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References

1. Margos G, Wilske B, Sing A, Hizo-Teufel C, Cao WC, Chu C, et al. *Borrelia bavariensis* sp. nov. is widely distributed in Europe and Asia. Int J Syst Evol Microbiol. 2013;63:4284–8. https://doi.org/10.1099/ijs.0.052001-0
2. Strnad M, Hönig V, Růžek D, Grubhoffer L, Rego ROM. Europe-wide meta-analysis of *Borrelia burgdorferi* sensu lato prevalence in questing *Ixodes ricinus* ticks. Appl Environ Microbiol. 2017;83:e00609-17. https://doi.org/10.1128/AEM.00609-17
3. Coipan EC, Jahfari S, Fonville M, Oei GA, Spanjaard L, Takumi K, et al. Imbalanced presence of *Borrelia burgdorferi* s.l. multilocus sequence types in clinical manifestations of Lyme borreliosis. Infect Genet Evol. 2016;42:66–76. https://doi.org/10.1016/j.meegid.2016.04.019
4. Courtney JW, Kostelnik LM, Zeidner NS, Massung RF. Multiplex real-time PCR for detection of *anaplasma phagocytophilum* and *Borrelia burgdorferi*. J Clin Microbiol. 2004;42:3164–8. https://doi.org/10.1128/JCM.42.7.3164-3168.2004
5. Heylen D, Tijsse E, Fonville M, Matthysen E, Sprong H. Transmission dynamics of *Borrelia burgdorferi* s.l. in a bird tick community. Environ Microbiol. 2013;15:663–73. https://doi.org/10.1111/1462-2920.12059
6. Michel H, Wilske B, Hettche G, Göttner G, Heimerl C, Reischl U, et al. An ospA-polymerase chain reaction/restriction fragment length polymorphism-based method for sensitive detection and reliable differentiation of all European *Borrelia burgdorferi* sensu lato species and OspA types. Med Microbiol Immunol (Berl). 2004;193:219–26. https://doi.org/10.1007/s00430-003-0196-8
7. Jahfari S, Ruyts SC, Frazer-Mendelewska E, Jaarsma R, Verheyen K, Sprong H. Melting pot of tick-borne zoonoses: the European hedgehog contributes to the maintenance of various tick-borne diseases in natural cycles urban and suburban areas. Parasit Vectors. 2017;10:134. https://doi.org/10.1186/s13071-017-2065-0
8. Medlock JM, Vaux AGC, Gandy S, Cull B, McGinley L, Gillingham E, et al. Spatial and temporal heterogeneity of the density of *Borrelia burgdorferi*-infected *Ixodes ricinus* ticks across a landscape: a 5-year study in southern England. Med Vet Entomol. 2022;36:356–70. https://doi.org/10.1111/mve.12574
9. Olsthoorn F, Sprong H, Fonville M, Rocchi M, Medlock J, Gilbert L, et al. Occurrence of tick-borne pathogens in questing *Ixodes ricinus* ticks from Wester Ross, Northwest Scotland. Parasit Vectors. 2021;14:430. https://doi.org/10.1186/s13071-021-04946-5
10. Mahase E. UK confirms two cases of tickborne encephalitis acquired within the country. BMJ. 2023;381: p799. https://doi.org/10.1136/bmj.p799

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Emergence of Novel Type C Botulism Strain in Household Outbreak, Japan

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In 2021, an outbreak of food poisoning caused by *Clostridium botulinum* type C occurred in Kumamoto, Japan. Analysis of the isolated strain revealed that it possessed the *bont/C* gene and was slightly different from the reference *bont/C* gene. The risk for human infection with this new toxin type may be low.

Botulism is a neuroparalytic disease caused by the botulinum toxin, which is produced by *Clostridium botulinum*. *C. botulinum* is physiologically divided into groups I–IV, and botulinum neurotoxins (BoNT) are classified into 7 types, BoNT/A–G. Human botulism is caused primarily by toxin types A, B, and E, and cases of human infection with *C. botulinum* group III, which produces toxin types C and D, are rare. Only 5 foodborne botulism outbreaks caused by *C. botulinum* group III (4 outbreaks caused by type C and 1 outbreak caused by type D) have been reported to date (*1*), and in Japan, only 1 infant botulism case caused by type C has been reported (*2*). *C. botulinum* group III is primarily known as an animal infection, and many of its toxin types have been reported as mosaic types (primarily in birds with toxin type CD and cattle with toxin type DC).

