

## ***Salmonella enterica* Serovar Abony Outbreak Caused by Clone of Reference Strain WDCM 00029, Chile, 2024**

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A *Salmonella enterica* serovar Abony outbreak occurred during January–April 2024 in Chile. Genomic evidence indicated that the outbreak strain was a clone of reference strain WDCM 00029, which is routinely used in microbiological quality control tests. When rare or unreported serovars cause human infections, clinicians and health authorities should request strain characterization.

**N**ontyphoidal *Salmonella enterica* is a major cause of foodborne disease worldwide (1). Although a few serovars, such as Enteritidis and Typhimurium, produce most infections (2,3), uncommon serovars can cause clinical cases. Characterization may contribute to early identification of emerging strains. We report a multiregional outbreak of salmonellosis caused by *Salmonella* Abony and characterization of clinical isolates collected during the outbreak.

During January 19–March 16, 2024, two healthcare centers in Santiago, Chile, diagnosed 134 human salmonellosis cases: 29 at UC-Christus and 105 at Clínica Alemana. All isolates were submitted to Instituto de Salud Pública de Chile for serotyping; serovar Abony (antigenic formula 1,4,[5],12:b:e,n,x) was found in 57% (56/97) of cases with culture (Appendix 1 Figure 1, <https://wwwnc.cdc.gov/EID/article/31/1/24-1012-App1.pdf>). Among those cases, 33 (58.9%) were in male patients and 23 (41.1%) in female patients; 40 (71.4%) patients were <18 years of age, 17 (30.4%) required hospitalization, and 10 (17.9%) had bacteremia (Appendix 2 Table 1, <https://wwwnc.cdc.gov/EID/article/31/1/24-1012-App2.xlsx>).

Whole-genome sequencing was performed for 18 of 56 outbreak isolates, 13 from UC-Christus and 5

from Clínica Alemana (Appendix 1 Figure 1). Isolates comprised 6 blood, 2 urine, and 10 feces samples. Hierarchical clustering (HC) of global *Salmonella* Abony genomes identified 150 HC50 ( $\leq 50$  core genome multilocus sequence typing allele differences) clusters; outbreak isolates belonged to the HC50\_20673 cluster (Appendix 1 Figure 2; Appendix 2 Table 2). That cluster encompassed isolates from the United Kingdom, United States, Brazil, Nigeria, and France collected during 2008–2024. A core single-nucleotide polymorphism (SNP) phylogeny grouped all HC50\_20673 genomes into 3 clades corresponding to 3 HC10 clusters (Figure 1). The genomes from Chile grouped within HC10\_20673, differing by only 0–3 pairwise SNP differences. That cluster also included 4 genomes from reference strain *Salmonella* Abony WDCM 00029 (4) (Figure 1; Appendix 2 Table 2). The 4 WDCM 00029 genomes differed by 0–19 SNPs from the remaining HC10\_20673 isolates and by 0–7 SNPs from the isolates from Chile, indicating high relatedness (Appendix 2 Table 3).

To confirm the high genomic similarity was not limited to the core genome, we calculated average nucleotide identity and alignment fraction for all HC50\_20673 isolates using the WDCM 00029 genome provided by the American Type Culture Collection (strain BAA-2162; <https://www.atcc.org>) (5) as the reference (Figure 2, panel A). Of note, isolates from Chile had median alignment fraction (97.85%) and average nucleotide identity (99.99%) values greater than those of the other HC10 clusters ( $p < 0.0001$ ) (Figure 2, panels B, C), in line with core SNP data and further suggesting an almost complete genomic identity between WDCM 00029 and the outbreak genomes. We observed similar findings when we included all HC10\_20673 genomes, except WDCM 00029 genomes, in the analysis (Appendix 1 Figure 3).

