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A pan-coronavirus RT-PCR assay for rapid viral screening of animal, human, and environmental specimens

Xinye Wang [a,j], Leshan Xiu [b,c,d], Raquel A. Binder [b,c], Teck-Hock Toh [e,f], Jeffrey Soon-Yit Lee [e,f], Jakie Ting [e], Son T. Than [g], Wenhao Qi [a], Kristen K. Coleman [g], David Perera [h], Maijuan Ma [i], Gregory C. Gray [a,b,c,g,*]

[a] Global Health Research Center, Duke Kunshan University, Kunshan, China
[b] Division of Infectious Diseases, Duke University School of Medicine, Durham, NC, USA
[c] Duke Global Health Institute, Duke University, Durham, NC, USA
[d] NHC Key Laboratory of Systems Biology of Pathogens, Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China
[e] Faculty of Medicine, SEGi University, Kota Damansara, Selangor, Malaysia
[f] Clinical Research Center, Sibu Hospital, Ministry of Health Malaysia, Sibu, Sarawak, Malaysia
[g] Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore
[h] Institute of Health and Community Medicine, Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, Malaysia
[i] State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing 100,071, China
[j] School of Medical Sciences, Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia

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ABSTRACT

We examined a collection of 386 animal, 451 human, and 109 archived bioaerosol samples with a new pan-species coronavirus molecular assay. Thirty-eight (4.02%) of 946 specimens yielded evidence of human or animal coronaviruses. Our findings demonstrate the utility of employing the pan-CoV RT-PCR assay in detecting varied coronavirus among human, animal, and environmental specimens. This RT-PCR assay might be employed as a screening diagnostic for early detection of coronaviruses incursions or prepandemic coronavirus emergence in animal or human populations.

1. Introduction

As we endure the mortality, morbidity, and societal disruptions of the COVID-19 pandemic, public health and research administrators are beginning to think about how we might detect and possibly mitigate future pandemics. Experts are in disagreement regarding the best approach, with some proposing complex and comprehensive efforts focused upon the detection and characterization of an estimated 1.67 million of the world's viruses [1,2], and intense immunological study and vaccine construct development targeting large numbers of potential human viral threats [3,4], and others proposing aggressive epidemic and human clinical viral surveillance [5,6]. The comprehensive viral and immunological approaches are expensive in both cost and effort, [5,6] and given recent US history of how pandemic preparedness funding waxes and wanes, it may be difficult to sustain for the long term. In this paper, we examine how one might reduce such costs and effort by employing a One Health approach in searching for new pandemic threats in geographical areas thought to be at risk of novel virus emergence. We demonstrate this by searching for coronaviruses using a low-cost viral diagnostic [7] in a large panel of archived samples collected from people, animals, and animal environments.

2. Materials and methods

We sought to detect possible cryptic coronaviruses (α-, β-, γ-, and δ-coronaviruses) from a collection of archived animal, human, and environmental samples using a previously reported conventional RT-PCR assay [7]. The archived samples were collected by our multinational teams in China [8], Malaysia [9,10], and Vietnam [11] during the years 2015 to 2019.

* Corresponding author at: Duke University School of Medicine, DUMC Box 102,359, Durham, NC 27710.
E-mail address: gregory.gray@duke.edu (G.C. Gray).

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2.1. Biorepository

A total of 386 animal samples were screened with the pan-coronavirus (pan-CoV) assay in this work, including 58 pig oral secretion and fecal specimens (collected from pig farms in Sibu, Malaysia) [9], 200 pig oral secretion specimens (collected from pig farms in Shandong, China) [8], as well as 128 pig nasal swab, respiratory tract swab, and lung swab specimens (collected from pig farms in Jiangsu, China). A total of 451 human samples were also tested with the pan-CoV assay, including 45 animal workers' nasal wash specimens (collected from pig farms in Sibu, Malaysia) [9], 350 pneumonia patients' nasopharyngeal (NP) samples (collected from hospitals in Hanoi, Vietnam) [11] and 56 patients' nasopharyngeal (NP) samples (collected from hospitals in Sibu and Kapit, Malaysia) [10]. Additionally, 109 bioaerosol samples collected using National Institute for Occupational Safety and Health (NIOSH) 2-stage bioaerosol cyclone samplers in a live poultry market in Kunshan (China) were tested with the pan-CoV assay [12]. Detailed information about all specimens is provided in supplemental document.

