SARS-CoV-2 transmission in intercollegiate athletics not fully mitigated with daily antigen testing

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Summary: High frequency, rapid turnaround SARS-CoV-2 testing continues to be proposed as a way of efficiently identifying and mitigating transmission in congregate settings. However, here we

describe two SARS-CoV-2 outbreaks occurring among intercollegiate university athletic programs during the fall 2020 semester.

Running title: SARS-CoV-2 in intercollegiate athletics

Abstract

Background

High-frequency, rapid-turnaround SARS-CoV-2 testing continues to be proposed as a way of efficiently identifying and mitigating transmission in congregate settings. However, two SARS-CoV-2 outbreaks occurred among intercollegiate university athletic programs during the fall 2020 semester despite mandatory directly observed daily antigen testing.

Methods

During the fall 2020 semester, athletes and staff in both programs were tested daily using Quidel's Sofia SARS Antigen Fluorescent Immunoassay (FIA), with positive antigen results requiring confirmatory testing with real-time reverse transcription polymerase chain reaction (RT-PCR). We used genomic sequencing to investigate transmission dynamics in these two outbreaks.

Results

In Outbreak 1, 32 confirmed cases occurred within a university athletics program after the index patient attended a meeting while infectious despite a negative antigen test on the day of the meeting. Among isolates sequenced from Outbreak 1, 24 (92%) of 26 were closely related, suggesting sustained transmission following an initial introduction event. In Outbreak 2, 12 confirmed cases occurred among athletes from two university programs that faced each other in an athletic competition despite receiving negative antigen test results on the day of the competition. Sequences from both teams were closely related and distinct from viruses circulating in Team 1's community, suggesting transmission during intercollegiate competition in Team 2's community.

Conclusions

These findings suggest that antigen testing alone, even when mandated and directly observed, may not be sufficient as an intervention to prevent SARS-CoV-2 outbreaks in congregate settings, and highlight the importance of supplementing serial antigen testing with appropriate mitigation strategies to prevent SARS-CoV-2 outbreak in congregate settings.

Keywords (up to 5): SARS-CoV-2, antigen testing, genomic epidemiology

Introduction

Timely reporting of SARS-CoV-2 test results is critical for controlling transmission through prompt public health action, yet at times during 2020, turnaround times for SARS-CoV-2 test results in the United States have averaged 4 days, with some individuals waiting 10 days or more [1]. While turnaround times in early 2021 have improved, the lag between specimen collection and receipt of a test result continues to represent a window in which the risk of viral spread from SARS-CoV-2-infected individuals is high. Rapid antigen tests, like Abbott's BinaxNow COVID-19 Ag Card and Quidel's Sofia SARS Antigen FIA, can reduce this lag between testing and results reporting [2–5]. Because of these qualities, high-frequency, rapid-turnaround SARS-CoV-2 antigen testing has been proposed as a prevention strategy in many congregate settings where SARS-CoV-2 infection risk is elevated [6–8].

In data submitted for emergency use authorization, the Sofia SARS Antigen FIA antigen test reported a sensitivity of 97% and specificity of 100% when used for symptomatic patients within five days of symptom onset [9,10]. It therefore follows that serial antigen testing could rapidly identify persons with symptomatic infections enabling rapid isolation of such individuals [2,4]. Recent studies, however, have found that the Sofia SARS Antigen FIA antigen test was less sensitive (41.2% sensitivity) in asymptomatic individuals [10–13]. Use of Sofia SARS Antigen FIA on asymptomatic patients is not included in the FDA authorization and is considered an "off-label" use of the test. Nonetheless, many universities and other congregate settings have used the tests for asymptomatic screening. The potential for false-negative antigen results among asymptomatic patients may

present a significant risk in that a negative test could result in risk disinhibition behavior in an individual who may be infectious during their pre-symptomatic period, leading to sustained and increased viral spread[14] (**Figure 1**).

