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Review

The relationship between encephalitis lethargica and influenza: A critical analysis

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Since encephalitis lethargica's (EL) prevalence in the 1920s, epidemiologic and clinical debate has persisted over whether EL was caused by, potentiated by, or merely coincident with the Spanish influenza pandemic. Epidemiologic analyses generally suggest that the disorders were coincidental. Beginning in the 1970s, modern experiments on archival brain samples mainly failed to confirm a direct relationship between influenza and EL. These experimental studies have technical limitations, e.g., the appropriateness of antibodies, polymerase chain reaction (PCR) primers and controls, and the extreme paucity and age of available material. These factors render the case against influenza less decisive than currently perceived. Nevertheless, there is little direct evidence supporting influenza in the etiology of EL. Almost 100 years after the EL epidemic, its etiology remains enigmatic, raising the possibility of a recurrence of EL in a future influenza pandemic. *Journal of NeuroVirology* (2008) **14,** 177–185.

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Introduction

Encephalitis lethargica (EL) was a mysterious and devastating early 20th century epidemic that killed by one estimate 500,000 people worldwide (Ravenholt and Foege, 1982). Some survivors suffered sequelae such as postencephalitic parkinsonism (PEP) popularized in the book and movie, *Awakenings*. Today's prevailing view is that the EL and the approximately contemporaneous Spanish influenza pandemic were not etiologically related (Reid *et al*, 2001). Although we recognize (and two of us helped to develop) the evidence against the influenza hypothesis,

it is *prime facia,* not conclusive. This paper examines the case for and against the influenza hypothesis. An open mind about the influenza hypothesis avoids complacency about the potential for another EL-like epidemic accompanying a new influenza pandemic.

Influenza and encephalopathy/encephalitis

Influenza-related encephalopathy, mainly observed in pediatric populations in Asia, can occur at the peak of viral illness and may be fatal (Mizuguchi *et al*, 2007). The cerebrospinal fluid (CSF) is usually normal and the brain shows minimal histopathology, but has severe congestion at autopsy. The virus has been reported to have been recovered at low titers in extrapulmonary tissues, including meninges (Hoult and Flewett, 1958; Delorme and Middleton, 1979). A more severe condition, acute necrotizing encephalopathy, has also been linked to influenza virus infection and other viral pathogens (Mizuguchi *et al*, 1995; Weitkamp *et al*, 2004).

Postinfluenzal encephalitis syndrome is extremely rare and, in contrast to encephalopathy, occurs 2

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to 3 weeks after recovery from influenza. The CSF shows inflammatory changes and recovery is the rule. An autoimmune mechanism has been proposed, but the association with influenza infection is tenuous because virus has not been recovered from the brain or CSF. Nevertheless, influenza virus RNA has been reported by reverse transcriptase–polymerase chain reaction (RT-PCR) of the CSF, and initial serology may already reflect a rising titer (Hayase and Tobita, 1997; Hoult and Flewett, 1958).

Frankova and Jirasek (1974) reported the presence of influenza virus by immunofluorescence in the brains of H3N2 flu victims. Avian derived influenza strains can invade the brain via the trigeminal nerve and other pathways after intranasal inoculation (Reinacher *et al*, 1983; Shinya *et al*, 2000). However, experimental infection with the reconstituted 1918 virus in mice did not identify virus outside the lung, including brain (Tumpey *et al*, 2005).

Influenza is a known cause of viral meningoencephalitis, but it is rare. An 11-year study of over 22,000 cultures of CSF samples in meningoencephalitis recovered 1270 viral isolates, but not a single case with influenza (Polage and Petti, 2006). The shell vial cultures including A549 and Rhesus monkey kidney as well as other cell lines were monitored for ten days and should have been permissive for growth of influenza A (Bartholoma and Forbes, 1989; Lee *et al*, 1992).

Indirect evidence relating EL to influenza

Baron Constantin von Economo, who typically is credited with originally characterizing EL in 1917 (von Economo, 1917), did not think the same organism caused influenza and EL (others credit Cruchet with defining the disease slightly earlier; see Vilensky, *et al*, 2008). However, von Economo believed influenza may have "paved the way for the assumed virus of encephalitis lethargica." He also believed, "A connection is obvious not only from the recrudescence of the encephalitis epidemics in the wake of the influenza pandemic of 1918–1921 and its simultaneous subsidence in the years 1922–1925, but also from historical considerations" (von Economo, 1931 p.98). Similar to von Economo, few other contemporary observers believed EL was caused by influenza, but many believed influenza might "predispose to infection with the virus of encephalitis lethargica" (e.g., Symonds, 1921; Flexner, 1923; Wilson-Smith, 1920; Kramer, 1924).