In 2021, foodborne botulism occurred in Kumamoto, Japan. A meal eaten in a domestic residence was the assumed cause, and 4 patients were affected. Botulinum toxin and *C. botulinum* were detected in 3 of the 4 specimens. A commercially prepared chicken dish was suspected to be the cause, but because no food was remaining, we were unable to conduct tests on it. We neutralized the toxin present in the specimens with type C botulinum antitoxin serum, and the isolated strain was found to carry the *bont/C* gene using PCR targeting the *bont* genes (*3*). Next-generation sequencing data revealed full-length coding regions of the *bont*

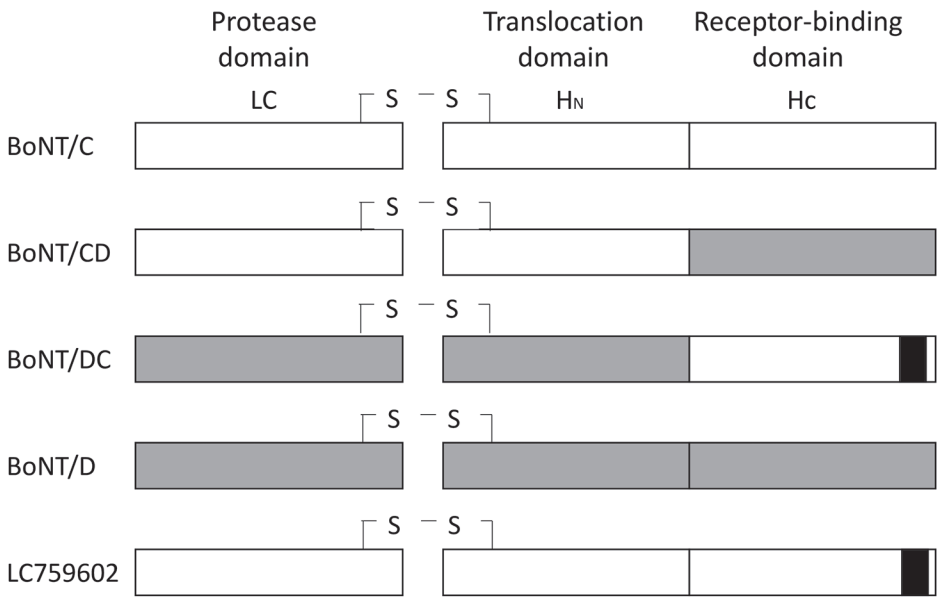

Figure. Schematic diagrams of each functional domain between BoNT (C, CD, DC, D, and LC759602) in study of novel type C botulism strain in household outbreak, Japan. Gray shaded areas indicate the partial sequence of the reference bont/D gene, white areas indicate the partial sequence of the cont/C gene, and black areas indicate the partial sequence of the reference bont/DC gene. BoNT, botulism neurotoxin.

gene of the isolated strains (GenBank accession no. LC759602). The next-generation sequencing method was as follows: after treating *C. botulinum* with 20 mg/mL lysozyme in 20 mM Tris-HCl, 2 mM EDTA, and 1% Triton X-100 (pH 8.0), we extracted DNA using a QIAamp DNA Mini Kit (QIAGEN, https://www.qiagen.com). We prepared genome-sequencing libraries using the QIAseq FX DNA Library Kit (QIAGEN) and sequenced the samples on the Illumina iSeq 100 (https://www.illumina.com). We analyzed sequencing data using CLC Genomics Workbench 22.0.2 (QIAGEN). The obtained contig was assembled from reads of 59× coverage and 29 kbp in size. In comparison with other known *bont* genes, the *bont* gene of the strain sequenced in this study had the highest amino acid sequence similarity with the *bont/C* gene (90%) but was partially different from the reference *bont/C* gene (Table). Detailed analysis revealed that the *bont* gene (LC759602) had the *bont/C* gene or the *bont/CD* gene in the protease domain (LC) and the translocation domain (H_N), as well as the *bont/DC* gene in the receptor-binding domain (H_C) (Table; Figure). The *bont* gene (LC759602) has not been previously reported, and we propose its designation as a new subtype of *C. botulinum* toxin.

The H_C domain is involved in neurotoxin binding to specific receptors in peripheral nerve terminals. The *bont* gene (LC759602) possesses the *bont/DC* gene in the H_C domain, suggesting that human susceptibility to this gene might differ from that of the reference BoNT/C toxin. Unlike other BoNTs, BoNT/C interacts only with gangliosides, and no protein receptor for this toxin has been identified (*4*). However, BoNT/DC has been reported to interact with gangliosides and protein receptors (synaptotagmin I and II) (*5*).

