

Genomic Epidemiology of a Severe Acute Respiratory Syndrome Coronavirus 2 Outbreak in a US Major League Soccer Club: Was It Travel Related?

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Background. Professional soccer athletes are at risk of acquiring severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). United States Major League Soccer (MLS) uses protocol-based SARS-CoV-2 testing for identification of individuals with coronavirus disease 2019.

Methods. Per MLS protocol, fully vaccinated players underwent SARS-CoV-2 real-time polymerase chain reaction testing weekly; unvaccinated players were tested every other day. Demographic and epidemiologic data were collected from individuals who tested positive, and contact tracing was performed. Whole genome sequencing (WGS) was performed on positive specimens, and phylogenetic analyses were used to identify potential transmission patterns.

Results. In the fall of 2021, all 30 players from 1 MLS team underwent SARS-CoV-2 testing per protocol; 27 (90%) were vaccinated. One player who had recently traveled to Africa tested positive for SARS-CoV-2; within the following 2 weeks, 10 additional players and 1 staff member tested positive. WGS yielded full genome sequences for 10 samples, including 1 from the traveler. The traveler's sample was Delta sublineage AY.36 and was closely related to a sequence from Africa. Nine samples yielded other Delta sublineages including AY.4 (n = 7), AY.39 (n = 1), and B.1.617.2 (n = 1). The 7 AY.4 sequences clustered together, suggesting a common source of infection. Transmission from a family member visiting from England to an MLS player was identified as the potential index case. The other 2 AY.4 sequences differed from this group by 1–3 nucleotides, as did a partial genome sequence from an additional team member.

Conclusions. WGS is a useful tool for understanding SARS-CoV-2 transmission dynamics in professional sports teams.

Keywords. SARS-CoV-2; molecular epidemiology; transmission; professional sports.

Major League Soccer (MLS) is the most diverse North American professional sports league, with a roster of 224 internationally

born players among 536 total players (mlssoccer.com). Players participate in frequent domestic travel during the regular MLS season, which runs from early March through November. There are breaks throughout the MLS season for international competitions, and players often engage in international travel beyond their MLS team duties to compete in these tournaments throughout the year as required by their national teams. Because global travel has been an important factor in the spread of emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants, MLS players who engage in frequent international travel may have increased risk of acquiring SARS-CoV-2 infection and spreading variants of SARS-CoV-2.

Professional sports leagues, including MLS, have enacted mitigation strategies to reduce the risk of acquiring SARS-CoV-2 infection, including vaccination and intensive

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screening for early identification of individuals with coronavirus disease 2019 (COVID-19) [1–3]. As a part of these efforts, MLS collects data on COVID-19 exposure history, travel history, and clinical symptoms. In this study, we leveraged these data and used whole genome sequencing (WGS) to investigate a SARS-CoV-2 outbreak within an MLS club following the return of a player from international travel.

MATERIALS AND METHODS

Data collection was facilitated by GeoSentinel, a collaboration between the United States (US) Centers for Disease Control and Prevention (CDC) and the International Society of Travel Medicine. GeoSentinel is a global clinical care-based surveillance system that monitors infectious diseases and other adverse health events that may impact international travelers and migrants. This activity was reviewed by CDC and determined to be nonresearch; it was conducted consistent with applicable federal law and CDC policy.

Patient Consent Statement and Ethics Approvals

This design of this study was approved by the University of Delaware institutional review board. It was determined that this study did not include factors necessitating patient consent.