2.2. Conventional RT-PCR

The conventional RT-PCR assays were run at corresponding collaborator site (China, Singapore, and Malaysia) with slight variations in methods (details in Supplementary Data). This RT-PCR assays that all teams used targeted the conserved RNA-dependent RNA polymerase (RdRp) genome region common to all members of the *Orthocoronavirinae*. This assay has previously been shown to be effective in detecting different strains of coronaviruses [7]. All sites yielded the expected amplicon sizes for the first and second amplification step, which were 670–673 bp and 559–602 bp respectively. To avoid possible contamination, negative controls [7] were used in each step. Positive controls, subsequently identified at the correct molecular size, were also included in each step.

2.3. Sequencing and sequence alignment

Sanger sequencing of the amplicons from the RT-PCR assays was performed by Genewiz Inc. (Genewiz, Suzhou, China), Bio Basic Asia Pacific Pte. Ltd. (Singapore), or Institute of Health and Community Medicine at the Universiti Malaysia Sarawak (Malaysia). The obtained sequences were assembled and edited using Geneious R11 Software (Biomatters Ltd., Auckland, New Zealand), and then compared to the NCBI sequence database using online BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). GenBank sequence information is recorded in Table S1 (details in Supplementary Data).

3. Results

Among the 946 archived field samples evaluated with the conventional pan-CoV RT-PCR assay, thirty-eight (4.02%) were positive for coronaviruses (See Table 1 and Table S1).

Among the animal samples, sixteen (8.00%) out of the 200 swine oral secretion samples (China) were positive for coronaviruses. Among these sixteen specimens, eight (50.00%) were identified as porcine hemagglutinating encephalomyelitis virus (PHEV), five (31.25%) were identified as porcine epidemic diarrhea virus (PEDV), and three (18.75%) were identified as porcine respiratory coronavirus (PRCV). Thirteen (10.16%) out of 128 pig nasal swab, respiratory tract swab, and lung swab specimens (China) were positive with the expected amplification size. But after sequencing, only one specimen tested positive for porcine epidemic diarrhea virus (PEDV). One (1.72%) out of 58 pig oral secretion and fecal samples (Malaysia) demonstrated sequence data consistent with human coronavirus 229E.

Among 451 human samples (Malaysia and Vietnam), we identified four (0.89%) positives for coronavirus. Sequencing of these positive samples identified one human coronavirus HKU1 (Vietnam) and three human coronavirus 229E (Malaysia).

Of the 109 bioaerosol samples from live poultry markets, twenty-four (22.02%) were positive with amplicons of the expected amplification size. Sequencing of these positive samples identified seven duck coronaviruses and nine infectious bronchitis viruses (IBVs). The remaining eight positive samples could not be sequence-typed might be due to their low viral concentration.

4. Discussion

As a supplement to the original report of the pan-species assay [7], we detected seven unique coronaviruses in thirty-eight (4.02%) of the 946 archived field samples studied. These viruses included porcine hemagglutinating encephalomyelitis viruses (PHEV), porcine epidemic diarrhea viruses (PEDV), porcine respiratory coronaviruses (PRCV), human coronavirus (229E), human coronavirus (HKU1), duck coronavirus, and infectious bronchitis viruses (IBV). These findings supplement

Table 1
Types of samples tested with the pan-CoV assay and their aggregated laboratory results.