A common argument against an influenza etiology of EL is that von Economo's first description of EL preceded the pandemic. Oxford (2000) reviewed respiratory disease outbreak reports from World War I and concluded that there were sporadic influenza outbreaks in Europe in 1916 and 1917 that might represent early limited circulation of the pandemic influenza virus. These outbreaks would have been roughly contemporaneous to the first reported EL cases. An earlier review, however, suggested that they were the final sputtering of the preexisting influenza subtype (Jordon, 1927). Unfortunately, in the absence of archevirologic analyses of autopsy material from these and other earlier outbreaks, it is impossible to determine their etiology.

Another objection to the influenza theory of EL is that whereas the influenza pandemic apparently spread from North America to Europe in 1918, EL appears to have spread from Europe to North America. However, it is conceivable a variant influenza virus spread from East to West during the early phase of the pandemic, or that pandemic influenza circulated early in at least low levels in Europe. Furthermore, EL may have existed in North America prior to its recognition there as a nosological entity. This is plausible due to poor information exchange between World War I belligerents, censorship, only annual indexing of the medical literature, and the publication of initial case reports in German and French.

The time lag in outbreaks of the two diseases at the same locations also weighs against the influenza/EL hypothesis. Possibly EL was a delayed autoimmune response triggered by influenza as hypothesized for postinfluenza encephalitis (cf. above). Experimental support for the autoimmune theory is provided by a single immunization study in rabbits. Infection with H1N1 influenza viruses (A/NWS/33, A/WSN/33, A/Bellamy/42, and A/New Jersy/76), but not other influenza A and B strains, produced antibodies to a 37-kDa neural protein also present in humans. These antibodies were produced by antigen challenge alone without clinical infection (Laing *et al*, 1989).

The difficulty of proving an autoimmune etiology for influenza encephalitis is demonstrated by the recent claim that modern "EL" is caused by poststreptococcal disease (Dale *et al*, 2004). Even assuming that these sporadic cases represent true EL, there are no data on duration of disease, 60% did not have magnetic resonance imaging (MRI), and 35% did not have anti–streptolysin O elevation. Furthermore, the putative anti–basal ganglia antibodies detected were not documented to cross-react with streptococcal antigens. They also varied in specificity between patients, suggesting they are secondary to an immune condition rather than causative (Vincent, 2004).

It is also reasonable to suggest that neural damage could be a direct effect of influenza, but with a delayed clinical onset because symptoms do not appear until extensive neural destruction occurs. Fearnley and Lees (1991) estimated that Parkinson's disease (PD) has a 5-year preclinical phase with onset only after striatal dopamine content is reduced by 80%. Although chronic influenza infection is unknown clinically, Takahashi and Yamada (1999) argued influenza could persist in neurons by evading immune surveillance and cite evidence of influenza persistence in cell culture and detection of influenza sequences for

long periods in children born to mothers with gestational influenza.

A further objection to an etiological relationship between influenza and EL is the lack of influenza history in two thirds of EL patients. Modern testing, however, demonstrates the unreliability of clinical judgment either to diagnose or exclude influenza. An influenza case definition of fever and cough identified 80% to 86% of cases detected by RT-PCR or viral culture, but sensitivity was poor in children (63% to 65%) and after age 65 (58% to 77%). Specificity of the clinical case definition was also low (35% to 58%), as was positive predictive value (41% to 60%). Results should be much worse in a population prospectively monitored for asymptomatic infection (Ruest *et al*, 2003).

Mild influenza infections preceding EL might be undiagnosed or under reported. Recently, two children died of probable highly pathogenic avian H5N1 influenza virus-associated encephalitis apparently without respiratory presentation (DeJong *et al*, 2005). On the other hand, asymptomatic infections probably reflect low respiratory viral titers (Richman *et al*, 1976; Zambon *et al*, 2001). Subclinical Spanish influenza infection seems an unlikely etiologic basis for subsequent EL pathology given the marked acute inflammatory response ('cytokine storm') characterizing the pandemic, at least in animal models (Kash *et al*, 2006; Kobasa *et al*, 2007). If an autoimmune mechanism is responsible for EL, then fever and symptoms of vigorous immune response in the initial influenza infection should be evident.

