A Guide to Investigating Outbreak Origins: Nature versus the Laboratory

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Contents

Introduction

COVID-19 has exposed key gaps in the global community's ability to assess infectious disease outbreaks of international concern, in particular the ability to differentiate between natural and laboratory sources of infection. The risk of natural outbreaks is increasing as unchecked population growth, industrial expansion, and corresponding ecological disruption increases the likelihood that novel disease agents will come into contact with naïve human populations.[1] Likewise, the risk of laboratory accidents is increasing as more high-containment laboratories are built and higher risk experiments are conducted around the world.[2] Meanwhile, a deliberate biological attack may resemble an outbreak of natural or accidental origin, and a natural or accidental outbreak may be misattributed as an attack.

The purpose of this Occasional Paper is to outline a readily adoptable, stepwise methodology to guide the investigation of corresponding outbreak origins, building upon traditional epidemiological principles. We have sought to remain minimally intrusive at all times; however, an increasing level of need-to-know information, site, and personnel access becomes necessary as attention shifts toward potential laboratory sources. Accordingly, we include recommendations to ensure such access under existing international regimes, primarily the World Health Organization (WHO)'s International Health Regulations.

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[1] See, for example, Madhav N, Oppenheim B, Gallivan M, et al. Pandemics: Risks, Impacts, and Mitigation. In: Jamison DT, Gelband H, Horton S, et al., editors. Disease Control Priorities: Improving Health and Reducing Poverty. 3rd edition. Washington (DC): The International Bank for Reconstruction and Development / The World Bank; 2017 Nov 27. Chapter 17. Available from: https://www.ncbi.nlm.nih.gov/books/NBK525302/ doi: 10.1596/978-1-4648-0527-1_ch17

[2] While the formal definition varies, high-containment laboratories are commonly identified as those laboratories that contain the most dangerous infectious diseases and thus operate at the highest biosafety levels (BSL), namely BSL-3 and BSL-4. See, for example, https://www.gao.gov/products/GAO-08-108T

Background

The origin of the SARS-CoV-2 virus that causes COVID-19 has yet to be determined. On May 19, 2020, the 73rd World Health Assembly adopted a resolution that, among other items, calls on the WHO to "identify the zoonotic source of the virus and the route of introduction into the human population."[3] The United States applauded this "mandate given by the resolution to the WHO to investigate the origins of the virus."[4]

One origin hypothesis is that SARS-CoV-2 escaped from a biological laboratory in China rather than naturally spreading from animal to human hosts.[5] In support of this hypothesis are the following facts:

1. Laboratories around the world have accidents, including in China;

2. Prior to COVID-19, one laboratory in close proximity to the initially identified cases, the Wuhan Institute of Virology *(Figure 1)*, had come under scrutiny for reported safety lapses;

3. That same laboratory, as well as another laboratory in Wuhan, was responsible for studying unknown, potentially zoonotic disease agents in animal populations, including bat coronaviruses genetically related to SARS-CoV-2; and

4. If SARS-CoV-2 was being studied there, and an accident happened, it could plausibly have been introduced into the neighboring human population by any number of well-documented routes of laboratory "escape," including on or in laboratory workers or in infectious waste.

Against the laboratory-origin hypothesis: there is a well-documented mechanism for animal-to-human spillover of biological agents like SARS-CoV-2, including in the area where COVID-19 was initially detected, and the ensuing spread follows expected epidemiological characteristics of a natural event.

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[3] https://apps.who.int/gb/ebwha/pdf_files/WHA73/A73_R1-en.pdf, pg. 6, item 9(6).
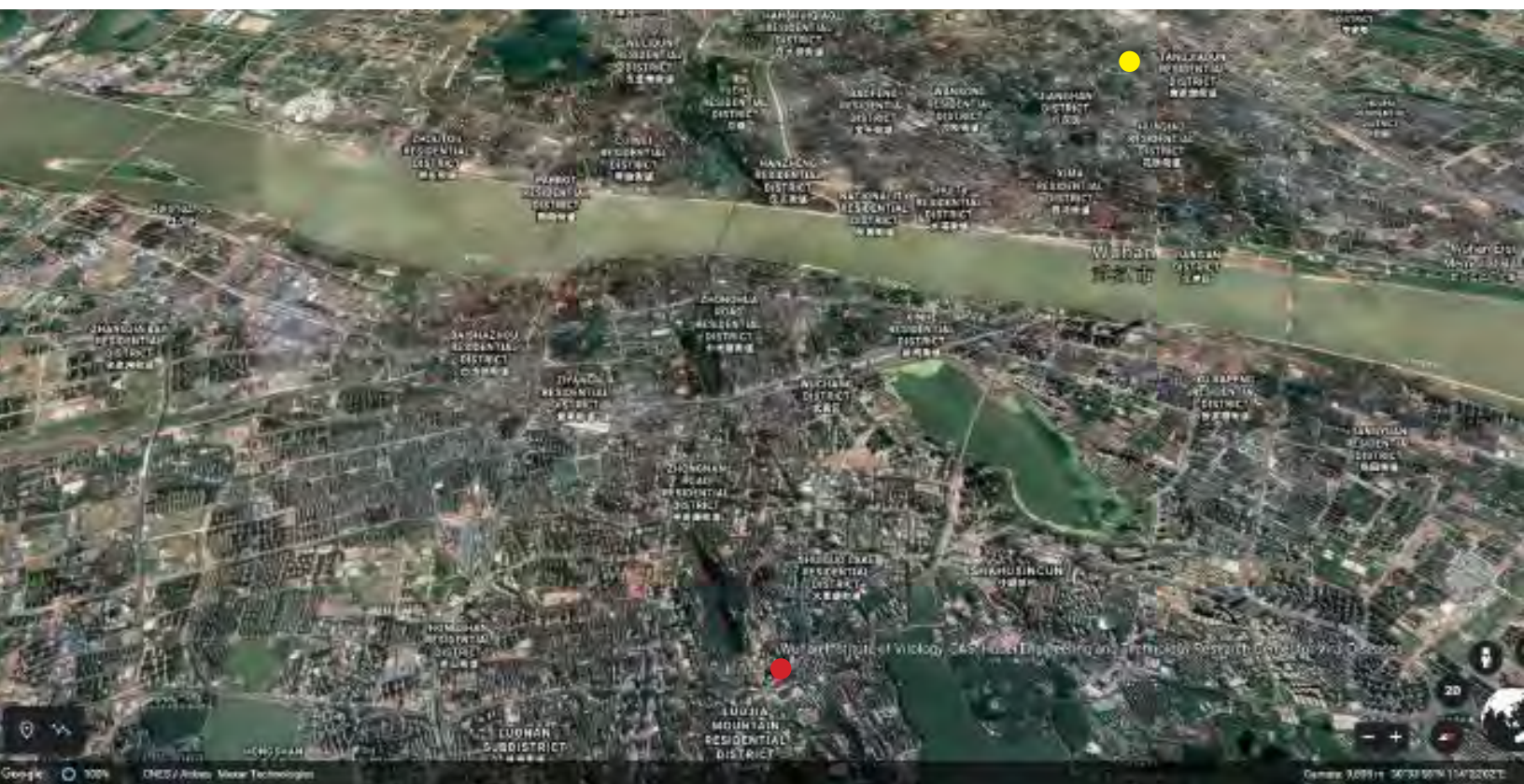[4] https://geneva.usmission.gov/2020/05/19/explanation-of-position-covid-19-response-resolution/
[5] See, for example, https://www.forbes.com/sites/jackbrewster/2020/05/10/a-timeline-of-the-covid-19-wuhan-lab-origin-theory/#638e124a5aba; https://www.nytimes.com/2020/05/03/us/politics/coronavirus-pompeo-wuhan-china-lab.html; https://foreignpolicy.com/2020/05/01/coronavirus-trump-pandemic-pompeo-attack-china/

