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Adenovirus Type 3 Outbreak in Connecticut Associated with A Novel Variant

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Abstract

An adenovirus outbreak occurred in New Haven, Connecticut in 2006-2007. Molecular typing revealed a 2-fold increase in adenovirus type 3 infections. Restriction enzyme analysis indicated that the CT outbreak was largely due to a marked increase in the novel Ad3a51 strain. This outbreak represents the first detection of Ad3a51 in the United States. While most infections were mild, children under 3 were at increased risk for severe disease and one patient with underlying disease died.

Keywords

Adenoviridae; Adenovirus; epidemiology; respiratory tract diseases; communicable diseases; infectious diseases; emerging; outbreak

Adenovirus infections are associated with a variety of clinical syndromes, and are usually self-limited. However, severe illness has been reported, especially in military recruits and compromised hosts, but has also been associated with new genomic variants [Kajon et al., 1990; Louie et al., 2008]. In order to investigate the current epidemiology of adenovirus infections in the United States, the University of Iowa Center for Emerging Infectious Diseases (CEID) and 22 US laboratories collaborated in a national surveillance program for emerging adenovirus infections from July 2004 through April of 2007. Laboratories routinely sent adenovirus-positive clinical specimens to the University of Iowa where the specimens were sequence typed using a hexon gene typing strategy [Lu and Erdman, 2006]. The collaboration resulted in the characterization of more than 3000 clinical adenovirus isolates. In November 2006, the Clinical Virology Laboratory at Yale New Haven Hospital (YNHH) in New Haven, Connecticut, experienced a marked increase in the number of clinical specimens that were adenovirus positive. This report summarizes an investigation into this increase.

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Methods

Patient samples

Samples were submitted to clinical laboratories for diagnostic testing and were not solicited for the purposes of the study. Specimens positive for adenovirus by direct fluorescent antibody staining (DFA), PCR, other rapid test, or culture were sent to the University of Iowa from 22 participating laboratories, including 14 civilian and 8 military sites, for typing and further analysis. Prior to participation, the study was approved by each institution's Institutional Review Board.

Clinical information

Limited demographic and clinical data was also submitted by each laboratory: collection date, collection site, specimen source, patient birth date, gender, hospitalized when specimen was collected, and if the subject had received a cellular or solid organ transplant during the previous six months. Because of their risk for severe disease, additional information was obtained from adenovirus patients <7 yrs of age or who had received a transplant procedure within six months of their adenovirus detection: race and ethnicity, clinical presentation, hospitalization, intensive care, disposition, cancer, and chronic disease.

After recognition of the CT adenovirus outbreak, supplemental clinical information was obtained on the 218 Ad3 infected YNHH patients regardless of age and transplant status: diagnoses of tonsillitis, otitis media, bronchiolitis, croup, or gastrointestinal symptoms, and history of asthma.

Laboratory analysis

At the University of Iowa's CEID, viral DNA was extracted from submitted specimens or cultures, and analyzed according to published procedures to determine adenovirus type and genotype [Adrian et al., 1989; Arens and Dilworth, 1988; Golovina et al., 1991; Guo et al., 1988; Li and Wadell, 1988; Lu and Erdman, 2006].

Statistical analyses

The χ^2 test and Fisher's exact test were used to examine potential risk factor associations. The proportional odds model was used to examine risk factors for severe adenovirus infection. Analyses were performed using SAS, version 9.1 (SAS Institute).

Results

From November 2006 to April 2007, compared to the previous two years, YNHH experienced a nearly 2-fold increase in Ad3 observed among YNHH adenovirus positive specimens (Fig. 1). While the number of other adenovirus types detected was stable over time, the sudden increase in Ad3 specimens explained the overall increase in adenovirus detections. Prior to November 2006, YNHH submitted 214 adenovirus specimens of unique patient/event to the national adenovirus surveillance network and 75 (35.0%) were typed as Ad3 (data not shown). From November 2006 to April 2007, YNHH submitted 183 adenovirus specimens and 143 (78.1%) were typed as Ad3 (data not shown). A total of 193 of the 218 Ad3 identified at the YNHH Clinical Virology Laboratory were analyzed by restriction enzyme (RE) digest patterns and revealed all to be of the 3a genome group. The remaining 25 Ad3 identified were either typed following the April 2007 cutoff or were unable to yield viable precipitated DNA for RE analysis (REA).

In a recent study we reported the identification of two new Ad3 variants Ad3a50 and Ad3a51 (LeBeck MG, Capuano AW, Schnurr DP, Kajon AE, Setterquist SF, Heil GL, et al. Two new

genotypes of adenovirus type 3 identified from a US National Surveillance Study, 2004-2007, submitted). The CT outbreak is largely explained by a marked increase in the clinical detections of the novel Ad3a51 strain (Table 1 and Figure 1). To the authors' knowledge, Ad3a51 has never been detected in the United States before the CT outbreak (Table 1).