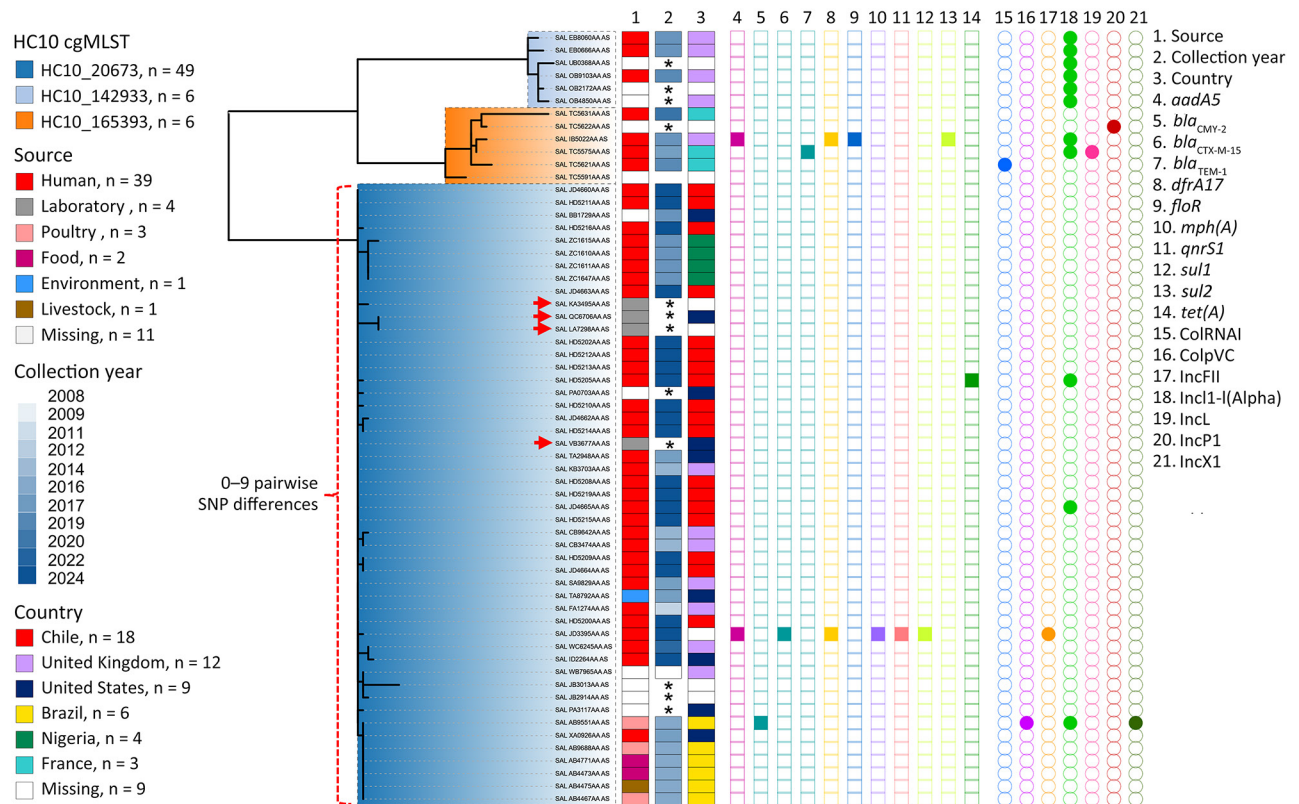
According to official data requested from the Chile government (Appendix 1 Figure 4; Appendix 2 Table 4), 287 *Salmonella* Abony isolates were collected during January 24–April 21 from 12 of 16 administrative regions of Chile, corresponding to infections occurring in persons 0–82 years old. Most (79.8%; 229/287) isolates came from Región Metropolitana, and 57.5% (165/287) were obtained during February 2024. Because we did not have additional epidemiologic information (e.g., food consumed), we did not investigate the source of the outbreak.