The bottom line: regardless of COVID-19's origin, the risk of both high-consequence laboratory mishaps and spillover at the animal-human interface is real, and the disease is now affecting populations around the world.[6]

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[6] Only a few months before COVID-19, a different laboratory – and outbreak – dominated American headlines, when the US House of Representatives launched a formal investigation into renewed allegations that the 1975 emergence of Lyme disease in Lyme, Connecticut may have been linked to the nearby Plum Island Animal Disease Center (PIADC) (https://chrissmith.house.gov/uploadedfiles/final_lyme_ig_amendment.pdf). Despite the questionable veracity of these allegations, the supporting evidence aligns with that of the SARS-CoV-2 laboratory origin hypothesis: (1) Laboratories around the world have accidents, including in the United States; (2) One laboratory in close proximity to the initially identified cases, PIADC, had come under scrutiny for reported safety lapses; (3) That same laboratory was responsible for studying unknown, potentially zoonotic disease agents in animal populations; and (4) If the causative agent of Lyme disease *(Borrelia burgdorferi)* was being studied there, and an accident happened, it could plausibly have been introduced into the neighboring human population by any number of well-documented routes of laboratory "escape," including on/in a laboratory worker traveling on one of only two ferries serving PIADC, birds migrating along the north-south Atlantic Flyway, or wildlife known to move between the island and mainland (See, for example, https://www.gao.gov/assets/130/120213.html; https://www.nytimes.com/2004/08/22/nyregion/plum-island-reports-disease-outbreak.

Figure 1. Wuhan Institute of Virology

The Institute is located at the red marker (lower middle); the Huanan Seafood Market, where the first identified cases were initially traced, is located at the yellow marker (upper right). Earlier cases have since been reported. Source: Google Earth.

Investigating Outbreak Origins

The SARS-CoV-2 example underscores the need for a systematic approach to investigating outbreaks of unknown origin that occur near laboratories, for example among inhabitants of a city where a high-containment laboratory is located.[7] Our recommended methodology for this purpose is depicted in *Figure 2*. The investigation initially focuses on natural sources (blue boxes), following a typical outbreak investigation approach. If a natural source is not identified, the search for a natural source continues as the investigation expands to include laboratory sources (tan boxes), with the goal of remaining minimally intrusive at all times. However, as need-to-know information, site, and personnel access becomes increasingly necessary throughout the course of investigation, we provide recommendations to ensure such access under the WHO's revamped International Health Regulations.

Step 1. Descriptive Epidemiology

An outbreak investigation typically begins with defining the *who*, *what*, *when*, and *where* of infection. Investigators perform case histories and interviews to determine who is being infected, by what disease agent, when infection occurred, and in what location; this is called "descriptive epidemiology." By first describing the epidemiology in this way, investigators can ask the following key questions to explore potential indicators of a laboratory accident or unusual source:

- Is the infecting agent unusual for the location, or time of year?
- Is it affecting unusual populations, in unusual ways?

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html). Against the laboratory origin hypothesis, again like SARS-Co-V: there is a well-documented mechanism for animal to human spillover of biological agents like *B. burgdorferi*, including in the area where Lyme disease was initially detected (babesiosis, for example; see Diuk-Wasser MA, Liu Y, Steeves TK, et al. Monitoring human babesiosis emergence through vector surveillance New England, USA. Emerg Infect Dis. 2014; 20(2):225-231. doi: 10.3201/eid2002.130644), and the ensuing spread follows expected epidemiological characteristics of a natural event. The bottom line: as with SARS-CoV-2, regardless of Lyme disease's origin, the risk of both high-consequence laboratory mishaps and spillover at the animal-human interface is real, and the disease is now affecting populations around the world.

[7] The need for a credible investigative approach to COVID-19's origin and what that might entail was described by Dr. Filippa Lentzos in a pair of May 2020 articles in the Bulletin of the Atomic Scientists, both of which informed our proposed methodology. Dr. Lentzos's articles are available at: https://thebulletin.org/2020/05/natural-spill-over-or-research-lab-leak-why-a-credible-investigation-in-needed-to-determine-the-origin-of-the-coronavirus-pandemic/; https://thebulletin.org/2020/05/will-the-who-call-for-an-international-investigation-into-the-coronaviruss-origins/

Figure 2. Investigation Methodology

Our recommended methodology for investigating outbreaks of unknown origin that occur near laboratories builds upon a typical outbreak investigation approach to assess potential natural (blue boxes) and laboratory (green boxes) sources in stepwise fashion.

Case Study. Inhalational anthrax in Sverdlovsk, 1979

In April–May 1979, an anthrax outbreak involving livestock and humans occurred in and around the city of Sverdlovsk.[8] Anthrax is caused by *Bacillus anthracis*, which is naturally present in soil in an environmentally stable form called a spore. When animals such as cattle ingest or inhale anthrax spores while grazing, they become infected and can subsequently infect humans in one of three ways: through a cut or scrape in the skin when handling the infected animals or their byproducts, for example hides, which causes a minimally lethal cutaneous infection that accounts for approximately 95% of all human anthrax cases; by inhaling spores when similarly handling infected animals or byproducts, which causes a highly lethal respiratory infection that accounts for nearly 5% of all human anthrax cases; or by ingesting spores via contaminated meat, which causes a moderately lethal gastrointestinal infection that accounts for less than 1% of all human anthrax cases. The ability of anthrax spores to cause a highly lethal respiratory infection has led to its longtime development as a biological weapon and use in the "anthrax letter" attacks of 2001.

The 1979 outbreak was initially reported to involve livestock south of the city, with resulting cutaneous and gastrointestinal infections among humans due to handling and consuming contaminated meat. A total of 96 human cases (79 gastrointestinal, 17 cutaneous) and 64 deaths (all due to gastrointestinal infection) were reported. Because anthrax had long been documented to infect animals in the region, the explanation appeared plausible. However, the 81% mortality rate for gastrointestinal anthrax (64 deaths out of 79 cases) exceeded its accepted lethality range of 25-75%, and—unbeknownst to Western analysts at the time—autopsies of the deceased demonstrated significant lung pathology consistent with respiratory infection.

