The Reemergent 1977 H1N1 Strain and the Gain-of-Function Debate

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ABSTRACT **The 1977-1978 influenza epidemic was probably not a natural event, as the genetic sequence of the virus was nearly identical to the sequences of decades-old strains. While there are several hypotheses that could explain its origin, the possibility that the 1977 epidemic resulted from a laboratory accident has recently gained popularity in discussions about the biosafety risks of gain-of-function (GOF) influenza virus research, as an argument for why this research should not be performed. There is now a moratorium in the United States on funding GOF research while the benefits and risks, including the potential for accident, are analyzed. Given the importance of this historical epidemic to ongoing policy debates, we revisit the evidence that the 1977 epidemic was not natural and examine three potential origins: a laboratory accident, a live-vaccine trial escape, or deliberate release as a biological weapon. Based on available evidence, the 1977 strain was indeed too closely matched to decades-old strains to likely be a natural occurrence. While the origin of the outbreak cannot be conclusively determined without additional evidence, there are very plausible alternatives to the laboratory accident hypothesis, diminishing the relevance of the 1977 experience to the modern GOF debate.**

In 1977, an H1N1 influenza virus appeared and circled the globe. Colloquially referred to as the "Russian flu," as the USSR was the first to report the outbreak to the World Health Organization (WHO), the 1977 strain was actually isolated in Tientsin, Liaoning, and Jilin, China, almost simultaneously in May of that year (1). It was atypically mild for a new epidemic strain; the influenza mortality rate (IMR) of the 1977 flu was calculated to be <5 out of 100,000, less than typical seasonal influenza infections (IMR of 6/100,000 people) (2). In addition, the 1977 strain appeared to affect only those 26 years of age and younger (3). These odd characteristics turned out to have a simple scientific explanation: the virus was not novel. The 1977 strain was virtually identical to an H1N1 influenza strain that was prevalent in the 1950s but had since dropped out of circulation (4).

The first researchers to point out the unusual characteristics of the 1977 strain suggested multiple theories to explain the remarkable preservation of the genetic information in the resurgent strain. These possibilities included "sequential passage in an animal reservoir in which influenza viruses replicate without rapid genetic change" or perhaps a "frozen [reservoir] in nature or elsewhere" (4). However, given the extensive experience with typical influenza strain evolution, a natural origin for the 1977 strain is not likely.

There are multiple potential explanations that may explain the viral resurgence, but the possibility that the epidemic was the result of a laboratory accident has recently gained currency in discussions about the biosafety risks of gain-of-function (GOF) influenza virus research and has been used as an argument for why this research should not be performed. GOF studies aim to better understand disease pathways, but they have been controversial because they involve enhancing viral traits, such as pathogenicity or transmissibility, prompting biosafety concerns. There is now a moratorium in the United States on funding GOF research while the risks and benefits are being analyzed, and the possibility that a laboratory escape could lead to an epidemic will be considered and quantified (5). Given the importance of this historical epidemic to modern policy debates, we compared the sequences of 1977 strains to earlier strains and examined available evidence that could explain the 1977 H1N1 resurgence. We summarize these possible hypotheses, discuss informal evidence, and examine the trends of how the most popular explanation has changed over time in relation to political and world events. Explanations for the 1977 H1N1 reemergence include the deliberate release of the virus, a vaccine trial or challenge mishap, or a laboratory accident.

Confirmation that the 1977 strain was derived from a 1950s strain. In 1978, researchers demonstrated that an H1N1 influenza virus strain from 1950 and another strain from 1977 (Fort Warren [FW] and USSR/90, respectively) were unusually closely related, although they were isolated 27 years apart (4, 6, 7). Using the NCBI Influenza Virus Resource database, we analyzed the hemagglutinin (HA) sequences of all the late 1950s H1N1 strains (1947 to 1957) and compared them to the HA sequences of the 1977 isolates (Table 1). We found that the 1977 cluster has the closest degree of genetic similarity to strains isolated in Albany, NY, in 1948 and 1950, strains isolated in Rome, Italy, in 1949, and strains isolated in Fort Leonard Wood, MO, in 1951, instead of the FW 1950 strain examined previously (Fig. 1). These strains are 98.4% identical (Table 2), containing only four differences among the 566 amino acids that make up the protein, evidence that the 1977 H1N1 epidemic strain is derived from a 1950s virus.