Sample Type	Collection Year(s)	Collection Location	No. Tested	No. (%) Positive by Sequencing	Summary of Sequence Results
Animal Specimens					
Pig oral secretion specimens (rope swab sampling)	2015–2016	China	200	16 (8%)	Eight porcine hemagglutinating encephalomyelitis viruses (PHEV); five porcine epidemic diarrhea viruses (PEDV); and three porcine respiratory coronaviruses (PRCV)
Pig lung swab, respiratory track swab, and nasal swab specimens	2018–2019	China	128	1 (0.78%)	One porcine epidemic diarrhea virus (PEDV)
Pig oral secretion specimens (rope sampling)	2017	Malaysia	30	1 (3.33%)	One human coronavirus (229E)
Pig fecal specimens	2017	Malaysia	28	0 (0%)	–
Human Specimens					
Animal workers' nasal wash specimens	2017	Malaysia	45	1 (2.22%)	One human coronavirus (229E)
Pneumonia patients' nasopharyngeal specimens	2017	Malaysia	56	2 (3.57%)	Two human coronaviruses (229E)
Pneumonia patients' nasopharyngeal specimens	2017–2019	Vietnam	350	1 (0.29%)	One human coronavirus HKU1
Environmental Specimens					
Bioaerosol samples from a live bird market	2018	China	109	16 (14.68%)	Seven duck coronavirus; nine infectious bronchitis viruses (IBV)

Archived samples ($N = 946$) from One Health research network laboratories in China, Malaysia, and Singapore were selected for this study.

previous animal and human coronavirus detections recorded in the original study [7]. Incidentally, a novel recombinant canine-feline alphacoronavirus from a recent report has been isolated from human specimens and gnomically characterized [13].

All the coronaviruses detected in this project were previously recognized as animal or human pathogens. As we detected them in association with their natural host, their detection does not raise major alarms. However, having an assay capable of detecting new incursions with specific coronavirus or cross-species coronavirus infections is of marked value to veterinarian or public health professionals. Such an assay covers multiple threats and does not require a knowledge that a virus is enzootic or endemic.

We argue that such tools are necessary, the current COVID-19 pandemic making these tools more apparent than ever, as both domestic and wild animals and humans may easily experience cross-species infections from coronaviruses. Pepin et al. mentioned that the surveillance of divergent CoVs in the swine population was essential, considering the rapid evolution of coronaviruses and the high levels of contact between domestic pigs and humans in ordinary life [14]. Especially, they found in previous reports that human CoVs can replicate in porcine cells (e.g., SARS-CoV-2), which may increase the potential zoonotic transmission of CoVs in pigs and humans. Another recent study provided molecular evidence to support the argument that pigs were susceptible to SARS-CoV-2 infection [15]. The authors suggested that further investigations into the role of domestic animals in the spread of SARS-CoV-2 are needed.

If we only consider the porcine coronaviruses we detected in this study, it seems they too have spillover potential. Edwards et al. found that swine acute diarrhea syndrome coronaviruses (SADS-CoVs) could replicate efficiently in human liver and rectal carcinoma cell lines, which may increase the spillover risk of SADS-CoV from pigs into humans [16]. Another group of researchers reported that porcine deltacoronavirus (PDCoV) was capable of infecting human cells under laboratory conditions [17].

This pan-coronavirus assay also has added value as a broad screening tool. Compared to targeted primers (e.g., for the PHEV test only), the pan-CoV RT-PCR assay does not require a veterinarian or public health official to know which specific coronavirus type molecular assay to order. Through its use, veterinary personnel may discover pathogens in various field samples that they did not know were enzootic. Besides, this assay can be easily conducted in molecular laboratories, which may also help laboratory professionals save costs and effort during the initial stage of an outbreak investigating.

It is also interesting to note that we detected a human coronavirus (229E) from one pig oral secretion specimen. This animal specimen was previously collected from pigs at a human-pig interface in Sarawak (Malaysia), where two animal workers were infected with human coronavirus (one of their specimens was also successfully sequenced as HCoV 229E positive in this study) may explain this result [9]. But because no similar finding was reported in previous studies, future studies are needed to validate this finding and explore the possibility of reverse zoonosis in coronavirus transmission. Additionally, the discovery of animal coronaviruses (duck coronavirus and IBV) in bioaerosol samples suggests that the RT-PCR assay we used can also be used to screen environmental samples. As coronaviruses were detected in various animal, human, and environmental samples, the pan-species coronavirus assay seems to have potential for One Health-oriented surveillance where multiple sample types are collected.