When animal and human cases were eventually mapped on a grid of the region, a clear pattern emerged: nearly all human cases fell within a narrow exposure zone extending south-southeast from an origin point inside the city, while animal cases mapped to multiple neighboring villages along the zone's extended axis *(Figure 3)*. The origin point of the exposure zone approximated the location of a military microbiological facility called Compound 19. An analysis of archived meteorological data around the time of the outbreak determined that, on April 2, 1979, wind direction matched the south-southeast exposure

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[8] For detailed discussions of the Sverdlovsk outbreak, see Meselson M et al., "The Sverdlovsk Anthrax Outbreak of 1979," Science, 1994, Volume 266, pp. 1202-1208; Leitenberg, Milton, "Anthrax in Sverdlovsk: New Pieces to the Puzzle," Arms Control Today, vol. 22, no. 3, 1992, pp. 10–13.
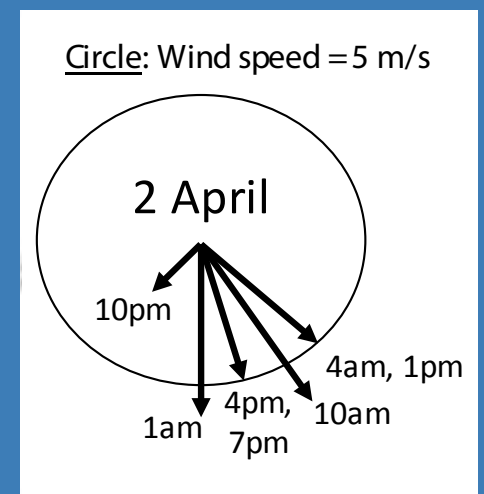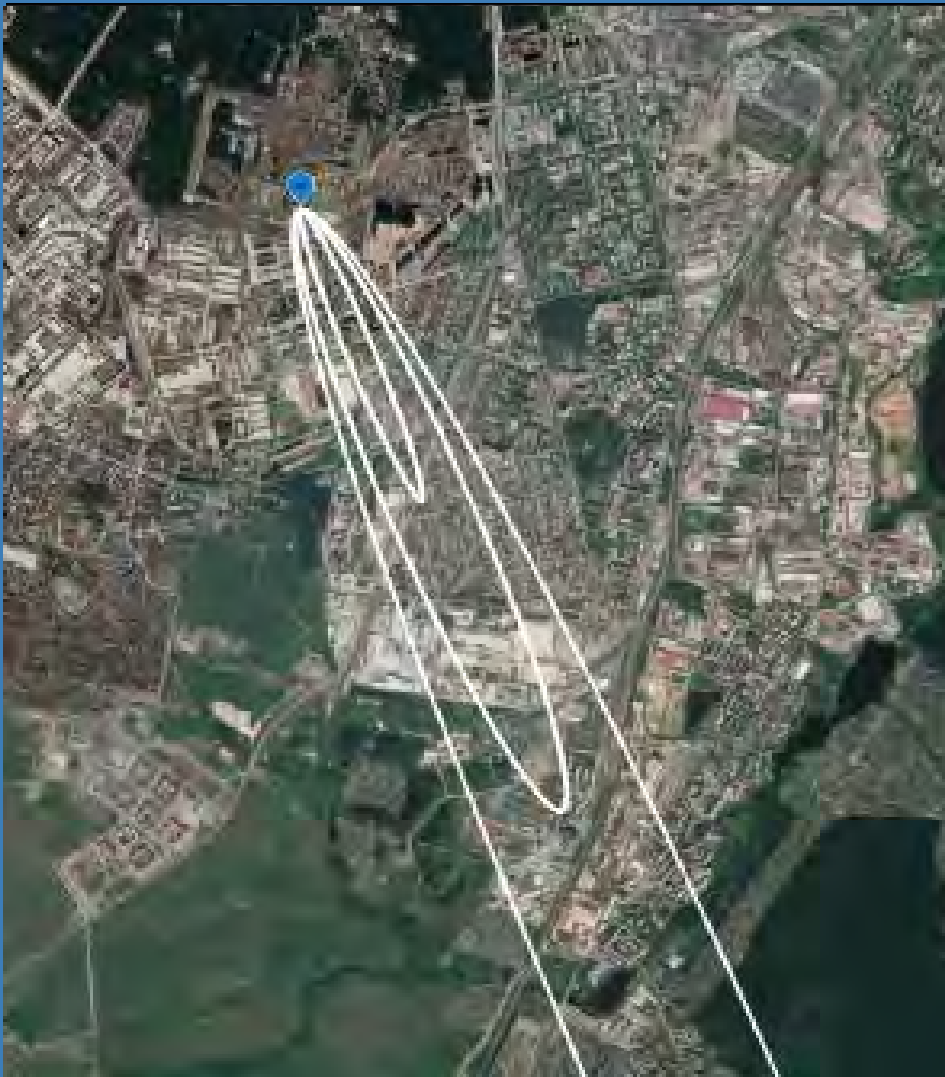
Figure 3. Sverdlovsk Anthrax Outbreak, 1979

Human cases were mapped to a narrow exposure zone extending south-southeast from an origin point inside the city (left image). Animal cases mapped to multiple neighboring villages along the zone's extended axis (upper right image). Wind direction matched the exposure trajectory on April 2, 1979, consistent with the expected range of incubation periods calculated for reported cases (lower right image). The origin point of the exposure zone was identified as Compound 19, a Soviet biological weapons production facility. The cause of the outbreak was ultimately determined to be a laboratory mishap at the facility involving removal of an exhaust filter and subsequent aerosol release of anthrax spores, which were carried by the wind along the exposure zone. Source: Meselson M et al., "The Sverdlovsk Anthrax Outbreak of 1979," *Science*, 1994, Volume 266, Pages 1202–1208.

zone. An April 2 exposure date aligned with the expected range of time to presentation of symptoms (*i.e.*, incubation period) and clinical course observed in reported cases. A 1999 insider account of the incident identified Compound 19 as a biological-weapons-production facility and cited a laboratory mishap involving the removal of an exhaust filter and subsequent aerosol release of anthrax spores, which were carried by the wind along the exposure zone, as the cause of the outbreak.[9]

Step 2. Analytical Epidemiology

Next, investigators seek to determine the *how* and *why* of infection. How did infection occur, and why—what circumstances enabled infection? To explore these questions, investigators typically assess (a) the epidemiological triangle for indicators of convergence that would enable spillover of the infecting agent from its natural reservoir to humans, and (b) the infecting agent genome for indicators of geographical and temporal spread; this is called "analytical epidemiology." Assessment results guide further sample analysis and, where warranted, exploration of alternate origin hypotheses such as laboratory accidents.

(a) Assess the Epidemiological Triangle

The epidemiological triangle is a simplified representation of the relationship between (1) a disease agent, typically in an animal reservoir; (2) a human host; and (3) the environment, which form the three points of a triangle. The lines that connect these points can be long or short, and can be lengthened or shortened. The goal of the assessment is to determine whether the lines have shifted in a way that has brought the infecting agent (or its animal reservoir) into contact with the human host. Initially, assessment focuses on tracing human cases to any known animal reservoirs, whether exposed through direct contact, consumption of byproducts, or another route. If no epidemiological link is apparent, investigators can seek to identify risk factors that might enable such exposure by asking the following key questions:

- Has the human population expanded into areas where the disease agent resides in animal reservoirs, for example due to wildlife trade, deforestation, or industrial farming?
- Has the disease agent expanded into human populations, for example due to animal reservoir overgrowth, vector population overgrowth (*e.g.*, ticks, fleas), or interspecies spillover?

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[9] Alibek, Ken. Biohazard: The Chilling True Story of the Largest Covert Biological Weapons Program in the World, Told from the inside by the Man Who Ran It. New York: Random House, 1999.

- Has the environment brought animal and human populations closer together, for example due to short-term meteorological shifts or longer-term climate shifts?