Methods

A university implemented daily SARS-CoV-2 antigen testing for college athletics

The two outbreaks occurred among athletes and staff affiliated with a university's intercollegiate athletics programs despite daily SARS-CoV-2 testing with Quidel's Sofia SARS Antigen FIA. Both sports involved in the outbreaks were considered "high-risk" by the National Collegiate Athletics Association (NCAA) due to frequent contact and collision between athletes during play. Students and staff affiliated with the two athletics programs began daily antigen testing for SARS-CoV-2 in September 2020. Prior to September 2020, all athletes were tested by RT-PCR once or twice a week. Outbreak 1 included 133 total individuals, and Outbreak 2 included 55 total individuals (32 on Team 1, and 23 on Team 2). Daily antigen testing was not required for persons with a RT-PCR-confirmed SARS-CoV-2 infection in the past 3 months, and persons experiencing symptoms consistent with COVID-19, as symptomatic persons received RT-PCR testing without initial antigen testing. For remaining asymptomatic students and staff, antigen testing was conducted using anterior nasal swabs that were self-collected each morning under the direct supervision of a nurse. Antigen test results were provided to athletics department medical staff who coordinated exclusion from team activities and confirmatory testing, but were not reported back to students and staff.

A negative antigen result meant an individual could engage in all sport-related activities, like indoor meetings, practices, scrimmages, and intercollegiate competitions. Athletes and staff with positive antigen results were immediately excluded from team activities by department medical staff and subject to confirmatory testing with RT-PCR using the TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific). Students and staff with positive RT-PCR results were excluded from team activities for 21 days and were interviewed by university staff to identify close contacts. Close contacts of RT-PCR-confirmed students or staff were required to self-quarantine for 14 days from the date of last contact per public health guidance [17]. Importantly, contact tracing for student athletes did not include contacts that occurred during practices, competitions, meetings, or other team activities, but could include contacts that occurred during social activities or at home (e.g., roommates). In addition to daily antigen testing, the athletic programs implemented a physical distancing policy requiring all students and staff to be at least six feet apart during meetings, and mandatory mask use during team activities.

Epidemiological investigation

Confirmed cases of COVID-19 were defined as students or staff affiliated with the two athletics programs who received a positive SARS-CoV-2 RT-PCR result during the outbreak period. False-negative antigen results were defined as a negative antigen test and a positive RT-PCR collected on the same day. During each outbreak, once the number of confirmed cases reached the threshold established by intercollegiate athletics conference protocols, in-person team activities were suspended, and all students and staff were tested by RT-PCR. Specimens that tested positive by RT-PCR confirmation were used for sequencing analysis.

University names, specific sports, and relevant dates have been removed from the report to protect the privacy of the students and staff involved. We used identifiers (Athletics-##) to denote individuals associated with these outbreaks. Dates are encoded as X-day-YY; 'X' indicates the outbreak investigated, and 'YY' indicates the day of that outbreak. The first notable event for each outbreak is "day 0" – in Outbreak 1, this was a negative antigen test for the index case (who later tested positive by RT-PCR), and in Outbreak 2, this was the date of the first competition between the two teams. This activity was reviewed by CDC and was conducted in a manner consistent with applicable federal law and CDC policy[1].

Laboratory Methods

We obtained a waiver of HIPAA Authorization and were approved to obtain the clinical samples along with a Limited Data Set by the Western Institutional Review Board (WIRB #1-1290953-1).

Sequences for this study were derived from 42 total nasopharyngeal (NP) swab samples collected from Outbreak 1 (n=32), Outbreak 2 team 1(n=5), and Outbreak 2 team 2 (n=5).

Outbreak 1 viral RNA isolation

Nasal swabs were collected and placed in 3mL phosphate buffered saline. RNA was extracted from 190 uL of sample using the MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit (Thermo Fisher Scientific, Waltham, MA) and eluted in a volume of 50 µL. 5µL of RNA was quantitated using a one-step RT-PCR using a TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific).

