Serologic testing of U.S. blood donations to identify SARS-CoV-2-reactive antibodies: December 2019-January 2020

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Article Summary: Retrospective SARS-CoV-2 serological testing of routine blood donations collected in nine U.S. states from December 13, 2019- January 17, 2020 suggests that the virus was present in the United States earlier than previously recognized.

Abstract:

Background: SARS-CoV-2, the virus that causes COVID-19 disease, was first identified in Wuhan, China in December 2019, with subsequent worldwide spread. The first U.S. cases were identified in January 2020.

Methods: To determine if SARS-CoV-2 reactive antibodies were present in sera prior to the first identified case in the U.S. on January 19, 2020, residual archived samples from 7,389 routine blood donations collected by the American Red Cross from December 13, 2019 to January 17, 2020, from donors resident in nine states (California, Connecticut, Iowa, Massachusetts, Michigan, Oregon, Rhode Island, Washington, and Wisconsin) were tested at CDC for anti-SARS-CoV-2 antibodies. Specimens reactive by pan-immunoglobulin (pan Ig) enzyme linked immunosorbent assay (ELISA) against the full spike protein were tested by IgG and IgM ELISAs, microneutralization test, Ortho total Ig S1 ELISA, and receptor binding domain / Ace2 blocking activity assay.

Results: Of the 7,389 samples, 106 were reactive by pan Ig. Of these 106 specimens, 90 were available for further testing. Eighty four of 90 had neutralizing activity, 1 had S1 binding activity, and 1 had receptor binding domain / Ace2 blocking activity >50%, suggesting the presence of anti-SARS-CoV-2-reactive antibodies. Donations with reactivity occurred in all nine states.

Conclusions: These findings suggest that SARS-CoV-2 may have been introduced into the United States prior to January 19, 2020.

Introduction:

SARS-CoV-2, the virus that causes novel coronavirus disease (COVID-19), was first identified in Wuhan, China, with notification to the World Health Organization on December 31, 2019, about a cluster of pneumonia cases of unknown etiology and release of the genomic sequence on January 10, 2020 [1]. Subsequent reports have identified a patient with confirmed SARS-CoV-2 hospitalized in Wuhan with symptom onset as early as December 1, 2019 [2]. In the United States, the first COVID-19 infection was reported on January 19, 2020 in a returned traveler from China, two days after domestic testing was initiated [3]. While the first confirmed case had a symptom onset date of January 19, 2020, two others within the first 12 U.S. cases identified had illness onset dates of January 14, 2020 [4]. Some reports have suggested the introduction of SARS-CoV-2 into the U.S. may have occurred earlier than initially recognized, though widespread community transmission was not likely until late February [5-7].

Simulation models used to predict COVID-19 case burden, subsequent healthcare utilization, and fatalities are reliant on accurately assessing date(s) of introduction of a pathogen into susceptible populations [8]. A number of strategies have been used to estimate the introduction of SARS-CoV-2, including retrospective molecular testing of clinical respiratory samples, nucleic acid testing (NAT), and, in some circumstances, phylogenetic analyses [6, 9-12]. Early phylogenetic analyses suggest that SARS-CoV-2 may have evolved between October–December 2019 [9-11]. While the first recorded COVID-19 case outside of China was identified in Thailand on January 13, 2020 [13], retrospective NAT identified a respiratory specimen with molecular evidence of SARS-CoV-2 from a patient hospitalized in France on December 27, 2019 [12]. Similarly, in the U.S., retrospective NAT of archived respiratory samples in the Seattle region have suggested introduction of SARS-CoV-2 virus into the Seattle, Washington, area between January 18- February 9, 2020 [6].

Serologic testing has been previously used to estimate introduction of viral infections into populations, including for HIV [14]. Retrospective serologic testing may augment results obtained from testing archived respiratory specimens with molecular methods when trying to identify the introduction of SARS-CoV-2 into a population. For several reasons, infections may not be fully captured by surveillance conducted using respiratory specimens collected from symptomatic people in healthcare settings. Patients infected with SARS-CoV-2 may not seek medical care because infections could be mild or asymptomatic [15]. For those with symptomatic infections who may have sought medical care before SARS-CoV-2 was known to be circulating in the U.S., clinical samples may not have been collected and therefore respiratory virus testing may not have been performed; even fewer specimens would likely be archived and available for retrospective molecular testing. To determine whether serologic testing can provide further insight into SARS-CoV-2 introduction into the U.S., U.S. blood donation specimens from an existing repository collected by the American Red Cross from December 13, 2019 to January 17, 2020 were sent to CDC for retrospective testing for SARS-CoV-2 reactive antibodies. Implications for future SARS-CoV-2 seroprevalence surveys are discussed.