Collection of Data and Samples

Throughout the pandemic, MLS developed protocols for testing which were modified, based on infectious disease expert recommendations, over time. Starting in June 2020, surveillance testing was performed on a regular basis and every other day at the “MLS Is Back” tournament. These protocols were modified to accommodate those who tested positive as well as those who had been vaccinated. In addition, testing was performed when athletes returned from international travel. During the time of the data collected for this report, 2 doses of messenger RNA vaccines and 1 dose of the Janssen vaccine was considered fully vaccinated. All vaccinated players and staff with 1 booster were tested for SARS-CoV-2 by real-time polymerase chain reaction (rt-PCR) weekly, those fully vaccinated with 2 doses but no booster were tested twice weekly, and unvaccinated players and staff were tested every other day. This routine testing complemented protocols for other testing, such as for travel, symptoms, and according to state and federal guidelines. Samples were collected by a trained medical professional in all cases. Demographic characteristics (eg, age and sex), epidemiologic characteristics (eg, travel history), and clinical data (eg, symptoms) were collected from individuals who tested positive for SARS-CoV-2, and these data were managed in Excel software version 16.63.1. Symptom tracking was done via a survey for daily reports, and a follow-up standardized interview was performed with all confirmed positive cases. Contact tracing was performed by the county department of

health with jurisdiction in the county in which the club was based. To protect participant privacy, dates and exact locations were omitted.

SARS-CoV-2 Whole Genome Sequencing

Residual samples were collected in viral transport media from SARS-CoV-2-positive specimens from MLS traveler 711 and MLS team members 718, 719, 720, 729, 730, 731, 734, 735, 736, 737, and 738. RNA was extracted, and SARS-CoV-2 RNA was amplified using the Illumina COVIDSeq Assay using Artic 4 primers; libraries were sequenced on an Illumina NextSeq 500. Full-length genome sequences for each amplified sample were assembled through alignment to the Wuhan-Hu-1 reference sequence (NC_045512.2) using Bowtie2 [4]. Nucleotide substitutions, insertions, and deletions were identified with LoFreq version 2.1.3.1 [5]. Lineage assignment for each genome was carried out using Pangolin version 3.1.20 and Pangolin-data version 1.2.127-beta3 [6]. All sequences newly generated in this study were deposited in the National Center for Biotechnology Information under BioProject PRJNA874720 and the Global Initiative on Sharing All Influenza Data (GISAID) under EPI_SET_220831kt.

Subsampling

Traveler Tree

A total of 2424 AY.36 sequences, collected a week prior to the start of the MLS player’s international travel through a week after his positive SARS-CoV-2 rt-PCR, were downloaded from GISAID [7] and aligned with MLS traveler sequence 711 and reference strains Wuhan/Hu-1/2019 and Wuhan/WHO/2019 using Nextalign within the Nextstrain version 3.2.4 pipeline [8]. This dataset was subsampled in Nextstrain using a custom scheme in which the proximity filter was set to select up to 600 of the most genetically related sequences to the MLS traveler sequence. To facilitate data visualization, the resulting set of sequences was further downsampled to up to 150 sequences using the same proximity filter strategy.

Team Member Tree

A total of 64 083 AY.4 sequences, collected between the traveler’s return and 2 weeks after the last positive SARS-CoV-2 rt-PCR among his team members, were downloaded from GISAID and aligned with reference strains Wuhan/Hu-1/2019 and Wuhan/WHO/2019 and all MLS player AY.4 sequences. Subsampling of the dataset was performed in Nextstrain using a custom scheme: A custom location was created for the 7 team members with full AY.4 sequences, including players 719, 729, 730, 731, 734, 735, and 737. The proximity filter was set to focus on the custom location and select up to 300 sequences from the same region as the MLS club, 200 North American sequences, and 100 global sequences most genetically related to the 7 MLS team members. To facilitate data

