

eSCOUT - Environmental Surveillance for COVID19 in Ohio: Understanding Transmission

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Purpose

We aim to build a public-private partnership to conduct a large-scale surveillance of SARS-CoV-2 in the “wild” to *discover* the environmental and animal reservoirs that harbor the virus long-term, assess the hazard of virus entry to the human population from the environment (spillover), and *study* the virus adaptations/mutations in real-time to inform public health responses and preparedness.

The need

To date, COVID-19 has infected over 8.3 million people around the world and is responsible for over 450,000 deaths. In Ohio alone, the SARS-CoV-2 pandemic has claimed 2,377 lives and infected 39,303 individuals [1]. SARS-CoV-2 has shown an extremely high potential to rapidly spread in the human population globally, yet the non-human reservoirs, persistence, and means of spread for this virus remain largely unknown.

The origin of SARS-CoV-2 also remains uncertain; although, both pangolins and horseshoe bats have been touted as potential natural reservoirs due to high sequence homology between bat and pangolin SARS CoV species [2, 3]. This preliminary data suggests the virus first was transmitted as a spillover event to humans from animals.

Another piece of the puzzle, the environment, must be considered for the transmission of the virus between human and animal reservoirs. As SARS-CoV-2 is shed in feces [4, 5], wastewater offers promise for monitoring viral presence in the human population as well as an early-warning tool [6] and has proven itself as such a sentinel for chemical hazards and opioid use in the field of wastewater based epidemiology (WBE) [7, 8]. In the Netherlands, SARS-CoV-2 was identified in wastewater within four days of the first reported case [6]. Wastewater can also pose a potential health risk as SARS-CoV-2 can infect human gut cells [10], and infectious virus has been identified in the feces of a patient with severe COVID-19 [9]. Together, this strongly implies a fecal-oral transmission route for SARS-CoV-2, and humans or animals that come in contact with wastewater may be at risk for infection. Preliminary research with Hampton Roads Sanitation District is demonstrating that WBE methods for SARS-CoV-2 are effective at monitoring the development of COVID-19 equilibrium, and is currently being explored for COVID-19 and seropositive testing in sewersheds. Cross-contamination of urban runoff with wastewater has also been reported, and fecal bacteria from deer and human sources are quite prevalent in urban stormwater runoff around Columbus, Ohio [11]. Therefore, it is expected that viruses

found in humans or deer may be present in our watershed and directly or indirectly increasing SARS-CoV-2 infection risks to humans and animals in contact with runoff.

Sampling in wildlife and domestic / companion animals offers an opportunity to identify potential reservoir species and new mutations. 75% of zoonotic emerging diseases come from wildlife [12], and early evidence suggests SARS-CoV-2 has a wildlife origin [13]. Although not home to pangolins or horseshoe bats, Ohio is home to 10 other bat species [14]. In addition, SARS-CoV-2 entry into human cells occurs by binding to the human ACE2 receptor. When ACE2 binding residues from 410 other species were compared to human ACE2 binding residues, medium to high receptor similarity was observed in multiple species of primates, rodents, deer, and felines [15]. This suggests that these species may be at higher risk of infection with SARS-CoV-2 and could be acting as intermediate hosts or reservoir species. Indeed, laboratory studies have confirmed that primates and cats can be infected with *and transmit* SARS-CoV-2 [16, 17]. 40% of all U.S. households own cats, therefore, it is critical that we begin characterizing the potential prevalence of SARS-CoV-2 in cats so that we can provide more informed management recommendations for both human and animal health.

Additionally, per the Ohio Department of Natural Resources, there are over 600,000 deer in the state of Ohio, and many live in close proximity to humans and their companion animals. In the 2019-2020 season alone, 184,465 Ohio deer were harvested by hunters [18], and a majority of these deer were intended for human consumption. Notably, butchering, has been directly linked to previous viral pandemics including Ebola [19]. Agricultural animals, like pigs, are also intended for human consumption, and are recognized as effective reservoirs for viral reassortment of zoonotic diseases, such as influenza virus [20]. There is also growing evidence that pig coronaviruses like Porcine deltacoronavirus (PDCoV) can infect human cells [21]. While one small laboratory study suggests that pigs are not susceptible to SARS-CoV-2 [22], further surveillance is critical in non-laboratory settings. Swine exhibitions provide an interface where pigs have contact with many other pigs and humans, and where co-infection (e.g. with influenza) is feasible. Ohio is the eighth largest pig-producer in the U.S., and per the Ohio Pork Council, the Ohio swine industry represents \$1.8 billion of the Ohio economy [23]. Surveillance for COVID-19 in animals – including deer, bats, cats, and pigs – is essential to understanding if these animals can become infected with SARS-CoV-2, can serve as a mixing-vessel for this virus, can move SARS-CoV-2 around the landscape, and can be a source of infection to humans either through contact or consumption. These surveillance efforts together are important from public health, economic, management, and policy perspectives.

Presently, most surveillance efforts are devoted to human sampling of the virus (PCR) and exposure (antibody) [24] and are not assessing the environment or animals. Efforts are needed to holistically assess SARS-CoV-2 spread, reemergence, and mutation to consider humans AND the environment.

Thus, environmental and animal surveillance provides critical insights on current circulating SARS-CoV-2 strains as well as emerging mutations, and this monitoring is key to help predict and prevent future outbreaks.

Our Approach

In this study we will survey wastewater, wildlife, and domestic animals to test for presence and change in SARS-CoV-2 in Ohio. The following objectives are the data-driven thrust areas for the proposed seed funding, which provide numerous outgrowths for further research.