Methods:

Ethical considerations:

The study was approved by the American Red Cross Institutional Review Board. Data for this report were collected as part of public health emergency response and determined by the Centers for Disease Control and Prevention (CDC) office of the Associate Director for Science to not require additional CDC Institutional Review Board review. All blood donations were de-identified prior to shipment to CDC and subsequent testing.

Blood donor sample description:

Whole blood or blood products intended for transfusion are collected from volunteer donors in either fixed collection sites or as part of mobile collection drives. All blood donors are subjected to medical and social history questionnaires to ascertain risk factors associated with transfusion-transmissible infectious diseases, such as HIV [16]. Donors are questioned regarding travel outside of the U.S. and are deferred for travel to malaria affected areas [16]. SARS-CoV-2 risk-based donor deferral for travel to China was not implemented until February 2020 [17]. As part of the donation evaluation, donors undergo a basic physical exam which includes temperature, blood pressure, and heart rate measurements. Persons presenting to donate blood with signs or symptoms consistent with bacterial or viral respiratory infections, including influenza, are deferred and instructed to return for donation once symptoms have resolved. Serum specimens from all blood donations are tested for infectious disease markers as required by the Food and Drug Administration (FDA) [18].

Archived, residual serum specimens from routine donations collected by the American Red Cross from December 13, 2019, to January 17, 2020, from donors resident in California, Connecticut, Iowa, Massachusetts, Michigan, Oregon, Rhode Island, Washington, and Wisconsin were sent to CDC (Atlanta, GA) for additional testing (n=7,389). All donations collected during this period, for which residual serum specimens were available, were included in this study. These specimens were previously archived for potential future studies to identify emerging transfusion-transmissible infections but were re-purposed for the present study.

Laboratory Methods:

Once at CDC, sera were screened using a pan-Ig enzyme linked immunosorbent assay (ELISA) against the pre-fusion stabilized ectodomain of the spike protein (S) that includes both S1 and S2 domains [19, 20]. To ensure high-throughput screening capability, initial screening did not include background correction. Initial reactive specimens, defined as having an optical density (OD) of 0.5 or greater in the screening ELISA (tested at a 1:100 dilution), were then confirmed by reflex testing at 1:100 and 1:400 dilutions using the same ELISA with background correction. Specimens were considered confirmed reactive if there was a signal to threshold ratio of 1 or greater at a background corrected OD of 0.4. At a background corrected OD of 0.4 with serum diluted 1:100, specificity of this assay is 99.3% (confidence interval 98.32 – 99.88%) and sensitivity is 96% (confidence interval 89.98 – 98.89%) [20]. When paired sera from PCR-confirmed infections with other common coronaviruses were tested, 4 of 42 exhibited increasing signal between the acute and convalescent timepoints, but all were below the assay cutoff (18). Isotype-specific tests were performed using the same ELISA technique, but with IgG or IgM-specific secondary antibody (Kirkegaard and Perry Laboratories, Gaithersburg, MD).

Confirmed reactive specimens were further tested using a microneutralization test with live SARS-CoV-2 USA-WA1/2020 [21], SARS-SoV-1 S1 pan Ig ELISA (Ortho Clinical Diagnostics, Raritan NJ), and a surrogate neutralization assay that measures the ability of sera to block the interaction between the S receptor binding domain (RBD) and the cellular receptor, ACE2 (Genscript). For microneutralization, sera were serially diluted two-fold between 1:20 and 1:640, incubated with virus for 30 minutes at 37°, and used to inoculate Vero CCL-81 cells. After 5 days, cells were fixed and stained with formalin – crystal violet to observe live / dead cells. The highest dilution at which sera blocked viral infection was determined to be the neutralizing titer, with >40 designated as positive. For the

Ortho ELISA and surrogate neutralization assays, the manufacturers' instructions were followed.

Statistical analyses:

Descriptive analyses were performed to stratify reactive donations by state of residence, date of collection, and donor age and sex. Analysis was performed using SAS version 9.4 (SAS Institute Inc., Cary, NC). As these donations represent a convenience sample, additional tests to ascertain statistical significance or extrapolate findings to a broader population were not performed.

