10. Brienen NC, Timen A, Wallinga J, van Steenbergen JE, Teunis PF. The effect of mask use on the spread of influenza during a pandemic. Risk Anal. 2010;30:1210–8. https://doi.org/10.1111/j.1539-6924.2010.01428.x

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SARS-CoV-2 Variants BQ.1 and XBB.1.5 in Wastewater of Aircraft Flying from China to Denmark, 2023

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We analyzed wastewater samples from 14 aircraft arriving in Denmark directly from China during January 9–February 12, 2023. Wastewater from 11 aircraft was SARS-CoV-2–positive by PCR; 6 predominantly contained BQ.1 and XBB.1 subvariants. Wastewater-based surveillance can contribute to public health monitoring of SARS-CoV-2 and other emerging infectious agents.

Relaxation of China's zero-COVID policy in December 2022 led the European Centre for Disease Prevention and Control to recommend several nonpharmaceutical interventions to curb COVID-19 spread and monitor any emerging SARS-CoV-2 variants; those interventions included wastewater-based surveillance (*1*). We report results of subsequent wastewater surveillance of aircraft arriving at Copenhagen Airport in Copenhagen, Denmark, directly from Beijing or Shanghai, China.

During weeks 2–6 of 2023 (January 9–February 12), a total of 14 aircraft arrived at Copenhagen Airport from China. A service truck extracted waste from the aircraft by using vacuum pressure, after which a rinsing program was performed, and the disinfectant Idu-Flight (Brenntag Nordic A/S, https://www.brenntag.com) was added to the waste tank. Wastewater samples were collected as grab samples from the service truck and immediately transported to Statens Serum Institut in Copenhagen for analysis.

The pH value of the sample material ranged from 9–10 because of the addition of Idu-Flight. Idu-Flight contains the active ingredients glutaraldehyde and benzalkonium chloride; the disinfectant is expected to negatively affect the stability of virus particles and hinder amplification of RNA sequences. We adjusted the samples to pH 7.5–8.5 by using HCl and homogenized them by vigorous vortexing. We split the 14 samples into a total of 43 aliquots and then centrifuged those at either 4,000 × *g* or 10,000 × *g* for 10 min to pellet solid material. For the first aliquot from aircraft AC1, we analyzed 10 mL of sample material without any centrifugation; for all other samples, we analyzed 10 mL of supernatant after centrifugation. We purified viruses by using NanoTrap Microbiome A particles (Ceres Nanosciences Inc., https://www.ceresnano.com) and RNA by using Maxwell RSC Cartridges (Promega Corporation, https://www.promega.com). We performed quantitative reverse transcription PCR (qRT-PCR) in technical triplicate by using the GoTaq Enviro kit (Promega) and the US Centers for Disease Control and Prevention N2 primer/probe for SARS-CoV-2 detection (Table; Appendix Table, https://wwwnc.cdc.gov/EID/article/29/12/23-0717-App1.pdf).

Of the 43 qRT-PCR reactions, 31 (72%) were positive for SARS-CoV-2, representing 11 aircraft. We conducted whole-genome sequencing of samples from those 11 aircraft by using the Illumina MiSeq platform (https://www.illumina.com) according to the ARTIC protocol; we generated 2 × 150-bp paired-end reads by using the ARTIC 4.1 primer scheme (*2*). Wastewater raw reads are available from the European Nucleotide Archive (https://www.ebi.ac.uk/ena; accession no. PRJEB66221). We trimmed reads by using Trim Galore with default settings (*3*; https://zenodo.org/record/5127899). We removed human sequence reads by using the BWA-MEM alignment algorithm with default settings (H. Li, unpub. data, http://arxiv.org/abs/1303.3997) and the human genome reference build GRCh38. We then used BWA-MEM with default settings to map SARS-CoV-2 reads to the SARS-CoV-2 wild-type reference genome (GenBank accession no. MN908947.3). We performed

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Table. Sequencing results for SARS-CoV-2–positive wastewater samples in study of SARS-CoV-2 variants BQ.1 and XBB.1.5 in wastewater of aircraft flying from China to Denmark, 2023*