Previous studies from Brazil and Nigeria also reported human *Salmonella* Abony infections, and cases from Brazil were linked to consumption of food containing chicken meat (6,7). We found those

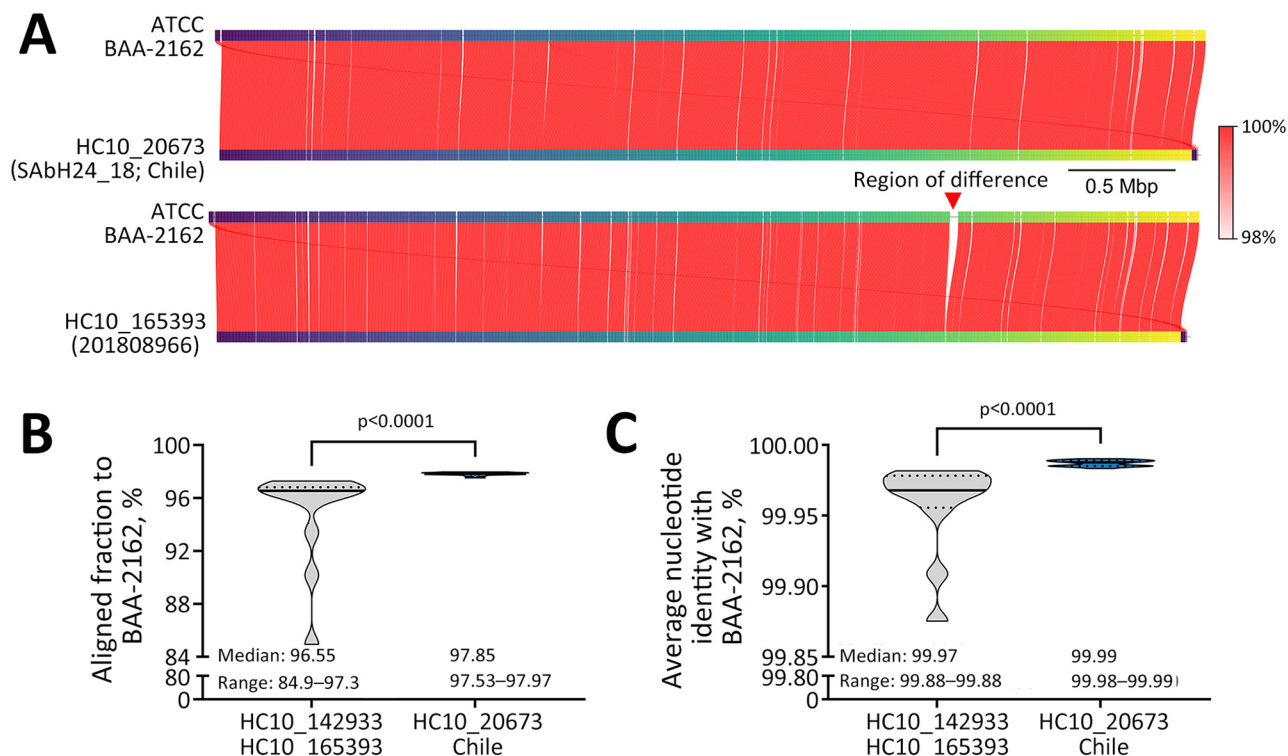
isolates also belonged to the HC10\_20673 cluster and were closely related to WDCM 00029 genomes (1–9 core SNPs differences) (Figure 1; Appendix 2 Table 3). One isolate from Brazil was resistant to third-generation cephalosporins because of a *bla*<sub>CMY-2</sub>-carrying IncI1 plasmid (Figure 1). Moreover, 2 isolates from Chile and 1 isolate from the National Center for Biotechnology Information Pathogen Detection database (<https://www.ncbi.nlm.nih.gov/pathogens>; strain PNUSAS428168, SNP cluster PDS000001617.32) also carried IncI1 and IncFII plasmids. Strain PNUSAS428168 harbored the *qnrS1* gene involved in resistance to fluoroquinolones and *bla*<sub>CTX-M-15</sub> gene involved in

resistance to third-generation cephalosporins (Figure 1), highlighting the capacity of HC10\_20673 *Salmonella* Abony to acquire plasmids conferring resistance to first-line antibiotic drugs used for treating severe salmonellosis.