Even at the peak of an influenza epidemic, only one third of patients with influenza-like illness actually have influenza (CDC, 2000). During an influenza epidemic, and applying a case definition of fever and two associated symptoms (headache, myalgia, sore throat, or cough), a study resulted in 23% false-positive cases unconfirmed by culture, serology, or multiplex RT-PCR collected within 48 h of onset (Zambon *et al*, 2001). In a pandemic setting, however, a higher percentage of influenza-like illness is likely to reflect infection with the pandemic strain.

The closest epidemiological inference between influenza and EL can be drawn from pediatric cases (Beverly and Sherman, 1924; Ebaugh, 1923; Jenkins and Ackerson, 1934). Neal (1920) reported EL in 58 children, "...in a large proportion of cases, ... the onset has been preceded by an attack of clinical influenza," (p. 337). Accordingly, influenza shedding in children occurs at higher levels and may persist for twice as long (up to 13 days after symptom onset as detected by culture) as in adults (Richman *et al*, 1976).

A final objection to the influenza/EL hypothesis is differences in prevalence, clinical features, infectivity, and sequelae between the diseases. Maurizi (1989), however, referred to other staged-diseases that have very different signs and symptoms within each stage, but the same etiology, e.g., primary and tertiary syphilis, measles, and subacute sclerosing panencephalitis.

Direct evidence

Experimental studies for and against the influenza hypothesis are presented in Table 1, with a summary of the EL/PEP subjects utilized. The studies are arranged by type: serology—examined antibodies against influenza present in the serum of PEP patients; RT-PCR—tests for influenza viral RNA in brain tissue samples; and, antigen (immunohistochemistry)—utilizing anti-influenza antibodies to search for influenza proteins still present in brain tissue samples. In our compilation of Table 1, we excluded studies of purported modern EL because, in the absence of pathognomonic symptoms, signs, or laboratory findings, diagnosis outside the epidemic is especially tenuous (Vilensky and Gilman, 2006). Even epidemic EL diagnosis was often questionable because it was based on exclusion of more common neurological diseases (cf. below).

Despite the strong apparent weight of direct evidence against the influenza hypothesis (all but one of the listed studies in Table 1 report negative results), they are all limited by the extreme rarity of acute EL material and by the difficulty of retrospective confirmatory diagnosis of acute EL. The Lo *et al* study appears strongest in this respect. Three of their cases had courses shorter than a week (24 h, 3 days, 1 week). However, the 3-day case was a 3-month infant and diagnosis of EL in such a patient seems questionable. Furthermore, the meaning of "fulminating EL" in the 1916 London case is unclear and that patient died before the first published case in 1917 in Vienna. Lo *et al*'s second 1916 case is suspect because the patient had a clinical diagnosis of botulism. Only two of McCall *et al*'s patients were deemed certain EL cases, only one had classic ocular signs, and their shortest courses were 7 and 11 days.

Presumably as a means to compensate for the shortage of EL brain tissue, many studies used more readily available PEP tissue or serum. PEP, which may have followed as many as 50% of EL patients (Duvoisin and Yahr, 1965), is easier to diagnose than EL and to distinguish from idiopathic PD. The best differentiators of PEP from PD are early age onset, symptoms persisting over 10 years, oculogyric crises, and palilalia (Hoehn and Yahr, 1967; Litvan *et al*, 1998).

The two serological studies found PEP patients no more likely to have influenza exposure than PD patients. Differences in circulating influenza strains detectable in hemagglutination-inhibition tests usually occur after only 2 to 5 years of antigenic drift (Wright *et al*, 2006). Therefore, these studies employed influenza strains that might not cross-react with the 1918 strain (cf. below). Of course, the 1918 influenza circulated before the advent of virology and technology to culture or freeze virus, or raise antibodies.