In general, the outbreak patients were healthy children <7 yrs of age, who suffered from acute respiratory infection but were not hospitalized. No statistically significant differences were identified in any of the variables examined before or during the outbreak (Table 1). Supplemental clinical information subsequently obtained on all YNHH Ad3 infected patients showed directionally higher incidence of wheezing, rash and asthma among patients infected with the newly described variant types, Ad3a50 and Ad3a51 compared to Ad3a2 and other Ad3. However, these differences did not reach statistical significance (data not shown).

Multivariable risk factor modeling (Table 2) for adenovirus 3 disease severity (death or intensive care unit stay vs. hospitalization vs. other disease) revealed that age was an important factor (controlling for chronic diseases). Specifically, children under 2 years of age (OR 5.9; 95% CI 1.9 to 19.0) and from 2 to 3 years of age (OR 3.7; 95% CI 1.1 to 12.2) were at increased risk of severe Ad3 clinical disease when compared to subjects above. When modeling for severe adenovirus infection or for pneumonia, no statistically significant risk factor associations were found.

While most infections were mild, ten of the outbreak patients developed pneumonia and one of the outbreak patients died (patient infected with Ad3a51). The one death reflects the only Ad3-associated death reported from among the 576 Ad3 isolates collected from among the 14 civilian sites during our surveillance. The patient was a child of 3 years and 11 months of age with a congenital oral-facial condition. She was doing well until she developed adenovirus pneumonia in January 2007. She was admitted to the hospital and died one month after admission.

Discussion

Adenovirus type 3 is most commonly recognized as a cause of pharyngoconjunctival fever, sometimes with gastrointestinal symptoms. Outbreaks associated with childrens' camps, swimming pools or lakes, chronic care facilities and hospitals have been reported [Brummitt et al., 1988; Chang et al., 2008; Kajon et al., 1990; Li and Wadell, 1988; Singh-Naz et al., 1993; Zarraga et al., 1992]. Although rare, severe morbidity and deaths due to Ad3 can occur [Barker et al., 2003; Brummitt et al., 1988; Singh-Naz et al., 1993; Zarraga et al., 1992]. Epidemic outbreaks of Ad3 involving several genome types of the same serotype have been documented [Li and Wadell, 1988]. Most recently a community outbreak of Ad3 was reported in Taiwanese children [Chang et al., 2008]. Only 15 of 172 isolates from the outbreak were typed by RFLP and all belonged to genotype Ad3a2.

In the present study, 161 Ad3 positive specimens were genotyped by REA. Our findings are consistent with findings of previous Ad3 outbreak investigations in its demonstration of Ad3 genomic variability [Barker et al., 2003; Brummitt et al., 1988; Foy et al., 1968; Price et al., 2001; Singh-Naz et al., 1993; Zarraga et al., 1992]. In particular, the discovery of two newly described variant types, Ad3a50 and Ad3a51 may explain not only the dramatic rise in incidence of Ad3 infections over such a brief period of time, but also the potential for these new variants to cause marked morbidity among susceptible populations.

This study has a number of limitations. Due to the specialized and tedious nature of whole genome REA, it is rarely performed. Thus there is no central repository of reference material with which to compare our findings. However, we comprehensively studied the literature and are convinced of the uniqueness of the reported novel viral strains. While it is possible that our

claim of the newness of the Ad3a51 strain is inaccurate, in another work we examined Ad3 specimens collected in California back to 1963 and found no evidence of this strain [Barker et al., 2003; Foy et al., 1968; Price et al., 2001] (LeBeck MG, Capuano AW, Schnurr DP, Kajon AE, Setterquist SF, Heil GL, et al. Two new genotypes of adenovirus type 3 identified from a US National Surveillance Study, 2004-2007, submitted). The application of this technology has the potential to advance our understanding of outbreaks and severe infections.

In summary, an outbreak of adenovirus disease occurred in New Haven, CT from November 2006 to April 2007. Molecular typing revealed a 2-fold increase in Ad3 infections. Restriction enzyme analysis identified three variant types, including two newly described variants, Ad3a50 and Ad3a51. The CT outbreak was largely explained by a marked increase in the novel Ad3a51 strain. This outbreak represents the first detection of Ad3a51 in the United States. Although most infections were mild, children less than 3 were at greater risk of severe disease.

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Fig. 1.

Total number of adenovirus-positive clinical specimens (bar), with all adenovirus type 3 (solid line), variant Ad3a50 and variant Ad3a51 positive specimens (dotted lines), Clinical Virology Laboratory, Department of Laboratory Medicine, Yale New Haven Hospital, New Haven, Connecticut, July 2004 to April 2007.