Aircraft ID	Arrival week	Raw sequence reads	Mapped sequence reads	Genome (spike) coverage,† %	Pangolin lineage‡ (% abundance)
AC1	2	16,699,590	948,407	85.5 (49.5)	XBB.1.1 (21), BA.5 (9), XBB.2 (7), XBB.1 (7)
AC2	2	15,892,650	122,882	48.5 (17.6)	NA
AC3	3	14,467,192	725,320	93.1 (90.7)	BQ.1 (60)
AC4	4	13,463,062	5,584	10.3 (8.1)	NA
AC5	4	25,193,066	607,011	83.9 (88.7)	XBB.1.5 (96)
AC6	4	5,215,440	135	16.7 (0.0)	NA
AC7	5	15,691,594	960,609	82.9 (91.4)	XBB.1.5 (99)
AC8	5	2,749,174	102	0.0 (0.0)	NA
AC9	6	5,880,410	14,457	14.0 (9.1)	NA
AC10	6	13,244,030	1,306,993	98.1 (95.2)	XBB.1.5 (59), XBB.2 (18), XBB.1 (18)
AC11	6	1,132,399	1,127,995	98.1 (95.2)	XBB.1.5 (97)
EC§	NA	4,050,388	370	0.5 (0.0)	NA

*Wastewater was collected from aircraft during January 9 (week 2)–February 12 (week 6), 2023. Samples from each aircraft were combined and reverse transcription quantitative PCR and sequencing were performed; only 1 sample each was collected from AC6, AC8, and AC9. Full table of results for each aliquot is available in the Appendix (https://wwwnc.cdc.gov/EID/article/29/12/23-0717-App1.pdf). EC, extraction control; ID, identification; NA, not applicable.
†Coverage percentages are provided for the full genome sequence and for the spike protein gene.
‡Lineages according to Pangolin software (https://cov-lineages.org).
§Water sample was extracted and sequenced as a control.

primer trimming by using iVar with a minimum read length of 30 nt (*4*) and estimated SARS-CoV-2 lineage abundance in each sample by using Freyja; depth cutoff was 10×, and the lineage abundance filter was 5% (*5*). We used a 50% coverage minimum across the genome as the threshold for lineage calling. We obtained sequencing results for 13 (42%) of 31 SARS-CoV-2–positive samples (Appendix Table).

We analyzed sequence reads for each sample aliquot and also after combining raw reads for each aircraft (Table; Appendix Table). When reads were combined for each aircraft, we found that the SARS-CoV-2 BQ.1 variant was dominant in wastewater of 1 aircraft, and XBB.1 variants were dominant in wastewater of 5 aircraft; the XBB.1.5 subvariant was dominant in 4 of those 5 aircraft (Table). The discovery of predominant XBB subvariants (dominant in Europe and the United States during the study period) in the aircraft samples contrasts with variant data uploaded to the GISAID database (https://www.gisaid.org) from China within the same time frame, which were mainly subvariants BA.5.2.48 and BF.7.14 (*6*).

For wastewater-based surveillance, limited information is generally available regarding the persons who contributed to the samples, and, consequently, data related to travel history and place of residence are lacking. Because of the lack of supporting information for passengers, the SARS-CoV-2 variants observed in wastewater-based surveillance of aircraft arriving in Copenhagen might have come from passengers infected outside of China.

In conclusion, our findings indicate that the largely infection-naive population of China might not have comprised a strong selective force driving SARS-CoV-2 toward variants with immune evasive features, such as BQ.1 and XBB. Thus, if BQ.1 and XBB.1.5 subvariants were indeed circulating in China to the extent suggested by our analysis, their dominance in wastewater samples might have occurred because of a founder effect in selected communities instead of those variants arising in China. Since January and February 2023, XBB has become the dominant variant in sequence data from China (*6*). No new variants have been identified, but our study highlights the potential for wastewater-based surveillance to monitor virus spread among airline passengers in a cost-effective, anonymous, and noninvasive manner and to potentially identify circulating variants. This method can be rapidly modified to include other emerging infectious agents and can contribute substantially to future public health surveillance.