Tree scale: 0.001

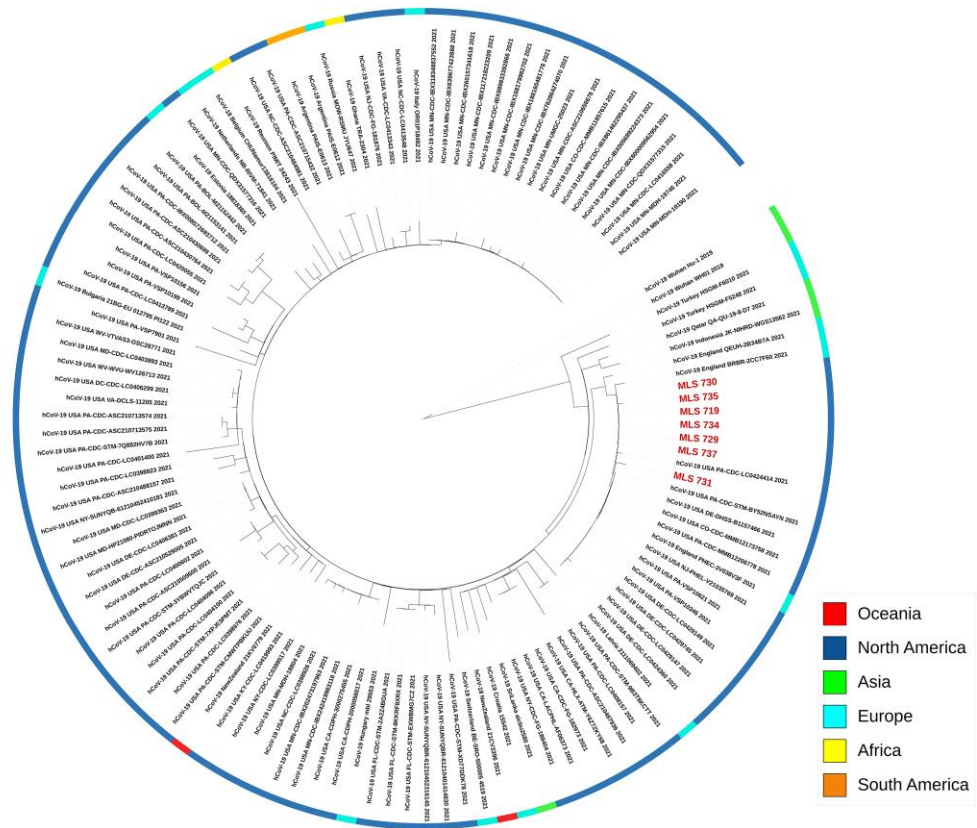


Figure 1. Phylogenetic analysis shows highly related sequences among US Major League Soccer (MLS) team members. A maximum likelihood phylogenetic tree including 7 MLS team members and 117 representative AY.4 lineage sequences, rooted to reference Wuhan/Hu-1/2019. MLS team members are colored red. This tree is a subsampled version of [Supplementary Figure 2](#), to facilitate data visualization. Abbreviation: MLS, Major League Soccer.

visualization, the resulting dataset was further downsampled to up to 150 sequences using the same proximity filter strategy at 75, 50, and 25 sequences per group, respectively. Fourteen GISAID sequences that were identical or near identical to the AY.4 MLS cluster but originally filtered out due to Nextstrain configurations for closely related sequences were added back to the dataset. The findings of this study are based on metadata associated with 949 sequences available on GISAID up to 18 March 2022, and accessible at doi.org/10.55876/gis8.221102hn.

Phylogenetic Analysis

Maximum likelihood phylogenetic trees were constructed using default settings of the Nextstrain SARS-CoV-2 Workflow with TreeTime version 0.8.6 [9]. The tree in [Figure 1](#) was constructed using IQtree version 2.2.0 [10]. Supplementary figure trees were visualized using Auspice version 2.37.3. All other trees were visualized and annotated using Interactive Tree of Life (iTOL) version 4 [11]. For the tree schematic in [Figure 2](#), a maximum likelihood tree of MLS team members involved in the outbreak investigation was generated using IQtree and a schematic representation of this (not exactly to scale) was generated by hand.

