Case Report

An unusual case of influenza-like illness after yellow fever vaccination

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ABSTRACT

Yellow fever (YF) is an important public health concern in areas where the disease is endemic. For more than 60 years a highly effective live attenuated vaccine has been available, its widespread use resulting in a dramatic decrease in the number of cases. On rare occasions, YF vaccine can cause mild to severe disease and rare adverse vaccine-associated events have been reported. Additionally, an average viremia of 3–5 days after administration of the YF vaccine has been published. Here we present a case where YF vaccine was isolated in cell culture from a respiratory swab collected from a patient presenting with influenza-like illness. To the best of our knowledge, this is the first report finding replicating YF vaccine in the respiratory sample of a post inoculated individual.

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1. Why this case is important

Influenza-like illness (ILI) is a major health issue during winter months in temperate climates and can be caused by a large range of pathogens including influenza, rhinovirus, human metapneumovirus, parainfluenza viruses and some bacteria [1]. The use of culture to detect respiratory pathogens became increasingly common in clinical virology laboratories during the last 50 years with the commercial availability of cell cultures. However, with the introduction of commercially manufactured and FDA-approved molecular assays, including multiplexed PCR assays, the preference for testing viral pathogens using cell culture systems is diminishing [2]. In this case study we describe the utility of cell culture followed by electron microscopy (EM) to identify an unknown pathogen causing ILI in a healthy 19 year old woman. Moreover, the case highlights the importance of providing relevant case history in order to avoid unnecessary testing.

2. Case description

A 19 year old female was seen in a college student health clinic, presenting with fever of 103 °F (39 °C) for 3 days, nausea, vomiting, cough and myalgia. The patient history did not note any recent travel, vaccination, animal contact or insect bites. ILI was the initial diagnosis and a respiratory swab was sent for influenza testing.

2.1. Investigation of causative agent

The testing algorithm included both molecular assays and conventional cell culture. The respiratory swab was inoculated into primary Rhesus monkey kidney cells (Diagnostic Hybrids, Athens, OH), human lung adenocarcinoma cells (A549), human embryonic lung cells (HEL) and epithelial colorectal adenocarcinoma (Caco-2) cells. All inoculated cell lines were examined for cytopathic effect (CPE) three times a week for 2 weeks. In parallel, the primary sample was extracted using the bioMérieux easyMAG, tested for influenza A and B using the Centers for Disease Control and Prevention (CDC) Human Influenza Virus Real-time RT-PCR Diagnostic Panel (unpublished protocol, details are available from the CDC on request), and for Influenza A and B, respiratory syncytial virus A and B, human metapneumovirus, rhinovirus, enterovirus, parainfluenza viruses 1–4, and human coronaviruses OC43, 229E, NL63 and HKU1 with the Qiagen ResPlex II kit (Qiagen, Germantown, MD). Multiple real-time (RT)-PCR assays were used for the detection of cytomegalovirus, Epstein–Barr virus, human herpesvirus 6, adenovirus, enterovirus, herpes simplex virus 1, herpes simplex virus 2, varicella zoster virus [3], rhinovirus [4], *Mycoplasma pneumonia* [5], *Legionella pneumophila* [6], and *Chlamydia pneumonia* (unpublished assay provided by K. Musser).

2.2. Results

CPE was observed at 9 days in the Caco-2 cells and all other cell lines exhibited no viral changes after a total of 14 days incubation. Further, the Qiagen ResPlex II assay and all of the real-time (RT)-PCR assays were negative.

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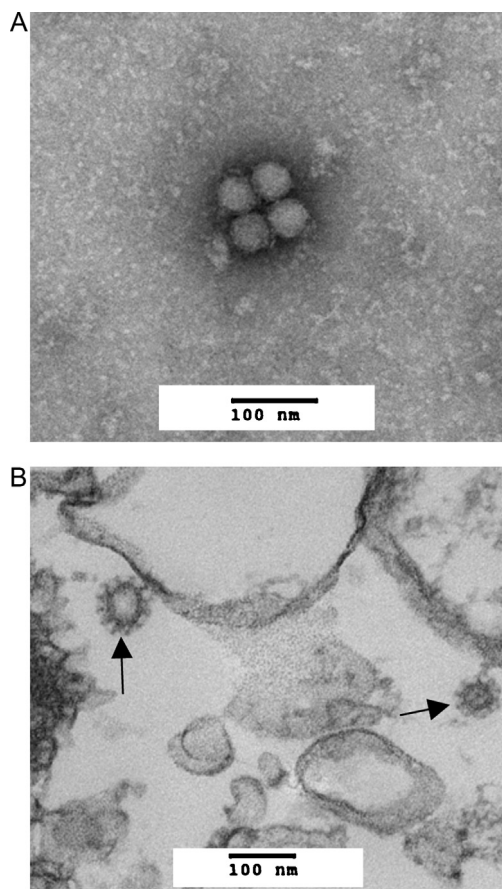E-mail address: dml20@health.state.ny.us (D.M. Lamson).