*Salmonella* Abony WDCM 00029 (also known as strains BAA-2162, NCTC 6017, CIP 80.39, CECT 545, and DSM 4224, among others) is a strain with >80 years of history. Originally isolated from human feces in Hungary before 1940, it was part of Fritz Kauffmann’s collection and was later deposited in different culture collections (4,5,8). WDCM 00029 is widely used as a control strain for testing culture media performance, detailed in



**Figure 1.** Phylogenetic analysis of *Salmonella enterica* serovar Abony from an outbreak caused by a WDCM 00029 Clone, Chile, 2024. A core SNP maximum-likelihood phylogenetic tree was constructed by using RAxML version 8 (<https://github.com/stamatak/standard-RAxML>) and genomes of *Salmonella* Abony isolates from the HC50\_20673 cluster with the ATCC 6017 genome as the reference (Enterobase Barcode SAL\_BA5138AA; Sequence Read Archive accession no. SRR1786283). The tree was constructed by using the Enterobase pipelines refMasker, refMapper, refMapperMatrix, and matrix\_phylogeny, which together masked repeated regions, tandem repeats, and CRISPR regions in the reference genome, aligned genomes to reference, called nonrepetitive core SNPs, and built the maximum likelihood tree. Metadata regarding HC10 clusters ( $\leq 10$  allele differences) include isolation source, collection year, country of origin, antibiotic drug resistance genes (AMRFinderPlus version 3.12.8; database version 2024-05-02.2; <https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/AMRFinder>), and plasmid replicons (ABRicate version 1.0.1, <https://github.com/tseemann/abricate>; PlasmidFinder database, updated June 4, 2024). Red arrows indicate the WDCM 00029 genomes found in the public databases (Enterobase, <https://enterobase.warwick.ac.uk/species/index/senterica>; National Center for Biotechnology Information Sequence Read Archive, <https://www.ncbi.nlm.nih.gov/sra>) that were made public in 2013 (accession no. SRR955283), 2016 (accession no. SRR1815498), 2019 (accession no. SRR8599079), and 2021 (accession no. SRR15145673). Asterisks (\*) in the collection year column indicate that information was missing. The figure was made by using iTOL version 6.9 (<https://itol.embl.de>). ATCC, American Type Culture Collection; cgMLST, core genome multilocus sequence typing; CRISPR, clustered regularly interspaced short palindromic repeats; SNP, single-nucleotide polymorphism.



**Figure 2.** Whole-genome comparisons of *Salmonella enterica* serovar Abony from an outbreak caused by a WDCM 00029 clone, Chile, 2024. A) Example whole-genome comparison between *Salmonella* Abony WDCM 00029 (genome provided by ATCC; strain BAA-2162) and an isolate from the HC10\_20673 cluster (strain SAbH24\_18) from Chile, or the most closely related isolate (strain 201808966) outside the HC10\_20673 cluster (mean difference to HC10\_20673 isolates: 73 SNPs). Red lines connect regions of genome identity between each pair of compared genomes, with color indicating the percent identity. The red vertical arrow points to a region of difference between the compared genomes. B, C) Truncated violin plots of AF (B) and ANI (C) to the WDCM 00029 genome of the *Salmonella* Abony isolates ( $n = 18$  genomes) from Chile and other isolates from the same HC50 cluster (HC10\_142933 and HC10\_165393;  $n = 12$  genomes). In the violin plots, black horizontal lines represent medians and dotted lines represent 25% and 75% quartiles. Differences between the median values were assessed by using Mann-Whitney tests. AF and ANI calculations were made with FastANI version 1.34 (<https://github.com/ParBLISS/FastANI>). AF, alignment fraction; ANI, average nucleotide identity; ATCC, American Type Culture Collection; SNP, single-nucleotide polymorphism.

pharmacopeial texts from the United States, Europe, and Japan that are accepted by the International Council of Harmonization (9,10). Accordingly, WDCM 00029 is sold by many suppliers as certified lyophilized or ready-to-use reference material for quality control of food, water, and environmental testing (Appendix 2 Table 5).

In summary, evidence suggests the 2024 *Salmonella* Abony outbreak in Chile was caused by contamination of an unknown vehicle with the widely used WDCM 00029 reference strain. Our findings raise concerns about safety of bacterial quality control strains. When rare or unreported serovars cause human infections, clinicians and health authorities should request strain characterization.

