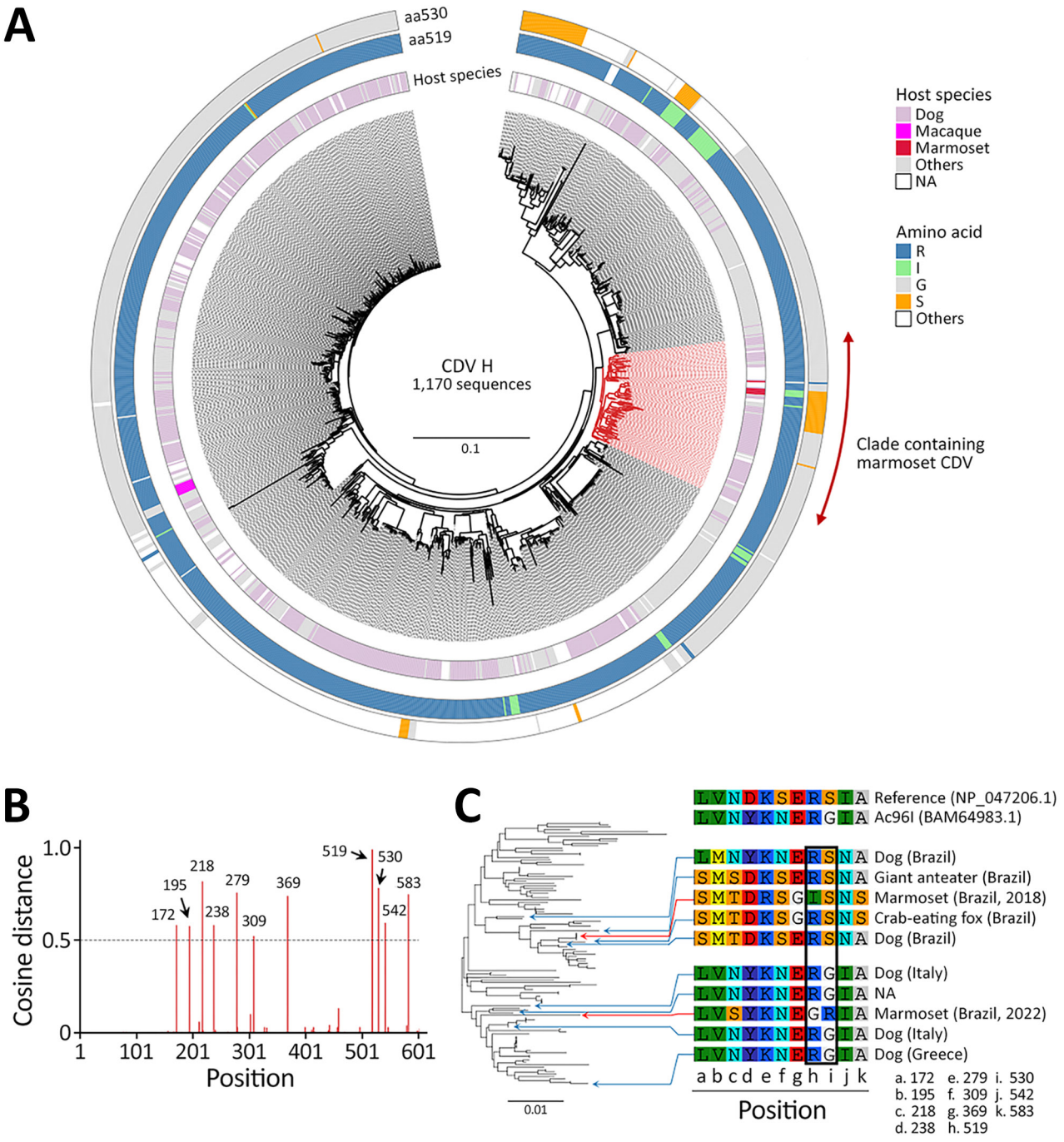


Figure 3. Phylogenetic analysis of marmoset CDV H protein in study of natural occurrence of primate SLAM-adapted CDV, Brazil, 2018. A) Phylogenetic tree of CDV H proteins. Alignment was performed by using MAFFT (https://mafft.cbrc.jp). The tree was constructed by using the neighbor-joining method according to the Jones-Taylor-Thornton amino acid substitution model. All positions with <99% site coverage were eliminated, resulting in a total of 601 positions in the final dataset. Red indicates clade containing marmoset CDVs. B) Bar plot showing cosine distances between CDV H protein sequences from marmoset and other CDV host species. Analysis included only the sequences within the marmoset CDV-containing clade highlighted in panel A. C) Amino acid alignment at positions with large cosine distances identified in panel B. Phylogenetic tree is identical to that in panel A but shows only the clade containing marmoset CDVs. Sequence sources (Genbank accession nos. or country source) are indicated in parentheses. CDV NHP1 strain belongs to the Europe 1/South America 1 lineage. Scale bars indicate amino acid substitutions per site. CDV, canine distemper virus; H, hemagglutinin; NA, not applicable.