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References

1. European Centre for Disease Prevention and Control. EASA/ECDC provide guidelines for aviation as part of European response to COVID-19 developments in China. 2023 [cited 2023 Mar 13]. https://www.ecdc.europa.eu/en/news-events/easaecdc-provide-guidelines-aviation-part-european-response-covid-19-developments-china
2. Quick J. nCoV-2019 sequencing protocol V.1. 2020 Jan 22 [cited 2023 Apr 17]. https://www.protocols.io/view/ncov-2019-sequencing-protocol-bbmuik6w
3. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J. 2011;17:10–12. https://doi.org/10.14806/ej.17.1.200
4. Grubaugh ND, Gangavarapu K, Quick J, Matteson NL, De Jesus JG, Main BJ, et al. An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar. Genome Biol. 2019;20:8. https://doi.org/10.1186/s13059-018-1618-7
5. Karthikeyan S, Levy JI, De Hoff P, Humphrey G, Birmingham A, Jepsen K, et al. Wastewater sequencing reveals early cryptic SARS-CoV-2 variant transmission. Nature. 2022;609:101–8. https://doi.org/10.1038/s41586-022-05049-6
6. Khare S, Gurry C, Freitas L, Schultz MB, Bach G, Diallo A, et al. GISAID's role in pandemic response. China CDC Wkly. 2021;3:1049–51. PubMed https://doi.org/10.46234/ccdcw2021.255

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Systemic Erysipelas Outbreak among Free-Ranging Bottlenose Dolphins, San Diego, California, USA, 2022

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We diagnosed fatal *Erysipelothrix rhusiopathiae* sepsis in 3 stranded bottlenose dolphins (*Tursiops truncatus*) during summer 2022, in San Diego, California, USA. The previously undetected disease in this relatively small, regional population of dolphins most likely indicates an environmental or biological change in the coastal ocean or organisms.

Erysipelas is a disease of animals caused by the bacterium *Erysipelothrix rhusiopathiae*, which can be transmitted via exposure to feces, urine, saliva, and nasal secretions from infected animals and contaminated food, water, and soil (*1*). Human infection with this bacterium most often involves occupational exposure (*1*). In cetaceans, the disease is thought to be caused by ingesting infected fish, tooth raking from infected conspecifics, or infected wounds. Chronic cutaneous and acute fatal septicemic forms of the disease have been reported for captive and free-ranging cetaceans (*2*) but not for free-ranging cetaceans along the Pacific Coast of the United States.

Two stocks of bottlenose dolphins (*Tursiops truncatus*) inhabit the waters of California, USA: coastal and offshore. The coastal population comprises ≈500 dolphins that range from San Francisco, California, USA, to San Quintin, Mexico (latitudinal distance = 802 km), with little site fidelity (*3*). In southern California, coastal bottlenose dolphins are typically found within 500 meters of the land.

During summer 2022 (June–September), 3 coastal bottlenose dolphins, of mixed sex and age class, were found stranded within 46 km of each other in San Diego, California, USA; we diagnosed sepsis caused by *E. rhusiopathiae*. The diagnoses coincided with increased strandings for this species in the region. In 2022, a total of 8 bottlenose dolphins were stranded, compared with a 20-year average of 4.35 per year (K. Danil, unpub. data; calculated by using Southwest Fisheries Science Center stranding records).

We determined cause of death for 6 of the 8 dolphins: 3 systemic erysipelas, 1 brucellosis, 1 trauma, and 1 malnutrition (Table). Gross necropsy findings for the 3 with erysipelas included open rake wounds (Appendix, https://wwwnc.cdc.gov/EID/article/29/12/23-0811-App1.pdf), mottled livers, distended urinary bladders, empty stomachs, and pulmonary edema; 2 dolphins also had ascites and icterus. Histopathologic examination for the 3 dolphins with erysipelas indicated vasculitis associated with multiorgan inflammation, necrotizing adrenalitis and nephritis for 1, and gastroenteritis for 1. Intracellular bacteria were identified (Figure), and *E. rhusiopathiae* were cultured

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SARS-CoV-2 Variants BQ.1 and XBB.1.5 in Wastewater of Aircraft Flying from China to Denmark, 2023

Appendix

Appendix Table. Extended sequencing results for individual SARS-CoV-2–positive wastewater samples in study of SARS-CoV-2 variants BQ.1 and XBB.1.5 in wastewater of aircraft flying from China to Denmark, 2023*