Fig. 1. (A) Negative stain electron micrograph of isolate from Caco-2 cells (bar represents 100 nm). (B) Thin-section electron micrograph of isolate obtained from Caco-2 cells. Virus particles can be seen adjacent to the arrows (bar represents 100 nm).

To further investigate the agent that had grown in the Caco-2 cells, the isolate was extracted using the Qiagen Viral RNA mini kit and tested with the same panel of molecular assays as those used for the primary sample; all results were negative. At this time the submitting laboratory was contacted to obtain additional patient information that may elucidate what could be propagating in cell culture. The clinician reported that there was nothing relevant of note in the history. The virus cultures were then prepared for shipment to the CDC for EM.

Briefly, 1:1 mixtures of infected scraped cells and 5% glutaraldehyde were centrifuged at $1000 \times g$ for 10 min. Supernatant of the centrifugate was adsorbed to formvar-carbon coated grids and negatively stained with 5% ammonium molybdate-1% trehalose, pH 6.9. Subsequently, thin-section electron microscopy was performed on the centrifuged cell culture precipitate using routine methods, similar to those described previously [7–9]. Specimens were viewed within a Tecnai BioTwin electron microscope operating at 80–120 kV (FEI Company, Hillsboro, OR). Images were captured digitally by using a 2K × 2K CCD camera (Advanced Microscopy Techniques Corp., Woburn, MA).

The images observed on EM were consistent with either a togavirus or a flavivirus (Fig. 1A and B). Consequent to this observation, nucleic acid from the Caco-2 isolate was amplified using a pan-flavivirus conventional RT-PCR assay that targets a portion of the NS5 region [10]. PCR products of the expected size were sequenced using the PCR primers. Resulting sequences were combined and BLAST analyzed, demonstrating the highest sequence similarity (99%) to YF virus. The student health clinic was contacted for further discussion on the patient history. They provided

Table 1
Primers used in RT-PCR to amplify the membrane (M) and envelope (E) genes of yellow fever virus.

Primer	Sequence	Region	Publication
CAG	CTGTCCCAATCTCAGTCC	M	Onyango et al. [11]
YF7	AATGCTTCCTTTCCCAAAT	M	Onyango et al. [11]
DL2	GAGTTTGTTTGAGGTTGATCAGAC	E	This Study
DL4	GAGCCAAACACCGTATGAATTC	E	This Study

M = membrane; E = envelope.

additional information, including their administration of the YF vaccine to the patient 8 days prior to the collection of the original respiratory specimen, and also that the patient had recovered.

To further characterize this YF virus, published primers [11] and primers designed in this study using Geneious Pro 5.6 (Biomatters, San Francisco, CA), were used to further sequence the Membrane (M), and envelope (E) gene regions (Table 1). These targets further identified the virus as having the highest similarity to YF vaccine.

3. Other similar and contrasting cases in the literature

Multiple papers have been published on adverse events associated with the YF vaccine; however, the literature focuses on viscerotropic disease and multisystem organ failure that mimic wild-type disease progression [12–14]. Here we present a case suffering ILI with fever and cough, eight days after receiving the YF vaccine. YF vaccine was isolated in Caco-2 cells from a respiratory swab. The virus was further prepared, stained and imaged by EM before it was identified by sequence analysis of the NS5 gene. Additional sequence analysis of the E and M gene verified highest identity to YF vaccine (strain 17D), reversions to wild-type YF virus (Asibi strain) were not present [15].

4. Discussion

YF vaccine is a highly effective vaccine that has the ability to greatly decrease the incidence of YF in people traveling to endemic areas. Despite the possibility of an adverse event, the risk of a severe event post-vaccination is low (http://www.cdc.gov/vaccines/pubs/vis/downloads/vis-yf.pdf). Since replicating YF vaccine was isolated and propagated in cell culture, and in the absence of detection of other known infectious agents, the potential cause of the ILI was the YF vaccine the patient received 8 days previously. Although a known viremia occurs 3–5 days after inoculation of the YF vaccine [16], this is the first report, to our knowledge, on isolation of YF vaccine from the respiratory sample of a recently vaccinated individual.

This case highlights the importance of the collection and submission of an appropriate case history, and the ongoing availability of cell culture and electron microscopy to assist in the identification of unusual or unexpected agents. EM and cell culture isolation have been utilized to identify many previously unknown and unsuspecting viruses associated with disease outbreaks [17]; such examples include Zaire ebolavirus, Reston ebolavirus, Hendra virus, Monkeypox virus, and SARS coronavirus. In this case, like the other viruses mentioned, the pathogen was detected by cell culture and was subsequently identified by EM. Without the assistance of EM the relevant molecular assay would not have been chosen to further identify the virus.

Competing interests

None declared.

Ethical approval

Ethical approval was given and covered under the New York State Department of Health Institutional Review Board study #07-022 and #02-054.

Acknowledgments

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