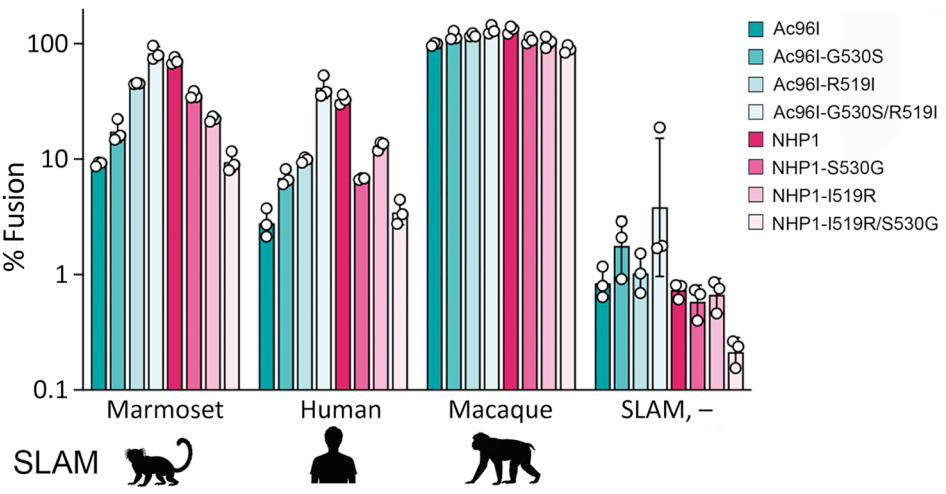


Figure 4. Quantitative analysis of SLAM-dependent cell–cell fusion in study of natural occurrence of primate SLAM-adapted canine distemper virus, Brazil, 2018. We quantified cell–cell fusion mediated by hemagglutinin (H) proteins derived from virus strains Ac96I and NHP1 by using dual-split reporter protein (DSP) assays, as described previously (*4*). H proteins were unchanged or had amino acid substitutions at positions 519 or 530. Fusion protein was derived from the Ac96I virus strain. We used Vero cells expressing canine, marmoset, human, or macaque SLAM, or parental Vero cells lacking exogenous SLAM (SLAM, –) as target cells. For each H protein, fusion activity with canine SLAM was defined as 100%, and fusion activities for the other SLAM orthologs were expressed relative to this value. Data are shown on a logarithmic scale. Bars represent means ±SDs from 3 independent experiments. Open circles are individual data points. SLAM, signaling lymphocytic activation molecule.

increased usage. The corresponding I519R or S530G reverse substitutions in the H protein from the NHP1 strain reduced human SLAM usage (Figure 4). Our findings indicate that CDV adaptation to marmoset SLAM can occur under natural conditions and that this adaptation can concurrently enhance the ability to use human SLAM.

The NHP1 strain can be characterized as a primate SLAM-adapted CDV because the H protein efficiently uses SLAM from macaque, marmoset, and human cells. The adaptations did not reduce the ability of the H protein to use canine SLAM (Figure 2, panel B), which indicates no apparent trade-off in SLAM usage was associated with adaptation to primate SLAMs.

Conclusions

Recently, CDV has rapidly expanded its host range to include diverse noncanine species (*13*), including primates. Although CDV can cause severe neurologic disease in susceptible animal hosts, the clinical consequences of potential CDV infection in humans remain unknown. Animal studies have suggested that existing immunity to measles virus might provide some degree of cross-protection against CDV (*14*,*15*). Therefore, our findings should not be interpreted as evidence that CDV currently poses an imminent risk for neurologic disease in humans. Continued surveillance of circulating CDV strains will be critical for monitoring virus evolution and cross-species transmission. Maintaining high measles vaccination coverage might also be critical, especially in regions where SLAM-adapted CDV strains circulate, because of the partial cross-protection against CDV demonstrated by measles immunity in animal studies (*14*,*15*).

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During the preparation of this manuscript, the authors used Google Gemini (https://gemini.google.com) to translate the draft from Japanese to English, refine the grammar, and improve the clarity and overall flow of the text.

About the Author

Ms. Tominaga is a researcher at the Graduate School of Medicine and Faculty of Medicine at The University of Tokyo, Japan. Her research interests focus on acute viral infections in humans and animals.