Outbreak 2 viral RNA isolation

Nasopharyngeal swabs were received in 3mL of transport medium (VTM). Viral RNA (vRNA) was

extracted from 100 µl of VTM using the Viral Total Nucleic Acid Purification kit (Promega, Madison, WI, USA) on a Maxwell RSC 48 instrument, using manufacturer guidelines, and was eluted in 50 µL of nuclease-free H_2O.

Library preparation and sequencing

Complementary DNA (cDNA) was synthesized using a modified ARTIC Network approach[15]. SARS-CoV-2-specific multiplex PCR for nanopore sequencing was performed, similar to amplicon-based approaches as previously described[15,16]. Amplified product was made compatible for deep sequencing using the one-pot native ligation protocol with Oxford Nanopore kit SQK-LSK109 and its Native Barcodes (EXP-NBD104 and EXP-NBD114)[15]. Up to 24 samples were pooled prior to being run on a flow cell (FLO-MIN106) using the 24hr run script.

Processing raw ONT data

Sequencing data was processed using the ARTIC bioinformatics pipeline (https://github.com/artic-network/artic-ncov2019) [17]. Consensus sequences were assembled for samples with greater than 400x coverage. Samples were excluded from analysis if gaps in the consensus sequence totaled ≥20% of the genome. The entire ONT analysis pipeline is available at https://github.com/gagekmoreno/SARS-CoV-2-in-Southern-Wisconsin.

Phylogenetic analysis

Phylogenetic analysis was completed using tools implemented in Nextstrain custom builds (https://github.com/nextstrain/ncov)[18,19]. We used custom python scripts to filter and clean metadata. Sequences names were coded as OB#-T#-A#. Where OB signifies the outbreak, T

represents the team that the sequence came from, and A is the athlete from which the sample that the sequence was derived originated.

Data availability

Source data after mapping to SARS-CoV-2 reference genome (Genbank: MN908947.3) have been deposited in the Sequence Read Archive (SRA) under bioproject PRJNA614504.

Results

Outbreak 1: Cases can be linked to a single viral introduction

An athlete (Athletics-1) received a negative antigen test result the morning of day-0, and attended an indoor meeting with approximately 10 other student-athletes and staff in which attendees reportedly sat six feet apart and wore masks at all times. The following morning (day-1), Athletics-1 received a positive antigen test result followed by RT-PCR swab for confirmation (Ct 15.9), and began experiencing symptoms by mid-afternoon of day-1. During day-3 through day-7, four attendees of the initial day-0 meeting developed symptoms and received subsequent positive RT-PCR results. Additionally, three roommates of Athletics-1 who did not attend the meeting developed symptoms on day-4 and received positive RT-PCR results on day-4 and day-5. In-person team activities were suspended on day-8 to prevent additional transmission.

Program-wide RT-PCR and antigen testing was conducted seven times throughout the outbreak period (day-7, 10, 13-17, and 20). Mass RT-PCR testing identified 21 new SARS-CoV-2 infections among students and staff. Of these, 18 (86%) were negative on contemporaneous rapid antigen tests. Among 11 positive antigen results obtained during mass testing, 4 (36%) were confirmed with

RT-PCR and 7 (64%) received negative RT-PCR results. For samples with available Cts, Outbreak 1 contained 13 individuals that were antigen-negative and RT-PCR positive, with Ct values ranging from 21.3 to 35.3 (avg. 29.5). Conversely, there were 9 individuals who were antigen-positive and RT-PCR positive, with Ct values ranging from 15.9 to 31.3 (avg. 24.9).

Overall, during Outbreak 1, 32 individuals (22 students and 10 staff) from the program had laboratory-confirmed SARS-CoV-2 infections (**Figure 2a**). Of confirmed cases, 4 (13%) were tested by RT-PCR because they were symptomatic, 7 (22%) were antigen-positive and received RT-PCR confirmation, and 21 (66%) were positive during mass RT-PCR testing. Contact tracing interviews found that 13 (40%) of 32 confirmed cases attended a team meeting where someone with confirmed COVID-19 was present and in their infectious period; 6 (13%) had close contact with a roommate with COVID-19; and 8 (25%) had no documented exposures (**Table 1)**.