Table 1 Experimental studies of the influenza hypothesis

Source	Type	Findings	Clinical details	Comments
Marttila *et al*, 1977a	S	No difference between 23 PEP and 421 PD and controls for antibodies to influenza strain PR/8/34	No details provided on how PEP was distinguished from PD	Authors indicate results do not support the findings of Gamboa *et al* (1974)
Marttila *et al*, 1977b	S	No significant differences between 20 PEP and 55 PD and matched controls in antibodies to influenza strains PR/8/34 and Sw/1976. Same PEP patients as Marttila *et al* 1977a.	PEP patients either had reliable history of encephalitis or oculogyric crises	Authors consider patient antibody levels to be associated with the virus most likely to have caused pandemic influenza
Isaacson *et al*, 1995	R	Could not detect influenza RNA in 7 PEP patients	No clinical data provided	Implies that influenza virus may have been detectable during the EL phase of disease
McCall *et al*, 2001	R	Detected β2-microglobulin mRNA from 2 EL, 3 possible EL and 2 PEP victims but unable to detect any influenza RNA fragments	Only 1/5 EL patients had ocular signs; lethargy definite in 3/5.	Authors noted the possibility that secondary effects of influenza, e.g., autoimmunity was responsible for EL
Lo *et al*, 2003	R	Detected mRNA of the β-actin gene in 8 EL brains EL but unable to detect flu RNA	Clinical information provided is scanty; 2/8 EL cases from 1916; 1 patient died in 24 h; another died of influenza; one was 3-month-old infant	One of the authors, Oxford (2000), suggests the PCR technique utilized may have lacked sensitivity to detect influenza RNA
Gamboa *et al*, 1974	A	NWS and WSN antigens detected in hypothalamus and midbrain in 6 PEP patients, but not PD patients	4/6 PEP had oculogyric crises; all had encephalitic features consistent with von Economo	Authors state results are not conclusive proof of the influenza etiology of EL
Elizan and Casals, 1989	A	Negative immunostains for several flu strains including in WS/33, Sw/1976 and NWS in one acute EL and one PEP brain	PEP had oculogyric crises; EL case was 20-years-old male who died 1 week following headache, drowsiness, fever, and coma	Authors suggest negative results possibly because antigen detection not sensitive, proper antigen not tested for, or virus was not present at death

S = serology type study; R = RTPCR type study; A = antigen type study.

The 1918 influenza virus (A/South Carolina/1/18 and A/Brevig Mission/1/18) was sequenced from 1997 to 2005 (Taubenberger *et al*, 1997; Reid *et al*, 1999, 2000, 2002, 2003a, 2003b, 2004, ; Basler *et al*, 2001; Taubenberger and Reid, 2002; Tumpey *et al*, 2004; Taubenberger *et al*, 2005) and has now undergone a remarkable laboratory anabiosis. Serologic ambiguity could partly be resolved by retesting directly the antibodies used in these studies against the 1918 virus. Another technique would be to raise antibodies to the 1918 virus and retest acute EL material.

Additionally, there is new information on the cross-reactivity of several of the antibodies used in the serology studies. Antisera raised in ferrets with 1918 HA/NA recombinant virus produced a titer of 2560 by hemagglutination inhibition. In contrast, A/swine/Iowa/30 antisera had an HI titer of 1280 against the 1918 HA/NA recombinant, but only 320 for A/WS/33 and 40 for A/PR/8/34 (Tumpey *et al*, 2004). All of the H1N1 virus antibodies cross-reacted to some extent, but the antibodies used in the postmortem tissue studies thus likely reacted only weakly against the 1918 influenza virus, compatible with the significant antigenic drift that occurred in humans after the pandemic (Taubenberger, 2006). These data may reduce the negative predictive value of the results obtained.

It would be theoretically possible to test the reconstructed 1918 virus against any surviving PEP serum samples. Unfortunately, this would not likely be helpful because almost all humankind was exposed to the 1918 influenza virus and its descendent early antigenic variants in the 1920s. For cohorts who experienced it as their first influenza infection, nearly 100% still had detectable antibodies over half a century later (Rekart *et al*, 1982; Masurel *et al*, 1983).

A possible flaw in the RT-PCR studies listed in Table 1 is primer mismatch due to influenza viral mutation between 1918 and the later sequences against which primers were designed. The McCall *et al* (2001) primers were aligned to A/Puerto Rico/8/34 (H1N1) and preliminary 1918 sequence information. The Lo *et al* (2003) work was also performed before publication of the most relevant 1918 sequences. Both groups appropriately amplified influenza genes that evolve very slowly because they do not encode surface antigens. Comparison of primers to the 1918 sequence reveals few mismatches. Two of the McCall *et al* primer sets are perfect matches for 1918 matrix and nucleoprotein sequences. These amplify segments of 112 and 104 base pair (bp) *vis-à-vis* the successfully amplified beta-2-microglobulin control gene amplicon of 158 bp. Effectiveness of the McCall *et al* primers was confirmed against RNA from positive 1918 influenza lung material at the end of the project. The working positive controls were A/Puerto Rico/8/34 to allow exclusion of false positive results by sequence. The Lo *et al* primers have at

least one mismatch in the outer primers of each set compared to the 1918 influenza sequences. Whereas these primers would likely have detected the 1918 influenza virus, their efficiency against the 1918 virus can only be determined empirically, and this was not done because the authors did not possess a 1918 influenza sample.