Ethics approval for this study was provided by Comité Ético Científico de Ciencias de la Salud de la Pontificia Universidad Católica de Chile (approval no. 240422008) and Comité Ético

Científico Facultad de Medicina Clínica Alemana Universidad del Desarrollo (approval no. UIEC 779).

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Dr. Piña-Iturbe is a microbiologist and postdoctoral researcher at Pontificia Universidad Católica de Chile. His research interests are focused on genetics and genomics of bacterial pathogens, with a special emphasis on the genomic epidemiology of *Salmonella* serovars, antibiotic resistance, and the potential of bacteriophages as control agents.

## References

- Ikuta KS, Swetschinski LR, Robles Aguilar G, Sharara F, Mestrovic T, Gray AP, et al.; GBD 2019 Antimicrobial Resistance Collaborators. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. 2022;400:2221–48. [https://doi.org/10.1016/S0140-6736\(22\)02185-7](https://doi.org/10.1016/S0140-6736(22)02185-7)
- Katz TS, Harhay DM, Schmidt JW, Wheeler TL. Identifying a list of *Salmonella* serotypes of concern to target for reducing risk of salmonellosis. *Front Microbiol*. 2024; 15:1307563. <https://doi.org/10.3389/fmicb.2024.1307563>
- European Food Safety Authority (EFSA); European Centre for Disease Prevention and Control (ECDC). The European Union One Health 2022 zoonoses report. *EFSA J*. 2023;21:e8442. <https://doi.org/10.2903/j.efsa.2023.8442>
- World Data Centre for Microorganisms. *Salmonella enterica* serovar Abony WDCM 00029 [cited 2024 Jun 7]. [https://refs.wdcm.org/browse/getinfo?sid=WDCM\\_00029](https://refs.wdcm.org/browse/getinfo?sid=WDCM_00029)
- American Type Culture Collection. *Salmonella enterica* serovar Abony ATCC BAA-2162 [cited 2024 Jun 7]. <https://www.atcc.org/products/baa-2162>
- Monte DFM, Nethery MA, Barrangou R, Landgraf M, Fedorka-Cray PJ. Whole-genome sequencing analysis and CRISPR genotyping of rare antibiotic-resistant *Salmonella enterica* serovars isolated from food and related sources. *Food Microbiol*. 2021;93:103601. <https://doi.org/10.1016/j.fm.2020.103601>
- Akinyemi KO, Fakorede CO, Linde J, Methner U, Wareth G, Tomaso H, et al. Whole genome sequencing of *Salmonella enterica* serovars isolated from humans, animals, and the environment in Lagos, Nigeria. *BMC Microbiol*. 2023;23:164. <https://doi.org/10.1186/s12866-023-02901-1>
- National Collection of Type Cultures. *Salmonella enterica* serovar Abony NCTC 6017 [cited 2024 Jun 7]. <https://www.culturecollections.org.uk/nop/product/salmonella-enterica-subsp-enterica-serotype-abony>
- International Council for Harmonization. Q4B evaluation and recommendation of pharmacopoeial texts for use in the ICH regions – annex 4B(R1) microbiological examination of nonsterile products: tests for specified microorganisms general chapter [cited 2024 Jun 7]. <https://www.fda.gov/media/71267/download>
- US Pharmacopoeia. <62> Microbiological examination of nonsterile products: tests for specified microorganisms [cited 2024 Jun 7]. [https://www.usp.org/sites/default/files/usp/document/harmonization/gen-method/q05a\\_pf\\_ira\\_34\\_6\\_2008.pdf](https://www.usp.org/sites/default/files/usp/document/harmonization/gen-method/q05a_pf_ira_34_6_2008.pdf)

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## Endogenous Endophthalmitis Caused by *Prototheca microalga* in Birman Cat, Spain

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We identified *Prototheca* spp. microalga in ocular samples of a cat in Spain with nontreatable endogenous endophthalmitis. Within 2 years, the eye lesions progressively worsened and neurologic signs appeared, suggesting systemic spread of the infection. On multitarget sequence analysis, the feline pathogen could not be assigned to any known *Prototheca* species.