Results:

Serum specimens were sent to CDC for anti-SARS-CoV-2 testing from 7,389 unique donations (Table). Of these, 106 (1.4%) were confirmed reactive by the pan-Ig S ELISA screen and then a confirmatory assay with background correction (Table). These confirmed reactive sera included 39/1,912 (2.0%) donations collected between December 13-16, 2019, from residents of California (23/1,912) and Oregon or Washington (16/1,912). Sixty seven confirmed reactive (67/5,477, 1.2%) donations were collected between December 30, 2019, and January 17, 2020, from residents of Massachusetts (18/5,477), Wisconsin or Iowa (22/5,477), Michigan (5/5,477), and Connecticut or Rhode Island (33/5,477). During validation of the assay, 3 of 519 true negative sera had reactivities above the signal: threshold cutoff of 1 ranging from 1.46 – 2.11. These true negative sera were collected from healthy adults between 2016 and 2019 (n = 377), suspected hanta virus patients between 2016 and 2019 (n = 101), HIV positive individuals between 2011 and 2012 (n = 10), hepatitis B

positive individuals between 2011 and 2012 (n = 10), or hepatitis C positive individuals between 2011 and 2012 (n = 10). True positive sera from 99 PCR-confirmed COVID-19 patients collected >10 days post symptom onset ranged from 0.11 - 6.99, with a median of 6.10 and a standard deviation of 1.91 (19). Of the 106 confirmed reactive sera, 67 had threshold cutoffs between 1.0 and 2.11, which is within the same range of true negative sera that were above the assay cutoff. In contrast, 32 and 4 had threshold cutoffs of 2.12-4.08 and > 4.30, respectively, well above the true negative sera that tested above cutoff limits.

Of the 106 confirmed-reactive specimens, 90 were available for further testing. These sera were tested using isotype-specific spike protein ELISAs, Ortho pan-Ig S1 assay, microneutralization tests, and a surrogate neutralization assay that detects the ability of sera to block receptor binding domain binding to ACE-2. Of the 90 sera tested by microneutralization, 84 had an endpoint titer >40. When the anti-spike protein isotype responses were examined, 39 of the 90 had both S-reactive IgG and IgM (43.3%), 8 were IgM positive, but IgG negative, 29 were IgG positive but IgM negative, and the remaining14 were just positive using a pan-Ig secondary. By the Ortho S1 pan Ig assay, one reactive serum had a signal to threshold cutoff of 1.89 (with a retest of 1.10), and by surrogate neutralization, 21 sera exhibited 20-30% inhibition, 1 at 45% inhibition, and 1 at 71% inhibition. When results in all tests were compared by individual specimen, there was not an obvious pattern of specimens with higher signals in ELISA, surrogate neutralization or live virus microneutralization tests clustering together (Figure 1), indicating that each donation had a unique pattern of test results. These data might indicate that there is no clear delineation between potentially cross reactive specimens, and those that were obviously from SARS-CoV-2 infected individuals.

The mean age of repeat reactive donors was 52 years (range 16-95 years). More donations occurred among males than female donations (55.1% male specimens from

December 13-16, 2019; 53.6% male specimens from December 30, 2019 and January 17, 2020). The proportion of reactive donations was higher among males than females among donations from December 13-16, 2019 (2.6% among males, 1.4% among females) and from December 30, 2019 -January 17, 2020 (1.1% among males, 0.7% among females). Among donations collected in California, Washington and Oregon, the proportion of reactive donations was higher among donors aged ≥ 40 years.

Discussion

These findings indicate that SARS-CoV-2 reactive antibodies were detected in 106 specimens, a small percentage of blood donations from California, Oregon, and Washington as early as December 13–16, 2019. The presence of these serum antibodies indicate that isolated SARS-CoV-2 infections may have occurred in the western portion of the United States earlier than previously recognized or that a small portion of the population may have pre-existing antibodies that bind SARS-CoV-2 S [3]. Similarly, antibodies to SARS-CoV-2 were identified among donations occurring in early January in Connecticut, Iowa, Massachusetts, Michigan, Rhode Island, and Wisconsin prior to known introduction of SARS-CoV-2 into those states.

A key question raised by these findings is whether the detection of reactive antibodies in these specimens from December and January indicate infections with SARS-CoV-2 in the U.S. population earlier than currently recognized. As the COVID-19 epidemic has evolved, several serological assays for detection of SARS-CoV-2 have become available to determine whether persons may have had previous infection. One recent report described cross-reactive serum antibody responses between SARS-CoV-2 and a small proportion of common human coronaviruses, particularly OC43 when using ELISA[22] Neutralizing activity in sera from

individuals with prior common human coronavirus infections has been described against SARS-CoV-2, specifically targeting the S2 portion of the S protein [23, 24]. The S2 subunit of the spike protein is more conserved across coronaviruses and thus may play a role in the cross-reactivity observed during ELISA testing when the whole S protein is used as an antigen [23]. The S2 region is involved in membrane fusion, and cross neutralizing monoclonal antibodies from SARS-CoV-1 have been identified that bind S2 [25].