Aircraft ID*	Arrival week	Ct	Raw sequence reads	Mapped sequence reads	Genome (spike) coverage,† %	Pangolin lineage‡ (% abundance)
AC1	2	37.2	4,424,432	199,962	62.2 (20.5)	BA.2.85 (58), XBB.1.7 (27)
		36.8	4,446,070	340,210	54.3 (38.8)	BA.2.10.1 (32), BA.2.10 (32)
		38.3	2,760,420	23,213	5.1 (10.5)	NA
		35.4	5,068,668	385,022	47.7 (20.8)	NA
Combined AC1 samples	2	NA	16,699,590	948,407	85.5 (49.5)	XBB.1.1 (21), BA.5 (9), XBB.2 (7), XBB.1 (7)
AC2	2	35.4	4,602,774	107,184	42.1 (17.6)	NA
		36.8	4,167,118	11,265	6.0 (0.0)	NA
		37.8	3,629,122	2,983	2.4 (0.0)	NA
		37.6	3,493,636	1,450	1.2 (0.0)	NA
Combined AC2 samples	2	NA	15,892,650	122,882	48.5 (17.6)	NA
AC3	3	34.9	1,486,100	125,201	87.6 (51.1)	BQ.1 (37), BQ.1.17 (8), BQ.1.1.28 (6)
		34.2	4,561,054	240,781	63.1 (67.3)	BQ.1.24 (15), BQ.1.19 (15), BQ.1.11 (15), BQ.1.15 (15), BQ.1.20 (15), BQ.1 (15)
		35.4	3,868,320	139,310	77.4 (75.6)	BQ.1.17 (15), BQ.1.26 (15), BQ.1.19 (15), BQ.1.11 (15), BQ.1 (15), BQ.1.20 (15)
		34.3	4,551,718	220,028	63.5 (8.1)	BE.1.1 (32), BQ.1 (14), BQ.1.26 (14), BQ.1.11 (14), BQ.1.15 (14)
Combined AC3 samples	3	NA	14,467,192	725,320	93.1 (90.7)	BQ.1 (60)
AC4	3	38.4	3,655,710	662	1.2 (8.1)	NA
		38.4	3,194,498	225	1.1 (0.0)	NA
		38.4	3,676,300	3,474	2.2 (0.0)	NA
		38.3	2,936,554	1,223	2.4 (0.0)	NA
Combined AC4 samples	3	NA	13,463,062	5,584	10.3 (8.1)	NA
AC5	4	38.9	6,993,416	105,986	52.9 (43.7)	XBB.1.5 (99)
		37.8	5,253,686	204,214	32.8 (41.5)	NA
		37.2	5,716,928	66,675	17.0 (18.0)	NA
		37.9	7,229,036	230,136	51.2 (79.8)	XBB.1.5 (96)
Combined AC5 samples	4	NA	25,193,066	607,011	83.9 (88.7)	XBB.1.5 (96)
AC6	4	38.4	5,215,440	135	16.7 (0.0)	NA
AC7	5	38.7	3,306,180	3,2013	39.8 (31.8)	NA
		38.4	4,204,666	124,643	51.2 (34.5)	XBB.1.5 (99)
		37.9	4,719,822	601,107	56.8 (49.4)	XBB.1.5 (97)
		38.3	3,460,926	202,846	26.4 (55.6)	NA
Combined AC7 samples	5	NA	15,691,594	960,609	82.9 (91.4)	XBB.1.5 (99)
AC8	5	38.3	2,749,174	102	0.0 (0.0)	NA
AC9	6	38.0	5,880,410	14,457	14.0 (9.1)	NA
AC10	6	34.4	5,970,934	102,160	68.5 (57.7)	XBB.1.5 (97)

Aircraft ID*	Arrival week	Ct	Raw sequence reads	Mapped sequence reads	Genome (spike) coverage,† %	Pangolin lineage‡ (% abundance)
		35.4	7,273,096	1,204,834	97.2 (95.2)	XBB.1.5 (59), XBB.1 (18), XBB.2 (18)
Combined AC10 samples	6	NA	13,244,030	1,306,993	98.1 (95.2)	XBB.1.5 (59), XBB.2 (18), XBB.1 (18)
AC11	6	36.1	5,092,066	222,781	93.4 (82.4)	XBB.1.5 (96)
		35.1	6,231,930	905,214	41.7 (95.2)	NA
Combined AC11 samples	6	NA	1,132,399	1,127,995	98.1 (95.2)	XBB.1.5 (97)
EC§	NA	NA	4,050,388	370	0.5 (0.0)	NA

*Wastewater was collected from aircraft during January 9 (week 2)–February 12 (week 6), 2023. For combined samples, all raw reads for that aircraft were pooled before analysis. Only 1 sample each was collected from AC6, AC8, and AC9. Ct, cycle threshold; EC, extraction control; ID, identification; NA, not applicable.

†Coverage percentages are provided for the full genome sequence and for the spike protein gene.

‡Lineages according to Pangolin software (https://cov-lineages.org).

§Water sample was extracted and sequenced as a control.