RESULTS

On day 0, an MLS player (player 711) traveled from the US to Africa to participate in matches in the fall of 2021. Before international travel, the MLS player had received 1 dose of the Janssen vaccine (considered to be fully vaccinated at the time) and tested negative by rt-PCR for SARS-CoV-2. His itinerary included several layovers on both the departure and return legs of his trip. Player 711 traveled within the continent, including to South Africa, to compete in matches against other African teams. During his travels, the first case of the Omicron variant was detected in South Africa [12]. On day 10, the player tested negative for SARS-CoV-2 by rt-PCR and returned to the US. The player reported to his MLS club's training facility the next day (day 11). During point-of-care testing for entry into the facility following international travel, his SARS-CoV-2 PCR test returned positive. There was no documented contact with other teammates or staff. The player was instructed to immediately isolate for 10 days, during which time he reported no symptoms.

From day 12 to day 25, all 30 players from the team underwent SARS-CoV-2 testing per protocol; 27 (90%) were vaccinated. During this time, 10 additional MLS players and 1 staff member tested positive for SARS-CoV-2 ([Figure 2](#)).

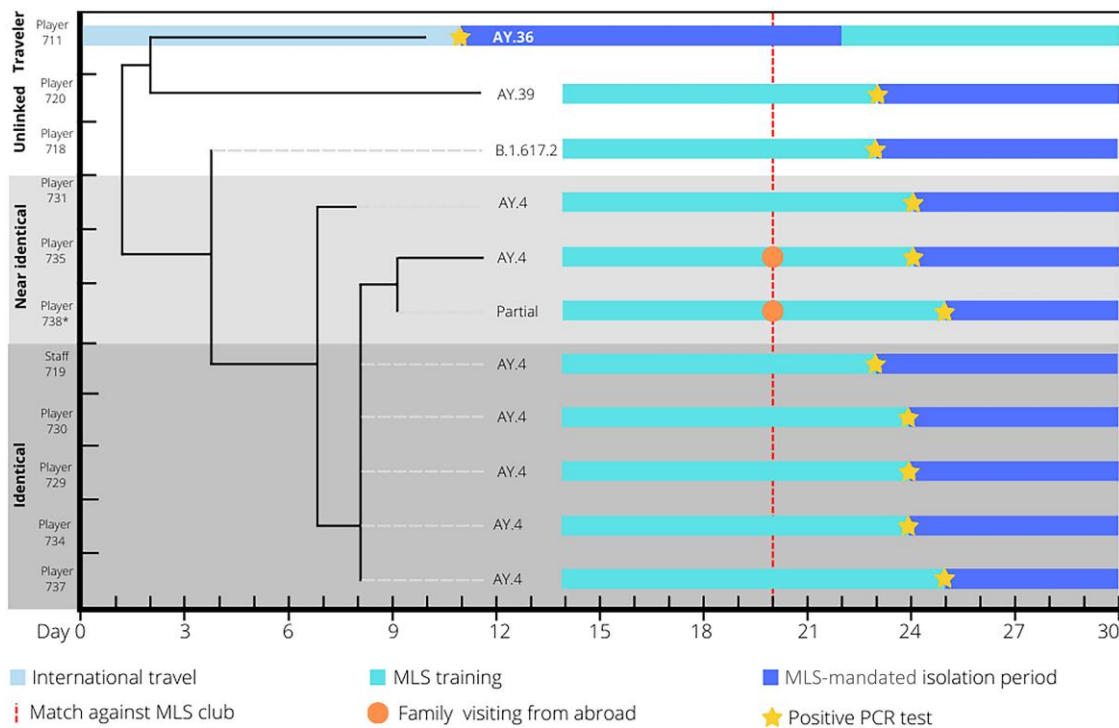


Figure 2. Epidemiologic and clinical timeline for SARS-CoV-2 positive US Major League Soccer (MLS) players and staff. *Partial sequence was used for player 738. Abbreviations: MLS, Major League Soccer; PCR, polymerase chain reaction.

Among the additional 11 team members who tested positive, all but 1 reported having symptoms, which included congestion (10 [100%]), headache (8 [80%]), body aches (8 [80%]), fever (8 [80%]), and wet cough (8 [80%]). Nine of the 11 (82%) additional players and staff who tested positive were fully vaccinated (Table 1). Individuals who were unvaccinated or considered high-risk close contacts followed all MLS policies at the time and protocols, including wearing facemasks, daily testing, and avoiding off-field activities (eg, meetings, meals).