References

1. Sakai K, Nagata N, Ami Y, Seki F, Suzaki Y, Iwata-Yoshikawa N, et al. Lethal canine distemper virus outbreak in cynomolgus monkeys in Japan in 2008. J Virol. 2013;87:1105–14. https://doi.org/10.1128/JVI.02419-12
2. Qiu W, Zheng Y, Zhang S, Fan Q, Liu H, Zhang F, et al. Canine distemper outbreak in rhesus monkeys, China. Emerg Infect Dis. 2011;17:1541–3. https://doi.org/10.3201/eid1708.101153
3. Sun Z, Li A, Ye H, Shi Y, Hu Z, Zeng L. Natural infection with canine distemper virus in hand-feeding Rhesus monkeys in China. Vet Microbiol. 2010;141:374–8. https://doi.org/10.1016/j.vetmic.2009.09.024
4. Hyodo A, Seki F, Fukuda K, Tashiro K, Kitai Y, Akahori Y, et al. Evolutionary and structural basis of SLAMF1

utilization in morbilliviruses—implications for host range and cross-species transmission. PLoS Pathog. 2025;21:e1012990. https://doi.org/10.1371/journal.ppat.1012990
5. Yamamoto Y, Nakano S, Seki F, Shigeta Y, Ito S, Tokiwa H, et al. Computational analysis reveals a critical point mutation in the *N*-terminal region of the signaling lymphocytic activation molecule responsible for the cross-species infection with canine distemper virus. Molecules. 2021;26:1262. https://doi.org/10.3390/molecules26051262
6. Sakai K, Yoshikawa T, Seki F, Fukushi S, Tahara M, Nagata N, et al. Canine distemper virus associated with a lethal outbreak in monkeys can readily adapt to use human receptors. J Virol. 2013;87:7170–5. https://doi.org/10.1128/JVI.03479-12
7. Bieringer M, Han JW, Kendl S, Khosravi M, Plattet P, Schneider-Schaulies J. Experimental adaptation of wild-type canine distemper virus (CDV) to the human entry receptor CD150. PLoS One. 2013;8:e57488. https://doi.org/10.1371/journal.pone.0057488
8. Rota PA, Moss WJ, Takeda M, de Swart RL, Thompson KM, Goodson JL. Measles. Nat Rev Dis Primers. 2016;2:16049. https://doi.org/10.1038/nrdp.2016.49
9. de Campos BH, Dos Santos DO, Duarte JR, Amaral VHB, Figueiredo CCC, Vieira AD, et al. Lethal canine distemper virus (*Morbillivirus canis*) outbreak in free-ranging black-tufted marmosets (*Callithrix penicillata*) in Brazil: clinical, pathological, genotypical evaluation, and assessment of viral tropism. Transbound Emerg Dis. 2025;2025:4701926. https://doi.org/10.1155/tbed/4701926
10. Santos RL, Dos Santos DO, de Campos BH, de Oliveira AR, de Carvalho MPN. Emergence of canine distemper virus in Brazilian neotropical primates. J Med Primatol. 2025;54:e70048. https://doi.org/10.1111/jmp.70048
11. Wilson TM, de Sousa DER, Macêdo IL, Machado M, de Toledo Costa GR, Fair P, et al. Emergence of a novel canine distemper virus variant in urbanized free-ranging marmosets (*Callithrix penicillata*). Transbound Emerg Dis. 2025;2025:4818076. https://doi.org/10.1155/tbed/4818076
12. Mares-Guia MAMM, Furtado MC, Chalhoub FLL, Portugal MD, de Oliveira Coelho JMC, de Filippis AMB, et al. Coinfection with canine distemper virus and yellow fever virus in a neotropical primate in Brazil. Viruses. 2024;16:1670. https://doi.org/10.3390/v16111670
13. Takeda M, Seki F, Yamamoto Y, Nao N, Tokiwa H. Animal morbilliviruses and their cross-species transmission potential. Curr Opin Virol. 2020;41:38–45. https://doi.org/10.1016/j.coviro.2020.03.005
14. de Vries RD, Ludlow M, Verburgh RJ, van Amerongen G, Yüksel S, Nguyen DT, et al. Measles vaccination of nonhuman primates provides partial protection against infection with canine distemper virus. J Virol. 2014;88:4423–33. https://doi.org/10.1128/JVI.03676-13
15. Appel MJ, Shek WR, Shesberadaran H, Norrby E. Measles virus and inactivated canine distemper virus induce incomplete immunity to canine distemper. Arch Virol. 1984;82:73–82. https://doi.org/10.1007/BF01309369

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