Possible origin. (i) Deliberate release. There are historical and epidemiological aspects of the 1977 influenza epidemic that can be considered suspicious. During that time, the Soviet Union employed tens of thousands of scientists to make biological weapons, and as the 1979 release of aerosolized anthrax in Sverdlovsk, Soviet Union, demonstrated, the safety record for the weapons program was not perfect (8). In addition, influenza was considered to be an incapacitating agent, especially to those without previous exposure to a specific virus strain. The lack of immunity to the resur-

Published 18 August 2015

Citation Rozo M, Gronvall GK. 2015. The reemergent 1977 H1N1 strain and the gain-of-function debate. mBio 6(4):e01013-15. doi:10.1128/mBio.01013-15.

Editor Mark R. Denison, Vanderbilt University Medical Center

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TABLE 1 Influenza virus strains from the late 1950s (1947 to 1957) used to compare to the 1977 isolates[a]

Accession no.	Yr isolated	Strain name
U02085	1947	A/Fort Monmouth/1/1947(H1N1)
JN540082	1947	A/USA/L3/1947(H1N1)
CY009612	1947	A/Fort Monmouth/1/1947(H1N1)
CY045780	1947	A/Fort Monmouth/1/1947(H1N1)
CY147342	1947	A/Fort Monmouth/1-JY2/1947(H1N1)
CY019947	1948	A/Albany/4835/1948(H1N1)
CY019971	1949	A/Roma/1949(H1N1)
CY077763	1949	A/Netherlands/002K1/1949(H1N1)
CY147358	1949	A/Roma/JY2/1949(H1N1)
CY077719	1950	A/Netherlands/001G1/1950(H1N1)
CY009332	1950	A/Fort Warren/1/1950(H1N1)
CY021701	1950	A/Albany/4836/1950(H1N1)
CY147334	1950	A/Fort Warren/50-JY2/1950(H1N1)
CY077768	1951	A/Netherlands/002P1/1951(H1N1)
CY021821	1951	A/Albany/12/1951(H1N1)
CY021901	1951	A/Albany/1618/1951(H1N1)
CY022021	1951	A/Albany/14/1951(H1N1)
CY022093	1951	A/Albany/13/1951(H1N1)
CY077889	1951	A/Liverpool/1951(H1N1)
CY147374	1951	A/FLW/1951(H1N1)
CY077748	1953	A/Netherlands/001R1/1953(H1N1)
CY009340	1954	A/Malaysia/1954(H1N1)
CY021053	1954	A/Malaysia/302/1954(H1N1)
CY077725	1954	A/Netherlands/001H1/1954(H1N1)
CY146785	1954	A/Malaysia/JY2/1954(H1N1)
CY008988	1957	A/Denver/1957(H1N1)
CY125862	1957	A/kW/1/1957(H1N1)
CY146793	1957	A/Denver/JY2/1957(H1N1)
CY009292	1977	A/Hong Kong/117/1977(H1N1)
CY020573	1977	A/Tientsin/78/1977(H1N1)
CY121878	1977	A/USSR/90/1977(H1N1)
CY009284	1977	A/USSR/92/1977(H1N1)

[a] Strains were downloaded from the NCBI Influenza Virus Resource database via the accession number.

gent strain was clearly evident by the affected population: individuals who were 26 years of age or younger were especially vulnerable to infection. As this is the predominant age range of the active-duty military population, influenza virus could have been used as a biological weapon to target this group.

Indeed, outbreaks of A/USSR/90/77(H1N1) in military academies were described in official memos as "explosive" (9, 10). The Royal Air Force in Upper Heyford, England, was first affected in January 1978, followed by the U.S. Air Force Academy (USAFA) in Colorado in February. The outbreak at the USAFA was so severe—over the course of 9 days, 76%, or 3,280 cadets, became ill—that all academic and military training was suspended. This was the "first such interruption in training due to influenza illness in the cadet population" (9). An epidemiological investigation at USAFA revealed no link to other outbreaks nor a temporal association between the onset of cases and athletic competitions with other institutions with influenza cases. It should be noted, however, that the investigations of illness at military academies were likely better investigated and documented than similar outbreaks at other universities and colleges.