Detecting and mitigating future pandemic threats is a complex issue and various US government agencies and philanthropic organizations have championed different approaches. Since 2009, the US Agency for International Development (AID) has sponsored a PREDICT program which has expended more than $170 M in efforts to predict and preempt the next pandemic through viral surveillance and infrastructure development. PREDICT teams have collected 140,000 biological samples from various animals in numerous geographical areas and identified ~1200 novel viruses and trained thousands of professionals in 60 labs in 30 countries. In 2018, a more ambitious Viral Genome Project [1] was initiated with a 10-year goal to study an estimated 70% of the world's 1.67 million viruses to learn which are likely to crossover to man. While the effort is said to have won some funding from Chinese and Thai governments, its total support of $3.4 billion has yet to be reached. Similarly, administrators at National Institute of Allergy and Infectious Diseases have begun to talk about a 20-year plan for the institute's pandemic prevention effort [4]. The not well-described prospective human cohort surveillance and intense immunological study of 120 viral targets would likely be very expensive. Other scientists have criticized the intense viral surveillance strategies and would also likely criticize the immunological approach as too expensive in cost and effort. They point out that spillover events are rare, and mention that viruses are continually changing, requiring repeated analytical efforts. They underscore that predicting spillover events (and attempting to mitigate them immunologically) is very challenging involving many factors and thus much effort could be misdirected, never found to be practical, and funding misspent. Instead, other scientists argue for increased epidemiological and clinical surveillance for novel pathogens in high-risk settings [5,6,18].

It will be interesting to observe how policy makers decide to invest future government funding in pandemic prevention. However, it is becoming increasingly clear that a One Health Approach will be a key element for future work. The One Health Approach advocates for collaborations between human, animal, and environmental health. It often involves professionals from these diverse disciplines working together on a specific complex problem like emerging infectious diseases at the human-animal nexus. The One Health Approach is now embraced by almost all branches of the US government.

In this work, we examined archived specimens from a series of our One Health research projects in China, Malaysia, and Vietnam. We coached three different laboratory teams in adapting the conventional RT-PCR assay [7] that was developed at Duke University. They successfully ran the nested assay and found it relatively easy to use. This rather non-complex and inexpensive assay [7], other conventional pan-species RT-PCR and PCR assays [19], and next-generation sequencing laboratory assessments [20] could be powerful screening tools in assisting laboratory professionals in developing countries to better to identify novel respiratory viruses. Moreover, we also found such conventional pan-species assays to be a valuable supplement to focused real-time clinical assays in the detection of novel agents [19] among pneumonia patients. For instance, in Vlasova et al., we recently reported the detection of a novel canine-like recombinant alphacoronavirus from human pneumonia patients in Malaysia using the pan-species CoV RT-PCR assay [13,]. This finding underscores the value of the pan-species CoV assay in novel virus discovery. Hence, we strongly recommend public health and veterinary health officials include pan-species or next-generation sequencing assessments for novel virus detections in planning pre-pandemic virus surveillance among high-risk populations such as animal workers [22,23]. Early detection of known and unknown coronaviruses is the key to preventing transmission among animals and humans.

5. Limitations

The results reported herein should be considered in light of the following limitations. First, this study represents a convenience sample that was not representative of particular animal host species, specimen types or geographical areas. Second, this study was limited in that we could not link detected coronaviruses to clinical manifestations in humans or animals from which the specimens were taken. Third, while the veracity of the pan-species assay was presented in detail in the original report by Xiu et al. [7], we sought no estimations of sensitivity, specificity, and accuracy for the assay work reported here. We are just adding supplemental data to the Xiu et al., report demonstrating the

epidemiological value of the assay. Still, we took considerable care to verify our findings. We employed positive and negative controls in each pan-CoV assay step. Most of the CoV-positive specimens' amplicons were sequenced twice (no disagreements detected). The human specimen with evidence for HCoV HKU1 found in this study was previously tested and found to be HCoV HKU1-positive in a previous study using a different RT-PCR assay [11]. Our findings support the notion that the pan-species CoV assay could be an inexpensive and useful screening tool for coronaviruses surveillance among animal, human, and environmental samples, especially in developing countries. This assay could supplement other molecular testing methods in the detection of novel agents.

Ethical approvals

The collection of samples from humans and animals in China [8], Malaysia [9,10], and Vietnam [11] were approved by multiple human and animal ethical committees as documented in the original reports.