Case Study. Hantavirus cardiopulmonary syndrome in the United States, 1993–94

A 1993–94 outbreak of hantavirus cardiopulmonary syndrome in the Four Corners region of the United States illustrates how the relationships of the epidemiological triangle drive infectious disease emergence and re-emergence *(Figure 4)*.[10] After a multi-year drought diminished the local predator population, the 1991–92 El Niño-southern oscillation caused increased precipitation that resulted in extensive pine nut overgrowth, which in turn fueled an explosion of the local deer mouse population (*Peromyscus maniculatus*). This deer mouse is the animal reservoir for Sin Nombre virus, a novel hantavirus that is excreted in the rodents' feces, urine, and saliva. Subsequent inhalation of this excreta by humans leads to the life-threatening disease hantavirus cardiopulmonary syndrome. During the 1993–94 outbreak, the exploding deer mouse population brought the viral reservoir into closer contact with nearby human populations, increasing the probability of zoonotic transmission and ultimately causing 52 cases of human disease.[11]

(b) Assess the Genome

The infecting agent's genome may also hold vital clues to its origin. This is especially true for viral agents, and RNA viruses in particular, where mutations routinely occur as the virus replicates (*i.e.*, reproduces, which requires infection of a host cell). Mutations that offer a selective advantage for the virus are more likely to survive, providing a geospatial and temporal map of the outbreak based on prevailing mutations. By comparing the infecting agent's genome with the genomes of well-characterized reference strains in the public domain, investigators can (1) identify the closest known relative of the infecting agent; and (2) determine whether the infecting agent's

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[10] Hjelle B, Glass GE. Infection in the Four Corners region of the United States in the wake of the 1997-1998 El Nino-southern oscillation. J Infect Dis. 2000 May;181(5):1569-73. Epub 2000 May 15.

[11] Another example of convergence on the epidemiological triad is the 1942 tularemia outbreak among German and Soviet troops during the Battle of Stalingrad. Despite some allegations that the outbreak was caused by a Soviet biological attack, detailed analyses have revealed that the outbreak originated due to disruption of the local grain harvest due to the war and resulting population overgrowth of infected rodents that passed the disease to both armies. See, for example, Leitenberg M and Zilinskas RA. The Soviet Biological Weapons Program. Cambridge, Harvard University Press, 2012; and Geissler E. Alibek, Tularemia, and the Battle of Stalingrad. CBWCB 69+70, September/December 2005.
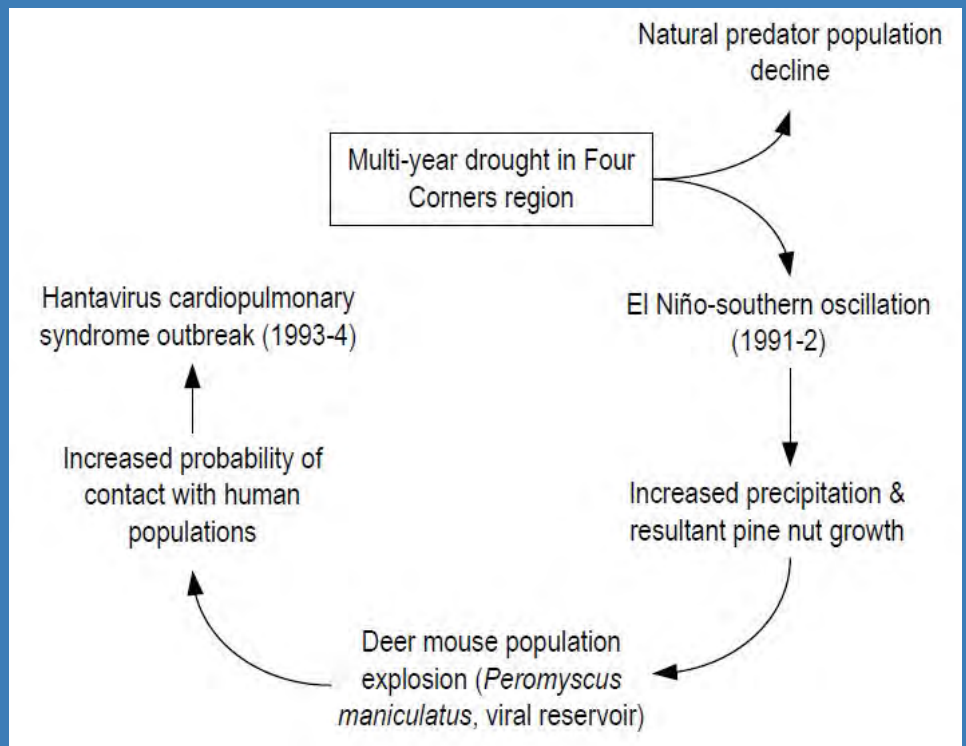
Figure 4. Hantavirus Cardiopulmonary Syndrome in the Four Corners region, 1993–94

In accordance with the epidemiological triangle, environmental changes brought the animal reservoir into contact with the human host, resulting in a localized outbreak of zoonotic disease.

genome has amassed mutations consistent with known patterns of natural emergence. For example, SARS-CoV-2's genome closely resembles that of a bat coronavirus,[12] but a small section of the genome called the "polybasic cleavage site," believed to provide a selective advantage for disease transmission,[13] would have been expected to evolve over time but instead is present in the earliest sequences of the virus.[14] Investigators can further determine whether the infecting agent's genome so closely resembles a given reference strain that a period of limited or no replication is likely. Such so-called "frozen evolution," when an infecting agent's genome lacks the expected accumulation of mutations over time, suggests that alternate origin hypotheses such as a laboratory accident must be explored.[15]

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[12] Zhou, P., Yang, X., Wang, X. et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579, 270–273 (2020). https://doi.org/10.1038/s41586-020-2012-7
[13] While the role of the polybasic cleavage site has yet to be confirmed, the polybasic cleavage site differs from typical monobasic cleavage sites in that it offers multiple ("poly," as opposed to "mono," meaning one) nucleic acids where the viral binding protein, called the spike or "S" protein, can be cut and thus activated to enable infection of human cells.
[14] Zhen, Deverman, and Chan. SARS-CoV-2 is well adapted for humans. What does this mean for re-emergence? bioRxiv, 2 May 2020. doi: https://doi.org/10.1101/2020.05.01.073262.
[15] See, for example, Pascall DJ, Nomikou K, Bréard E, Zientara S, Filipe AdS, Hoffmann B, et al. (2020) "Frozen evolution" of an RNA virus suggests accidental release as a potential cause of arbo-

Case Study. Foot-and-mouth disease in the United Kingdom, 2007

On August 2, 2007, a cluster of foot-and-mouth disease (FMD) was identified on a farm near Normandy, United Kingdom, a short distance from the Institute for Animal Health in Pirbright *(Figure 5)*. FMD is a debilitating and highly contagious viral illness of cloven-hoofed animals such as cattle that had been absent from the United Kingdom since 2001; because the disease spreads so readily, countries with FMD typically have their agricultural exports restricted, often with severe economic consequences. Over the course of the next nearly two months, additional farms were affected in the area surrounding Pirbright, leading to the slaughter of 2,160 animals in order to control the disease.