The *bont* gene (LC759602) was determined to be BoNT/C using PCR, which can easily distinguish between types C, D, CD, and DC of *C. botulinum* group III (*6*). It should be noted that, because not all type C strains were subjected to sequencing, the presence of the *bont* gene (LC759602) as type C, as determined by typing PCR, might already exist in other samples. Further investigation is needed to determine the proportion of *C. botulinum* carrying the *bont* gene reported in this study. The risk for human infection with this new toxin type should also be investigated in

Table. Amino acid percentage similarity between BoNTs (C, CD, DC, D, and LC759602) in study of novel type C botulism strain in household outbreak, Japan*

	LC759602			
BoNT serotype (accession no.)	BoNT gene	Protease domain	Translocation domain	Receptor-binding domain
BoNT/C(BAA14235)	89.79	97.73	93.57	78.01
BoNT/CD(BAA08418)	79.31	97.73	98.33	40.55
BoNT/DC(ABP48747)	72.87	48.05	74.45	98.57
BoNT/D(EES90380)	56.48	47.83	73.72	47.74

*BoNT, botulism neurotoxin.

future research. However, given that human infections with a similar toxin type, *C. botulinum* group III, have rarely occurred, this new toxin type might pose little threat to human health.

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References

1. Rasetti-Escargueil C, Lemichez E. Michel, Popoff MR. Public health risk associated with botulism as foodborne zoonoses. Toxins (Basel). 2019;12:17.
2. Oguma K, Yokota K, Hayashi S, Takeshi K, Kumagai M, Itoh N, et al. Infant botulism due to *Clostridium botulinum* type C toxin. Lancet. 1990;336:1449–50. https://doi.org/10.1016/0140-6736(90)93157-K
3. Takeshi K, Fujinaga Y, Inoue K, Nakajima H, Oguma K, Ueno T, et al. Simple method for detection of *Clostridium botulinum* type A to F neurotoxin genes by polymerase chain reaction. Microbiol Immunol. 1996;40:5–11. https://doi.org/10.1111/j.1348-0421.1996.tb03310.x
4. Strotmeier J, Gu S, Jutzi S, Mahrhold S, Zhou J, Pich A, et al. The biological activity of botulinum neurotoxin type C is dependent upon novel types of ganglioside binding sites. Mol Microbiol. 2011;81:143–56. https://doi.org/10.1111/j.1365-2958.2011.07682.x
5. Peng L, Berntsson RP, Tepp WH, Pitkin RM, Johnson EA, Stenmark P, et al. Botulinum neurotoxin D-C uses synaptotagmin I and II as receptors, and human synaptotagmin II is not an effective receptor for type B, D-C and G toxins. J Cell Sci. 2012;125:3233–42. https://doi.org/10.1242/jcs.103564
6. Nakamura K, Kohda T, Umeda K, Yamamoto H, Mukamoto M, Kozaki S. Characterization of the D/C mosaic neurotoxin produced by *Clostridium botulinum* associated with bovine botulism in Japan. Vet Microbiol. 2010;140:147–54. https://doi.org/10.1016/j.vetmic.2009.07.023

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Plasmodium knowlesi Infection in Traveler Returning to Canada from the Philippines, 2023

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A 55-year-old man sought treatment for an uncomplicated febrile illness after returning to Canada from the Philippines. A suspected diagnosis of *Plasmodium knowlesi* infection was confirmed by PCR, and treatment with atovaquone/proguanil brought successful recovery. We review the evolving epidemiology of *P. knowlesi* malaria in the Philippines, specifically within Palawan Island.

In February 2023, a 55-year-old man sought care at the emergency department of Vancouver General Hospital, Vancouver, British Columbia, Canada, for daily fevers, headache, and abdominal pain 5 days after returning from a 3-week trip to the Philippines. He stayed mostly in Manila but spent 4 days on Palawan Island in the western Philippines 4 days before his return to Canada; he had not taken malaria chemoprophylaxis. Bloodwork was notable for platelet nadir of 48 × 10^9/L (reference range 150–450 × 10^9/L), alanine transaminase of 329 U/L (reference range 10–55 U/L), and alkaline phosphatase of 177 U/L (reference range 30–135 U/L). Results of abdominal computed tomography were unremarkable and of a single-target *Plasmodium falciparum* histidine-rich protein 2 rapid diagnostic test were negative. Peripheral blood thin smear demonstrated variable intraerythrocytic parasite morphology, including band-like forms suggestive of *P. malariae* (<0.1% parasitemia) (Figure, panels A–C). Loop-mediated isothermal amplification testing was