Authorship contributions

X.W. conducted laboratory analysis and drafted the manuscript. L.X. designed the pan-species coronavirus RT-PCR assay, guided the laboratory work, and revised the manuscript. R.A.B. performed the laboratory work in the U.S. site and revised the manuscript. T.T. guided the laboratory work in the Malaysia site and revised the manuscript. J.L. and J.T. participated in the laboratory work on the Malaysia site. D.P. provided technical support for laboratory analysis in the Malaysia site. S.T. and K.K.C. conducted the laboratory work in the Singapore site. W.Q. participated in the laboratory work on the China site. M.M. performed the laboratory work in Beijing (China) site and provided the swine achieved specimens. G.C.G conceived the study, guided the work, and revised the manuscript.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2021.100274.

References

[1] R. Jim, Before the next pandemic, an ambitious push to catalog viruses in wildlife, Yale Environmental 360 Magazine (2020). https://e360.yale.edu/features/before-the-next-pandemic-an-ambitious-push-to-catalog-viruses-in-wildlife (accessed April 22, 2020).
[2] J. Morrison, Can virus hunters stop the next pandemic before it happens? Smithsonian Magazine (2018). https://www.smithsonianmag.com/science-nature/how-to-stop-next-animal-borne-pandemic-180967908/ (accessed January 25, 2020).
[3] B.S. Graham, K.S. Corbett, Prototype pathogen approach for pandemic preparedness: world on fire, J. Clin. Invest. 130 (7) (2020) 3348–3349.
[4] B. Graham, COVID-19: a wake-up call for more fundamental research in immunology, in: Human Vaccines Project, 2020.
[5] J.L. Geoghegan, E.C. Holmes, Predicting virus emergence amid evolutionary noise, Open Biol. 7 (10) (2017).
[6] E.C. Holmes, A. Rambaut, K.G. Andersen, Pandemics: spend on surveillance, not prediction, Nature 558 (7709) (2018) 180–182.
[7] L. Xiu, R.A. Binder, N.A. Alarja, K. Kochek, K.K. Coleman, S.T. Than, E.S. Bailey, V. N. Bui, T.H. Toh, D.D. Erdman, G.C. Gray, A RT-PCR assay for the detection of coronaviruses from four genera, J. Clin. Virol. 128 (2020) 104391.
[8] M.J. Ma, G.L. Wang, B.D. Anderson, Z.Q. Bi, B. Lu, X.J. Wang, C.X. Wang, S. H. Chen, Y.H. Qian, S.X. Song, M. Li, J.A. Lednicky, T. Zhao, M.N. Wu, W.C. Cao, G. C. Gray, Evidence for cross-species influenza a virus transmission within swine farms, China: a One Health, prospective cohort study, Clin. Infect. Dis. 66 (4) (2018) 533–540.
[9] L.K. Borkenhagen, K.A. Mallinson, R.W. Tsao, S.J. Ha, W.H. Lim, T.H. Toh, B. D. Anderson, J.K. Fieldhouse, S.E. Philo, K.S. Chong, W.G. Lindsley, A. Ramirez, J. F. Lowe, K.K. Coleman, G.C. Gray, Surveillance for respiratory and diarrheal pathogens at the human-pig interface in Sarawak, Malaysia, PLoS One 13 (7) (2018) e0201295.
[10] T.H. Toh, K.C. Hii, J.K. Fieldhouse, J. Ting, A. Berita, T.T. Nguyen, S.C. Wong, T. M. Wong, W.H. Lim, S.J. Ha, C.Z. Lau, S.L. Kong, E.S. Bailey, T.E. Warkentien, T. S. Husain, G.C. Gray, High prevalence of viral infections among hospitalized pneumonia patients in Equatorial Sarawak, Malaysia, Open Forum Infect. Dis. 6 (3) (2019) ofz074.