We generated consensus sequences for 26 (81%) of 32 RT-PCR positive samples [15,16]. Samples from the remaining six RT-PCR test-positive individuals in Outbreak 1 were not available at the time of sequencing and were excluded from this analysis. We found that 24 (92%) of these 26 genomic sequences cluster tightly in the Nextstrain 20A clade on a time-resolved tree and are separated by 0-2 fixed consensus nucleotide differences (**Figure 3**). These sequences differ from the closest phylogenetic relative in the same state by 5-7 mutations. The limited diversity of viruses detected in the 24 individuals suggests sustained transmission of SARS-CoV-2 following a single introduction [20–22]. Viruses from Athletics-3 and Athletics-26 did not appear to be part of the primary transmission cluster. Athletics-26 consensus sequence was too divergent, differed by 8-10 mutations, and was filtered out of **Figure 3**. As of 1-day-40, there was no evidence for onward spread within the program originating from Athletics-3 or Athletics-26. The viruses infecting these individuals cluster more closely with sequences seen in the community.

Outbreak 2: SARS-CoV-2 transmission during intercollegiate competition

Two teams from different universities engaged in intercollegiate competitions on consecutive days (day-0 and day-1). Both teams underwent daily antigen testing and received all negative antigen results in the week preceding the competitions, including both competition days (day-0 and day-1). No testing was conducted on day-2. On day-3, an athlete from Team 2 received a positive antigen test result, which was confirmed by RT-PCR. No athletes or staff on either team were quarantined from contact with the index athlete that occurred during competition on day-0 and day-1. During day-5 through day-10, multiple athletes on both teams developed symptoms and received positive antigen and RT-PCR results. On day-6, all athletes on Team 1 were tested by only RT-PCR, with 2 individuals testing positive, and in-person team activities were suspended. Overall, 12 athletes (seven from Team 1 and five from Team 2) had confirmed SARS-CoV-2 infections during this outbreak (**Figure 4**).

To determine whether the source of these infections could be linked to competition despite negative antigen results on the day of competition, we generated eight consensus sequences from ten available samples. All eight virus sequences (four from each team) clustered tightly in the 20G clade on a time-resolved tree and were separated by 0-2 fixed consensus nucleotide differences (**Figure 5**). Given the known epidemiological associations between these teams, this likely represented a single transmission cluster [20–22].

The sequences of the viruses infecting the individuals in Outbreak 2 were distinct from those of viruses circulating within the community where Outbreak 2 occurred. Sequences in Outbreak 2 contained a unique mutation, encoding Spike P26Y, which was not otherwise seen in the county where Team 1 was located. Given the depth of surveillance community sequencing in Team 1's

county available during the outbreak period (~4.7% of test-positive cases), it is unlikely that this unique signature arose independently in the county where Team 1 is located.

Discussion

The SARS-CoV-2 testing strategy of daily, directly observed, rapid antigen testing implemented by intercollegiate athletics programs nationwide has been resource-intensive, yet its impact on SARS-CoV-2 transmission in this setting has not been evaluated. In this report, we described two outbreaks within intercollegiate athletics programs in which daily antigen testing was unable to interrupt SARS-CoV-2 transmission. Sustained transmission within the program followed when additional exposures from presymptomatic and undetected SARS-CoV-2 infections occurred – at least 13 of the 32 outbreak-associated cases attended team meetings with individuals who had received negative antigen results yet were in their infectious period.

Transmission within the program was not interrupted until the program implemented serial RT-PCR testing, a strategy that led to identification of 21 new confirmed SARS-CoV-2 infections, 18 of which were negative on contemporaneous antigen tests. Our findings suggest that serial antigen testing as a control strategy may have limited sensitivity for detecting early asymptomatic infections.