**P**rotothecosis is an uncommon disease caused by the unicellular microalga *Prototheca* spp., described in humans and animals and associated with systemic disease, cutaneous lesions, or both (1,2). *Prototheca* spp. has been identified as the cause of cutaneous lesions and in 1 case of disseminated neurologic disease in cats (2–4). Diagnosis of protothecosis can be challenging and usually is based on observation of the organism in tissues and body fluids (5). Culturing or PCR is required for a definitive diagnosis and species identification (2,4).

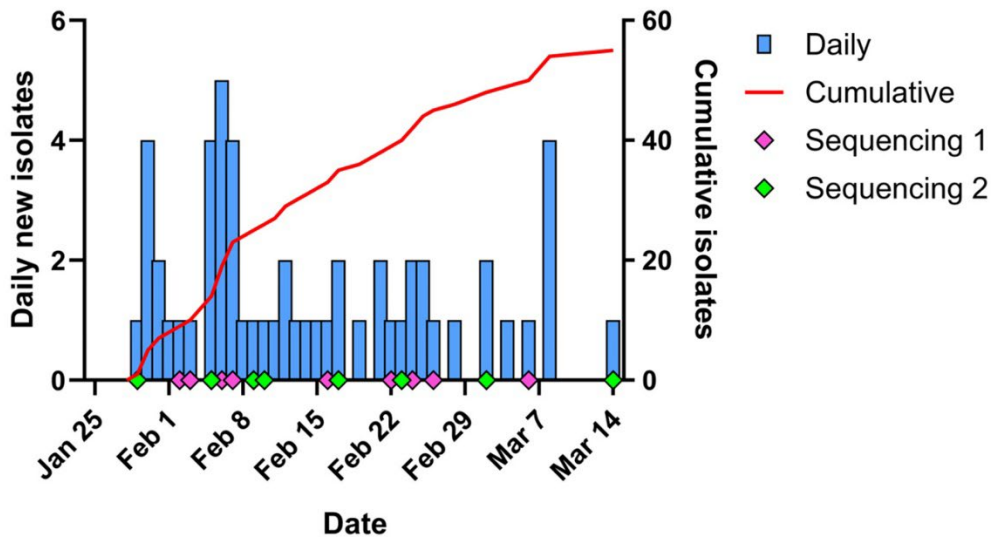
A 5-year-old female Birman cat, spayed and maintained indoors, was referred to our veterinary hospital for a 1.5-month history of uveitis in the right eye. Neuro-ophthalmic evaluation revealed that the right eye was blind and had severe signs of uveitis,

<sup>1</sup>These first authors contributed equally to this article.