WGS and Pangolin lineage assignment revealed that the international traveler was infected with Delta sublineage AY.36. A maximum likelihood phylogenetic tree was generated and phylogenetic analysis revealed that the traveler's sequence was most closely related to a sequence from Africa (Figure 3, Supplementary Figure 1). Contact tracing revealed that the international traveler had visited friends and was mostly unmasked. After return to the US, contact tracing confirmed that the traveler abided by isolation protocols after testing positive for SARS-CoV-2.

WGS was attempted on the 11 team member samples; 9 yielded complete, high-quality genomes, 8 from players and 1 from staff (Table 1, Supplementary Table 1). The team members were infected with Delta sublineages, including AY.4 (n = 7 [64%]), AY.39 (n = 1 [9%]), and B.1.617.2 (n = 1 [9%]) (Table 1, Figure 2). The sample from 1 team member, player 738, yielded a partial sequence (coverage = 60.6%), which

aligned closely with the other AY.4 samples (Supplementary Table 1, Figure 2). Phylogenetic analysis of the team member sequences identified highly related sequences (Supplementary Figure 2), including 5 identical AY.4 sequences among staff and players 719, 729, 730, 734, and 737, suggesting likely transmission among those team members (Figure 1, Figure 2). Three additional players, 731, 735, and 738, had near-identical sequences to the 5 players, differing only by 1–3 nucleotides.

All infected players and staff were interviewed by contact tracers to investigate the outbreak, and several potential transmission events were identified (Figure 2). The team participated in daily training in which close contact occurred on the field and within the training facilities. The team played a match against another MLS club on day 20, after which both players and staff celebrated a victory both indoors and outside of team facilities, breaching social distancing rules. Players 734 and 735 had close contact outside the MLS facility, as did players 731 and 736. Players 731, 734, and 735 were part of the AY.4 cluster; the specimen for player 736 was unable to be sequenced. Players 735 and 738 had lockers directly next to each other in the locker room and were also part of the AY.4 cluster; their sequences differed by 2 nucleotides, but the sequence from player 738 was incomplete.

Contact tracing also identified potential routes of transmission from family members. Players 735 and 738 hosted relatives