Collected samples were sent to the local Institute for Animal Health for analysis, which identified the infecting agent as FMD virus strain O_1BFS 1860. The strain was strikingly similar to a 1967 reference strain used at the same Pirbright laboratory for vaccine production.[16] Because the strain lacked the years of amassed mutations that would be expected in a naturally occurring outbreak, a laboratory origin was

virus re-emergence. PLoS Biol 18(4): e3000673. https://doi.org/10.1371/journal.pbio.3000673.
[16] Because three different laboratories at Pirbright worked with the strain, the sequence of each differed very slightly due to ongoing mutations.
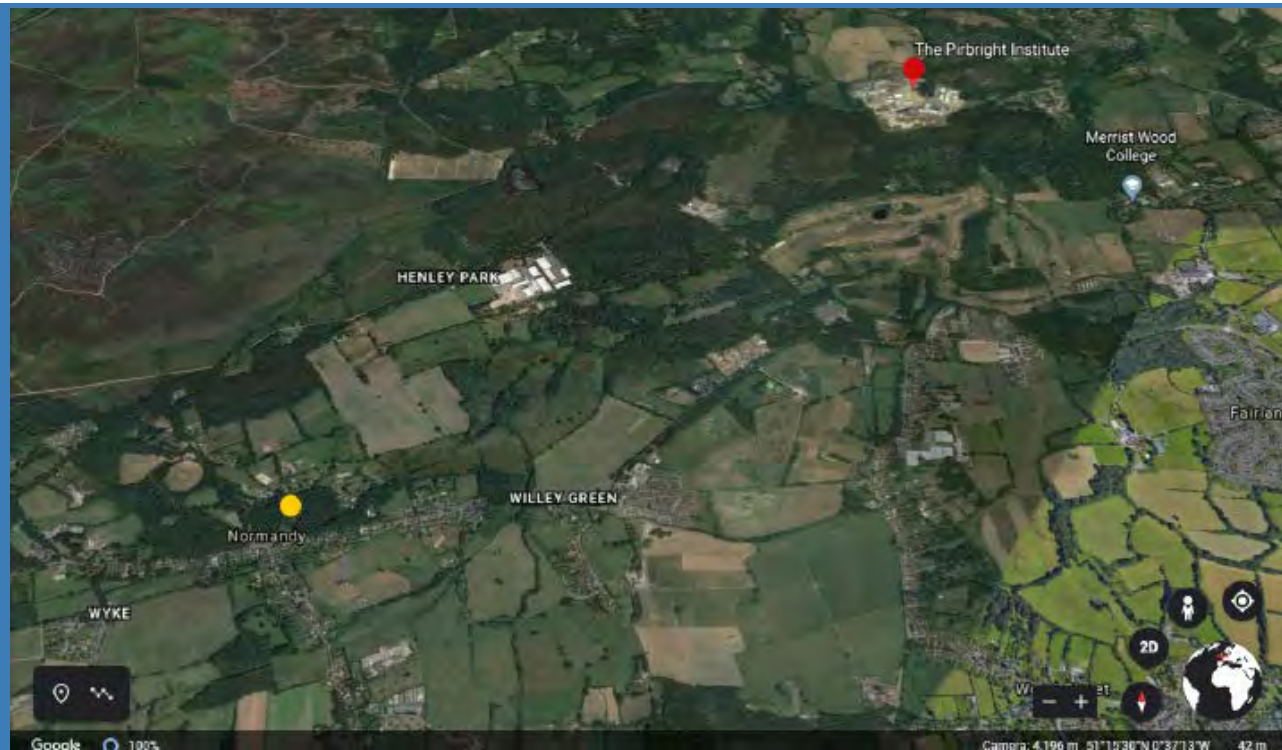
Figure 5. Institute for Animal Health, Pirbright

The Pirbright laboratory is located at the red marker (upper right); Normandy, where the first case was identified, is located at the yellow marker (lower left). Sample analysis identified the infecting agent as foot-and-mouth disease virus strain O_1BFS 1860, nearly identical to a strain held at the Pirbright laboratory. Source: Google Earth.

all but assured. Investigators continued to monitor the outbreak's spread from farm to farm by monitoring mutations in the same way. Investigators ultimately determined that the outbreak was caused by leakage of infectious waste from the facility drainage system, where it contaminated the soil and was inadvertently transferred by vehicles to the area of the first affected farm before spreading from animal to animal by the respiratory route.[17]

Step 3. Additional Sample Analysis

Based on descriptive and analytical epidemiology findings, investigators may collect and/or analyze additional animal, human, or environmental samples with the goal of closing information gaps in the prevailing origin hypothesis. If contact with an animal reservoir is suspected, investigators may collect animal or environmental samples at the suspected animal-human interface, whether a market, farm, abattoir, or in the wild. Analysis of these samples may identify the reservoir or provide additional clues that can be traced back epidemiologically and genetically.

"Banked" human samples predating the outbreak may also be tested to this end; often, such clinical samples are retained for extended periods of time, and may be revisited for further analysis, for example if they came from patients with clinical presentations resembling the current outbreak.[18]

In addition, investigators may actively collect human samples that might indicate exposure or infection in so-called sentinel populations at the animal-human interface. For example, serological testing of hunters or wildlife traders may identify antibodies against the agent causing the current outbreak, indicating exposure that may then be traced back to an animal reservoir.[19] If a natural source is not identified, or if early evidence indicates a potential laboratory source, the search for a natural source continues as the investigation expands to include laboratory sources.

[17] Anderson I. Foot and Mouth Disease 2007: A Review and Lessons Learned. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/250363/0312.pdf (Accessed 6/29/2020)

[18] For example, banked samples from a December 2019 patient with influenza-like illness and pneumonia in France were retested after the emergence of COVID-19 and found to be positive for the pandemic coronavirus, thus predating all previously identified cases outside of China. See Deslandes A, Berti V, Tandjoui-Lambotte Y et al. SARS-CoV-2 was already spreading in France in late December 2019. International Journal of Antimicrobial Agents. Volume 55, Issue 6, June 2020, 106006. https://doi.org/10.1016/j.ijantimicag.2020.106006.

[19] See, for example, Dovih P, Laing ED, Chen Y, Low DHW, Ansil BR, Yang X, et al. (2019) Filovirus-reactive antibodies in humans and bats in Northeast India imply zoonotic spillover. PLoS Negl Trop Dis 13(10): e0007733. https://doi.org/10.1371/journal.pntd.000773.

Step 4. Laboratory Risk Assessment

If expansion of the investigation to include potential laboratory sources is warranted, the first step is to perform a risk assessment of proximal laboratories to identify: (a) what biological agents or unknown/suspect samples are being worked with; (b) using what techniques; and (c) at what level of biosafety.[20] These factors determine the potential risk to surrounding communities that a laboratory mishap may spark an outbreak, for example due to worker infection, incomplete decontamination of waste, or aerosol release. For the purposes of this analysis, any combination of factors that poses undue or unknown risk to the surrounding community is categorized as "high risk" requiring mitigation. Our proposed process for determining this information is as follows.

(a) Understand the laboratory's purpose

Understanding the laboratory's purpose provides useful insight into all three factors described above (agents, techniques, biosafety). Biological laboratories generally fall into two, non-exclusive categories: diagnostic laboratories and research laboratories.

Potential indicators of laboratory purpose and specific activities are as follows:

- Laboratory name, organization, and chain of command
- Website descriptions and social media pages/posts[21]
- Scientist publications and sequence submissions[22]
- Scientist posters or presentations at international conferences
- Laboratory or scientist grant descriptions.[23]

[20] Pilch R and Pomper M. A Guide to Getting Serious about Bio-Lab Safety. War on the Rocks. June 1, 2020. https://warontherocks.com/2020/06/a-guide-to-getting-serious-about-bio-lab-safety/

[21] Note the possibility of deliberate omissions or inaccuracies.

[22] PubMed consolidates the vast majority of scientific references into a single, searchable database (https://pubmed.ncbi.nlm.nih.gov/), while databases of the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) contain up-to-date information on all deposited genetic sequences, genome assemblies, and protein sequences and structures. The search process may begin with a scientist's name, for example from the laboratory's directory if one is available in the open source, or with the laboratory name as the search term, from which scientist names and research areas may be derived.