Contact tracing during Outbreak 1 identified interactions among individuals that may have contributed to at least 21 (66%) of the 32 confirmed cases (**Figure 2b**). In particular, the team continued to have physically distanced (6 feet apart) in-person meetings with cloth masks until all in-person team activities were suspended to prevent further spread. Per public health and university guidelines, attendees in these meetings were not quarantined, a step that might have prevented onward transmission during this outbreak. Roommates and household contacts of student-athletes

could represent additional sources of infection in Outbreak 1, as they were not required to quarantine due to the large size of the house and the university's assessment that physical distancing was achievable in this area. Continuing indoor in-person meetings and not quarantining potential contacts represent possible breaches in university's SARS-CoV-2 mitigation plan that, combined with the limitation of antigen testing, permitted viral spread throughout the team in Outbreak 1.

In Outbreak 2, we used genomic sequencing to demonstrate that SARS-CoV-2 transmission likely occurred between two teams during athletic competition despite both teams receiving negative antigen results immediately prior to competition. Supporting evidence for inter-collegiate transmission included detection of a unique mutation, encoding Spike P26Y, that was common to the samples from both teams and not otherwise seen in the county where Team 1 is located. Given these findings, the most parsimonious explanation is that an infection acquired in the community by the index athlete on Team 2 was transmitted to other individuals on both teams during the competition. However, it is also possible that athletes on Team 1 acquired SARS-CoV-2 infection in the community of Team 2, while visiting for competition. This can be seen with Athletics-31, as their viral consensus sequence is closely related to the cluster, but differs by 5 consensus mutations.

The potential for inter-collegiate transmission during an athletic competition has important implications for SARS-CoV-2 serial testing strategies and in-competition mitigation protocols. First, antigen testing on the competition dates failed to identify the index case, who may have been infectious and exposed other athletes. Like in Outbreak 1, more sensitive molecular tests could have identified the source case and allowed for exclusion from the competition. Second, this investigation showed that athletic competition may pose a risk for SARS-CoV-2 transmission, particularly in sports in which direct physical contact occurs. This outbreak occurred during an athletic competition that

included contact and collision, and is considered “high-risk” by NCAA. Despite the short duration of contact between athletes, transmission risk can be exacerbated by heavy breathing and shouting without masking, which regularly occurs in this setting and has been associated with SARS-CoV-2 outbreaks in other athletics competitions [23].

The findings in this report are subject to several limitations. First, we were not able to perform genomic sequencing on all positive samples from these outbreaks (34 of 44 samples were sequenced) either because of high Ct values on RT-PCR or lack of sample availability. Second, contemporaneous antigen and RT-PCR samples in Outbreak 1 were not collected as “paired” swabs (simultaneous swabbing of two nares) and may not be comparable to other antigen test evaluations. Similarly, the performance of antigen tests in this context of daily serial testing measured their ability to identify early presymptomatic infections, and may not be generalizable to antigen test performance in other settings. Third, there was a lack of testing prior to re-admittance to the team. While rare, individuals might still have been infectious after their 21 day quarantine. Similarly, the assumption that individuals who tested positive would remain uninfected for three months and were therefore exempt from testing may have been flawed because reinfection, although uncommon, does occur. Fourth, our ability to determine the source of infections in these outbreaks was limited by incomplete contact tracing data. Undocumented exposures between athletes and staff may have occurred outside of organized team activities that could have caused infections; although the strength of genomic clustering and epidemiologic evidence from these investigations suggests that such occurrences were rare.