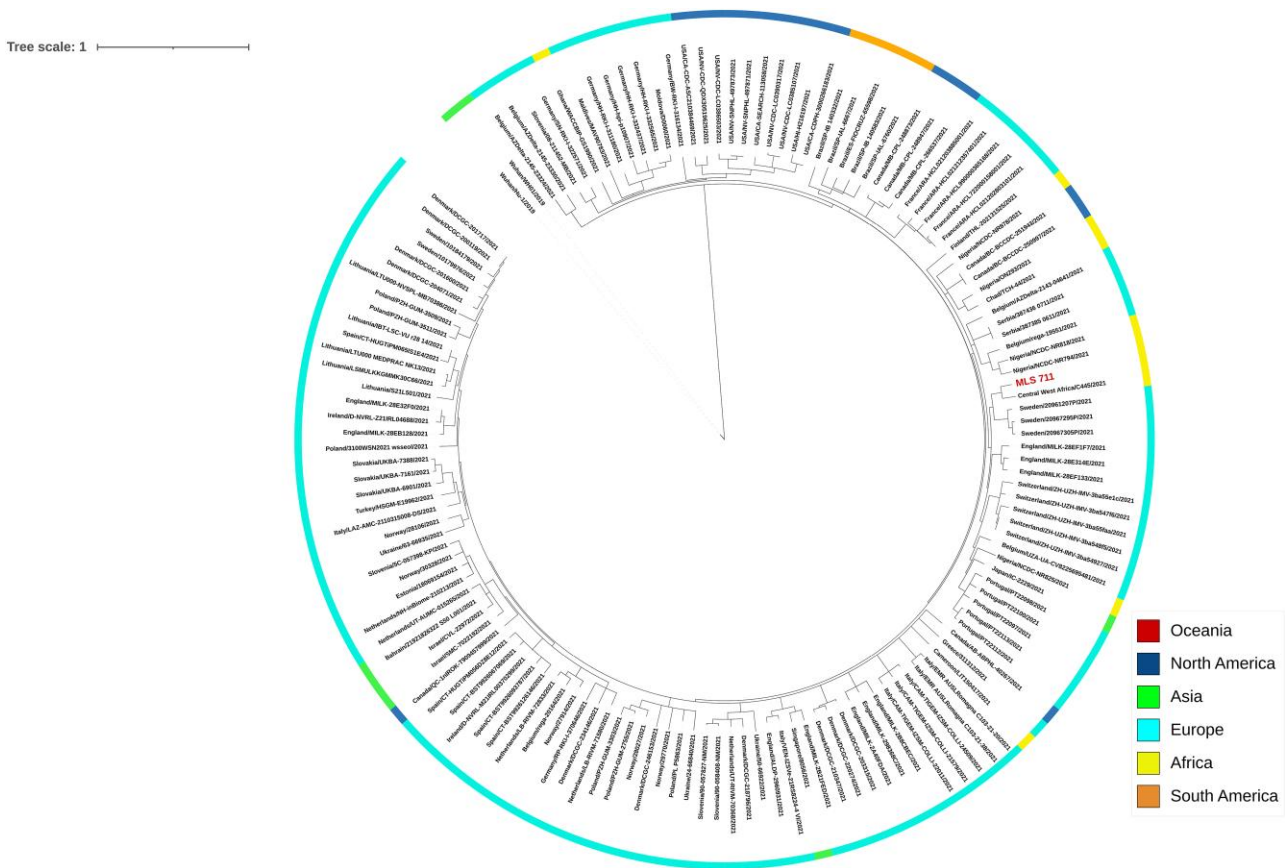


Figure 3. Phylogenetic analysis supports travel-associated infection in US Major League Soccer (MLS) traveler and confirms traveler was not source of outbreak among team members. Maximum likelihood phylogenetic tree including the MLS traveler and 142 globally representative AY.36 lineage sequences, rooted to reference Wuhan/Hu-1/2019. This tree is a subsampled version of [Supplementary Figure 1](#), to facilitate data visualization. Abbreviation: MLS, Major League Soccer.

from Europe who attended the match on day 20. A relative of player 738 visited from England, and the AY.4 cluster among MLS team members clustered with other AY.4 sequences from England and North America ([Figure 1](#), [Supplementary Table 2](#)). Dates of symptom onset were not available to assess potential directionality of transmission, but analysis of the sequences that clustered most closely with MLS team members revealed that the sequence was detected in England prior to detection within the US ([Supplementary Table 2](#)).

Players 718 and 720 did not share the same SARS-CoV-2 lineages of the others ([Table 1](#), [Figure 2](#)). Player 718 was living with a person infected with SARS-CoV-2 and was instructed to follow high-risk close contact procedures while at the MLS training facility, including masking and daily testing. A source of infection for player 720 was not identified.

DISCUSSION

This analysis describes 12 members of an MLS team who tested positive for SARS-CoV-2 within a 2-week period. A detailed genomic epidemiological investigation indicated that a player

returning from international travel was not the source of the outbreak. Although international travel is a conduit for introduction of emerging SARS-CoV-2 variants into the US, our analysis found that MLS practices to mitigate the spread of a travel-related infection among its players, including testing and isolation, were effective. However, close contacts who were recently abroad may import SARS-CoV-2, and transmission to players may occur in settings external to MLS facilities. This highlights the need for additional mitigation strategies for players when not at MLS facilities, such as masking, social distancing, and testing via rt-PCR or rapid antigen before and after community or family events.