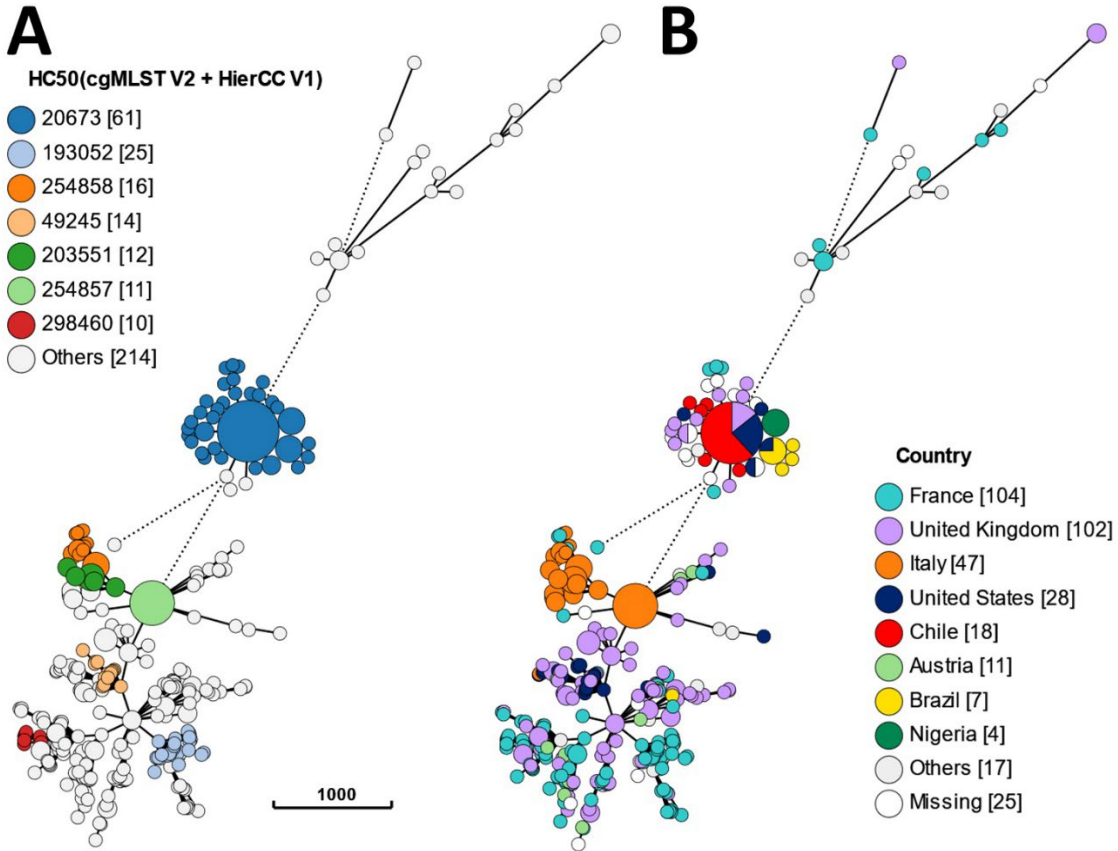
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# *Salmonella enterica* Serovar Abony Outbreak Caused by a Clone of Reference Strain WDCM 00029, Chile, 2024