In order to better characterize the specimens that were reactive on the pan-Ig ELISA containing whole SARS-CoV-2 spike protein as the capture antigen, and distinguish these from cross reactivity to common coronaviruses, additional, more specific SARS-CoV-2 testing was performed. The S1 subunit has been reported to be a more specific antigen for SARS-CoV-2 serologic diagnosis than the whole S protein [23]. Furthermore, in recent studies, sera from patients with confirmed human coronavirus infection only contained SARS-CoV-2 S-specific IgG antibodies and did not contain IgM or IgA antibodies; neutralizing activity in these sera was found to target only the S2 portion of the spike protein [23, 24]. Therefore, the presence of IgM or IgA antibodies and S1-specific binding activity may distinguish antibodies to SARS-CoV-2 from antibodies to human common coronaviruses [23, 24]. In the present study, 84 of 90 (>93%) reactive sera had neutralizing activity against SARS-CoV-2 virus, 39 (44.3%) had both IgG and IgM SARS-CoV-2 S-specific antibodies, 2 (2.2%) sera had surrogate neutralization activities, and 1/90 (1.1%) had SARS-CoV-2 S1-specific Ig. Collectively, these data suggest that at least some of the reactive blood donor sera could be due to prior SARS-CoV-2 infection. One serum, collected on January 10, 2020 in Connecticut, demonstrated a neutralization titer of 320, 6.75 signal to threshold ratio, and 70% inhibition activity by surrogate neutralization activity, but was Ortho S1 non-reactive. These data indicate that this donation was likely from an individual with a past or active SARS-CoV-2 infection.

In addition to potential cross reactivity with human common coronavirus infection other than SARS-CoV-2, the findings in this report are subject to the following limitations. First, none of the sera can be considered "true positives." A true positive would only be collected from an individual with a positive molecular diagnostic test, or paired acute – convalescent sera with rising titers [26, 27]. Second, the donations included in this report may not be representative of all blood donors or donations in these states and the findings may not be generalizable to all blood donors during the donation dates reported here. Therefore, population-based seroprevalence estimates or inference on magnitude of infections on a national or state level, cannot be made. Third, if some of these samples indicate antibody responses from undetected SARS-CoV-2 infections, it cannot be determined whether these infections were community- or travel-associated. A previous survey of blood donors to understand travel practices determined that less than 3% of respondents reported travel outside of the U.S. within the 28 days prior to donation [28]. Of those reporting travel, only 5% traveled to Asia [28]. Fourth, even with a highly specific test, false positives may occur, particularly in low prevalence areas [29]. However, the number of reactive specimens identified in this study was higher than expected given the specificity of the pan Ig spike ELISA. Furthermore, additional evidence including microneutralization, detection of both SARS-CoV-2 specific IgG and IgM, and SARS-CoV-2 S1-specific Ig reactivity, make it very unlikely that all reactive specimens represent false positives. Further studies involving retrospective analyses of human specimens with molecular or serologic methods are necessary to further corroborate the present findings, which suggest the presence of specific antibodies to SARS-CoV-2 in the U.S. as early as mid-December 2019.

The findings of this report suggest that SARS-CoV-2 infections may have been present in the U.S. in December 2019, earlier than previously recognized. These findings also highlight the value of blood donations as a source for conducting SARS-CoV-2 surveillance

studies. Data from U.S. blood donation screening have been previously used for population-based incidence and prevalence monitoring during infectious disease outbreaks, most recently the Zika virus epidemic [30]. CDC is continuing to work with federal and non-governmental partners to conduct ongoing surveillance using blood donations and clinical laboratory samples for SARS-CoV-2 infection in multiple sites across the U.S. Understanding the dynamics of SARS-CoV-2 pandemic from early introduction throughout further progression will advance understanding of the epidemiology of this novel virus and inform allocation of resources and public health prevention interventions to mitigate morbidity and mortality associated with COVID-19.

NOTES

Disclaimer:

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Names of specific vendors, manufacturers, or products are included for public health and informational purposes; inclusion does not imply endorsement of the vendors, manufacturers, or products by the Centers for Disease Control and Prevention or the US Department of Health and Human Services.

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Table. Total number of samples tested, number of samples that were reactive, number of samples with positive micro-neutralization and surrogate neutralization, and number of samples that were reactive for the S1 Ortho test. Summarized separately specimens collected between December 13-16th, 2019 those collected December 30th, 2019 – January 17th, 2020.