Lo *et al* employed a creative control of mouse brains infected with influenza A/NWS/33 by intracerebral inoculation. This has the virtue of possibly simulating the low central nervous system (CNS) viral titers that may have been present in EL and of performing extractions from fixed brain tissue. However, this control is considerably mutated from the Spanish influenza strain. Influenza A/NWS/33 and A/WSN/33 are not wild-type, naturally occurring viruses. Extraordinary measures were employed to artificially force the virus to mouse neurotropism (Stuart-Harris, 1939). Attempts to produce the effect in several related influenza viruses (e.g., A/swine/Iowa/30) subsequently failed (Francis and Moore, 1940). Forced adaptation of A/WS/33 produced mutations, including the loss of a glycosylation site in the neuraminidase stalk that is conserved in other related influenza A strains. This mutation has been associated with mouse neurotropism and pantropic brain infections (Li *et al*, 1993).

This mutation is not present in the neuraminidase of the 1918 virus (Reid *et al*, 2000). Tumpey *et al* (2005) performed experimental intranasal infections with 10^6 plaque-forming units (PFU) of the reconstituted 1918 strain and found at 4 to 5 days post infection that virus was undetectable in extrapulmonary tissues including brain, heart, liver, and spleen. This lack of pantropic or neurotropic spread is consistent with human autopsy findings during the 1918 pandemic and supports the conclusion that the 1918 virus replicated only in the respiratory tract (Taubenberger, 2006).

Comparison of primers used in the RT-PCR–based EL studies to A/NWS/33 (H1N1) produced similar results. The Lo *et al* matrix primers are a perfect match and indeed this was their positive control. The two matrix primer sets in the McCall *et al* study have one mismatch each. We may presume that all the primers should have worked, but this can only be determined empirically.

Unlike the 1918 situation, the Lo *et al* control was harvested at an optimal time of 3 days post infection. Given their small size, the mouse brains were also rapidly and efficiently fixed, being removed from the skull under 70% formal saline. Victims in 1918 died at various points in the disease course with variable postmortem intervals at room temperature before autopsy, which may have subjected the brains to autolysis. Autolysis has been shown to decrease housekeeping gene mRNA by 64% at 24 h (Abrahamsen *et al*, 2003) and up to 500-fold by 100 h (Smolinski *et al*, 2005). Various studies reporting RNA stability in brain employ modern postmortem refrigeration (Barrachina *et al*, 2006; Johnson *et al*, 1986; Schramm *et al* 1999; Yasjima *et al*, 2001). Refrigeration did not become prevalent in morgues until after chlorofluorocarbons became available in the 1930s (Washington Post, 1929; Krasner-Khait, 2000).

Additionally, the mouse brain control cannot simulate eight decades of paraffin block storage under uncontrolled temperature/humidity conditions. Low copy number mRNA can become undetectable by RT-PCR after only 5 years in storage (Smolinski *et al*, 2005). Both the McCall *et al* and Lo *et al* studies were limited by failure to test non-CNS tissues, especially lung, and thus cannot rule out a remote effect of influenza.

Amplification of RNA from formalin-fixed, paraffin-embedded tissue has been reported for fragments as large as 1000 bp (Krafft *et al*, 1997). During the sequencing of 1918 influenza material, however, the largest amplifiable fragment was 166 bp. Usually, only fragments of 100 to 120 bp could be amplified (Reid *et al*, 1999). This suggests the Lo *et al* influenza targets of 240 to 320 bp could probably not have been amplified from formalin-fixed, paraffin-embedded 1918 tissue. It is possible their positive control was present in high copy numbers and the random processes of RNA degradation and formalin cross-linking might allow longer templates to undergo RT-PCR amplification. Experimental evidence of this effect has been reported (Smolinski *et al*, 2005).