During the 2020 and 2021 sports seasons, a spotlight was placed on sporting games as SARS-CoV-2 superspreading events. While studies have found that large sporting events may facilitate the spread of infection among spectators [13–15], studies among players have found that transmission is due to internal club SARS-CoV-2 outbreaks, social interactions, and wider community spread rather than games played on the field [16, 17]. This analysis cannot rule out transmission during the game played on day 20, but contact tracing revealed

Table 1. Epidemiologic characteristics of US Major League Soccer (MLS) team members who tested positive via RT-PCR for SARS-CoV-2

ID	MLS Designation	Sex	Age Range, y	Fully Vaccinated	Vaccine Received	Symptoms	Ct	WGS Performed	Pangolin Lineage
711	Player/traveler	Male	20–25	Yes	Janssen	NA	28.4	Yes	AY.36
718	Player	Male	25–30	Yes	Pfizer	Congestion, headache, body ache, fever, wet cough	32	Yes	B.1.617.2
719	Staff	Male	30–35	No	NA	Congestion	31.1	Yes	AY.4
720	Player	Male	30–35	No	NA	Congestion	42	Yes	AY.39
729	Player	Male	30–35	Yes	Pfizer	Congestion, headache, body ache, fever, wet cough	20.9	Yes	AY.4
730	Player	Male	25–30	Yes	Janssen	Congestion, headache, body ache, fever, wet cough	32.7	Yes	AY.4
731	Player	Male	25–30	Yes	Pfizer	Congestion, headache, body ache, fever, wet cough	26.8	Yes	AY.4
734	Player	Male	30–35	Yes	Pfizer	Congestion, headache, body ache, fever, wet cough	18.7	Yes	AY.4
735	Player	Male	25–30	Yes	Pfizer	Congestion, headache, body ache, fever, wet cough	32.5	Yes	AY.4
736	Player	Male	30–35	Yes	Moderna	Congestion, headache, body ache, fever, wet cough	30.7	No	NA
737	Player	Male	15–20	Yes	Pfizer	NA	27.9	Yes	AY.4
738	Player	Male	25–30	Yes	Moderna	Congestion, headache, body ache, fever, wet cough	35.8	Partial sequence	UND

Abbreviations: Ct, cycle threshold; MLS, Major League Soccer; NA, not applicable; UND, undetermined; WGS, whole genome sequencing.

multiple interactions both in and external to MLS facilities as potential transmission events, so the event leading to transmission is unable to be identified. The results of this study also align with healthcare-acquired SARS-CoV-2 infection studies that find that mitigation strategies work for patient encounters [18, 19] but community encounters, household transmission, and breached mitigation strategies in shared break spaces lead to infections in healthcare workers [20, 21].

There were several limitations to this analysis. Complete genomes could not be sequenced from 2 (17%) specimens, so a linkage could not be ascertained. In addition, several players and staff (n = 4) who tested positive for SARS-CoV-2 during the outbreak timeframe were omitted from this investigation due to lack of clinical and genomic data. Lack of dates of symptom onset limited interpretation of potential transmission directionality. Finally, recall bias or partial recollection of events during contact tracing interviews may have impeded identification of additional potential transmission events.

This study highlights the benefit of supplementing traditional epidemiological outbreak investigations with a genomic approach. While 11 SARS-CoV-2 cases presented as a cluster within the MLS club, genomic epidemiology provided the resolution to identify one 5-player cluster and several independent transmissions. It is possible, given only a 1- to 3-nucleotide difference in the genetic sequence for 3 additional players, that up to 8 players were associated with this cluster. WGS could be performed when resources permit on clusters of COVID-19 in which there is interest or need in defining a common source. This study also highlights the benefit of genomic baseline surveillance initiatives, such as large-scale WGS sponsored by the CDC, and the value of public genomic databases such as GISAID that make these data available for epidemiologic investigations.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the

posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We gratefully acknowledge all data contributors (ie, the authors and their originating laboratories responsible for obtaining the specimens) and their submitting laboratories for generating the genetic sequence and metadata and sharing via the GISAID Initiative, on which this project is based (see [Supplementary Table 3](#)).

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC).

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Potential conflicts of interest. The authors: No reported conflicts of interest.

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