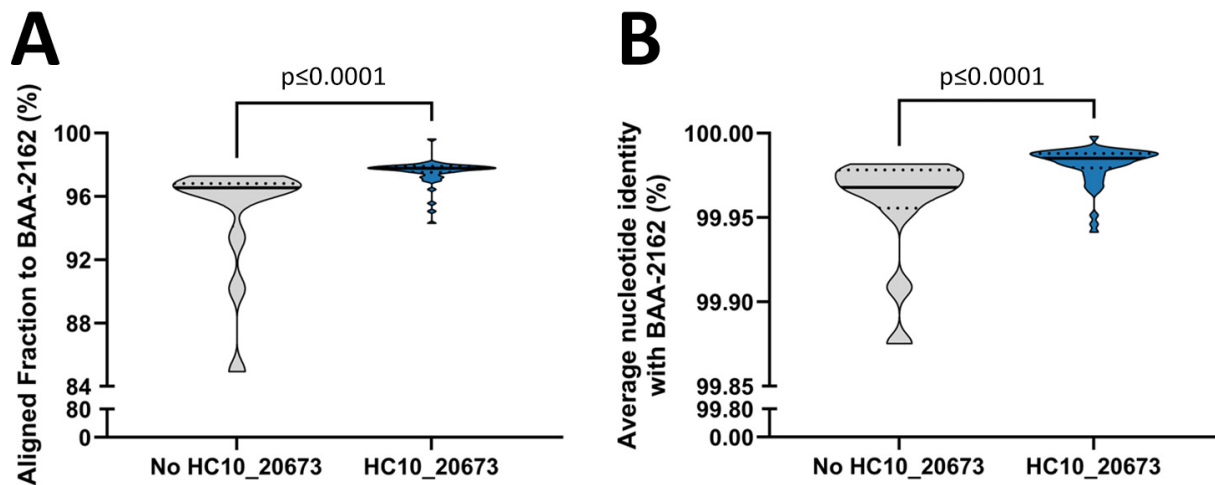
## Appendix



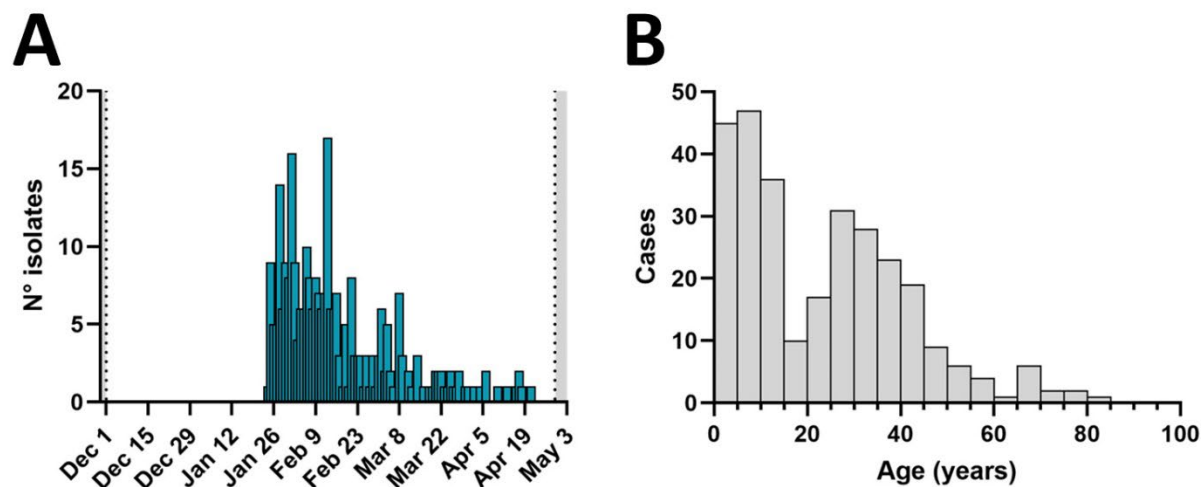
**Appendix 1 Figure 1.** Temporal distribution of *Salmonella* Abony isolates included in this study. The histogram (blue bars) shows the number of isolates per date of isolation. Information about dates of sample collection date or symptoms onset was not available. Only one isolate per case of infection was included. The red line indicates the cumulative number of *Salmonella* Abony isolates from 0 to 56. The pink and green diamonds indicate the isolation date of the sequenced *Salmonella* Abony (18 isolates) from the 2 microbiology laboratories that were included in this study, respectively.



**Appendix 1 Figure 2.** Structure of *Salmonella* Abony global population. A minimum spanning tree (MST) was constructed in Enterobase based on cgMLST (3002 alleles). Node size is proportional to the number of genomes included. Edges represent the number of allele differences. Nodes separated by more than 1000 allele differences are linked by broken-line edges. Nodes were colored by A) HC50 cluster according to HierCC v1 or B) country of origin. The Chilean *Salmonella* Abony isolates group in the HC50\_20673 cluster together with isolates from France, United Kingdom, Brazil, and Nigeria.



**Appendix 1 Figure 3.** Aligned fraction and average nucleotide identity of HC50\_20673 genomes to the WDCM 00029 genome. Truncated violin plots of A) the aligned genome fraction (AF) and B) average nucleotide identity (ANI) of HC10\_20673 and no HC10\_20673 (HC10\_142933 and HC10\_165393) isolates to the WDCM 00029 genome provided by the American Type Culture Collection (ATCC BAA-2162). The black horizontal line and dotted lines in the violin plots represent the median and the 25% and 75% quartiles, respectively. The AF and ANI were calculated with FastANI v1.34. Differences between the median values were assessed with the Mann-Whitney test (\*\*\*\* $p < 0.0001$ ).



**Appendix 1 Figure 4.** *Salmonella* Abony isolates received by the Instituto de Salud Pública (ISP) of Chile. A) Histogram of the *Salmonella* Abony isolates received by the ISP (287 isolates) from December 1, 2023 to April 29, 2024, and B) age distribution of infection cases according to data requested to the ISP. Some isolates may be obtained from the same patient.