Table 1
Characteristics of patients infected with adenovirus type 3 in US national surveillance, July 2004 – Apr 2007, from medical record information based upon unique clinical events associated with a positive adenovirus specimen among recent transplant patients or patients younger than 7 years of age

Variables	All US clinical laboratory sites (n=576) n (%)	Yale New Haven Hospital, Connecticut	
		Before outbreak Jul. 2004 - Oct. 2006 (n=60) n (%)	Outbreak period Nov. 2006 - Apr. 2007 (n=121) n (%)
Age group			
< 7 years old	562	59 (98.3)	121 (100)
≥ 7 years old	14	1 (1.7)	0 (0)
Gender			
male	324	35 (58.3)	71 (58.7)
female	252	25 (41.7)	50 (41.3)
Immunocompromised			
Cancer	2	0 (0)	0 (0)
Cancer and transplant	8	1 (1.7)	0 (0)
Nothing reported	545	59 (98.3)	121 (100)
Transplant	21	0 (0)	0 (0)
Cancer types			
None reported	566	59 (98.3)	121 (100)
Bone, connective tissue, skin or breast	1	0 (0)	0 (0)
Lymphatic and hematopoietic tissue	8	1 (1.7)	0 (0)
Respiratory or intrathoracic organs	1	0 (0)	0 (0)
Transplant type types			
None reported	561	59 (98.3)	121 (100)
Bone marrow	12	1 (1.7)	0 (0)
Kidney	1	0 (0)	0 (0)
Liver	1	0 (0)	0 (0)
Lung	1	0 (0)	0 (0)
Upper respiratory tract infection			
Not reported	279	8 (13.3)	8 (6.6)
Yes	297	52 (86.7)	113 (93.4)
Pneumonia			
Not reported	520	54 (90)	111 (91.7)
Yes	56	6 (10)	10 (8.3)
Conjunctivitis			
Not reported	496	54 (90)	95 (78.5)
Yes	80	6 (10)	26 (21.5)
Encephalitis			
Not reported	572	60 (100)	119 (98.4)

Variables	All US clinical laboratory sites (n=576) n (%)	Yale New Haven Hospital, Connecticut	
		Before outbreak Jul. 2004 - Oct. 2006 (n=60) n (%)	Outbreak period Nov. 2006 - Apr. 2007 (n=121) n (%)
Yes	4	0 (0)	2 (1.7)
Days in the hospital			
Missing	22	0 (0)	0 (0)
No, unknown	304	46 (76.7)	104 (86)
Yes, 0 - 3 days	144	8 (13.3)	10 (8.3)
Yes, 4 - 506 days	104	6 (10)	7 (5.8)
Yes, number of days unknown	2	0 (0)	0 (0)
Intensive care unit days			
Missing	1	0 (0)	0 (0)
No, unknown	545	58 (96.7)	118 (97.5)
Yes, 1 - 11 days	16	1 (1.7)	2 (1.7)
Yes, 12 - 763 days	11	1 (1.7)	1 (0.8)
Yes, number of days unknown	3	0 (0)	0 (0)
Death associated			
Missing	1	0 (0)	0 (0)
No/unknown	572	60 (100)	120 (99.2)
Yes, other cause or unknown	2	0 (0)	0 (0)
Yes, primarily due to adenovirus	1	0 (0)	1 (0.8)
Chronic disease			
Missing	1	0 (0)	0 (0)
No	452	57 (95)	113 (93.4)
Yes	73	3 (5)	8 (6.6)
Unknown	50	0 (0)	0 (0)
Adenovirus 3 variant type*			
Ad3a2	134	14 (23.3)	11 (9.1)
Ad3a50	100	21 (35)	25 (20.7)
Ad3 a51	74	12 (20)	60 (49.6)
Other Ad 3	59	12 (20)	6 (5)
Not Typed	209	1 (1.7)	19 (15.7)

* Statistically significant difference (before outbreak versus outbreak period, excluding "Not Typed") with Chi-Square.

Table 2

Risk factor for ordinal adenovirus severity (Died/ICU, hospitalized vs. others) using the proportional odds model among Yale New Haven Hospital recent transplant patients or patients younger than 7 years of age infected with REA typed adenovirus type 3, with medical record information and based upon unique clinical events associated with a positive adenovirus specimen.

Variables	n	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Age group			
0-1	47	4.1(1.4-11.9)	5.9(1.9-19)
2-3	41	2.9(0.9-9)	3.7(1.1-12.2)
≥4	73	reference	reference
Gender			
Male	95	1.2(0.5-2.8)	---
Female	66	reference	---
Respiratory disease			
Yes	147	0.3(0.1-1)	---
No/Unknown	14	reference	---
Period			
Outbreak	102	0.7(0.3-1.6)	---
Before outbreak	59	reference	---
Adenovirus 3 variant type			
Ad 3a2	25	reference	---
Ad3 a50	46	6.9(0.8-57.2)	---
Ad3 a51	72	4.3(0.5-35.5)	---
Others Ad3	18	8.9(0.9-84.6)	---
Conjunctivitis			
Yes	29	0.5(0.1-1.8)	---
No/Unknown	132	reference	---
Chronic disease			
Yes	10	6.1(1.7-22.1)	10.5(2.5-43)
No/Unknown	151	reference	reference