While it is possible that the 1977 influenza was caused by deliberate release of the virus, the Soviet Bioweapons program, Biopreparat, tended to use influenza preparedness as a cover story for some of the more nefarious work that was being performed (11). For example, the Omutninsk Chemical Factory manufactured large amounts of influenza vaccine and crop production bacteria aboveground, while plague and tularemia were researched in heavily guarded underground facilities. The Omutninsk Chemical Factory's capacity to mass produce viruses and bacteria allowed the production of 100 tons of each weapon annually (11). While Biopreparat has not been judged by experts to have seriously investigated influenza as a bioweapon, there were documented attempts to find the 1918 pandemic H1N1 strain in old icehouses where victims were buried, and studies were performed attempting to create radiation-resistant and aerosolized influenza virus (11). Thus, the likelihood of a biological weapons explanation for the 1977 epidemic cannot be completely ruled out, though it may not be considered likely.

(ii) Vaccine trial or challenge. There are two factors that point to the 1977 epidemic as resulting from vaccine challenge or trials: (i) live attenuated influenza virus (LAIV) research was extensive at the time, and (ii) a 1976 H1N1 swine flu outbreak was feared to have pandemic potential and led to a resurgent interest in H1N1 protection and research (12).

Between 1962 and 1973, almost 40,000 children participated in eight LAIV trials in the USSR (13). Scientists at the Peking Vaccine and Serum Institute in China also carried out clinical trials using live vaccines during the same time period (1). Additionally, there are records of the mass production of a live H1N1 vaccine in Odessa, USSR, in 1977 (14, 15). In the early days of research in the 1940s, LAIVs were often able to regain virulence upon administration to humans and cause disease (16). In addition, many strains isolated from the 1977 outbreak (for example, the A/Tientsin/78/77 isolate) were temperature sensitive (*ts*), meaning that the virus could not replicate at higher temperatures. Temperature sensitivity generally occurs only after a series of laboratory manipulations, typical in generation of LAIVs, and is used as a biological marker of attenuation. While not all of the 1977-1978 strains were temperature sensitive, a comparison of all 1977 strains shows a higher prevalence of the *ts* phenotype than in 1950 strains, supporting the claim that the outbreak may have resulted from attempts at attenuation for vaccine purposes (1, 17). The possibility that the 1977-1978 strain could have resulted from a LAIV trial was also mentioned in a personal communication from C. M. Chu, renowned virologist and the former director of the Chinese Academy of Medical Sciences to Peter Palese, who described "the introduction of this 1977 virus [as] the result of vaccine trials in the Far East involving the challenge of several thousand military recruits with live H1N1 virus" (18). Whether this involved an ineffectively attenuated vaccine or a laboratory-cultivated challenge strain, the deliberate infection of several thousand people with H1N1 would be a plausible spark for the outbreak.

The timing is probably not coincidental. In 1976, the swine H1N1 epizootic influenza virus infected 230 soldiers at Fort Dix, NJ, causing severe respiratory illness in 13 and one death (12). Edwin Kilbourne and others led a campaign that resulted in President Gerald Ford announcing a program to inoculate everyone in the United States against swine flu, and the concomitant production of 150 million doses of influenza vaccine. However, the program was halted soon after, as it became clear that A/New Jersey/1976 was not spreading outside the basic training group. It is possible that an archival H1N1 strain from the early 1950s was used as a challenge virus to evaluate the efficacy of the H1N1 vaccines prepared in response to the 1976 swine flu outbreak. If this virus were not attenuated properly, it may have been able to spread and cause a global epidemic.