	N tested	N reactive (% of tested)	N reactive with further testing (% of tested)	N reactive with positive micro-neutralization (% of tested)	N with surrogate neutralization (% of reactive with positive micro-neutralization)	N S1 reactive (Ortho)
All specimens	7389	106 (1.4)	90 (1.2)	84 (1.1)	23 (27.4)	1
All specimens from December 13-16, 2019	1912	39 (2.0)	39 (2.0)	37 (1.9)	9 (24.3)	1
American Red Cross Blood Services region						
Northern California (CA)	508	12 (2.4)	12 (2.4)	11 (2.2)	7 (63.6)	1
Pacific Northwest (OR, WA)	763	16 (2.1)	16 (2.1)	15 (2.0)	1 (6.7)	0
Southern California (CA)	641	11 (1.7)	11 (1.7)	11 (1.7)	1 (9.1)	0
Donor sex						
Female	859	12 (1.4)	12 (1.4)	11 (1.3)	1 (9.1)	0
Male	1053	27 (2.6)	27 (2.6)	26 (2.5)	8 (30.8)	1
Donor age group						

16-29 years	254	3 (1.2)	3 (1.2)	2 (0.8)	2 (100.0)	1
30-39 years	298	3 (1.0)	3 (1.0)	3 (1.0)	1 (33.3)	0
40-49 years	291	6 (2.1)	6 (2.1)	6 (2.1)	1 (16.7)	0
50-59 years	397	9 (2.3)	9 (2.3)	8 (2.0)	2 (25.0)	0
60-69 years	483	14 (2.9)	14 (2.9)	14 (2.9)	3 (21.4)	0
70 years or older	189	4 (2.1)	4 (2.1)	4 (2.1)	0 (0.0)	0
All specimens from December 30, 2019-January 17, 2020	5477	67 (1.2)	51 (0.9)	47 (0.9)	14 (29.8)	0
American Red Cross Blood Services region						
New England (MA)	1963	18 (0.9)	11 (0.6)	11 (0.6)	1 (9.1)	0
Badger-Hawkeye (WI, IA)	1556	22 (1.4)	17 (1.1)	16 (1.0)	6 (37.5)	0
Great Lakes (MI)	416	5 (1.2)	5 (1.2)	3 (0.7)	0 (0.0)	0
Connecticut (CT, RI)	1542	22 (1.4)	18 (1.2)	17 (1.1)	7 (41.2)	0
Donor sex						
Female	2541	23 (0.9	19 (0.7)	16 (0.6)	6 (37.5)	0

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Male	2936	44 (1.5)	32 (1.1)	31 (1.1)	8 (25.8)	0
Donor age group						
16-29 years	641	7 (1.1)	4 (0.6)	3 (0.5)	2 (66.7)	0
30-39 years	587	9 (1.5)	8 (1.4)	8 (1.4)	3 (37.5)	0
40-49 years	779	11 (1.4)	9 (1.2)	9 (1.2)	1 (11.1)	0
50-59 years	1447	15 (1.0)	11 (0.8)	9 (0.6)	3 (33.3)	0
60-69 years	1410	16 (1.1)	12 (0.9)	11 (0.8)	3 (27.3)	0
70 years or older	613	9 (1.5)	7 (1.1)	7 (1.1)	2 (28.6)	0

Figure legends

Figure 1. Combined results of confirmatory tests from 90 spike-reactive routine blood donations donations collected in nine U.S. states between December 13, 2019-January 17, 2020. Each line indicates a single serum that was already confirmed to bind SARS-CoV-2 spike by ELISA. A) Signal to threshold ratios of anti-spike ELISA assay using a pan-Ig secondary antibody are shown on the X axis. A signal : threshold >1.0 is positive, and greater values indicate more reactivity. B) The Y axis shows surrogate neutralization data. Ace-2 and spike receptor binding domain binding were assay in the presence and absence of sera. The percent inhibition was calculated by comparing the interaction with and without sera. C) Endpoint microneutralization titers are shown on the Z axis. The number indicates the dilution at which serum blocked live-virus induced CPE in all three replicative wells. Higher numbers indicate more neutralizing activity. The shape and color of each line indicate isotype-specific spike ELISA results and results using Ortho Vitros total Ig S1 assay. The ELISAs were performed the same as the pan Ig assay, but isotype specific secondary antibodies were used. Grey circles indicate that the serum was negative by Ortho Vitros total Ig, positive for either IgG or IgM, but not both. Blue triangles indicate negative by Ortho Vitros total Ig, and positive for both IgG and IgM spike ELISA. Red cloverleaf indicates positive for all three.

Figure 1