[23] See, for example, the National Institutes of Health's Project Reporter; a representative grant report of coronavirus funding related to the Wuhan Institute of Virology: https://bit.ly/35w8yq2

Diagnostic laboratories

Diagnostic laboratories typically receive samples[24] and perform tests to determine what biological agents or indicators of infection may be present. Received samples may be clinical (*i.e.*, human healthcare-related), animal, or environmental, depending on the laboratory. To determine whether the sample contains genetic material of an agent of interest, a polymerase chain reaction (PCR) test is typically performed first.[25] If the PCR test gives a positive result, the laboratory may attempt to grow the infecting agent in culture or—as an outdated practice that nonetheless continues in many countries—deliberately infect, or "challenge," an animal. The culturing approach depends on whether the sample has tested positive for bacteria or a virus. Bacteria are typically cultured using either solid or liquid media ("broth"), with liquid media posing the far greater documented biosafety risk due to an increased risk of aerosol generation, a major cause of laboratory-acquired infections.[26] Viruses are most commonly cultured in cell or tissue culture because viruses must infect cells in order to reproduce. Culturing increases the quantity of infectious agent whether bacteria or virus, increasing the potential inoculum in the case of a laboratory accident, splash, or spill.

On a biosafety scale of 1 to 4, with 4 being the highest level of safety, diagnostic laboratories typically operate at biosafety level 2. Culturing—liquid bacterial culture and viral culture in particular—and animal challenges should often be performed at biosafety level 3, but such practice is not consistent from laboratory to laboratory. With each increasing biosafety level, laboratory practices, safety equipment, and facility controls grow more robust.

However, such practices, equipment, and controls are only capable of reducing the risk of laboratory mishaps if implemented properly, and there is currently no international accreditation system or standard to this end (though the WHO's Laboratory Biosafety Manual represents the *de facto* albeit non-binding standard outside the United States). Therefore, the safety of laboratory activities cannot be assumed by identifying the biosafety level alone, and must be either validated by inspection (if laboratory access is granted) or assessed based on the

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[24] Diagnostic laboratories typically receive "suspected" samples for presumptive diagnosis and/or "presumptive" samples for confirmatory diagnosis.
[25] PCR enables the identification and speciation of very small amounts of genetic material (i.e., nucleic acid) in a sample by amplifying specific, conserved sequences of one or multiple target organisms.
[26] Kimman TG, Smit E, Klein MR. Evidence-based biosafety: a review of the principles and effectiveness of microbiological containment measures. Clin Microbiol Rev. 2008;21(3):403-425. doi:10.1128/CMR.00014-08. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories: Recommendations of a CDC-convened, Biosafety Blue Ribbon Panel. MMWR January 6, 2012;61(Suppl).

safety history of the laboratory itself, related in-country laboratories, and laboratories worldwide that perform similar activities.

Research laboratories

Research laboratories typically work with known biological agents to better understand and/or develop and test medical countermeasures against them, including diagnostics, treatments, and vaccines. Such research often involves growing the biological agent in culture and exposing an animal to it (*e.g.*, via injection or aerosol) in order to evaluate either the biological agent itself or countermeasures against it, thus mirroring the key risks of a diagnostic laboratory. In addition, research may involve genetic manipulation of the biological agent to alter its characteristics, whether by passaging (*i.e.*, the sequential infection of animals to enable genetic change via natural mutations), direct genome editing, plasmid or gene insertion, or recombination with other biological agents. Altered characteristics may include enhanced transmissibility or pathogenicity (so-called "gain of function" experiments), altered pathophysiology, resistance to prophylaxis or treatment, or other potentially harmful differences.[27]

The required biosafety level is determined by risk assessment of the research being performed (*i.e.*, what biological agent, using what techniques), but commonly defaults to level 2 or level 3 depending on what level is available in a given laboratory. There is an exponentially higher number of biosafety level 2 versus level 3 laboratories globally, but level 3 laboratories still number in the thousands. As with diagnostic laboratories, a certain safety level designation only reduces the risk of laboratory mishaps if implemented properly. Safety therefore must be validated by inspection or assessed based on safety history.

Given the broad range of potential biological agents, activities, and biosafety measures at any given research laboratory, risk must be assessed at their convergence. We define "high risk" research by the presence of three characteristics (1) it involves known pathogens of relevance to the outbreak (*e.g.*, genetic relatives or near neighbors); (2) it involves either culturing, animal studies, or genetic manipulation; (3) it occurs in a lab that lacks a strong safety profile.

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[27] See, for example, National Research Council. 2004. Biotechnology Research in an Age of Terrorism. Washington, DC: The National Academies Press. https://doi.org/10.17226/10827. ("Fink Report")

(b) Determine what samples or specimens are present

The goal is to identify samples or specimens that are relevant to the outbreak, whether presenting similarly, related genetically, or otherwise associated by person, place, or time. In a diagnostic laboratory, incoming samples are typically registered in an electronic or paper-based sample receiving log using a unique case identifier. Electronic systems are able to tag the sample to a case record that provides corresponding clinical details (*e.g.*, history, symptoms, and signs suggestive of a particular infectious disease, etc.) and/or sample collection data (*e.g.*, collection source, site, and GPS location), both of which provide clues regarding the sample's contents. Paper-based systems, which remain the standard of practice in many developing countries, contain this same information in aggregate but require investigators to manually trace back the sample from the receiving log to the collection activity and corresponding case data using the unique case identifier. In either case, the primary investigative hurdle is gaining access to the log itself. While there is no international mechanism or body identified for this purpose, we recommend that, in the case of an origin investigation, sample receiving log access be provided on a need-to-know basis to an approved international investigative body by amendment to the WHO's International Health Regulations ("IHR 2.0"). In August 2020, the WHO established an expert Review Committee to examine the effectiveness of the Regulations and recommend any necessary amendments.[28] There is a precedent for such an investigative body under the International Health Regulations' Joint External Evaluation process, where an invited team of international experts evaluates a given country's self-reported global health security data and conducts coordinated site visits and interviews.[29]

Research laboratories maintain a similar record of specimens called an accessioning log, which typically includes "strain passports" of every biological agent in the laboratory's repository. Researchers use this repository to grow "working cultures" of a particular agent to be used in their experiments, such that most research activities can be traced back to the repository specimens and thus accessioning log. As with the sample receiving log, the accessioning log may be electronic or paper-based, and the primary investigative hurdle is access, which we again recommend be provided on a need-to-know basis to an approved international investigative body under IHR 2.0.

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[28] "Committee to review global treaty on response to health emergencies," UN News, August 27, 2020, <https://news.un.org/en/story/2020/08/1071132>.
[29] https://apps.who.int/iris/bitstream/handle/10665/259961/9789241550222-eng.pdf?sequence=1

Additional resources that may indicate what samples or specimens are present in a given laboratory include fieldwork artifacts to determine if/what field samples were collected by the laboratory; purchase records of relevant control strains and/or culture media; publications, sequence submissions, posters, presentations, and grant descriptions; and laboratory descriptions in the open source domain (*e.g.*, websites, social media pages/posts). In practice, reviewing publications and sequence submissions may be the most accessible way to initially and unobtrusively determine whether relevant biological agents are present;[30] however, the laboratory's scientists must be permitted to publish openly in order for such information to be available.

> **Recommendation:** Enable need-to-know access to laboratory sample receiving and accessioning logs by an approved international investigative body under IHR 2.0.