- **Objective 1:** Identify environmental reservoirs of SARS-CoV-2 in Ohio through systematic sampling of wastewater, wildlife, and domestic animals. We will use qPCR or ddPCR to confirm the presence of SARS-CoV-2 in water or animal samples.
- **Objective 2:** Define SARS-CoV-2 mutation rates across environments in Ohio. We will use phylogenetic analysis of full-length SARS-CoV-2 genome sequences to calculate the mutation rate.

- **Objective 3:** Characterize the full metagenome - including virome - on a subset of SARS-CoV-2 positive animal samples to identify other potentially pathogenic and circulating co-infections (e.g. other coronaviruses, influenza virus).

To achieve these objectives, The Ohio State University is combining forces across multiple surveillance projects and technical programs whose collective efforts will yield data for 1) presence of the virus in numerous environmental and animal types and ii) virus genetic data to inform transmission models, and mutation/adaptation. The following surveillance teams will contribute to the program:

1. **Clinical Feline Surveillance** – OSU Veterinary Medical Center (J. Winston, Bowman, Diaz-Campos, Yaxley, Nolting, McLaine)
2. **Shelter Animal Surveillance** – OSU Veterinary Preventive Medicine (Bowman, Nolting)
3. **Wildlife Surveillance** – OSU Veterinary Preventive Medicine, Ohio Wildlife Center, The Wilds, Columbus Zoo & Aquarium, ODNR, USDA APHIS, and local hunters (M. Flint, J. Flint, Bowman, Nolting, Pesapane, Junge, Hale, Lombardi, Ramer)
4. **Wastewater Surveillance** – systems throughout Ohio, including daily sampling (Wittum, Lee, Bowman, Weir)
5. **Stormwater Surveillance** – Central Ohio watershed (R. Winston, Lee)
6. **Agricultural (Pig) Surveillance** - State and local fairs around Ohio, OSU Veterinary Preventive Medicine (Bowman, Nolting)
7. **Built Environment** – Indoor homes and urban environment (Dannemiller, Weir, Anderson, Lee)

Our teams will work in the seven areas above to collect and test samples. The request for seed funding will support identifying nearly 200 SARS-CoV-2 positive samples (estimated ~1,300 total samples collected), but our goal is to eventually obtain extramural support to collect and test >1,000,000 samples. For some collection activities (e.g., agricultural animals, wildlife, and wastewater), IACUC, IBCs, and other permits have been already been submitted or obtained. For any new protocol (e.g., animal collection, water collection), all appropriate approvals will be sought to perform collections and analysis.

Water samples will be tested with ultra-sensitive digital droplet polymerase chain reaction (ddPCR) technique developed at OSU (Lee lab). Animal swabs (swine, feline, wildlife) will be analyzed with a quantitative polymerase chain reaction (qPCR) test (Bowman lab) that is robust to give true positive results even while virus mutations have occurred. For all samples, metadata will be collected (e.g., location, species, time/date, sample type), and geomodelling will be applied to assess potential reservoirs and transmission routes within the Ohio region.

Positive samples will be split and one portion will undergo whole virus genome analysis to produce high quality SARS-CoV-2 sequence data by external partner Ginkgo BioWorks. The other portion will be Biobanked for future whole metagenome analysis. Phylogenetic analysis (Kubatko Lab) will be performed to measure genetic similarity and will be combined with geomodelling data. Evolutionary studies will include whole genome and independent gene analysis, as well as recombination potential following models such as those earlier used to assess influenza virus. A subset (n=10) of the animal samples (pigs, cats, wildlife) that test positive for SARS-CoV-2 will undergo shotgun metagenomic analysis including full virome characterization. This will allow us to look for other potentially pathogenic / zoonotic and circulating viruses (e.g. influenza virus or other coronaviruses) in these animals and begin examining SARS-CoV-2 mutation and mutation dynamics in the presence of other viruses and bacteria.

We will use a growing partnership with Amazon Web Services (AWS) to first assess implementation of shared bioinformatics tools, and second create a long term data storage and sharing environment/plan that is a critical resource for the Center of Microbiome Science (CoMS). We are targeting the Research as a Service (RaaS) toolkit that AWS wants to beta test with the CoMS.

Outcomes and impact

Since COVID-19 emerged in late 2019, the community has focused heavily and necessarily on understanding and preventing human-to-human transmission. This virus almost certainly originated in wildlife, and it is now time for us to establish active surveillance efforts in the environment around humans to uncover other potential sources of SARS-CoV-2 transmission. Identifying SARS-CoV-2 in the environment, domestic animals, and wild animals has critical public health and economic ramifications that could be mitigated through early detection, as well as inform on transmission of the virus to stop its spread and develop tactics for mitigation and prevention.

This project also will provide a rich biorepository and genomic sequence data that can be proposed for additional studies. We will also develop outreach mechanisms through IDI / CoMS and College / University social media, and press / online / WOSU media. We will develop and dedicate a webpage (within the CoMS website) to disseminate eSCOUT activities, reports, and findings, and we will work with our partner organizations and the Ohio Department of Health to share information, data, and develop opportunities for public outreach focused on SARS-CoV-2 transmission risks around Ohio.

This revolutionary project will provide a rich biorepository and RNA/DNA sequence data that will be shared as open-source knowledge, identifying SARS-CoV-2 in the environment, domestic animals, and wild animals. The contributions to public and animal health are numerous: demonstrating approaches for early detection, informing on transmission and persistence, guiding policies for stopping its spread, and developing tactics for mitigation and prevention. This results in more lives saved and enhanced global health.

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