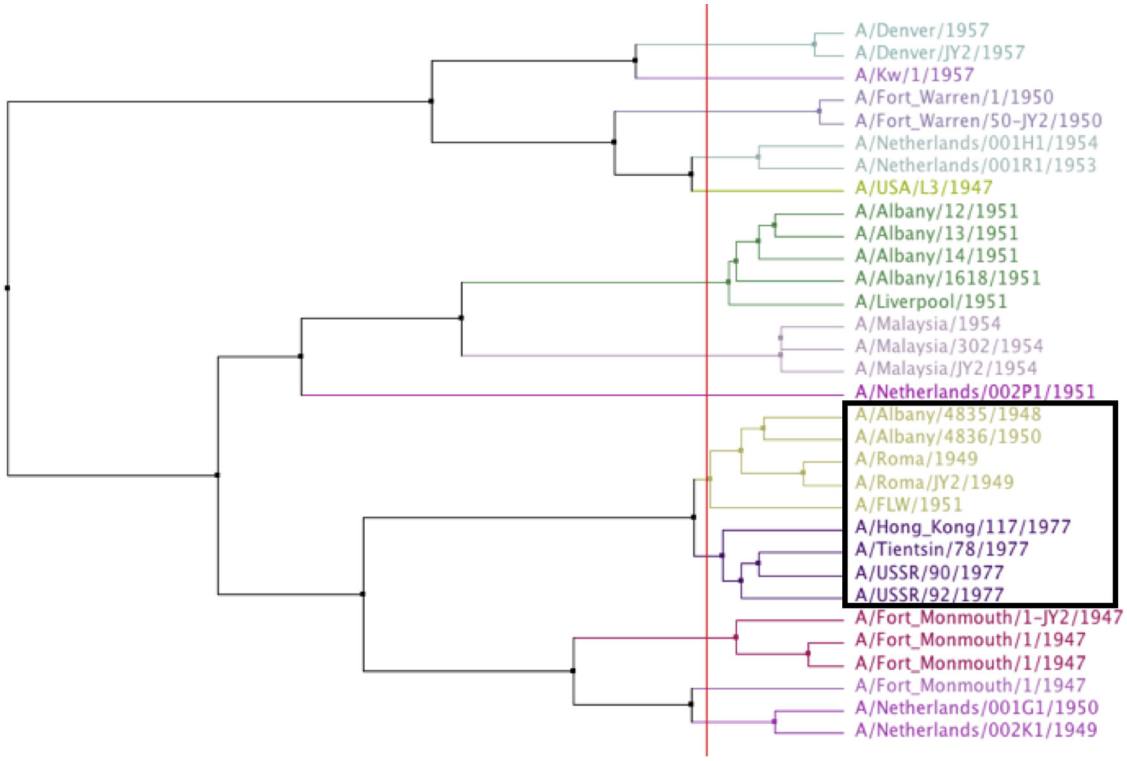

FIG 1 Phylogenetic tree of late 1950s and 1977 influenza virus strains. Distance was calculated using BLOSUM 62 based on HA amino acid sequence. Black box indicates 1977 strains and the most similar late 1950s strains.

(iii) Laboratory accident. A biosafety lapse in a research laboratory is now most often cited as the cause of the 1977-1978 reemergence of the H1N1 influenza virus strain (Fig. 2). The evidence in favor of this possibility is the clear unnatural origin of the virus and its temperature sensitivity, suggesting laboratory manipulations. At the time of the epidemic, however, the World Health Organization excluded the lab accident possibility after discussions with influenza virus laboratory researchers in the Soviet Union and China, finding that "the laboratories concerned either had never kept H1N1 virus or had not worked with it for a long time" (1). It is likely that the swine flu scare the previous year prompted the international community to reexamine their stocks of the latest previously circulating H1N1 strains to attempt to develop a vaccine. However, the tripartite origin of the outbreak in northeast China that produced almost identical isolates is not supportive of the conclusion that this was a single laboratory accident.

It is more likely that either the vaccines produced from these stocks or the viruses themselves used in tests of vaccine development were virulent enough to spark the 1977 epidemic. The bulk of the evidence rests with this possibility: the unnatural origin, mildness of presentation of the virus, widespread dissemination of cases in a short amount of time, temperature sensitivity of the samples, contemporary observations, and existence of live-virus vaccine trials which were occurring at that time.