(c) Determine what high–risk activities are being performed

The goal is to identify high–risk culturing (liquid bacterial culture or viral culture), animal studies, or genetic manipulation of relevant samples or specimens identified in the previous step (4b). Diagnostic laboratories, while far less likely than research laboratories to house such activities, may describe their diagnostic activities on websites or social media pages/posts in the open–source domain, in particular if advertising a commercial testing service. Research laboratories may similarly describe their activities on the Internet, mention a vivarium indicating that animal studies are performed onsite, or enumerate research objectives or departments indicating that genetic manipulation is performed onsite. Publications, sequence submissions, posters, presentations, and grant descriptions are likely to be more informative, most notably with respect to genetic manipulation of relevant biological agents.[31] Definitive sources would be expected to include internal biosafety documents, laboratory risk assessments, standard operating procedures , and other oversight/ governance documentation.

[30] See the following for an example of how the Wuhan Institute of Virology was linked to coronavirus gain of function experiments: Menachery VD, Yount BL Jr, Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence [published correction appears in Nat Med. 2016 Apr;22(4):446]. Nat Med. 2015;21(12):1508-1513. doi:10.1038/nm.3985.

[31] For an example of how such sources might be applied in an origin investigation, see point #8 in Leitenberg, M. Did the SARS-CoV-2 virus arise from a bat coronavirus research program in a Chinese laboratory? Very possibly. Bulletin of the Atomic Scientists. June 4, 2020. https://thebulletin.org/2020/06/did-the-sars-cov-2-virus-arise-from-a-bat-coronavirus-research-program-in-a-chinese-laboratory-very-possibly/

(d) Determine laboratory safety profile

The goal is to identify unsafe or uncertain safety conditions where high-risk activities with relevant samples or specimens—identified in the previous step (4c)—are being performed. There is no international system or standard for documenting laboratory safety incidents, but some nations report certain safety records in the open-source domain.[32] Similarly, training records, procedural compliance, and equipment and facility maintenance records may be kept at the facility- or national-level but are rarely reported.[33] In the absence of such records, news reports may provide an indication of laboratory safety in a specific facility or country.[34] Surrogate data may also be utilized to gain insight into the safety of certain activities with certain biological agents regardless of laboratory, enabling some level of safety estimation.[35] Ultimately, as with sample receiving and accessioning logs, an international mechanism must be established to enable safety record access on a need-to-know basis by an approved international investigative body, ideally under IHR 2.0. For completeness, security records should also be collected and evaluated.

Recommendation: Enable need-to-know access to laboratory safety records by an approved international investigative body under IHR 2.0.

[32] For example, the US Federal Select Agent Program, which controls the possession, use, and transfer of dangerous biological agents (and toxins) deemed by the US government as holding the potential to pose a severe threat to public, animal, or plant health, releases an annual safety report of laboratories under its purview; the 2018 report documented 193 accidents the majority of which were the result of conducting work without appropriate safety equipment. See: https://www.selectagents.gov/resources/FSAP_Annual_Report_2018_508.pdf.

[33] For example, laboratories accredited to the International Organization for Standardization's (ISO) "General Requirements for the Competence of Testing and Calibration Laboratories" standard (ISO/IEC 17025:2017) are required to maintain a range of records spanning training, maintenance, and other relevant activities. See: https://www.iso.org/standard/66912.html

[34] See for example, (a) reports on laboratory safety in Wuhan: Josh Rogin, "State Department cables warned of safety issues at Wuhan lab studying bat coronaviruses," Washington Post, April 14, 2020; John Xie, "Chinese Lab with Checkered Safety Record Draws Scrutiny over Covid-19," VOA News, April 21, 2020, https://www.voanews.com/covid-19-pandemic/chinese-lab-checkered-safety-record-draws-scrutiny-over-covid-19; and (b) reports on accidental releases of SARS from a related Beijing laboratory: Robert Walgate, "SARS escaped Beijing lab twice: Laboratory safety at the Chinese Institute of Virology under close scrutiny," The Scientist, April 24, 2004, https://www.the-scientist.com/news-analysis/sars-escaped-beijing-lab-twice-50137. Rosie McCall, "Almost 100 Lab Workers in China Infected With Potentially Deadly Pathogen," Newsweek, December 17, 2019, https://www.newsweek.com/almost-100-lab-workers-china-infected-potentially-deadly-pathogen-1477652.

[35] See, for example, https://thebulletin.org/2019/02/human-error-in-high-biocontainment-labs-a-likely-pandemic-threat/

Step 5. On-Site Laboratory Assessment

If the Laboratory Risk Assessment indicates that further investigation is warranted—i.e., relevant specimens or samples are present, high–risk activities are being performed, and safe working conditions are either lacking or uncertain—the next step is to perform a comprehensive onsite assessment. The assessment would ideally be performed under IHR 2.0 by the same approved international investigative body identified above, which would require unrestricted access to the laboratory and its personnel, conditional upon the findings of the Laboratory Risk Assessment.

The onsite assessment would be expected to include the following activities:

- A review of the overall nature of the research and development program, including aims, objectives, and timelines

- A detailed review of sample receiving and/or accessioning logs, repository contents and strain passports, and sequence libraries

- A review of laboratory access and visitor logs (names, dates, and times)

- A comprehensive laboratory audit, including a review of employee training and performance records, standard operating procedures, and equipment and facility maintenance logs

- A comprehensive biosafety audit, including a review of laboratory and field risk assessments and biosafety protocols, employee medical surveillance and health and safety records, accident and near-miss records, and building automation system archives/alarms (if applicable)

- An animal care and use audit, including types of animals held, experimental and ethical considerations, and disposal procedures for experimental animals

- A biosafety inspection, including validation of biosafety cabinet performance, autoclave performance, laboratory decontamination, effluent decontamination, heating, ventilation, and air conditioning system performance, and building automation system performance (if applicable)

- Employee interviews and observation of regular work duties

- Sample collection from work surfaces, equipment, and—ideally—employees for subsequent analysis in a WHO-accredited laboratory.

The above activities would be expected to maximize the likelihood of a conclusive determination regarding the origin of the outbreak. If results remained inconclusive, field-sample collection and analysis activities would continue until a source was confirmed.

Recommendation: Enable conditional access to the laboratory and its personnel by an approved international investigative body under IHR 2.0.

Differentiating Deliberate Outbreaks

As with outbreaks of natural and laboratory origin, the global community must be able to differentiate between intentional and unintentional outbreaks in order to optimally identify, manage, and attribute deliberate events. Such investigation differs from distinguishing between outbreaks of natural and laboratory origin in two important ways: (1) law enforcement must become involved either at the national level, possibly with support from other states, or at the international level under the UN Secretary-General's Mechanism, likely in coordination with other relevant international organizations such as the WHO or World Organization for Animal Health; and (2) chain-of-custody must be maintained to ensure the integrity of evidence, requiring careful documentation and quality control of sample collection, transport, and analysis.

While a range of scenarios is possible, there are two commonly considered scenarios in which a deliberate event might play out.[36] First, a detection system may be triggered. Second, human or animal populations may begin to fall ill.