[11] Y.H. Le, K.C. Nguyen, K.K. Coleman, T.T. Nguyen, S.T. Than, H.H. Phan, M. D. Nguyen, N.D. Ngu, D.T. Phan, P.V.M. Hoang, L.P. Trieu, E.S. Bailey, T. E. Warkentien, G.C. Gray, Virus detections among patients with severe acute respiratory illness, Northern Vietnam, PLoS One 15 (5) (2020) e0233117.
[12] X. Wang, E.S. Bailey, X. Qi, H. Yu, C. Bao, G.C. Gray, Bioaerosol sampling at a live animal market in Kunshan, China: a noninvasive approach for detecting emergent viruses, Open Forum Infect. Dis. 7 (5) (2020) ofaa134.
[13] A.N. Vlasova, A. Diaz, D. Damtie, L. Xiu, T.H. Toh, J.S. Lee, L.J. Saif, G.C. Gray, Novel canine coronavirus isolated from a hospitalized pneumonia patient, East Malaysia, Clin. Infect. Dis. Off. Pub. Infect. Dis. Soc. Am. (2021), https://doi.org/10.1093/cid/ciab456. In press.
[14] K.M. Pepin, R.S. Miller, M.Q. Wilber, A framework for surveillance of emerging pathogens at the human-animal interface: pigs and coronaviruses as a case study, Prev. Vet. Med. 188 (2021) 105281.
[15] B.S. Pickering, G. Smith, M.M. Pinette, C. Embury-Hyatt, E. Moffat, P. Marszal, C. E. Lewis, Susceptibility of domestic swine to experimental infection with severe acute respiratory syndrome coronavirus 2, Emerg. Infect. Dis. 27 (1) (2021) 104–112.
[16] C.E. Edwards, B.L. Yount, R.L. Graham, S.R. Leist, Y.J. Hou, K.H. Dinnon 3rd, A. C. Sims, J. Swanstrom, K. Gully, T.D. Scobey, M.R. Cooley, C.G. Currie, S. H. Randell, R.S. Baric, Swine acute diarrhea syndrome coronavirus replication in primary human cells reveals potential susceptibility to infection, Proc. Natl. Acad. Sci. U. S. A. 117 (43) (2020) 26915–26925.
[17] W. Li, R.J.G. Hulswit, S.P. Kenney, I. Widjaja, K. Jung, M.A. Alhamo, B. van Dieren, F.J.M. van Kuppeveld, L.J. Saif, B.J. Bosch, Broad receptor engagement of an emerging global coronavirus may potentiate its diverse cross-species transmissibility, Proc. Natl. Acad. Sci. U. S. A. 115 (22) (2018) E5135–e5143.
[18] C.J. Carlson, From PREDICT to prevention, one pandemic later, Lancet Microbe 1 (1) (2020) e6–e7.
[19] J.K. Fieldhouse, E. Bailey, T.H. Toh, K.C. Hii, K.A. Mallinson, J. Ting, J. A. Lednicky, A. Berita, T.T. Nguyen, D.G. Donlo, S.T. Than, S.C. Wong, T.M. Wong, P.J. Blair, G.C. Gray, Panspecies molecular assays detect viral pathogens missed by real-time PCR/RT-PCR among pneumonia patients, Sarawak, Malaysia, Trop. Dis. Travel Med. Vaccines 6 (2020), 13, https://doi.org/10.1186/s40794-020-00114-2.
[20] E. Crawford, J. Kamm, S. Miller, L.M. Li, S. Caldera, A. Lyden, D. Yokoe, A. Nichols, N.K. Tran, S.E. Barnard, P.M. Conner, A. Nambiar, M.S. Zinter, M. Moayeri, P. H. Serpa, B.C. Prince, J. Quan, R. Sit, M. Tan, M. Phelps, J.L. Derisi, C.M. Tato, C. Langelier, Investigating transfusion-related sepsis using culture-independent metagenomic sequencing, Clin. Infect. Dis. Off. Pub. Infect. Dis. Soc. Am. (2020) 1537–6591 (Electronic).
[22] G.C. Gray, E.R. Robie, C.J. Studstill, C.L. Nunn, Mitigating future respiratory virus pandemics: new threats and approaches to consider, Viruses 13 (4) (2021).
[23] G.C. Gray, A. Abdelgadir, While we endure this pandemic, what new respiratory virus threats are we missing? Open Forum Infect. Dis. 8 (3) (2021) ofab078.