Explanations for the origin of the 1977 epidemic. Explanations for the 1977 epidemic have varied over time and have likely

TABLE 2 Percent identity of hemagglutinin amino acid sequence of 1977 isolates compared to selected strains from late 1950s[a]

	Alb;1948	Alb;1950	Roma;1949	FLW:1951	T;1977	USSR;1977	FW;1950
A/Albany/4835/1948							
A/Albany/4836/1950	99.65						
A/Roma/1949	99.47	99.29					
A/FLW/1951	99.12	98.94	99.29				
A/Tientsin/78/1977	99.12	98.94	99.29	99.29			
A/USSR/90/1977	99.12	98.94	99.29	99.29	99.65		
A/Fort Warren/1/1950	93.82	93.64	93.82	93.64	93.46	93.46	

[a] The human HA amino acid sequences were downloaded from the NCBI Influenza Virus Resource database. Strains were aligned using MAFFT, and the resulting alignment was visualized and annotated in Jalview. Jalview was used to calculate a pairwise alignment to determine percent identities between the sequences, which are listed in the table. The 1977 strains are more similar to strains from Albany (NY), Rome (Italy), and Fort Leonard Wood (MO), than the original reference Fort Warren (NJ) 1950 strain, highlighted in yellow. The name of the influenza virus strain as it appears in the NCBI Influenza Virus Resource database is listed in the left column. The locations are abbreviated as follows: Alb, Albany; FLW, Fort Leonard Wood; T, Tientsin; FW, Fort Warren.