In the first instance, detection systems may identify an increased level of a biological agent over "background" levels that exist naturally in the environment, suggesting a deliberate biological event. Investigation would thus immediately fall under the purview of law enforcement in

[36] For a further discussion, see Pilch, R., "Arms Control Measures," in Heggenhougen, H.K., International Encyclopedia of Public Health, 2nd Edition (San Diego: Elsevier Inc., 2016). Other possible ways in which a biological attack may be detected include law enforcement interdiction, whether pre-attack, at the time of attack, or postattack; an allegation by a state or non-state entity that it has been subjected to an attack; notification or tipoff, possibly on the part of the perpetrator; or identification and subsequent characterization of a visible substance such as a powder.

coordination with public-health officials. Of note, most detection systems are only intended to identify aerosol releases of a subset of traditional biological-warfare agents such as *B. anthracis*, such that they are of limited utility versus atypical agents or alternative forms of attack.

In the second and arguably more likely instance, ill human and/ or animal populations would be expected to trigger an outbreak investigation following the initial steps of the methodology described in this article, namely: (1) descriptive epidemiology to determine the *who*, *what*, *when*, and *where* of the outbreak; and (2) analytical epidemiology, including an assessment of the epidemiological triangle and infecting agent genome, to determine the *why* and *how* of the outbreak. Findings at each step may suggest the possibility of a deliberate origin *(Figure 6)*, prompting immediate assessment of potential nefarious sources.

If findings of the outbreak investigation suggest the possibility of a deliberate origin, law enforcement must become involved at the next step of the investigation—additional sample analysis—to ensure systematic sample collection, careful documentation of chain-of-custody, and analysis in an accredited laboratory. In parallel, a law-enforcement investigation would likely be initiated, involving additional evidence collection and examination; patient and witness interviews; coordination with intelligence officials regarding adversary capabilities and motivations; and targeted intelligence-gathering activities.

The corresponding investigative methodology would be expected to resemble the flow diagram presented in *(Figure 7)*.

Step 1. Descriptive Epidemiology

- Multiple, geographically-dispersed index cases are identified
- Infecting agent is a traditional biological warfare agent
- Infecting agent is unusual for location or time of year
- Symptoms are unusual or unexpected (*e.g.*, pulmonary symptomatology)
- Animal populations are affected in concert with humans
- Animal effects are unusual or unexpected for the species

Step 2. Analytical Epidemiology

(a) Epidemiological Triangle Assessment

- Lack of recognizable animal-human interface

  (*e.g.*, exposure to sick animal, tick bite)
- Epidemiological traceback of multiple cases to a common location or exposure

(b) Genome Assessment

- Infecting agent genome matches known weapons strain
- Infecting agent genome displays "frozen evolution"
- Infecting agent genome has been engineered / edited

Figure 6. Indicators of Deliberate Origin

As the epidemiological investigation proceeds, certain findings may suggest the possibility of an intentional source warranting law enforcement involvement.
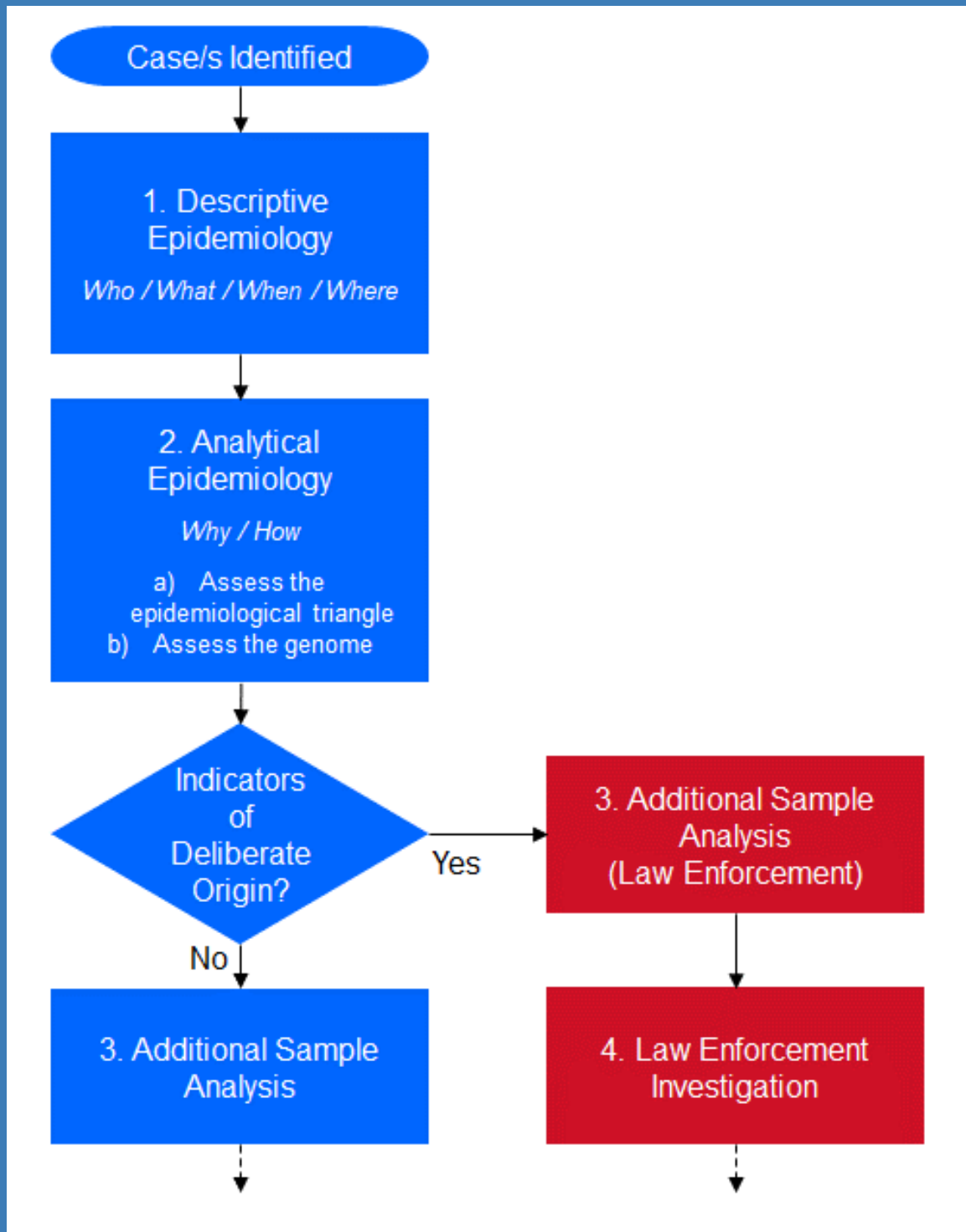
Figure 7. Differentiating Deliberate Outbreak

Any investigation seeking to differentiate between intentional and unintentional outbreaks would probably begin with a typical outbreak investigation (blue boxes), the findings of which may trigger law enforcement involvement (red boxes).

Conclusion

We have outlined a stepwise methodology to guide the assessment of outbreak origins, in particular those seeking to differentiate between natural and laboratory sources of infection. Our methodology builds on a traditional outbreak investigation approach to include a dedicated laboratory assessment component in the event that a natural source is not identified. The goal is to remain minimally intrusive at all times; however, an increasing level of need-to-know information, site, and personnel access becomes necessary as attention shifts toward potential laboratory sources. Accordingly, we recommend that the following measures be implemented by amendment to WHO International Health Regulations—"IHR 2.0"—to ensure the global community's ability to assess infectious disease outbreaks of international concern in the wake of COVID-19:

- Enable need-to-know access to laboratory sample receiving and accessioning logs by an approved international investigative body under IHR 2.0.

- Enable need-to-know access to laboratory safety records by an approved international investigative body under IHR 2.0.

- Enable conditional access to the laboratory and its personnel by an approved international investigative body under IHR 2.0.

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