Among athletics programs and other congregate settings where outbreaks may spread rapidly after introduction of SARS-CoV-2, serial antigen testing alone may not be sufficient to prevent outbreaks. However, serial antigen testing can help to rapidly identify outbreaks as they happen, but might fail

to detect every positive individual. Instead, they work to detect outbreaks and can inform policy decisions to slow the spread of SARS-CoV-2. Any robust testing strategy should be supplemented with multilayered prevention strategies that includes correct and consistent mask use, physical distancing, increased hand hygiene and disinfection, avoiding crowds and poorly ventilated spaces, and isolation of symptomatic individuals regardless of antigen test result[13,24–26]. Serial testing with RT-PCR may identify additional cases that were not detected by antigen testing, but the increased sensitivity would have to be balanced with laboratory resources and increased turnaround times.

NOTES

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Disclosures

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. Use of trade names is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention.

Footnotes

[1] See e.g., 45 C.F.R. part 46.102(l)(2), 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C.

§3501 et seq.

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Figure Captions

Figure 1. Graphical abstract of cryptic transmission that could occur when a person is asymptomatic and the amount of virus remains below the limit of detection for antigen tests despite the person being potentially infectious to others. This is a schematic and is meant to represent general, not quantitative, relationships among these variables. LOD = limit of detection.

Figure 2. Overview of Outbreak 1 A) Epidemic curve for confirmed COVID-19 cases (n = 32) among students and staff associated with the athletics program during Outbreak 1. Abbreviations: Ag = antigen; COVID-19 = coronavirus disease 2019; RT-PCR = reverse transcription–polymerase chain reaction. B) Graphical representation of known interactions between all persons in the athletics program affected by Outbreak 1. Roommates are shown by a solid black line; confirmed close contact with a positive case as identified through contact tracing interviews is shown with a red line; r persons who attended indoor team meetings together while following physical distancing policies (> 6 feet apart and wearing masks) are shown with a dashed gray line; persons who received false negative antigen results are shown with a red circle.

Figure 3. Phylogeny of Outbreak 1. A) Time-resolved phylogenetic tree created using Nextstrain tools and nomenclature showing the team sequences contextualized with all available community sequences (gray) for 25 (78%) of 32 confirmed cases associated with Outbreak 1; tips affiliated with Outbreak 1 are colored red. B) Zoomed in time-resolved phylogeny showing all of these samples are part of the same athletics cluster. C)

Divergence tree showing the number of mutations each sequence has relative to Wuhan/WH01/2019 (Genbank: MN908947.3), a standard reference comparison sequence.

Figure 4. Overview of Outbreak 2. A) Epidemic curve for Outbreak 2 showing confirmed (n = 12) COVID-19 cases within the two intercollegiate teams. Testing was not conducted on day-2.

Figure 5. Phylogeny of Outbreak 2. A) Time-resolved phylogenetic tree created using Nextstrain tools and nomenclature showing 8 (67%) of 12 available samples from Outbreak 2 sequences contextualized with all available community sequences (light gray). Tips affiliated with Team 1 are colored red, and Team 2's sequences are colored dark gray. B) Zoomed in time-resolved phylogeny showing all these samples are part of the same athletics cluster. C) Divergence tree showing the number of mutations each sequence has relative to Wuhan/WH01/2019 (Genbank: MN908947.3), a standard reference comparison sequence.

Tables

Table 1. Characteristics and exposure details for confirmed COVID-19 cases (n=32) during Outbreak 1.

Characteristics	**No. (%)**
Confirmed cases of COVID-19 associated with Outbreak 1	**N=32**
Program affiliation	
Student-athlete	22 (69%)
Staff	10 (31%)
Symptomatic	27 (84%)
Possible contact with a confirmed COVID-19 case during exposure period	
Housemate	6 (19%)
Team meeting	13 (40%)
Social gathering (outside of program)	3 (9%)
Other	2 (6%)
No known exposure	8 (25%)
Source of positive RT-PCR result	
Symptom-based testing (RT-PCR only)	4 (13%)
Antigen-based screening with confirmatory RT-PCR	7 (22%)
Mass combined testing (paired RT-PCR and antigen testing)	21 (66%)
Antigen-positive	3 (14%)
Antigen-negative	18 (86%)

Total tests performed after pause of activities	
Antigen	1921
RT-PCR	1012