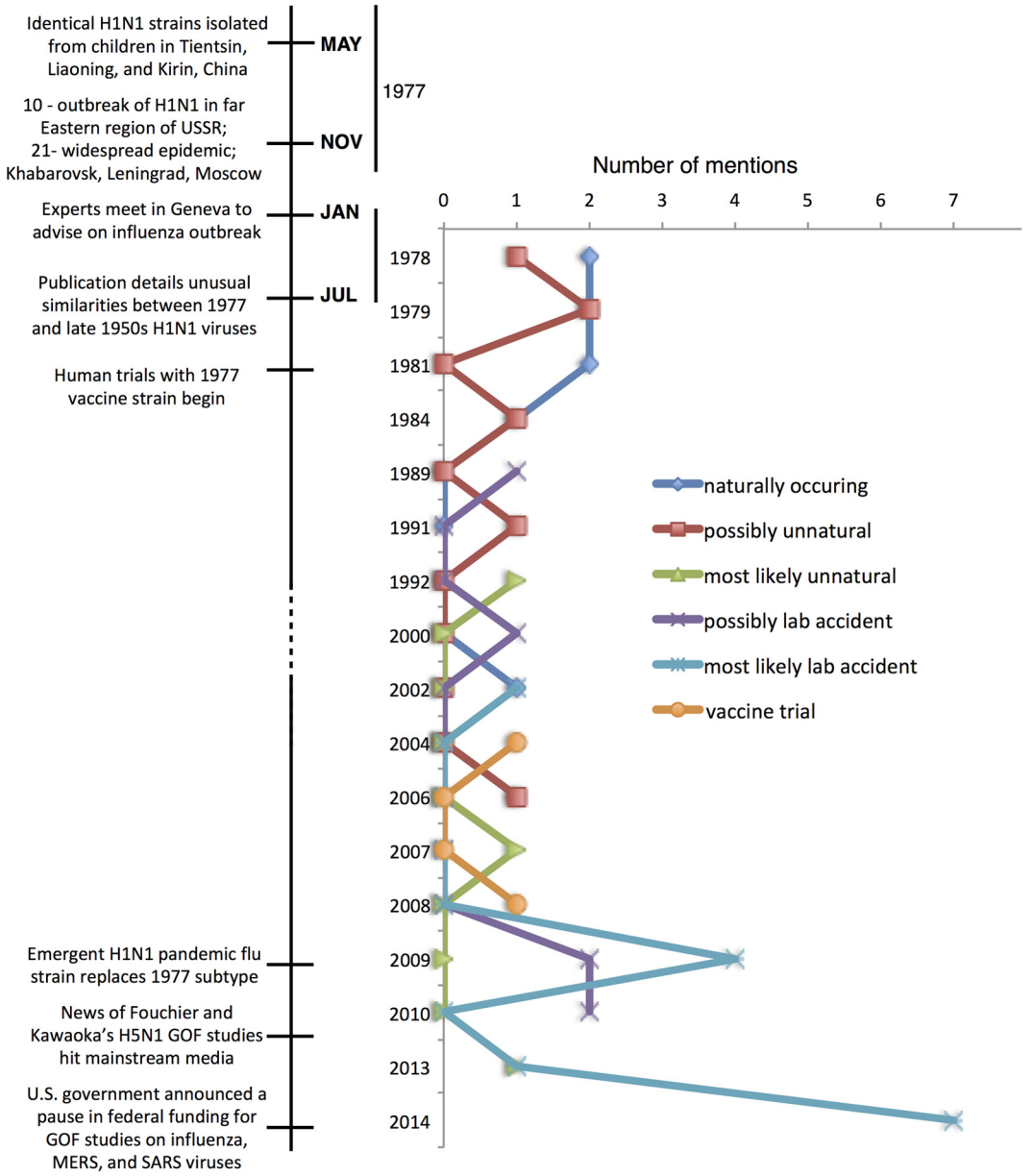

FIG 2 Explanations for the origin of the 1977 influenza epidemic have changed over time. Timeline of the 1977 H1N1 epidemic and relevant modern influenza events (left) correlated with explanations of the origin of the 1977 viral strain (right). A comprehensive search was performed using PubMed, searching for "1977 AND H1N1," which produced 159 results, published between 1977 and 2015. Additionally, "1977 H1N1" was placed in Google search engine, and the first 100 results were examined. Non-English publications were excluded. Out of these results, 41 publications that listed a conclusion regarding the reemergence of the 1977 H1N1 strain were identified. The 41 publications are listed in the Appendix. The conclusion was subcategorized into six types, and the frequencies of these were plotted over time. Both the number of mentions (*y* axis) and the conclusion ("most likely lab accident") increased in prominence over time. MERS, Middle East respiratory syndrome; SARS, severe acute respiratory syndrome.

been influenced by political considerations (Fig. 2). In 1991, in the last days of the Soviet Union, researchers suggested that the virus was "potentially frozen" in nature until its reemergence, an unsatisfying explanation that places no blame on China or Russia for the incident (see reference 13 in the Appendix). In 2008, it was suggested that the epidemic was "probably" the result of an influenza vaccine trial (19, 20). The 2009 H1N1 flu (pandemic H1N1/09 virus) brought the 1977 epidemic back to the forefront, as there were soon-discredited rumors that it was the result of a lab accident (21, 22). This reenergized the discussion on the origin of the epidemic, with explanations now "assum[ing] that the virus was kept frozen in a yet unidentified laboratory," although how it was released was left in doubt (23, 24). However, more-recent publications focusing on the GOF debate have strengthened this stance, concluding that it was "almost certainly due to an escape from a virology lab" (25, 26) (also see references 34 to 41 in the Appendix). Additionally, proponents against GOF research continue to use the potential reemergence from a laboratory accident in their slides and presentations at debates and public forums as a cautionary tale; some examples include the Risks and Benefits of

Gain-of-Function Research Symposium held in December 2014, the keynote presentation at the American Biological Safety Association (ABSA) Biological Safety Conference in 2013, and a Viewpoint article published in *Nature Reviews Microbiology* in December 2014 (27–29).

GOF studies are performed with the aim to better understand disease pathways, but have been controversial because they involve enhancing viral traits, such as pathogenicity or transmissibility, prompting biosafety concerns. Coupled with recent laboratory accidents at the U.S. Centers for Disease Control and Prevention (CDC), the controversy over the potential risks of GOF research led to the recent decision by the U.S. Government to pause federal funding for GOF influenza research and severe acute respiratory syndrome (SARS) research, until an assessment can be made of its risks and benefits (30). The moratorium for MERS and other coronavirus research was lifted, but the evaluation of GOF influenza research risks and benefits is expected to take nearly a year (31, 32).

While the use of the 1977 influenza epidemic as a cautionary tale for potential laboratory accidents is expedient, the relevance to GOF research is greatly diminished if the 1977 epidemic was the result of a vaccine trial or vaccine development gone awry; these are both more plausible explanations than a single laboratory accident. In addition, in 1977, influenza research was performed without modern biosafety regulations and protective equipment, making the lab accident hypothesis much less relevant to the modern GOF debate. While the events that led to the 1977 influenza epidemic cannot preclude a future consequential accident stemming from the laboratory, it remains likely that to this date, there has been no real-world example of a laboratory accident that has led to a global epidemic.

ACKNOWLEDGMENTS

We thank Yoshihiro Kawaoka and Peter Roman for helpful comments and perspectives.

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APPENDIX

Figure 2 bibliography

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