Only the Gamboa *et al* immunohistochemistry study supported the influenza hypothesis experimentally. However, their detection of A/WSN/33 and A/NWS/33 antigens is inconsistent with the fact that A/PR/8/34 and A/WS/33 antibodies (one known to cross-react with 1918 influenza and the second the virus from which A/WSN/33 and A/NWS/33 were derived) did not react at all. If this were a simple neutropism effect, then why would antibodies to A/swine/Iowa/30 (the virus most closely related to 1918) react in one but not the other five cases? These authors did not consider their study to be conclusive and it could not be replicated (Elizan and Casals, 1989).

Furthermore, all of Gamboa *et al*'s material was derived from PEP patients more than 45 years after EL onset. Viral detection after half a century suggests chronic influenza infection but the occasional persistent or abortive influenza infection observed in cell culture does not occur clinically (Stuart-Harris and Schild, 1976). The length of persistent influenza cell culture infection is also far too short to be consistent with EL (Gavrilov *et al*, 1972; Tyrrell, 1959; Wilkinson and Borland, 1972).

Viral antigen can be detected several days longer in infection than the culturable virus (Richman *et al*, 1976; Taubenberger and Layne, 2001). Thus, it seems likely that proteins would better survive formalin-fixation than labile viral RNA. Nevertheless, it would be surprising if influenza viral RNA was undetectable

by RT-PCR in acute cases, but influenza viral proteins would be detectable by immunohistochemistry in PEP cases decades after the acute EL event.

Negative results from most PEP cases weigh against chronic influenza infection analogous to the defective measles virus in subacute sclerosing panencephalitis (Cattaneo *et al*, 1988). Cases of rapid EL demise are crucial to rule out acute influenza infection. Influenza viral isolation by culture peaks on nasopharyngeal washes 2 days after experimental human infection and declines slowly with little virus detectable 6 to 8 days after infection (Richman *et al*, 1976; Taubenberger and Layne, 2001). Compared to culture, RT-PCR increased detection of influenza in nasal swabs 45%. These were true positives confirmed by a second method. Because genetic material can be present in the absence of infectious viral particles, RT-PCR methods can detect influenza later in the disease course than culture (Krafft *et al*, 2005). Among 50 influenza patients with initial RT-PCR–positive throat swabs, 68% were still positive on the second day, only 32% on day 3, 25% on day 5, and 20% by day 7 (Zitterkopf *et al*, 2006). These are days of hospitalization, doubtless some time after disease onset. Because the patients tested on each day were still hospitalized, they were the sickest and possibly immunodeficient with prolonged shedding.

Applying this information to the available EL cases, if they were true cases and if influenza directly invaded the brain to survive fixation, storage, and extraction, then viral RNA were theoretically detectable by RT-PCR. Given the technical difficulties, however, the probably low CNS viral titers and small number of acute cases, negative predictive value may be limited.

Conclusions

It is frustrating that after almost a century of effort, we know very little about the etiology of EL. Pertaining to the influenza hypothesis, it is invariably difficult to prove a negative case. There are myriad technical limitations that potentially could cause experimental false negatives. Contemporary morgues were not refrigerated so autolysis likely caused viral degradation, after which brain is notoriously difficult to fix, and formalin fixation is suboptimal for molecular studies. A 1-day delay of formalin fixation reduces measurable mRNA 99% and the ratio between measured quantities of different genes could vary over 10-fold. This effect is most pronounced for large amplicons; reducing the amplicon from 497 to 136 bp increased quantifiable mRNA 100-fold (Abrahamsen *et al*, 2003; Smolinski *et al*, 2005). Additionally, there is the difficulty of lysate processing from formalin-fixed, paraffin-embedded tissue and the common problem of PCR inhibitors. All of these factors may adversely affect sensitivity.

Furthermore, cellular housekeeping mRNAs may be more plentiful and therefore statistically more likely to survive postmortem degradation in amplifiable lengths than influenza RNA. This effect might lower the power of negative RT-PCR studies such as those of McCall *et al* and Lo *et al.* Most available cases had clinical courses long enough before their death that an acute viral infection might no longer be detectable.

Influenza causation might provide a convenient explanation for EL's disappearance because the 1918-like influenza strains ceased human circulation sometime before 1933 when the first human strain was cultured (Taubenberger, 2006). Empirical studies provide little evidence of influenza causation; but, as we have demonstrated, technical limitations and the shortage of appropriate material for testing limit the degree of confidence. Therefore, unless another cause of classical EL is positively identified, its return in the context of another influenza pandemic remains formally possible. Such a recurrence would provide an opportunity to establish the etiology of EL using modern methods.

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